

# XX<sup>TH</sup> WORLD CONGRESS OF PSYCHIATRIC GENETICS

*Confronting the Complexity of Brain and Behavior*



## PRESENTATION ABSTRACTS



**October 14-18th, 2012**  
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**ISPG**

The International Society of Psychiatric Genetics



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**EDUCATIONAL  
SESSION  
ABSTRACTS**

## Educational Sessions

### Educational Session 1

#### Genetics of Bipolar Disorder

Sven Cichon<sup>1,2</sup>, Nick Craddock<sup>3</sup>

<sup>1</sup>University of Bonn, <sup>2</sup>Research Center Juelich, <sup>3</sup>Cardiff University

Bipolar disorder (BD) is a highly heritable disorder of mood with a lifetime prevalence of approximately 0,5-1% in all populations world-wide. The disorder is characterized by recurrent episodes of mania and depression which are often accompanied by behavioural and cognitive disturbances. In this educational session, Nick Craddock will provide an overview of the phenotype definition and diagnostic issues, followed by results from formal genetic studies that allow an estimation of the heritability of BD. Subsequently, Sven Cichon will summarize efforts to identify the genetic factors involved in the disorder, with a focus on recent genome-wide association studies. At the end of the session, attendees should have an overview of the phenotype, our current understanding of the genetic architecture of BD, as well as the challenges clinicians and geneticists are facing.

### Educational Session 2

#### Imaging Genomics for Psychiatric Disorders

Sophia Frangou<sup>1</sup>

<sup>1</sup>Institute of Psychiatry, King's College London

Advances in psychiatric genetics, including large genome-wide association studies (GWAS) have identified multiple genetic variants that increase the risk for psychiatric disorders. Neuroimaging provides a unique tool for the assessment of the impact on identified risk genes on brain structure and function. Overview of key imaging genomics studies focusing on reported genotype-phenotype associations and on the methodological approaches used. The most extensive information available describes the structural and functional impact of risk genes for schizophrenia, bipolar disorder, autism spectrum disorders and neurodegenerative disorders (e.g. Alzheimer's disease). Initial studies have focused on single genetic risk factors while the field is now moving towards greater methodological sophistication that will permit multimodal and multi-layered approaches to modelling multiple risk factors or genetic networks to neuroimaging data. Advances in genetics and neuroimaging offer new avenues for the identification of pathophysiological pathways into mental illness.

### Educational Session 3

#### Schizophrenia Genetics

Michael O'Donovan<sup>1</sup>

<sup>1</sup>*Cardiff University*

It has long been known that schizophrenia has high heritability, but as for other complex disorders, identifying the specific genes responsible has been a major challenge. However, through the technological revolution in genomics, and by the deployment of very large samples through international collaboration, highly significant evidence for the involvement of around 15 common genetic variants has emerged, each making a small contribution to risk of the disorder, and it also clear from multi-locus tests that perhaps a thousand or more such risk variants exist. There is also substantial evidence that these common variants overlap with those that confer risk for bipolar disorder, and emerging evidence that they also overlap with those conferring risk of other disorders. The same developments that have allowed GWAS have also identified a number of abnormalities of chromosome structure with clear evidence for their involvement in schizophrenia and that these occur as de novo mutations in about 5% of cases. Although rare, the CNVs confer a substantial increase in risk of schizophrenia to carriers as well as to other neurodevelopmental phenotypes including ADHD, Autism and Intellectual Disability. Early whole-exome sequencing studies have not yet identified additional variation, but show some promise to do so. Although only a small amount of the total genetic variation that contributes to schizophrenia has been allocated to specific DNA variants, recent findings have strongly implicated specific aspects of neuronal function that are of importance in the pathogenesis of psychosis.

### Educational Session 4

#### How to Make GWAS Successful

Stephan Ripke<sup>1,2</sup>, Sara Pulit<sup>3</sup>

<sup>1</sup>*Massachusetts General Hospital*, <sup>2</sup>*The Broad Institute*, <sup>3</sup>*Brigham and Women's Hospital*

We are now more than 5 years into the era of the genome-wide association study. Especially in psychiatric research, these broad, unbiased attempts were promising but unsuccessful in the beginning. It soon became clear that, given the modest impact of any one gene in disorders with hundreds of contributing factors, a major key to success is increasing sample size. Assembling such sample sizes could only be achieved in a collaborative effort with the formation of international consortia and widespread, unprecedented levels of data sharing among investigators. This picture is consistent across all "complex" disease, where only through the combined power of many research groups has much of the formerly unknown genetic structure of disease been revealed. In this session the presenters will give a personal overview on how to approach GWAS datasets. We will give a general overview over quality control, population stratification, sample overlap testing, association, polygene scoring, replication, presentation of results, and testing across classic phenotype boundaries. Additionally we will present common problems and pitfalls analysts of every experience level might face. Throughout the session we will show comparisons to other complex diseases to display the similarities and the generalization of GWAS. Based on those we will also try to have a glimpse at future projections for psychiatric genetics.

## Educational Session 5

### Evolutionary Aspects of Psychiatric Disorders

Matthew Keller<sup>1</sup>

<sup>1</sup>*University of Colorado at Boulder*

Given that natural selection is so powerful at optimizing complex adaptations, why does it seem unable to eliminate genes (susceptibility alleles) that predispose to common, harmful, heritable mental disorders, such as schizophrenia or bipolar disorder? In this talk, I assess three leading explanations for this apparent paradox from evolutionary genetic theory: ancestral neutrality (susceptibility alleles were not harmful among ancestors), balancing selection (susceptibility alleles sometimes increased fitness), and polygenic mutation-selection balance (mental disorders reflect the inevitable mutational load on the thousands of genes underlying human behavior). Data on mental disorder prevalence rates, fitness costs, the likely number and allelic spectra of susceptibility alleles, and the increased risks of mental disorders with brain trauma, inbreeding, and paternal age provide important information about the relative merits of these three evolutionary explanations for mental disorders.

## Educational Session 6

### Analysis of Rare Variants

Benjamin Neale<sup>1</sup>

<sup>1</sup>*Analytic and Translational Genetics Unit, Massachusetts General Hospital*

With the development of sequencing technologies near comprehensive capture of genetic variation is now a reality. Much of this newly discovered variation is rare and as a consequence requires different analytic strategies to those traditionally used to assess evidence for association for common variation. Recent developments in the analysis of rare variants can be classed into two domains: burden approaches such as GRANVIL and the combining and collapsing method which assess differences in the rate or amount of rare variation across groups and distributional approaches such as SKAT and C-alpha which assess whether the pattern of rare variation observed in the sample correlates with phenotype. The distributional approaches are robust to variation that may increase or decrease risk or a phenotypic value while the burden approaches assume rare variation in the region affect the phenotype in a consistent direction. Beyond these methods, other classes of rare variation such as spontaneously arising mutations necessitate tailored analytic methods. In this session, all of these methods will be reviewed to provide a global view of how best to leverage rare variation for identifying regions of interest in psychiatric disease and behavioral phenotypes.

## Educational Session 7

### Psychiatric Epigenetics: An Introduction

James Potash<sup>1</sup>

<sup>1</sup>University of Iowa

The field of epigenetics explores the molecular mechanisms behind differential gene expression and the way in which related molecular marks are passed on during cell division. The most studied of these marks involve DNA methylation and histone modifications. The study of brain epigenetics can advance our understanding of the etiology of psychiatric illnesses and also illuminate the essential interrelatedness of “nature” and “nurture,” through integrating the effect of genetic and environmental variation. Inherent challenges for psychiatric epigenetics include the inability to easily study the primary site of pathology, and the variability that exists between regions and cell types even within the brain. Progress has been made in the study of a few particular genes, such as BDNF in a stress model of depression, and RELN in schizophrenia. There is some evidence in both of these cases that medications that can reverse the epigenetic changes observed. Other intriguing findings have been those of stress-induced epigenetic modifications of genes involved in the HPA axis stress response. Differential DNA methylation of the glucocorticoid receptor gene was observed in mice subjected to early-life stress, and childhood abuse in humans has been associated with similar persistent DNA methylation changes. Another central HPA axis gene, FKBP5, whose product is a regulator of glucocorticoid receptor activity, has been shown to be up-regulated by glucocorticoid exposure in mice through a decrease in DNA methylation. Recent work aims to further dissect these relationships by distinguishing between different cell types, as, for example, neurons and glial cells may vary in their baseline methylation patterns. Genome-wide approaches are now being employed in these investigations. Some ongoing work seeks to integrate epigenomic with genomic variation. The question of how epigenomic insights might translate into new therapies is also being studied.

## Educational Session 8

### Next-Generation Data Integration

Alexander Niculescu<sup>1</sup>, Thomas Schulze<sup>2</sup>

<sup>1</sup>Indiana University School of Medicine, <sup>2</sup>University Medical Center Göttingen

We will provide an overview for how convergent genomic and phenomic approaches may lead to better outcomes, for psychiatric research in general, and for psychiatric genetics in particular.

### Convergent Functional Genomics of Psychiatric Disorders: From Comprehensive Understanding to Genetic Risk Prediction

Alexander B. Niculescu, MD, PhD

We have developed and used over the last 12 years a translational Convergent Functional Genomics (CFG) approach to identify genes involved in psychiatric disorders, by gene-level integration of genetic and gene expression studies in humans and animal models. Using this polyevidence scoring we have identified and prioritized top genes, blood biomarkers, pathways and mechanisms. In addition, we show how the top candidate genes identified by CFG can be used to generate a genetic risk prediction score (GRPS) and blood biomarker scores, to aid diagnostics, with predictive ability in independent cohorts. We also show increasing overlap, reproducibility and consistency of findings from SNPs to genes, then genes prioritized by CFG, and ultimately at the level of biological pathways and mechanisms. Lastly, we compared top candidate genes for schizophrenia, bipolar disorder and anxiety disorders from CFG analyses conducted by us. Overall, our work helps map the genomic and biological landscape for psychiatric disorders, providing leads towards a better understanding of illness, diagnostics, and therapeutics. It also reveals the significant genetic overlap among major psychiatric disorder domains, suggesting the need for an improved multi-dimensional nosology.

### The Phenotype—The Still Unknown Quantity in Psychiatric Genetics?

Thomas G. Schulze

Research into the genetic basis of psychiatric disorders has reached a turning point. Genome-wide association studies (GWAS), encompassing several thousand samples, have produced replicated evidence for some novel susceptibility genes; however, the genetic variants implicated so far account for only a fraction of disease liability, a phenomenon not limited to psychiatric phenotypes but characteristic of all complex genetic traits studied to date. It appears that pure genomic approaches, such as GWAS alone, will not suffice to unravel the genetic basis of psychiatric phenotypes. Apart from sophisticated genomic, epigenomic, and neurobiological approaches, the comprehensive study of the phenotype will be a hallmark of 21st century psychiatric genetics. The analysis of cross-sectional, categorical disease phenotypes by traditional statistical tools alone will have to be replaced by novel approaches modeling the complexity of psychiatric phenotypes. A framework is presented that encompasses strategies (1) to ascertain longitudinal phenotypes, (2) to systematically and prospectively assess environmental influences on phenotypic presentations, (3) to delineate phenotypes for pharmacogenetic studies, and (3) to define robust genotype-phenotype signatures or patterns using novel data-mining tools. This presentations will be given in a clearly educational style. The focus will be on concepts rather than data. At the end of the session we will reserve 10 minutes for general discussion/Q&A.

## Educational Session 9

### Pharmacogenomics in the Post-GWAS Era: Study Design and Analysis Approaches for the Next Generation of Pharmacogenomics

Marylyn Ritchie<sup>1</sup>, Wolfgang Sadec<sup>2</sup>, Brooke Fridley<sup>3</sup>, Eli Stahl<sup>4</sup>  
<sup>1</sup>Pennsylvania State University, <sup>2</sup>Ohio State University Medical Center,  
<sup>3</sup>Mayo Clinic, <sup>4</sup>Brigham and Women's Hospital

Personalized medicine has advanced as one of the predominant strategic initiatives and goals of the next decade for many pharmaceutical companies, biotech institutes, and academic medical centers. The primary goal of this type of initiative is to treat patients with the correct dose of the appropriate medication based on their individual demographic and genomic makeup. Pharmacogenetics and pharmacogenomics have made the dreams of personalized medicine a reality. Pharmacogenetics is the study of a single genetic variant with a drug response phenotype, such as treatment responders and non-responders or a serious adverse side effect. As molecular technologies to assay the entire genome developed and genome-wide association studies (GWAS) emerged, so did pharmacogenomics (surveying the entire genome for associations with drug response phenotypes). As with other genetic traits and diseases, it is hypothesized that variability in drug response is due to underlying individual variation in genetic architecture. This drug response can include efficacy, serious adverse events, or variability in target or maintenance dose. In general, pharmacogenetic and pharmacogenomic study designs and analysis approaches are very similar to standard human genetic association studies, however there are some subtleties that should be considered and will be described in this educational session.

In the first presentation, the general topic of Pharmacogenomics will be described. This will provide the motivation for the remainder of the educational session – namely the power of pharmacogenomics study designs for human traits. Wolfgang Sadec, Chair of Pharmacology from Ohio State University will give this presentation. The conclusions of this presentation will set the stage for the remaining three talks. Next, Brooke Fridley from the Mayo Clinic, will present general analysis strategies for pharmacogenomics. There are a number of subtleties in this area that provide greater power for gene\*drug interactions. She is an excellent presenter of biostatistical topics; therefore participants will learn a lot from this presentation. In the following presentation, Eli Stahl of the Brigham Women's Hospital will discuss the challenges of estimating the heritability of pharmacogenomics traits. A polygenic modeling approach can provide ample power to estimate this heritability and Eli will describe this approach and its current successes in pharmacogenomics. Lastly, Brooke Fridley of The Pennsylvania State University will describe systems pharmacogenomics, which includes data integration methods to combine genetic, genomic, and other “-omics” datasets to model complex trait architecture. Systems biology and network models have been implemented in many traits in human genetics to attempt to make sense of the missing heritability. Significant progress has been made in systems pharmacogenomics and these will be discussed in this presentation. These four presentations will result in a 60 minute educational session on the latest advances in pharmacogenomics. This session will provide participants with a wealth of knowledge for future pharmacogenomics studies.

## Educational Session 10

### Registry-Based Research in Psychiatric Diseases

Preben Bo Mortensen<sup>1</sup>

<sup>1</sup>National Centre for Register-based Research, Aarhus University

Registry-based research has been pivotal in studies of the occurrence of, and risk-factors for, a wide range of mental disorders. Studies from National registers, in particular from Scandinavia, have contributed to the genetic epidemiology of schizophrenia and other mental illnesses, studies of early risk factors, and also of mortality, somatic co-morbidity and other aspects of course and outcome. In this presentation I will provide examples of the use of registers in psychiatric research, both in pure register-based studies and in studies where registers provide a population based sampling frame for genetic and other biologically oriented studies. I will review strengths, limitations and caveats when using register data, give examples of efficient sampling strategies, and also give examples of some of the pitfalls and potential biases that can arise in this type of research. In particular I will highlight how the combination of registers and biobanks can provide key data to elucidate the relative importance and potential interaction of genetic and environmental risk factors for diseases like schizophrenia.

# **PLENARY ABSTRACTS**

## Plenary Sessions

### Plenary Session 1

#### Architecture of the Human Brain

Karl Zilles<sup>1</sup>

<sup>1</sup>*Institute of Neuroscience and Medicine*

The brain is an extremely inhomogeneous organ. Its complex organization is genetically determined, but modified by the environment. A combined multiscale (from molecules to large neural circuits) and multimodal (chemical, anatomical and physiological) analysis of spatially segregated but interconnected cerebral units is, therefore, necessary for understanding the structural and functional architecture of the brain. Segregation of cognitive functions (functional architecture), localization of structures underlying those functions (cyto- and myeloarchitecture), and their molecular basis (receptor architecture) will be demonstrated in the healthy and pathologically compromised human brain. Since cortical units are nodes in a net, the presentation will be finished by demonstrating the structural connectivity of the human brain using a novel approach.

### Plenary Session 2

#### The Genomic Architecture of Psychiatric Disorders

Mark J. Daly<sup>1</sup>

<sup>1</sup>*Massachusetts General Hospital*

Psychiatric genetics has undergone a revolution in the last decade, with unprecedented progress made in both our general understanding of genomic architecture of neuropsychiatric disease and, often for the first time, specific genetic variants conclusively linked to disease risk. Many factors, including dramatic advances in technology, our understanding of the genome, and a newfound commitment to collaborative science, have been integral to the progress made to date and even more so to ongoing efforts towards further discovery. In this talk I will provide an overview of the advances made primarily in the last five years and articulate how diverse studies designed to approach both rare and common genetic variation are pointing to a consistent view of the overall genomic architecture. I will then discuss the paths forward, focusing principally on two areas - how genome sequencing technologies are further advancing the available options for genetic studies and offer the potential to deliver more precise insights into the biological causes of disease, and how the results of collaborative efforts to study both rare and common variation are beginning to deliver hard targets for biological inquiry. Ultimately it is these insights that hold the best hope yet for delivering on the promise of human genetics - to identify potentially preventable or reversible root biological causes of disease.

### Plenary Session 3

#### What Can We Learn from the Study of Other Species for Human Brain Behavior Disorder?

Kerstin Lindblad-Toh<sup>1</sup>

<sup>1</sup>*The Broad Institute*

The search for genetic risk factors underlying human common disease has been complicated by the fact that many risk factors of small effect contribute to the disease, and that many loci identified by genome-wide association mapping fall outside protein coding genes. This diversity of risk factors makes development of personalized treatment options both necessary and complicated. The dog offers a complementary approach to studies in humans; this model system can be highly effective based on a unique breed structure that has led to the enrichment of risk factors of stronger effect within breeds. Briefly, the domestic dog encompasses hundreds of genetically isolated breeds, many of which show an increased risk for certain diseases. With the availability of the canine genome sequence, an understanding of the canine genome structure and availability of disease gene mapping tools, we are now in a unique position to map canine disease genes to inform human biology and medicine. The genetic homogeneity and enrichment for certain stronger genetic risk factors accumulated within specific breeds yields a cleaner picture of disease risk and greater ease of finding these risk factors (hundreds of samples suffice to map complex traits). In recent years, many monogenic and complex traits of human relevance have been identified. For some of these diseases, we find different genes and pathways predisposing to the disease in different breeds. For others a key pathway seems to prevail. For many of our studies the goal of creating a greater and more complete understanding of the different pathologic mechanisms leading to each disease. The dog has been proposed as an excellent model for behavioral studies including both pathological conditions such as Canine compulsive disorder (CCD) and for personality traits. Our group is working on both aspects. CCD manifests as time-consuming, repetitive behaviors, causing severe stress and functional impairment. CCD derives from normal canine activities such as grooming, predation and suckling and when exaggerated manifest as acral lick dermatitis, tail chasing/fly snapping, and flank/blanket sucking, respectively. These disorders affect many breeds, but the phenotypes varies depending on the breeds. For example, CCD in Doberman pinschers manifest as flank sucking, while bull terriers are vulnerable to spinning. We have performed a genome-wide association analysis using 92 rigorously phenotyped Doberman Pinschers cases and 68 controls and identified a major novel candidate gene for compulsive disorders, the neural cadherin CDH2. The pattern of inheritance in the breed suggests a complex inheritance. Not surprisingly, additional signals of associations are found and together implicate additional genes potentially linked to the cortico-striatal-thalamo-cortical (CSTC) circuit. Resequencing regions of CCD association regions in affected dogs and healthy controls, has identified candidate mutations that are now undergoing further analysis. Resequencing of the same candidate genes and their regulatory elements has also been performed in human patients and more in depth functional characterization of CDH2 is being performed in mouse models. Separately, we are building a program to map behavioural traits in the dog based on the unique Swedish resource, where thousands of two-year-old dogs undergo a standardized behaviour test each year. These personality traits measured include chasing, aggression, sociability, playfulness and curiosity. Based on the behavioural diversity within dogs this may help understand behaviour in an unprecedented way.

### Plenary Session 4

#### Novel Approaches to Psychiatric Drug Discovery: Impact of Psychiatric Genetics

Bryan L. Roth<sup>1</sup>

<sup>1</sup>*University of North Carolina at Chapel Hill*

GPCR functional selectivity—the process whereby ligands specifically engage distinct signaling pathways—is now a well-established and validated pharmacological concept. Thus, as we and many others have demonstrated, GPCR ligands can be identified which differentially activate canonical G-protein signaling pathways, b-arrestin-mediated signaling and non-G protein/non-arrestin-ergic signaling. With respect to D2-dopamine receptors, we have identified ligands which appear to possess extreme patterns of functional selectivity with bias for either Gi- or b-arrestin signaling pathways. In the first part of the talk I will highlight these results and show how they have revealed novel therapeutic approaches for psychiatric drug discovery. These findings, and similar results by many others, inspired us to develop a genome-wide platform suitable for interrogating the entire complement of druggable, non-olfactory GPCRs in a simultaneous and massively parallel fashion. Using this platform we hoped to discover new small molecules which can target, for instance, GPCR-b-arrestin signaling. To accomplish this task we synthesized a codon-optimized library encompassing essentially all the non-olfactory, druggable GPCRs in the human genome (NOD-GPCR). Using this resource, the entire genomic complement of NOD-GPCRs can be screened in a simultaneous and massively parallel fashion. Toward the end of the talk, I will present new and unpublished data whereby this unique resource has been used to profile essentially all known psychiatric medications (both approved and investigational) and will demonstrate that the overwhelming majority possess significant off-target b-arrestin signaling potentialities across the NOD-GPCR-ome. The relevance of these findings for psychiatric geneticists and the next era of psychiatric drug discovery will be highlighted. Supported by U19MH82441, the NIMH PDSP and the Michael Hooker Distinguished Chair to BLR.

## Plenary Session 5

### Missing Heritability Revisited

Peter Visscher<sup>1</sup>

<sup>1</sup>*Queensland Brain Institute*

To revisit the perceived problem of ‘missing heritability’ for complex traits, including psychiatric disorders, we will discuss the definition of heritability and how it is estimated, the definition of what is missing and the possible explanations of missing heritability in the light of empirical evidence from pedigree, association and sequencing studies.

## Plenary Session 6

### What Phenotypes Should Psychiatric Geneticists Focus On?

Trevor Robbins<sup>1</sup>

<sup>1</sup>*University of Cambridge*

Neurocognitive endophenotypes in neuropsychiatry refer to characteristic behavioural traits that can be linked to particular neural circuitry that may become dysfunctional in neuropsychiatric disorder. Defining such endophenotypes may eventually provide more accurate nosology and diagnosis, help to predict vulnerability or resilience, provide purer clinical populations for trials, rationalise comorbidities and enhance the power of psychiatric genetics. Endophenotypes may also help to define causal factors in diverse psychopathologies. Moreover, experimental animals can be utilised to facilitate this approach. I will illustrate this approach initially with the example of impulsivity in stimulant drug abuse. It will then be expanded to include other neuropsychiatric disorders including ADHD, obsessive-compulsive disorder, schizophrenia and dementia. Limitations in the approach will also be considered. Overall, the importance of defining refined phenotypes for investigations of psychiatric genetics will be argued, from the special perspective of using more sophisticated cognitive and behavioural measurements.

## Plenary Session 7

### **A Decade of Studies Investigating Gene X Environment Interactions and the Risk for Alcohol Abuse and Related Psychopathology: A Nonhuman Primate Model**

James Dee Higley<sup>1</sup>

<sup>1</sup>*Department of Psychology, Brigham Young University*

In 2003, landmark research, rated at the time as the second most important discovery of the year, showed that phenotypic outcomes in behavior and neurobiology depend on specific environments interacting with genotype. In one environment, two individuals may develop quite differently, depending on the specific genes that are inherited. On the other hand, two individuals with the same genetic heritage may show profoundly different developmental outcomes, depending on the different environments in which they develop. We will present more recent data from nonhuman primates showing that such gene X environment interactions in risk for alcohol abuse disorders and related psychopathology is not limited to early experience, but also to the current setting, sex, and other variables, and that the effects seem to generalize to other genotypes as well.

## Snow and Ming Tsuang Lifetime Achievement Plenary Session

### **45 Years Psychiatric Genetics: A Perspective from Iowa**

Raymond R. Crowe<sup>1</sup>

<sup>1</sup>*University of Iowa*

For 45 years the Psychiatry Department at the University of Iowa has played a major role in researching the genetics of mental disorders. Many of the faculty have been members of the ISPG. A number of them have played important roles in shaping the development of Psychiatric Genetics as a discipline. Their research has included family and adoption studies, linkage and association studies, and more recently participation in collaborations to search for genes. Thus, activity at Iowa has reflected state of the art strategies as Psychiatric Genetics has evolved over the past 45 years. This talk will review the growth of Psychiatric Genetics from the perspective of research activity at Iowa.

# **SYMPOSIA ABSTRACTS**

## Symposia Sessions

### Symposium 1

#### PGC Cross Disorder and Pathway Analysis Group: Results from SNPS to Pathways

**Chair: Gerome Breen**

**Co-Chair: Peter Holmans**

**Monday, 15 October, 2012 – 3:30 pm – 5:00 pm**

**Overall Abstract:** The Psychiatric Genetics Consortium has been bringing together datasets from across the world to attempt to find gene for the most common and severe psychiatric disorders. The size of the combined datasets is unprecedented in this field (Ripke et al., 2010, 2011). Here RIPKE will outline the current state of the analysis from the Cross-Disorder-Group of the psychiatric GWAS consortium. This study is based upon a combined GWAS of unprecedented size, consisting of 46 case-control-studies, for a combined study size of 56,867 independent individuals of European ancestry (N = 33,332 cases / 27,888 controls), derived from five distinct diseases: MDD (9,227 / 7,383), BIP (6,990 / 4,820), SCZ (9,370 / 7,736), AUT (4,949 / 5,314), ADD (2,787 / 2,635), where the latter two comprise mostly family-based trio datasets. We obtained genome-wide significant p-values for markers in ITIH3/4 (3), MIR-137 (1), MHC (6), CACNA1C (12), CACNB2 (10), CNNM2 (10) and TCF4 (18), confirming multiple genes previously implicated in either single-disease studies or previously performed cross-disorder studies. Following this main-focus analysis, several additional analyses were performed in order to statistically dissect the whole genome results, following in this section are the pathway efforts. LEE will present microRNA based pathway analyses of the Cross Disorder results of the PGC. Non coding RNA has emerged as a fundamental player in the coordinate regulation of neuronal gene expression. INRICH based analyses of gene-sets formed of the predicted targets of human miRNA revealed significantly associated miRNA regulated gene sets, including miRNA-9 targets which have been previously shown to regulate neuronal gene expression. ROSSLIN will present the protein based biological pathway results given by a combined analyses of 5 different algorithms from the 5 major PGC Disorders: Depression, Schizophrenia, Bipolar Disorder, ADHD and Autism. The results reveal both considerable evidence for the involvement of neurodevelopmental and neurotransmission pathways with considerable overlap in significant pathways across the schizophrenia, bipolar disorder and depression. Lastly, LEE (SANG-LEE) will present novel effect size analyses of biological pathways from GWAS data. He partitioned genomic variance annotating SNPs into genes preferentially expressed in the CNS, SNPs in other genes and SNPS not in genes, and show an excess of variance can be attributable to the CNS set. He has applied this method to all PGC data sets in bivariate analyses which allow us to estimate the partitioned variance shared within and between disorders for different biological pathways and the significant results from the PGC cross disorder pathway analysis.

### SS 1.1 Update on PGC Cross Disorder Analysis

Stephan Ripke<sup>1,2,3</sup>

<sup>1</sup>Massachusetts General Hospital; <sup>2</sup>Broad Institute of Massachusetts Institute of Technology, <sup>3</sup>Harvard University

Here we want to present the current state of the analysis from the Cross-Disorder-Group of the psychiatric GWAS consortium. This study is based upon a combined GWAS of unprecedented size, consisting of 46 case-control-studies, for a combined study size of 56,867 independent individuals of European ancestry (N = 33,332 cases / 27,888 controls), derived from five distinct diseases: MDD (9,227 / 7,383), BIP (6,990 / 4,820), SCZ (9,370 / 7,736), AUT (4,949 / 5,314), ADD (2,787 / 2,635), where the latter two comprise mostly family-based trio datasets. Access to raw genotypes was crucial for several analysis steps, e.g. creating a non-overlapping collection of samples, common imputation quality/format, and polygenic analysis. After demonstrating highly significant genetic commonalities across studies with polygene-analysis, described by Purcell 2009 (Nature), our primary analysis was a SE-weighted meta-analysis of five distinct effect-sizes coming from five within-disease meta-analyses. We obtained genome-wide significant p-values for markers in ITIH3/4 (3), MIR-137 (1), MHC (6), CACNA1C (12), CACNB2 (10), CNNM2 (10) and TCF4 (18), confirming multiple genes previously implicated in either single-disease studies or previously performed cross-disorder studies. Many genes within regions with suggestive p-values are undergoing further investigation. Following this main-focus analysis, several additional analyses were performed in order to statistically dissect the whole genome results, following in this section are the pathway efforts.

## SS 1.2 Network and Pathway Analysis for Deciphering the Pathogenetic Role of Micronas in Psychiatric Disorders

Phil Lee<sup>1,2</sup>

<sup>1</sup>Massachusetts General Hospital; <sup>2</sup>Harvard University

MicroRNAs are a family of small non-coding RNAs that function as repressors in post-transcriptional gene regulation<sup>1</sup>. Highly conserved across diverse species, microRNAs play an important role in the development of the central nervous system. Accordingly, mis-regulation of microRNAs has been implicated in the pathogenesis of several neuropsychiatric disorders, such as, schizophrenia and autism<sup>2</sup>. The present study investigates a pleiotropic role of microRNAs that might be shared across major neuropsychiatric disorders. This study analyzed the sample of 61,220 subjects assembled for the genome-wide studies of five psychiatric illnesses conducted by the Psychiatric Genetics Consortium (detailed elsewhere<sup>3</sup>). Primary statistical analysis was done using a fixed-effect meta-analysis of each single-disorder analysis, contrasting allele frequencies between a group of 27,888 healthy controls and a group of 33,332 patients with either autism (n=4,949), attention deficit hyperactivity disorder (n=2,787), bipolar disorder (n=6,990), major depressive disorder (n=9,227), and schizophrenia (n=9,379). In total, imputed SNP dosages from 1,250,922 autosomal SNPs were studied. First, we conducted pathway analyses using INRICH4 to look for aggregated association of 221 microRNA target gene sets shared by the five psychiatric illnesses. Secondary functional analyses include investigation of the gene expression profiles, common biological functions, and protein-protein interaction data of the microRNA target genes implicated in the pathway analyses. We found significantly enriched association signals from a set of 199 genes regulated by miR-9 (multiple-testing corrected  $p=1.9e-03$ ). There were 30 genes that are nominally associated with all of the five psychiatric disorders (i.e.,  $p < 1e-03$ ). Expression data analysis describes whole brain as the common gene expression tissue for the 30 genes, particularly, highlighting prefrontal cortex, amygdala, and cerebellum regions. The genes are also involved in key neuro-cellular mechanisms, such as axon guidance, neurite morphogenesis, and neuron development. PLCG1, CNTN4, and ATXN1 draw further attention due to their previous implication in multiple psychiatric disorders. The present study provides the first genome-wide evidence that miR-9 may play a significant pathogenetic role in a broad range of psychiatric illnesses, including both childhood and adult-onset mental disorders. This finding is consistent with recent model system studies that suggest a key regulatory role of miR-9 in neurogenesis as well as a causative role in several neurodegenerative diseases<sup>5</sup>. Further studies are essential to examine the potential of miR-9 as new diagnostic biomarkers and therapeutic tools for psychiatric disorders.

## SS 1.3 Combined Pathway Analysis from PGC Cross Disorder Datasets

Gerome Breen<sup>1</sup>

<sup>1</sup>Institute of Psychiatry, King's College London

Family and twin studies suggest that genetic contributions to psychiatric disorders do not map directly onto traditional diagnostic categories. We proposed that molecular heterogeneity might, at least in part, be better captured by pathway analysis. We conducted comprehensive pathway analyses of genome-wide association studies (GWAS) of five major psychiatric disorders: autism spectrum disorder, attention-deficit/hyperactivity disorder, bipolar disorder, major depressive disorder and schizophrenia. The combined GWAS meta-analysis of the five disorders comprised 61,220 case and controls. We applied five publicly available pathway methods (INRICH, FORGE, ALIGATOR, Set-Screen and MAGENTA) to these data. For all methods, we used a consensus set of pathways from a range of sources (GO, KEGG, Reactome, TargetScan and PANTHER) and a consensus set of genes with defined boundaries. We compared the intersections of the results across methods as well as across diseases. While we find that the five methods significantly overlap, none are entirely redundant from one another. Looking at the top results from the five methods, we see confirmation of previously observed disease overlap as well as provide novel insight into overlapping pathways affected by risk variation that are common among psychiatric phenotypes.

#### SS 1.4 Genomic Partitioning by Functional Annotation of Variance and Covariance Explained by SNPs

S. Hong Lee<sup>1</sup>, Naomi Wray<sup>1</sup>, Psychiatric Genomics Consortium-Cross Disorder Group

<sup>1</sup>Queensland Brain Institute; University of Queensland

Most methods that use GWAS data to explore contributions from biological pathways to the aetiology of disorders use, as their starting point, individual SNP associations. Small polygenic effects from individual SNPs make little contribution, even if their total contribution is important. In contrast, methods based on genome-wide similarity include contributions from all causal variants (associated with SNPs), whatever their effect size. We use GCTA, now updated to bivariate models, to partition the genomic variance attributable to SNPs in pathways. This is achieved by creating several estimates of genome-wide similarity for all pairs of individuals, based on different SNP sets defined by the annotation of SNPs to pathways. Variance attributable to each SNP set is estimated in a joint analysis. Non-zero genetic variance in risk of disease is estimated when case-case pairs of individuals are more similar genome-wide than case-control pairs. We have applied this method to all PGC data sets in bivariate analyses that allow us to estimate the partitioned variance shared between disorders. Sample sizes influence the standard errors on the estimates of the partitioned variance, and with current sample sizes only relatively crude partitioning can be achieved. We partitioned genomic variance annotating SNPs into genes preferentially expressed in the CNS, SNPs in other genes and SNPs not in genes, and show an excess of variance can be attributable to the CNS set. We find an excess of variance attributable to the CNS SNP set for most disorders.

#### Symposium 2

##### Next Generation Sequencing in Schizophrenia

**Chairperson: George Kirov**

**Co-Chair: Shaun Purcell**

**Monday, 15 October, 2012 – 3:30 pm – 5:00 pm**

**Overall Abstract:** Technological advances allow the sequencing of the whole protein-coding regions (exomes) of people. Studies are now underway to sequence the exomes of large numbers of probands affected with schizophrenia, their families, and control individuals. Sequencing of families allows the identification of de novo occurring mutations. It is expected that these will occur more frequently in genes that are involved in the pathogenesis of schizophrenia. This symposium will introduce novel results on studies on de novo mutations in schizophrenia in large collections of families from Canada and Bulgaria. We will also discuss data on rare variants from case-control studies from the UK and Sweden. As these are some of the first studies of their kind, we will focus on methodological issues as well as on the implications of the findings for disease pathogenesis.

## SS 2.1 Seeking De Novo Mutations in Schizophrenia by Whole Exome Sequencing of 600 Trios

Michael O'Donovan<sup>1</sup>, George Kirov<sup>1</sup>, Hywell Williams<sup>1</sup>, Sarah Dwyer<sup>1</sup>, Lyudmila Georgieva<sup>1</sup>, Elliott Rees<sup>1</sup>, Andrew Pocklington<sup>1</sup>, Peter Holmans<sup>1</sup>, Shaun Purcell<sup>2</sup>, Pamela Sklar<sup>2</sup>, Douglas Ruderfer<sup>3</sup>, Menachem Fromer<sup>3</sup>, Jennifer Moran<sup>4</sup>, Kimberly Chambert<sup>4</sup>, Janice Kranz<sup>4</sup>, Steve McCarroll<sup>4</sup>, Ed Scolnick<sup>4</sup>, Steve Hyman<sup>4</sup>, Aarno Palotie<sup>5</sup>, Pdraigh Gormley<sup>5</sup>, Priti Palta<sup>5</sup>

<sup>1</sup>Cardiff University, <sup>2</sup>Mount Sinai School of Medicine/Broad Institute of Massachusetts Institute of Technology and Harvard University/Massachusetts General Hospital, <sup>3</sup>Mount Sinai School of Medicine, <sup>4</sup>Broad Institute of Massachusetts Institute of Technology and Harvard University, <sup>5</sup>Wellcome Trust Sanger Institute

Most methods that use GWAS data to explore contributions from biological pathways to the aetiology of disorders use, as their starting point, individual SNP associations. Small polygenic effects from individual SNPs make little contribution, even if their total contribution is important. In contrast, methods based on genome-wide similarity include contributions from all causal variants (associated with SNPs), whatever their effect size. We use GCTA, now updated to bivariate models, to partition the genomic variance attributable to SNPs in pathways. This is achieved by creating several estimates of genome-wide similarity for all pairs of individuals, based on different SNP sets defined by the annotation of SNPs to pathways. Variance attributable to each SNP set is estimated in a joint analysis. Non-zero genetic variance in risk of disease is estimated when case-case pairs of individuals are more similar genome-wide than case-control pairs. We have applied this method to all PGC data sets in bivariate analyses that allow us to estimate the partitioned variance shared between disorders. Sample sizes influence the standard errors on the estimates of the partitioned variance, and with current sample sizes only relatively crude partitioning can be achieved. We partitioned genomic variance annotating SNPs into genes preferentially expressed in the CNS, SNPs in other genes and SNPs not in genes, and show an excess of variance can be attributable to the CNS set. We find an excess of variance attributable to the CNS SNP set for most disorders.

## SS 2.2 De Novo Mutations in Neurodevelopmental Disorders

Guy Rouleau<sup>1</sup>, Jacques Michaud<sup>1</sup>

<sup>1</sup>University of Montréal

Schizophrenia (SCZ), autism spectrum disorders (ASD) and intellectual deficiency (ID) are common, devastating and poorly treated neurodevelopmental brain disorders. The wide spectrum of symptoms and clinical variability in these disorders suggest a complex genetic etiology, which is consistent with the numerous loci thus far identified by linkage, copy number variation and association studies. Although heritability in all three disorders may be as high as ~80%, the genes responsible for much of this heritability remain to be identified. We, and others, hypothesized that a fraction of this missing heritability may be the result of the occurrence of de novo mutations affecting any of a large number of genes. In order to test this hypothesis we first sequenced over 400 synaptic genes in 148 subjects with SCZ, 148 subjects with ASD and 96 subjects with ID. Many likely de novo mutations were identified – these plus relevant functional studies will be presented. Next we sequenced the exomes of SCZ, ASD and ID probands, plus their parents, identifying numerous additional de novo mutations (DNMs). In addition, 1/4 of identified DNMs are nonsense mutations, which is more than what is expected by chance. Interestingly, some of the identified genes, such as SHANK3, show deleterious de novo mutations in patients from the three disease cohorts, suggesting close biological overlap in these disorders. Our study supports the notion that DNMs may account for some of the missing heritability SCZ, ASD and ID while providing a list of genes possibly involved in disease pathogenesis.

### SS 2.3 Sequence Analysis of Schizophrenia and Autism Spectrum Disorders in the UK10K Project

Aarno Palotie<sup>1</sup>, UK10K Consortium  
<sup>1</sup>*Wellcome Trust Sanger Institute*

The UK10K project aims at sequencing 10 000 individuals to provide a catalogue of low frequency variants and to study their association to disease traits. The entire genome is sequenced at 6x in 4000 individuals from two deeply phenotyped population based cohorts, the UK-Twin cohort and the ALSPAC cohort. Using the Agilent Genocode exome hybrid capture design, the whole exome is been sequenced in 6000 individuals with extreme clinical phenotypes related to obesity, neurodevelopmental and rare traits. In the neurodevelopmental arm sequencing of altogether 3000 cases with autism or schizophrenia is in progress. Altogether 1700 schizophrenia cases with a family history and/or detailed phenotypic description have been selected from three UK centers. About 200 of these cases have documented cognitive impairment as schizophrenia comorbidity. Additionally, 430 familial schizophrenia cases have been sequenced from the genetically and environmentally homogenous Finland, including 150 samples from the internal isolate of Kuusamo in the North Eastern boarder of the Country. All schizophrenia samples have previous GWAs data and samples for follow-up genotyping. Altogether about 800 non-syndromic autism spectrum disorder cases have been selected from three UK and two Finnish centers. The primary focus is on familial cases, where more than one first degree relative is affected with an autism spectrum disorder. Each autism family has participated in GWA studies to provide an imputation backbone for the sequencing study.

### SS 2.4 Whole-exome Sequencing in 5000 Swedish Schizophrenia Patients and Matched Controls

Shaun Purcell<sup>1</sup>, Douglas Ruderfer<sup>1</sup>, Menachem Fromer<sup>1</sup>, Jennifer Moran<sup>1</sup>, Kim Chambert<sup>1</sup>, Colm O' Dushlaine<sup>1</sup>, Giulio Genovese<sup>1</sup>, Patrick Sullivan<sup>1</sup>, Christina Hultman<sup>1</sup>, Steve McCarroll<sup>1</sup>, Pamela Sklar<sup>1</sup>  
<sup>1</sup>*Mount Sinai School of Medicine*

Previous genome-wide association studies (GWAS), focusing on both common single-nucleotide polymorphisms (SNPs) and rare copy number variants (CNVs), have pointed to a complex genetic basis for schizophrenia, in which a large number of loci are implicated in determining disease risk, although no individual variant explains even a moderate fraction of the total genetic variance. Here we report full results from whole-exome sequencing in a large Swedish sample to map specific variants, genes and/or networks related to rare alleles of moderate or large effect. This population-based case/control study comprises exome sequence data on over 5,000 individuals (cases with schizophrenia and matched controls). The sequencing of this sample is complete as of late April 2012 and analysis is underway. We will discuss the methodological challenges in analyzing these datasets and interpret the results in terms of emerging views on the genetic architecture of schizophrenia.

### Genetics of Imaging and Neuro-cognitive Phenotypes and their Relevance as Genetic Endophenotypes for Psychiatric Disorders

Chairperson: Stephanie Le Hellard

Monday, 15 October, 2012 – 3:30 pm – 5:00 pm

**Overall Abstract:** It has been recently established that genetic factors are responsible for up to 50% of the inter-individual variability in general cognition performance<sup>1</sup>. For brain morphology studies the heritability, estimated in twin studies, varies between 0.2 to 0.7 depending on the brain region<sup>2</sup>. Understanding which genetic factors are implicated in the inter-individual variability of these phenotypes will provide insight into the genetic factors implicated in the normal functioning of the brain and thus, will help understanding the dysfunctioning of the brain in psychiatric disorders. The approach of using cognitive and imaging phenotypes to better understand psychiatric disorders has also been proposed as a method to deconstruct the complexity of the psychiatric phenotypes, using these phenotypes as endophenotypes. As put by Gottesman and Gould in 2003, an “endophenotype-based approach has the potential to assist in the genetic dissection of psychiatric diseases”<sup>3</sup>. The term of intermediate phenotypes has also been proposed for phenotypes such as neuro-cognitive abilities or imaging traits in psychiatry. In the recent years, genetic studies in neuro-cognition and imaging have confirmed that genetic risk variants for psychiatric disorder can also have an effect on neuro-cognitive or imaging phenotypes. In this symposium, we will aim at presenting several of these studies in large samples of healthy individuals or in samples with healthy individuals and cases with schizophrenia and/or bipolar disorder, where genetic risk factor for psychiatric disorders have been followed up in neuro-cognitive and imaging traits (presentation by Ole A. Andreassen and Stephanie Le Hellard). We will also present studies of a German sample with imaging traits where using multivariate analyses, several genes were identified as correlating with brain morphology (Sven Cichon). We will also present the latest results from the ENIGMA consortium, the biggest consortium that has gathered images and genetic data for more than 10,000 individuals. The ENIGMA consortium recently published genome wide significant association between genetic variants and hippocampal volume<sup>4</sup>, follow-ups and secondary analyses will be presented (Sarah Medland). Finally we will discuss how using a sample of healthy individual with high phenotypic information we could identify sets of genes implicated in different cognitive abilities that were significantly associated with bipolar disorder or schizophrenia in several large GWASs (Stephanie Le Hellard). We propose to orientate the discussion towards the relevance of these phenotypes as endophenotypes for psychiatric disorders at the phenotypic and the genetic levels. <sup>1</sup>.Davies et al. *Mol Psychiatry*. 2011 Oct;16(10):996-1005. <sup>2</sup>.Rimol et al. *Biol Psychiatry*. 2010 Mar 1;67(5):493-9. <sup>3</sup>.Gottesman II, Gould TD. *Am J Psychiatry*. 2003 Apr;160(4):636-45. <sup>4</sup>.Stein et al. *Nat Genet*. 2012 Apr 15, epub.

### SS 3.1 TCF4 Sequence Variants and Mrna Levels are Associated with Neurodevelopmental Characteristics in Psychotic Disorders

Ole Andreassen<sup>1</sup>

<sup>1</sup>University of Oslo

*TCF4* is involved in neurodevelopment and intergenic and intronic variants in or close to the *TCF4* gene have been associated with the susceptibility to schizophrenia. However, the functional role of *TCF4* at the level of gene expression and relationship to severity of core psychotic phenotypes are not known. *TCF4* mRNA expression level in peripheral blood was determined in a large sample of patients with psychosis spectrum disorders (n=596) and healthy controls (n=385). The previously identified *TCF4* risk variants (rs12966547(G), rs9960767(C), rs4309482(A), rs2958182 (T) and rs17512836(C)) were tested for association with characteristic psychosis phenotypes including neurocognitive traits, psychotic symptoms and structural Magnetic Resonance Imaging brain morphometric measures, using a linear regression model. Further, we explored the association to these phenotypes of additional 59 SNPs covering the *TCF4* gene. The rs12966547 and rs4309482 risk variants were associated with poorer verbal fluency in the total sample. There were significant associations of other *TCF4* SNPs with negative symptoms, verbal learning, executive functioning and age at onset in psychotic patients and brain abnormalities in total sample. The *TCF4* mRNA expression level was significantly increased in psychosis patients compared to controls and positively correlated with positive and negative symptom levels. The increase in *TCF4* mRNA expression level in psychosis patients and the association of *TCF4* SNPs with core psychotic phenotypes across clinical, cognitive and brain morphological domains support that common *TCF4* variants are involved in psychosis pathology, probably related to abnormal neurodevelopment.

### SS 3.2 Systematic Search for Genetic Factors Influencing the Thickness of the Cerebral Cortex

Sven Cichon<sup>1</sup>

<sup>1</sup>*University of Bonn, Research Center Juelich*

The human cortex is structurally and functionally segregated. It varies more than any other part of the brain between subjects. The identification of gene variants contributing to this inter-subject variability will help to elucidate molecular mechanisms underlying brain functions. Through systematic correlation of each variant's genotype with the phenotypic variability, assessed through magnetic resonance imaging (MRI), those variants can be identified that explain the phenotypic variability. In the present study, we focused on cortical thickness (CT) which is a heritable, quantitative trait, assumed to reflect the architecture of neuronal and glial cells in the cortex. Because CT undergoes changes in neuropsychiatric disorders, it has become a promising endophenotype for many imaging genetic studies in our field. We analyzed 98 healthy volunteers using a 3T MRI scanner, obtained T1-weighted images, and performed genome-wide genotyping (HumanOmniExpress/ HumanOmni1S), resulting in 1.4 million single-nucleotide polymorphisms (SNPs) per subject after quality control. Thickness data were extracted using the FreeSurfer software. To determine those data which explain most of the thickness variability, we applied a principal component analysis (PCA) on the pooled data from all cortical regions. We selected the first 15 principal components which together explained 80% of the total variance and performed genome-wide association studies (GWAS) on each component. Across PCA GWAS, SNP P-values were combined using a meta-analysis approach. Overall, 31 SNPs showed P-values of less than 1E-04. The most significant finding was a cluster of SNPs located between CDYL2 and C16orf61 on chromosome 16q23.2 ( $P=9.48E-06$ ). The identification of genetic factors contributing to inter-individual variability in cortical thickness will provide important insights into the biological processes involved in cortico-genesis. Our study has identified several common genetic variants that show suggestive evidence for an involvement in cortical thickness. We are currently analyzing the respective genes and available knowledge of their function. The P-values do not exceed the formal threshold for genome-wide significance which may be a result of the relatively limited sample size and/or the small or medium effect-size conferred by each of these genetic variants. We have now initiated replication studies in suitable available samples to follow-up and further strengthen our results.

### SS 3.3 Enigma: Enhancing Neuro-imaging Genetics through Meta-analysis

Sarah Medland<sup>1</sup>

<sup>1</sup>*Queensland Institute of Medical Research*

The ENIGMA Network was created in 2010 to bring together researchers in imaging genomics to increase the understanding of brain structure and function. The flagship project from this consortium focused on total brain and hippocampal volume (a biomarker of incipient Alzheimer's disease which has been implicated in numerous psychiatric conditions). Through the consortium we were able to identify and replicate genetic variants influencing these traits. However, contrary to prior hypotheses regarding the magnitude of genetic effects on endophenotypes, imaging phenotypes have proven to be complex traits. The implications of these findings for psychiatric research using imaging endophenotypes will be discussed.

### SS 3.4 Polygenic Deconstruction of Psychiatric Disorders with Neuro-cognitive Gene Sets

Stephanie Le Hellard<sup>1</sup>

<sup>1</sup>University of Bergen

Since the heritability of psychiatric disorders appears to be highly polygenic, with probably many genetic variants of small effects being implicated, the power of classical Genome Wide Association Studies (GWAS) approaches to detect significant signal is limited. Alternative approaches to identify those genetic factors and their relevance are warranted. By using phenotypes that are associated with psychiatric disorders and that are heritable, it may be possible to identify common genetic susceptibility between these phenotypes. Cognitive deficits are common features of many psychiatric disorders, although discrepancies about the specificity of these deficits have been reported. Likewise changes in the brain morphology or connectivity in patients compared to controls have been reported in some studies but refuted by others. We chose to perform GWAS on a sample of 700 healthy individuals of Norwegian origin, which had been extensively phenotyped for neuro-cognitive abilities and brain imaging, and that we replicated in additional samples. We chose to analyze these phenotypes at the gene level rather than the genetic variant to allow for allelic heterogeneity due to the phenotypic and sample heterogeneity. We performed several studies to identify genes implicated in general cognition, in reaction time, speed of processing, memory and in brain imaging. We will present several of these studies and the relevance of the genes identified to psychiatric disorders. Finally, we selected gene sets associated to several cognitive domains (general cognition, verbal abilities, speed of processing, reaction time, memory), we identified and replicated significant enrichment across several large samples of patients with schizophrenia or bipolar disorder for specific neuro-cognitive associated gene sets.

### Symposium 4

#### Epigenetic Factors Influencing Neuropsychiatric Phenotypes and Disorders

**Chair: Melanie Carless**

**Co-Chair: Jimmy Potash**

**Monday, 15 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** We present here, an array of epigenetic studies, focusing on DNA methylation and microRNAs (miRNAs) involved in psychiatric disorders and the neuropsychiatric phenotypes associated with them. New large-scale epigenetic methodologies have brought about much excitement, and although several studies have implicated DNA methylation and miRNAs in the development of psychiatric disorders, these have generally relied on targeted approaches. Few published studies have integrated genome-wide methylation and miRNA profiles in their analysis of psychiatric disorders and related neuropsychiatric phenotypes. The proposed symposium not only addresses such genome-wide methylation data, but also incorporates related genetic data, such as DNA variation and gene expression. Targeted understanding of miRNA function is also presented. We begin by first exploring alterations in the prefrontal cortex that may contribute to schizophrenia, through both miRNAs and DNA methylation. Dr Murray Cairns will present his recent work outlining the expression of miR-137 in the prefrontal cortex and its role in cognition. We will then progress to recent work by Dr. Roel Ophoff, looking at the effect of genetic variation on DNA methylation and how this alters gene expression to influence schizophrenia. Dr Jonathan Mill will discuss the relevance of DNA methylation to the etiology of autism spectrum disorder. Finally, Dr Torsten Klengel will discuss the role of gene x environment interaction on DNA methylation and how this influences post-traumatic stress disorder.

#### SS 4.1 Genetic and Epigenetic Regulation of Schizophrenia Associated MicroRNA

Murray Cairns<sup>1</sup>

<sup>1</sup>The University of Newcastle Australia

Schizophrenia is a complex neuropsychiatric disorder involving disturbances in neural circuitry and synaptic function. While there are likely to be many genes and developmental pathways leading to the neurobehavioural syndrome, the redundancy of these networks means that many combinations of gene variants have the potential to play a role. Recent investigation has revealed that post-transcriptional gene regulation and associated small non-coding microRNA (miRNA) are likely to be important factors shaping the topography of these networks. miRNAs display complex temporospatial expression patterns in the mammalian brain and have the potential to regulate thousands of target genes by functioning as the specificity factor for intracellular gene-silencing machinery. Their dysregulation could also lead to pervasive changes in the network structure during development and in the mature brain that are significant in the pathophysiology of schizophrenia. This is now supported by compelling evidence that the underlying miRNA biogenesis machinery and miRNA genes themselves, are subject to disease-associated genetic mutation and epigenetic influence. We have examined the neurodevelopmental trajectory of miR-137 expression in the normal human and rat prefrontal cortex, and the effect of this variant on cognition and cortical expression of the mature miRNA. These analyses reveal a complex dynamic developmental expression pattern and significant mutation-associated change of cortical expression and cognitive phenotype in schizophrenia. These findings are supportive of a role for miRNA in schizophrenia and suggest that they may have significance as biomarkers or as targets for pharmacological manipulation.

#### SS 4.2 Combined Genetic Analysis of DNA Methylation and Gene Expression in Schizophrenia Identifies Disease Susceptibility Loci

Roel Ophoff<sup>1,2,3</sup>

<sup>1</sup>University of California Los Angeles School of Medicine, <sup>2</sup>Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, <sup>3</sup>University Medical Centre Utrecht

There is compelling evidence that epigenetic variation plays an important role in schizophrenia susceptibility. DNA methylation is known to affect gene expression and may thereby contribute to the development of schizophrenia. We performed a study in whole blood with the goal to gain a better understanding of causal relationships between genetic variation and DNA methylation profiles in schizophrenia patients and healthy controls. To examine the association between DNA methylation and genetic variants, genome-wide genotype and DNA methylation (27K Illumina array) data was obtained from whole blood of 260 schizophrenia patients and 240 healthy controls. For a subset of these subjects array-based gene expression data was available as well. By combining epigenetic and gene expression data we identified almost 700 CpG sites in the genome that are differentially methylated between schizophrenia patients and unaffected controls, and of which methylation status is associated with gene expression levels; moreover, these genes are also differentially expressed between cases and controls. Genetic analysis revealed that some 8% of these CpGs are under local (*cis*) genetic control. We hypothesized that identification of biological relevant links between DNA methylation and gene expression in the context of disease might serve as an efficient way to enrich for disease susceptibility loci if under genetic control (methylation QTLs, mQTLs). We used the available results of the large Psychiatric Genomics Consortium (PGC) schizophrenia genome-wide association (GWA) study to examine whether the observed mQTLs represent disease association signal. Overall, we observed significant enrichment of schizophrenia association signals of mQTLs with the most significant effects for those loci that are also associated with differential gene expression in cases and controls. While the enrichment effects were already visible when using nominal significant thresholds ( $p < 0.05$  in PGC) the effects were amplified at more stringent values (e.g.  $p < 1e-4$ ). We identified five disease-associated loci that control DNA methylation in *cis*, which in turn affect gene expression in a case/control study. Interestingly, one of these loci was previously identified to be involved in disease susceptibility in the recent PGC schizophrenia GWA study. Our results suggest that enrichment of biological signal by combining genetic, epigenetic and gene expression profiles from whole blood may be an efficient approach to identify disease susceptibility loci including neuropsychiatric traits.

### SS 4.3 Methylomic Profiling in Autism Spectrum Disorder

Chloe Wong<sup>1</sup>, Jonathan Mill<sup>1,2</sup>

<sup>1</sup>King's College London, <sup>2</sup>University of Exeter

Autism Spectrum Disorder (ASD) is a common, highly heritable neurodevelopmental disorder characterized by marked etiological heterogeneity. So far, forward genetic approaches, including linkage and candidate gene analysis, whole genome association and assessment of chromosomal structural variations have uncovered a range of genes with predisposing mutations and polymorphisms. It has been widely suggested, however, that non-genetic factors, acting at the level of epigenetic gene regulation, are also involved in the etiology of ASD. We have performed epigenome-wide profiling in a unique set of discordant monozygotic twin samples and post-mortem brain samples using a range of methylomic approaches. Our analyses revealed a number of ASD-associated DNA methylation changes in the vicinity of genes previously associated in psychiatric disorders as well as numerous plausible biological candidates. Our study represents the first in-depth epigenomic analyses of ASD and our findings highlight the potential imperative role of DNA methylation in the etiology of ASD.

### SS 4.4 Allele-specific DNA Demethylation in FKBP5: A Molecular Mediator of Gene X Environment Interactions With Childhood Trauma

Torsten Klengel<sup>1</sup>

<sup>1</sup>Max-Planck Institute of Psychiatry

For most psychiatric diseases neither a genetic disposition nor environmental factors on their own are sufficient to elicit a specific disorder. Rather, genetic variation and environmental exposure interact to shape the development and function of the human brain and ultimately moderate the risk to suffer from psychiatric disorders. Here, we delineate an epigenetic mechanism for the gene x environment (GxE) interaction of the FK506 binding protein 5 (*FKBP5*) gene with childhood abuse on the development of post-traumatic stress disorder (PTSD) in adulthood. Data from this study were collected as part of the Grady Trauma Project and replication was performed in data from the Conte Center Study for the Psychobiology of Early-Life Trauma (Emory University, Atlanta, GA, USA). We are able to show that *FKBP5* polymorphisms interact with child abuse exposure on the development of current PTSD symptoms in adulthood. The risk to suffer from lifetime PTSD is significantly increased by exposure to early trauma in *FKBP5* risk allele carriers, but not in carriers of the protective genotype. Pyrosequencing of bisulfite treated DNA of highly traumatized individuals and controls revealed a significant demethylation of CpGs around glucocorticoid responsive elements (GREs) of *FKBP5* in abused individuals. Further, we found a significant interaction of *FKBP5* genotype and childhood abuse on DNA methylation levels in 3 CpGs in intron 7 ( $F_{74,1}=37.8$ ,  $P_{\text{corr}} < 0.001$ ). Replication in an independent cohort from the Conte Center Study confirmed these findings. In a multipotent human hippocampal progenitor cell line we demonstrated that *FKBP5* demethylation is initiated by GR-activation with dexamethasone which led to a highly significant DNA demethylation in CpGs in intron 7 similar to the CpGs affected by early trauma in *FKBP5* risk allele carriers (average of 17.1% demethylation in the 3 CpGs,  $P < 0.001$ ). We extend these results comparing DNA methylation changes in dexamethasone treated hippocampal progenitor cells with trauma exposed individuals on Illumina's 450k methylation chip. In summary, *FKBP5* increases the risk of developing PTSD by allele-specific, childhood trauma-dependent demethylation of CpGs in functional GREs of *FKBP5*. For the first time, we delineate a molecular mechanism by which environmental impact in early life is encoded in epigenetic modifications and moderated by genetic predisposition influencing the development of psychiatric symptoms in later life. Our findings might be of particular relevance for the developing organism since the effects on DNA methylation seemed to be restricted to exposure to childhood trauma and were not influenced by traumatic experiences in adulthood, suggesting a possible sensitive period in early development for these epigenetic effects.

## Symposium 5

### Using Next Generation Sequencing to Unravel the Etiology of Mood and Psychotic Disorders

**Chair: Dick McCombie**

**Co-Chair: Fernando Goes**

**Tuesday, 16 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** Next-generation sequencing has revolutionized the ability to query the human genome at the single nucleotide level. As sequencing technologies evolve and costs fall, comprehensive approaches targeting the whole exome or whole genome are becoming increasingly feasible in the sample sizes necessary for the study of complex disorders. Next-generation sequencing studies will likely be one of the major methods of scientific inquiry of complex diseases, including many of the major mental illnesses. However, the deluge of data also poses a formidable analytical challenge in regard to study designs, data processing, variant annotation and functional inference. In this symposium, several investigators in the field of psychiatric genetics share their experience and unpublished results of next-generation sequencing studies of psychotic disorders. Highlighting the utility of family and case-control designs, this symposium will encompass several different techniques, including whole-genome, exome and targeted sequencing. In the initial presentation, Dr. David Porteus (Edinburgh University) will discuss large scale deep resequencing of the DISC 1 locus in a case-control study of the major mental illnesses, including controls from the Lothian Birth Cohort, a unique cohort of individuals with longitudinal neurocognitive measures. Dr. Fernando Goes (Johns Hopkins University) will discuss the interim results of a large case-control and family study of familial Bipolar Disorder in European Americans. A family based study of schizophrenia, using both extended pedigrees and trios, will be the focus of Dr. Shane McCarthy's presentation (Cold Spring Harbor Laboratory). Finally, Dr. Pamela Sklar (Mount Sinai School of Medicine) will present ongoing results from a large case-control exome sequencing study of Bipolar Disorder in the Swedish population. This symposium will end with Dr. Dick McCombie (Cold Spring Harbor Laboratory) leading a discussion of the future prospects and implications of next-generation sequencing for psychiatric disorders.

## SS 5.1 Next Generation Sequencing of the DISC1 LOCUS in Major Mental Illness and Cognition

David Porteus<sup>1</sup>

<sup>1</sup>*University of Edinburgh*

In 2000, we positionally cloned a completely novel gene, Disrupted in Schizophrenia 1, so named because it is disrupted by the breakpoint of a balanced translocation that segregates with schizophrenia, bipolar disorder and recurrent major depression in a large Scottish family. The translocation family is probably unique, but there is strong evidence from linkage, association, and now gene resequencing linking genetic variation in DISC1 to a broad spectrum of psychopathology. Common, rare and ultra-rare amino acid substitutions have been shown to be functional at the biochemical, cellular level or brain imaging level. Other evidence indicates that non-coding genetic variation is also important. Key to the role of DISC1 in the aetiology of major mental illness is its function as a scaffold protein, interacting with multiple protein partners involved in neurodevelopment, cytoskeletal function and cell signaling. I will present our findings following a comprehensive analysis of DISC1 structure-function and genotype-phenotype correlations from resequencing 528 kb of the DISC locus in over 1,500 subjects (240 with schizophrenia, 221 with bipolar disorder and 192 with recurrent major depression, plus 889 healthy controls who are part of the Lothian Birth Cohort of 1936 (IQ measures at age 11 and repeat IQ, plus extensive additional measures of behavior and cognition at ~70 years of age), with follow up of interesting results in relatives and replication cohorts, plus biological characterization of functional effects.

## SS 5.2 Family Studies of Psychiatric Disorders

W. Richard McCombie<sup>1</sup>, David Porteous<sup>2</sup>, Douglas Blackwood<sup>2</sup>, William Byerley<sup>3</sup>, Aiden Corvin<sup>4</sup>, Pippa Thomson<sup>2</sup>, Kathryn Evans<sup>2</sup>, Niamh Ryan<sup>2</sup>, Stewart Morris<sup>2</sup>, Shane McCarthy<sup>1</sup>, Jennifer Parla<sup>1</sup>, Jianchao Yao<sup>1</sup>, and Melissa Kramer<sup>1</sup>

<sup>1</sup>*Cold Spring Harbor Laboratory*, <sup>2</sup>*University of Edinburgh*, <sup>3</sup>*University of California, San Francisco*, <sup>4</sup>*Trinity University College of Medicine*

Understanding the genetics underlying major psychiatric disorders has proven to be one of the great challenges in human genetics research. While considerable progress has been made, much of the underlying genetic liability contributing to these disorders is yet unknown. In other areas of human genetics families have provided a unique resource to minimize the time to find genes causing diseases. While recognizing the value of larger case-control studies, our understanding of the genetic heterogeneity contributing to these disorders has led us to test family studies as a way of finding likely contributing genes. We explore several family-based strategies, using both trios and extended families to identify candidate genes. We have also developed technology to test these candidate genes in large numbers of unrelated patients and controls. We believe this combination of family studies followed by targeted sequencing in case control cohorts represents a powerful approach to understanding the genetics of psychiatric disorders.

## SS 5.3 Exome Sequencing of Familial Bipolar Disorder

Fernando Goes<sup>1</sup>

<sup>1</sup>*Johns Hopkins University School of Medicine*

Complex disorders such as bipolar disorder are likely to harbor both common and rare susceptibility alleles. While common variation has been widely studied in the last decade, identification of rare variants has only recently become feasible with the advent of next-generation sequencing. In this presentation we will report progress of a large ongoing exome sequencing study of familial bipolar disorder, employing complementary family-based and case-control approaches. In this interim analysis, we will present the results of exome sequencing of ten multiplex families, each with at least three sequenced affected subjects. We will also present the first stage of our case-control analysis with exome sequencing of over 350 subjects. Preliminary results suggest a potential increase in rare-variant burden in the protocadherin genes, which play important roles in neuronal cell adhesion and cell guidance. We will discuss these results in light of ongoing challenges in data analysis and bioinformatic annotation.

## SS 5.4 Whole-exome Sequencing in Swedish Bipolar Disorder Patients and Matched Controls

Pamela Sklar<sup>1</sup>

<sup>1</sup>Mount Sinai School of Medicine

Here we present data from a large-scale whole exome sequencing study of bipolar disorder in Swedish patients (N=500) and matched controls (N=2000) from an ongoing schizophrenia sequencing study. Previous genome-wide association studies (GWAS) focusing on common single-nucleotide polymorphisms have pointed to a complex genetic basis for bipolar disorder, in which a large number of loci are implicated in determining disease risk, although no individual variant explains even a moderate fraction of the total genetic variance. In this study, we focus on the role of rare coding variation (single point mutations, indels and structural variation), as assayed by high-depth next-generation sequencing. Sequencing is currently underway and expected to be completed mid-summer 2012: this project will generate a sequencing dataset for bipolar disorder far larger than any currently available. The analysis will aim to map specific variants, genes and/or networks related to rare alleles of moderate or large effect; we will also intersect this dataset with the parallel schizophrenia exome data. We will discuss the methodological challenges in analyzing these datasets and interpret the results in terms of emerging views on the genetic architecture of bipolar disorder and other neuropsychiatric disease.

## Symposium 6

### Immunogenetics of Affective Disorders and Cognitive Function

**Chair: Bernhard Baune**

**Co-Chair: Sarah Cohen-Woods**

**Tuesday, 16 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** The immune system has been shown to be involved in the etiology of neuropsychiatric disorders. The content of this symposium extends previous focus from humoral/inflammatory mechanisms, to cellular and genetic immunological mechanisms in affective disorders and cognitive function. The symposium will address a variety of molecular immunological mechanisms relevant to affective disorders and cognitive function, ranging from transgenic animal studies, molecular in-vitro studies, to translational aspects such as pharmacogenetics, immunogenetics in imaging and susceptibility to mental disorders following gene-environment interaction. The symposium will begin with an overview presentation describing immunological effects on molecular mechanisms of learning, memory, and emotion processes, and neuroplasticity relevant to physiological brain function as well as pathological conditions such as depression and cognitive dysfunction. This initial presentation will be followed by an imaging-genetics presentation on the role of immune genes in brain function and brain morphology in healthy individuals and in depression. It has been recently shown that immunological genetic make-up can predispose to morphometric brain changes, such as hippocampus volumes in health and disease. In addition, this imaging-immunogenetic approach shows promise in the prediction of antidepressant treatment response in depression. Since the hippocampus plays a critical role in both memory, learning and emotion processing, the next presentation will focus on the effects of interleukin 1 beta on hippocampus function, neurogenesis and pathways (e.g., kynurenine) involved in depression, using a clinically relevant model of human hippocampal progenitor cells. It will be specifically shown that IL-1b affects neurogenesis, by decreasing the production of neuroblasts and their maturation into neurons. Furthermore, that inhibition of the kynurenine pathway may provide a new therapy to revert inflammatory-induced reduction in neurogenesis. The final presentation will describe evidence for predisposition to affective disorder, following a model of childhood stress sensitivity and immunological genetic risk to major depressive disorder. Gene-environment (G-E) interaction models will be used to investigate the role interaction between immune genes and childhood maltreatment in the development of recurrent adult major depressive disorder in an initial discovery sample, and a similarly phenotyped replication sample (clinical case-control design). In summary, this symposium extends the immune theory of affective disorders and cognitive function by examining the role of immune genes in neurocircuitry, morphometry and brain function, treatment response and predisposition to major depression and cognitive dysfunction. This symposium is timely in addressing some issues faced in depression GWAS to date, such as including endophenotype (imaging) and environmental factors (G-E), and by bringing immunogenetics into the field of psychiatry. Research within this symposium presents novel and translational findings, with potential biomarkers and identification of novel immune-related pathways for the treatment of mood disorders and cognitive impairment.

## SS 6.1 Immune System in Emotion and Cognitive Processing

Bernhard Baune<sup>1</sup>

<sup>1</sup>University of Adelaide

The immune system has been shown to be involved in the pathogenesis of psychiatric disorders. Early studies made significant contributions to the identification of potential immunological and inflammatory blood biomarkers associated with symptoms and treatment of depression, schizophrenia and cognitive decline. More recently, it was suggested that an immunological imprint obtained through maltreatment during childhood might underpin the adult onset of depression. Although these studies being based on protein analyses are suggestive that immune factors are involved in complex CNS functions, research on the immunogenetic background of emotion and cognitive processes has only emerged recently. A contribution of immune genes is highly relevant since observation have been made that cytokines directly interact with molecular mechanisms of memory, learning and neuroplasticity in the hippocampus and the amygdala in particular, both of which are highly relevant to emotion and cognitive processing. In support of a genetic background of a complex immune-CNS interaction, transgenic animal models have shown that cytokines play a physiological role in normal cognitive function during neurodevelopment. Specifically, it was shown that the absence of Tumor necrosis factor alpha (TNF) in genetically modified mice is detrimental of cognitive function during neurodevelopment. In translational research, it was demonstrated that cytokines and genetic polymorphisms of cytokines play an important role in cognitive functioning in healthy elderly humans. In a study among healthy elderly from the general population, the chemokine IL-8 was significantly associated with poor cognitive performance in the memory, attention and motor domains. In a similar study among healthy elderly individuals, genetic variants of IL-1beta were related to poor memory, whereas a genetic variant of TNF was associated with better cognitive speed performance. In contrast, IL-6 showed no genetic association with cognitive performance in humans so far. Moreover, genetic variants of cytokines may play an important part in predicting anti-depressant treatment response. In different studies, it was shown that the genetic polymorphisms of IL-1beta and IL-11 may play a key role in antidepressant treatment response and emotion processing (IL-1beta) mediated by the amygdala in depression. Taken together, these findings suggest that genetic variants of factors of the immune system exert effects on complex cognitive processes at the molecular level, such as synaptic plasticity, neurogenesis, as well as neuromodulation, hence suggesting that these genes play an intimate role in the molecular and cellular mechanisms sub-serving learning, memory and emotion processing under physiological as well as pathological conditions. Immunogenetic research appears to be a promising area for characterizing the predisposition to psychiatric disorder, identifying disease and treatment biomarkers and developing interventions.

## SS 6.2 Immune System in Neuroimaging

Udo Dannlowski<sup>1</sup>

<sup>1</sup>University of Marburg

Cytokines such as tumor necrosis factor alpha (TNF) or interleukin 6 (IL-6) have been implicated in dual functions in neuropsychiatric disorders. Little is known about the genetic predisposition to neurodegenerative and neuroproliferative properties of cytokine genes. In the present ongoing neuroimaging project, we investigate the neurodegenerative or neuroproliferative roles of genetic polymorphisms in the TNF and IL-6 gene on brain morphology in healthy individuals. Furthermore, also variants in the Interferon gamma (IFG) gene and Interleukin 1 beta (IL-1b) gene were assessed. We particularly investigated the hippocampal formation due to its high susceptibility for neurodegenerative processes and due to its implication in several neuropsychiatric disorders, including affective disorders, schizophrenia, and Alzheimer's disease. We employed voxel-based morphometry (VBM) in a large sample of well characterized healthy individuals (N=303) to analyze the associations between genetic variants of TNF (rs1800629; rs361525), genetic variants of IL-6 (rs1800795; rs1800796, rs2069833, rs2069840) and brain morphology (gray matter concentration). All subjects underwent the SCID-Interview and had no life-time history of psychiatric disorders, auto-immune or chronic inflammatory disorders, chronic infections or any other relevant medical conditions. All analyses were conducted with age, gender, and IQ as covariates. In a Region-of-Interest (ROI) analysis of the hippocampus, for TNF rs1800629, we observed a strong genotype effect on bilateral hippocampus gray matter concentration. Carriers of one or two A-alleles had significantly smaller volumes compared to GG-homozygotes. For rs361525, a similar effect was observed at almost the same location, with the A-allele resulting in smaller hippocampus volumes compared to GG homozygotes. Regarding the IL-6 gene, the polymorphism IL-6 rs1800795 (-174C/G) showed a strong main effect of genotype within the right hippocampus head, even alpha-corrected for the entire brain. Homozygous carriers of the G-allele had significantly larger hippocampus gray matter volumes compared to heterozygous subjects. None of the other investigated IL-6 SNPs showed a significant association with gray matter volume in whole-brain analyses. The findings suggest a neurodegenerative role of the A-alleles of the TNF SNPs rs1800629 (-308G/A) and rs361525 (-238G/A) on hippocampal volumes in healthy individuals. However, a rather neuroprotective role of the G-allele of the SNP rs1800795 on hippocampal volumes could be discerned. Future imaging studies on the role of these SNPs in psychiatric populations of diseases with neurodegenerative components are warranted, especially in those with affected hippocampus (e.g., by maltreatment, stress) are warranted.

### SS 6.3 Molecular Aspects of the Immune System in Depression

Patricia Zunszain<sup>1</sup>

<sup>1</sup>*Centre for the Cellular Basis of Behaviour; King's College London*

Dysregulation of the immune system is recognized as playing an important role in the pathogenesis of depression. This is based on several observations: subsets of depressed patients have an altered peripheral immune system, with impaired cellular immunity and increased levels of pro-inflammatory cytokines; cytokines can influence neurotransmitter metabolism, neuroendocrine function and regional brain activity, all of which are relevant to depression; acute administration of cytokines causes sickness behavior which shares features with depression, and patients undergoing cytokine treatment develop depressive symptoms. Additionally, several lines of evidence suggest the involvement of altered neurogenesis in depression. It is known that the birth of new neurons in the hippocampus is impaired in stress-induced models of depression and in the brain of depressed patients. Conversely, antidepressants, electroconvulsive therapy and exercise have all been shown to enhance neurogenesis. By using a clinically relevant model of human hippocampal progenitor cells, we studied the effect of cytokines both on the regulation of neurogenesis and on possible neurobiological pathways that are involved in depression. Considering that levels of IL-1b have been repeatedly described as elevated in depressed patients, both in blood and in cerebrospinal fluid, we studied this cytokine. IL-1b affected neurogenesis, by decreasing the production of neuroblasts and their maturation into neurons. We then analyzed the cytokine effects on all enzymes involved in the kynurenine pathway. The enzyme indoleamine 2,3-dioxygenase (IDO) appears to be a key player, as it initiates the pathway by degrading tryptophan to produce kynurenine, which can then lead to neurotoxic or neuroprotective products. The differential regulation of these compounds is postulated to explain the behavioral changes experienced by some patients during exposure to inflammatory stimuli. By increasing IDO, IL-1b reduced the availability of tryptophan, precursor of serotonin. Additionally, it promoted the production of enzymes conducive to toxic metabolites. The detrimental effect of IL-1b on neurogenesis was partially recovered by blocking the neurotoxic pathway. Our results suggest that inhibition of the kynurenine pathway may provide a new therapy to revert inflammatory-induced reduction in neurogenesis. Finally, we investigated the anti-inflammatory effect of antidepressants from different chemical classes. Inflammation was induced in our cell model by incubation with IL-1b, and the inflammatory response was quantified by measurement of IL-6 secreted into the supernatant, and also by looking at auto-induction of IL-1b and IL-6 as mRNA. Our results add further evidence for the differential anti-inflammatory properties of antidepressants.

### SS 6.4 Childhood Stress-reactivity and Inflammatory Mechanisms in Clinical Major Depressive Disorder

Sarah Cohen-Woods<sup>1</sup>

<sup>1</sup>*Institute of Psychiatry, King's College London*

Major Depressive Disorder (MDD) is a leading cause of global disability, with heritability estimates ranging from 48 - 75%. However specific genes have yet to be consistently identified through linkage, candidate, and large-scale genome-wide association studies. This may be due to diagnostic and genetic heterogeneity, contribution from rare variants, gene-environment (G-E) interactions and epigenetic phenomena that go undetected in classic case-control association studies. The stress pathway interacts directly with the inflammatory system, and childhood maltreatment has been consistently associated with depression and with an enhanced inflammatory response. G-E studies in psychiatry investigating stress genes have yielded replicated findings in population studies (specific variation in the GR, CRHR1, and FKBP5 genes). Previous research will be briefly described, and novel research that aims to extend this research to a clinical case-control sample will be presented. In addition to this the first study to investigate variation in inflammatory candidate genes will be described. The sample consists of 227 individuals with recurrent moderate to severe MDD (ICD10/DSM-UV), and 228 unaffected individuals screened by questionnaire and telephone interview for absence of psychiatric disorder Child Trauma Questionnaire was completed to assess exposure to sexual, physical and emotional abuse, and physical and emotional neglect. DNA was extracted from blood or cheek swabs and genotyped externally using the Illumina Human 610-Quad bead chip, and imputed against the publically available 1000 genomes data. Candidate stress gene variation investigated was restricted to previously identified risk variants that interact with childhood maltreatment in GR, CRHR1, and FKBP5 in population studies. As genetic variation in inflammatory candidate genes (IL-1B, IL-6, IL-11, TNF, TNFR1, and TNFR2) has never been studied with childhood maltreatment and depression, SNPs were selected to tag the entire gene ( $r^2 > 1.0$ ) and to include functionally relevant SNPs. All forms of childhood maltreatment were increased in affected individuals relative to controls; main effects and G-E interaction effects will be described in detail, using dominant, additive, and recessive models. Genetic variation in inflammatory genes that present evidence for G-E interaction with childhood maltreatment to predict depression, were also genotyped and analyzed in a targeted replication study ( $n = 250$  cases and 250 screened controls). This study is the first to investigate the interaction of childhood maltreatment events with variation in stress genes in a clinical case-control (non-population) sample, and the first ever to explore potential interactions with cytokine (inflammation) genes in psychiatric disorder. This could lead to novel interventions and pharmacological targets; this is particularly salient as a history of childhood maltreatment is also associated with reduced pharmacotherapeutic response.

## Symposium 7

### **From GWAS Signals to Neural Mechanisms: Neurocognitive, Neuroimaging and Cellular Approaches to Characterizing the Functional Effects of Psychosis Risk Variants**

**Chair: Gary Donohoe**

**Co-Chair: Katherine Burdick**

**Tuesday, 16 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** Recent large-scale GWAS have identified more than a dozen novel risk alleles for both schizophrenia (SZ) and bipolar disorder (BPD), in addition to many more MHC class I variants. These include at least 8 convincing risk loci for SZ (Ripke et al. 2011) and ~4 for BPD (Sklar et al. 2011). Consistent with compelling family-based evidence of considerable genetic overlap between these two disorders, many of the variants initially identified as predisposing to SZ have subsequently been associated with BPD, and vice versa (Williams et al. 2011). Despite the evidence of partial sharing of molecular risk factors, BPD and SZ are not a single diagnostic entity. Rather, it is thought that the genetic variants that influence risk for both SZ and BPD may do so via their effects on overlapping intermediate phenotypes (e.g. psychosis; cognitive dysfunction; structural/functional brain abnormalities; mRNA/protein expression). This symposium will focus on approaches that have been utilized to follow up on GWAS results to better understand the functional relevance of variants identified using GWAS. Dr. Gary Donohoe (Trinity College, Dublin), will provide a brief overview of successes and challenges of the cognitive and imaging studies of GWAS signals. He will present new cognitive and imaging data on a CSMD1 psychosis risk variant recently identified in the Ripke et al GWAS mega-analysis, which provides evidence of the gene's involvement in brain structure and function. Dr. Matthew Hill (Institute of Psychiatry, King's College London) will describe studies in which the expression of TCF4 - another GWAS-identified susceptibility gene for SZ - has been experimentally altered in neural progenitor cells derived from human foetal cortex. Using genome-wide expression profiling, Dr. Hill and colleagues find changes in the expression of genes involved in cell cycling, providing the first molecular data on the neural functions of TCF4 and suggesting a mechanism by which variation within it may confer risk to SZ. Dr. Andrew McIntosh will describe a polygenic approach to characterizing the neural effects of GWAS identified risk variants for BPD on cortical activations during executive processing/language tasks in individuals at familial risk for BPD and healthy controls. He will discuss evidence that, in addition to a significantly higher polygene scores observed in BPD, increased polygenic risk allele load was associated with variation in cortical activations. Finally, he will discuss how this novel polygenic approach to examining brain imaging data may be a useful means of identifying endophenotypic traits relevant to BD risk mechanisms. Dr. Sophia Frangou (Institute of Psychiatry, London) will describe a series of studies in BPD focusing on the two leading candidate risk genes, ANK3 and CACNA1C. She will present data from her group that begin to elucidate the functional effects of these risk genes on neurocognition, structural brain morphology, and functional neuroimaging parameters in BPD patients, their unaffected relatives, and healthy individuals. Dr. Katherine Burdick (Co-chair; Mount Sinai, New York) will serve as the discussant for the session by summarizing these divergent approaches each focused on the common goal of gaining a better understanding of the functional effects of GWAS-identified risk loci on clinical, molecular, and brain-related phenotypes.

### **SS 7.1 CSMD1 Genome-wide Associated Risk Variant for Schizophrenia: Effects on Brain Function and Structure**

Gary Donohoe<sup>1</sup>, Emma Rose<sup>1</sup>, Derek Morris<sup>1</sup>, James Walters<sup>1</sup>, April Hargreaves<sup>1</sup>, Michael Gill<sup>1</sup>, Dan Rujescu<sup>1</sup>, Aiden Corvin<sup>1</sup>  
<sup>1</sup>Trinity College Dublin

The single nucleotide polymorphism rs10503253, located within the CUB and Sushi multiple domains-1 (CSMD1) gene on 8p23.2, was recently identified as genome wide significant for schizophrenia, but is of unknown function. The present study aimed investigate the effects of this CSMD1 variant *in vivo* in patients and healthy participants using behavioral and imaging measures of brain structure and function. We compared carriers and non-carriers of the risk 'A' allele on measures of neuropsychological performance in independent samples of Irish patients and controls and German patients and controls, and on measures of brain structure and function in Irish healthy participants using functional and structural MRI. Across the five samples investigated, the risk 'A' allele at CSMD1 was associated with deleterious effects on neurocognitive function. This was observed both on neuropsychological measures of general cognitive ability and memory function, and during an fMRI study of spatial working memory. These differences were observed in the absence of significant structural differences in grey or white matter volume. These data provide strong evidence that an effect on neurocognitive function is likely to be at least part of the mechanism by which CSMD1 associated risk for schizophrenia is mediated.

## SS 7.2 The Neural Basis of Polygenetic Risk to Bipolar Disorder

Andrew McIntosh<sup>1</sup>, Jessika Sussmann<sup>1</sup>, Heather Whalley<sup>1</sup>, Jeremy Hall<sup>1</sup>, Stephen Lawrie<sup>1</sup>

<sup>1</sup>*University of Edinburgh*

Growing evidence suggests a significant polygenetic contribution to affective disorders and schizophrenia, where the cumulative effects of many common low-penetrance alleles increase the risk of illness. We sought to determine the neural mechanisms underlying these associations using brain imaging. Using the Psychiatric GWAS consortium data for Bipolar Disorder as a training set, we have calculated a polygenetic 'risk' score in an independent sample of individuals with brain imaging data studies as part of a prospective high-risk cohort study. Polygenetic scores were regressed against brain activation in the cohort and compared between individuals who did and did not become unwell. Individuals who were at high risk of bipolar disorder had higher polygenetic loading scores for BD than controls. Within the high-risk sample, those people who later developed illness also had higher scores than those who did not. Polygenetic risk scores were also associated with increased activation of the amygdala and subgenual anterior cingulate cortex. Further structural imaging and cognitive analyses are underway and will be presented at the meeting. Polygenetic risk to bipolar disorder is associated with being at familial risk, converting to illness at a 2-year follow up assessment and with altered activation of an emotional brain circuit. These findings suggest that the use of a polygenetic risk score may help to identify the neural mechanisms underlying complex neuropsychiatric disorders and identify endophenotypes for these disorders that may aid gene identification.

## SS 7.3 Molecular Mechanisms of the Schizophrenia Susceptibility Gene TCF4

Matthew Hill<sup>1</sup>, Richard Killick<sup>1</sup>, Ricardo Sainz-Fuertes<sup>1</sup>, Nicholas Bray<sup>1</sup>

<sup>1</sup>*Department of Neuroscience, Institute of Psychiatry, King's College London*

Genome-wide association studies (GWAS) have identified common variants conferring susceptibility to schizophrenia with unprecedented confidence. Multiple single nucleotide polymorphisms (SNPs) residing in or near the TCF4 gene exceed criteria for genome-wide significance. In humans, TCF4 deletions and rare protein changing polymorphisms cause Pitt-Hopkins syndrome, a disorder characterized by mental retardation, distinctive facial features and intermittent hyperventilation. The importance of TCF4 in normal development is also evident from genetic manipulations in mice, where homozygous deletions are lethal. Mice over expressing *tcf4* postnatally have impaired fear conditioning and sensorimotor gating. TCF4 encodes a protein belonging to the basic helix-loop-helix family of transcription factors, which are capable of directly regulating the expression of other genes by binding to DNA elements. However, the specific gene targets and functions of TCF4 are unknown. To gain biological insight into the function of TCF4, we performed genome-wide expression profiling following TCF4 knockdown in neural progenitor cell lines derived from human foetal cortex. Primary experiments were performed using the CTXOE03 neural progenitor cell line, which we have recently used to explore the function of another schizophrenia susceptibility gene, ZNF804A (Hill et al, HMG 2012). TCF4 was experimentally reduced using two non-overlapping siRNAs and compared with a negative control siRNA, resulting in three experimental groups. Gene expression measurements were performed using Illumina HT-12 v4 BeadChip arrays. Differentially expressed probes were identified by t-test and nominally significant probes common to both target siRNA groups compared to control ( $P < 0.05$ , same direction of change) were subject to gene ontology and pathway analysis using DAVID Bioinformatics Resources. Replication experiments were then performed in an identical fashion using a second human neural progenitor cell line, CTXOE16. In CTXOE03 cells, each target siRNA reduced TCF4 protein expression by ~30%. Nominally significant ( $P < 0.05$  and same direction of change) gene expression changes common to both TCF4 siRNA groups, compared to control, were identified at 628 probes. Gene ontology (GO) analysis revealed these probes to be significantly enriched for various GO terms relating to the cell cycle, and, in particular, M-phase. In addition, the KEGG pathway 'cell cycle' was significantly over represented. Replication experiments using CTXOE16 cells identified nominally significant changes common to both TCF4 siRNA conditions at 197 probes. In agreement with our findings using the CTXOE03 cell line, these transcripts were again significantly enriched for cell cycle GO categories. Our unbiased assessment of gene expression changes induced by TCF4 knockdown suggests a role for TCF4 in mitosis. Variation in TCF4 might therefore increase risk for schizophrenia by altering neural cell number and/or differentiation.

#### SS 7.4 Functional Effects of Two Different Ank3 Alleles Associated with Psychosis

Sophia Frangou<sup>1</sup>

<sup>1</sup>*Institute of Psychiatry, King's College London*

Genome-wide association studies (GWAS) have implicated the ANK3 gene in the aetiology of psychosis particularly Bipolar Disorder and Schizophrenia. The gene encodes AnkyrinG, a cytoskeletal protein, located in the initial axonal segment and in the nodes of Ranvier that is involved in the clustering and cooperative activation of sodium gated channels which initiate and facilitate rapid and repetitive action potential firing in myelinated neurons. Fifty-two participants were genotyped for ANK3 rs9804190 and forty-six for ANK3rs10994336. All participants were of white British ancestry. Functional magnetic resonance imaging (fMRI) data were obtained from all participants while performing the 3-back working memory task and analyzed using statistical parametric mapping. For each SNP, homozygotes and heterozygotes for the respective risk alleles were grouped together. With respect to ANK3 rs9804190, the sample consisted of 17 risk allele carriers and 35 non-risk allele homozygotes. With respect to ANK3 rs10994336, the sample consisted of 14 risk allele carriers and 32 non-risk allele homozygotes. Compared to their respective non-risk homozygotes, ANK3 rs9804190 risk allele carriers during the 3-back vs, 0-back contrast showed increased activation in 2 prefrontal clusters centered in the left inferior frontal gyrus (BA 11/47) and the left middle frontal gyrus (BA 45/46). In the same contrast, compared to their respective non-risk allele homozygotes, ANK3 rs10994336 risk allele carriers showed increased activation in the right visual association cortex (BA19). Our results suggest that ANK3 may be a pleiomorphic risk locus with different variants impacting on disease risk via different pathways.

#### Symposium 8

##### Practical, Societal, Ethical, and Legal Challenges for Modern Biobanking and Brainbanking

**Chair: Thomas Schulze**

**Co-Chair: Peter Falkai**

**Tuesday, 16 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** Future success in biological psychiatric research will hinge on the availability of large and adequately characterized samples of patients and control individuals. Also, epidemiological cohorts are gaining more and more popularity for biological studies. Not only will ample phenotype data be needed but various types of biomaterial collected from study participants will be crucial for 21st century research. Biomaterial to be collected may include any type of tissue, in particular brain tissue or fibroblasts, whole blood, plasma, serum etc. For brain research, collection of whole brains has been and will always be key to successful research in neuroscience. Thus, brain banks and biobanks are gaining increasing importance in our field. Now that more and more research institutions and even countries as a whole are getting involved in setting up such infrastructure, there is a definite need to discuss the various practical, societal, ethical, and legal challenges involved and eventually find answers to the most ardent questions. How to set up a biobank or brain bank? How to join already existing banks into a network of banks that benefits the individual researcher? What IT solutions will be required? Can informed consent still be the basis for an individual's participation or will we have to move to a truly broad consent? How can this be regulated by legislatures? How can we involve patients' advocacy group? These and other questions will be addressed by a panel of international experts in biobanking, brain banking, bioethics and international leaders of major research and funding bodies. Thomas G. Schulze (University of Göttingen, Germany & Johns Hopkins University), who coordinates the biobanking efforts at the University of Göttingen will co-chair this study group together with Peter G. Falkai (Chairman, Department of Psychiatry, University of Munich, Germany). Heike Anderson-Schmidt (University of Göttingen, Germany) will present a German-wide effort to establish a huge cohort of psychiatric patients, the so-called "DGPPN-Kohorte", facilitated by the German Association for Psychiatry and Psychotherapy (DGPPN). This project, which is conceptualized to run for at least the next decade, will join phenotype and biomaterial, using a sophisticated IT platform that guarantees flexible management of data while adhering to highest data protection standards (e.g. through an honest broker approach). Peter Falkai will present experiences from BrainNet Europe. Camilla Stoltenberg (Deputy Director, Norwegian Institute of Public Health) will share the Scandinavian experience with large epidemiological, population-based health registries and biobanking using these unique resources. Shawn H.E. Harmon (School of Law, University of Edinburgh) will offer the lawyer's and bioethicist's point of view, drawing from ample experience with the UK and Taiwan Biobank. Finally, Marcella Rietschel (Central Institute of Mental Health, Mannheim) will serve as the discussant.

### **S 8.1 The DGPPN Cohort Study a National Initiative by the German Association for Psychiatry and Psychotherapy (DGPPN) for Establishing a Large-scale Cohort of Psychiatric Patients**

Heike Anderson-Schmidt<sup>1</sup>

<sup>1</sup>*Section on Psychiatric Genetics, Department of Psychiatry, University of Göttingen*

Results from genome-wide association studies (GWAS) have demonstrated the need for even larger samples than studied so far to comprehensively understand the complex genetic architecture of mental illness. Little is known about the course of various illnesses over time as information is usually collected retrospectively, and criteria and methodologies used in various studies are heterogeneous. Therefore, collaborations between different centers collecting the same information longitudinally are essential to understand the relationship between phenotypes and genotypes better. Ideally, any large-scale collaborative effort should bring together academic and non-academic centers. To foster such a research framework, the DGPPN has committed to establish a prospective national cohort of patients with major psychiatric disorders. A steering committee is supervising the collection of longitudinal data on a large national scale in centers spread across Germany, using existing information and re-recruiting patients to collect additional information, focusing on the course of illness over time. The use of uniform phenotyping, standardized measures, identical consent forms, shared databases and other resources, while simultaneously ensuring the highest standards of data protection and quality assurance, makes this a new and exciting attempt to advance our knowledge of psychiatric disorders. Another asset to this will be the use of a centralized biobank structure, achieved by networking already established “biobanks”. This will make it possible to pool already existing data from various centers, while meeting the highest data protection standards. By combining resources, the aim is to have a cohort of 100 000 patients by 2020. This cohort will not be restricted to genetic or other biological psychiatric research but constitute a valuable resource for research on epidemiological aspects, quality of care, and socio-demographic aspects of psychiatric morbidity.

### **SS 8.2 The Biological Psychiatrist’s View on Brainnet Europe II (BNE), A European-Wide Association of Brain Banks**

Peter Falkai<sup>1</sup>

<sup>1</sup>*Department of Psychiatry, Ludwig-Maximilians-University of Munich*

The development of new molecular and neurobiological methods, computer-assisted quantification techniques and neurobiological investigation methods which can be applied to the human brain, all have evoked an increased demand for post-mortem tissue in research. Funded by the European commission in the 6th framework, BrainNet Europe II (BNE) is an association of brain banks across Europe. P. Falkai is leader of the brain bank for psychiatric diseases, starting with the collections of brains of patients with affective disorders in BrainNet and extending to schizophrenia in BNE. The presentation will outline the consensus of the working group for neuropsychiatric brain banking organized in BNE, on ethical guidelines for brain banking, clinical diagnostic criteria, and a minimal clinical data set of retrospectively analyzed cases as well as neuropathological standard investigations to perform staging for neurodegenerative disorders in brain tissue. We will list regions of interest for assessments in psychiatric disorder, propose a dissection scheme and describe preservation and storage conditions of tissue. The main focus of our research was on assessment of neuromorphological aspects of schizophrenia.

### SS 8.3 “When the Entire Country is a Cohort.” Registries, Cohorts and Biobanks in Norway: Research Opportunities and Ethical, Legal and Societal Implications

Camilla Stoltenberg<sup>1</sup>

<sup>1</sup>*Norwegian Institute of Public Health*

The demand for scientific knowledge as a basis for health care increases steadily. Many of the most pressing research questions cannot be addressed through randomized controlled trials, and the quest for good observational studies is increasing. The Nordic countries have developed advanced research infrastructures based on nationwide routine registries, population-based longitudinal cohorts and biobanks that can be used in observational studies of genes, environment and health. Using Norway as an example, I will describe how these infrastructures provide unique research opportunities, particularly for discovering causes of diseases, but also for clinical, basic and translational research. Prerequisites for development of such research infrastructures are democratic societies with strong individual rights combined with regulatory systems ensuring privacy. In addition, it is of great value to have personal identification numbers assigned to all inhabitants and single payer public health care systems. Norway has (1) several nationwide registries recording health events from birth to death, (2) large, longitudinal cohorts in which more than 10% of the population has given consent to participate, and (3) population based and clinical biobanks organized in Biobank Norway. The largest population based cohorts are the Norwegian Mother and Child Cohort Study (MoBa) with about 270,000 participants and the Cohort of Norway (CONOR) with about 230,000 participants. Via the personal identification number, information from the cohorts can be linked with outcomes, exposures (for example folate intake at conception) and background variables (education, income, country of origin etc.) from the registries. Based on this information one can design nested case-cohort studies and access biological samples collected in the cohorts (for example plasma and RNA from cord blood) or in clinical biobanks (for example tumor tissue) to investigate specific hypotheses. Ethical, legal and societal governance is developing continuously to manage the challenges involved in such research.

### SS 8.4 Brain Banking: Ethical Issues and Legal Solutions the UK Experience

Shawn Harmon<sup>1</sup>

<sup>1</sup>*J. Kenyon Mason Institute for Medicine, Life Sciences & Law; School of Law Edinburgh University*

Like most forms of biobanking, brain banking is aimed at investigating and ultimately remediating a variety of inherited and acquired diseases and conditions. Also like most forms of biobanking, some of the key socio-ethical challenges revolve around effective recruitment of ‘participants’ and appropriate use of the resource. After briefly examining conceptions of the brain, this paper considers how the (ethical) obligations of (1) respecting people and operationalizing autonomy, (2) ensuring transparency of the science, and (3) encouraging legitimate trust in the undertaking are handled by the UK’s (legal and ethical) regulatory framework. Drawing on the Human Tissue Act 2004, the Human Tissue (Scotland) Act 2006, the HTA’s Code of Practice, the Mental Capacity Act 2005, and the Data Protection Act 1998, it considers capacity, consent, and privacy, and the management of brain banks in the UK. It suggests that UK Biobank’s ethics+ approach to governance is a positive model to emulate.

## Symposium 9

### Genome Sequencing in Bipolar Disorder

**Chair: John Kelsoe**

**Co-Chair: Francis McMahon**

**Wednesday, 17 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** Recent dramatic advances in sequencing technology have brought great increases in throughput and rapid reduction of costs. These developments now make possible an unprecedented level of detail in the search for disease genes and mutations for complex disorders. It also makes possible the testing of rare variant models of disease not previously possible. In this symposium, early results from several genome sequencing projects of bipolar disorder will be presented. Both family-based and case-control strategies will be represented. John Kelsoe will present results of whole genome sequencing of a family cosegregating for bipolar disorder and medullary cystic kidney disorder and the identification of a rare coding sequence variant implicating neurotrophin signaling. Jimmy Potash will present results from exome sequencing of both family and case control samples. Margit Burmeister will present results from a large case control whole genome sequencing study of bipolar disorder. Seth Ament will present results from the whole-genome sequencing of 40 multiplex bipolar families. Lastly, Francis McMahon will serve as discussant summarizing the early results and potential of this exciting technology

### S 9.1 Whole Genome Sequencing in an Unusual Family Identifies a Possible Pathogenic Variant in the NTRK1 Gene

John R. Kelsoe<sup>1,2</sup>, Szabi Szelinger<sup>3</sup>, David Craig<sup>3</sup>, Tatyana Shekhtman<sup>1,2</sup>, Martin Schalling<sup>4</sup>, Nicholas Schork<sup>5</sup>, The Bipolar Genome Study (BiGS)

<sup>1</sup>Department of Psychiatry, University of California San Diego, <sup>2</sup>VA San Diego Healthcare System, <sup>3</sup>Translational Genomics Institute, <sup>4</sup>Karolinska Institutet, Stockholm, <sup>5</sup>Scripps Genomic Medicine

We have previously reported an unusual family in which bipolar disorder co-segregates with medullary cystic kidney disease (MCKD). Linkage analysis suggested several genomic regions, one of which on 1q21 included a critical region reported for MCKD. Whole genome sequencing was conducted on the proband using Illumina technology and 30x coverage towards identifying possible causative mutations. Reads were mapped and filtered for quality control, and then variants detected using GATK. Predicted deleterious coding sequence variants were filtered for novelty and mapping to within linked regions in the family. Variants of interest were validated using Sanger sequencing of all family members to confirm co-segregation with disease. A missense mutation was identified in the NTRK1 gene that resulted in a glutamic acid to lysine substitution in the juxtamembrane region of the TrkA receptor. This variant produces a charge change near a phosphorylation site involved in SHC binding and signaling. A two stage strategy was employed for additional validation in which the exons in the NTRK1 gene were sequenced using a pooling strategy in 1000 bipolar I cases and 1000 controls. Results from this second stage sequencing, as well as, studies of receptor function in lymphoblastoid cell lines will be presented. These results are consistent with previous literature implicating multiple components of neurotrophin signaling in bipolar disorder and response to lithium. They also demonstrate the value of whole genome sequencing followed by targeted case control follow-up in identifying possible causative genes and rare variants. This strategy is currently being used by the Bipolar Genome Study (BiGS) consortium in a set of 50 additional families. This study and its design will be discussed.

## SS 9.2 Whole-exome Sequencing in Bipolar Disorder

Jimmy Potash<sup>5</sup>, Jennifer S. Parla<sup>1\*</sup>, Fernando S. Goes<sup>2\*</sup>, Mehdi Pirooznia<sup>2\*</sup>, Elena Ghiban<sup>1</sup>, Senem Mavruk<sup>1</sup>, Melissa Kramer<sup>1</sup>, Fayaz Seifuddin<sup>2</sup>, Eran Elhaik<sup>3</sup>, Yun-Ching Chen<sup>4</sup>, Virginia L. Willour<sup>5</sup>, Aravinda Chakravarti<sup>3</sup>, Rachel Karchin<sup>4</sup>, Peter P. Zandi<sup>2</sup>, W. Richard McCombie<sup>1</sup>

<sup>1</sup>Stanley Institute for Cognitive Genomics, Cold Spring Harbor Laboratory, <sup>2</sup>Department of Psychiatry, Johns Hopkins School of Medicine, <sup>3</sup>McCusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine, <sup>4</sup>Institute for Computational Medicine, Johns Hopkins University, <sup>5</sup>Department of Psychiatry, Carver College of Medicine, University of Iowa

\*These authors contributed equally

Bipolar disorder susceptibility is likely to be the result of both common and rare susceptibility alleles. While several common variants have recently been identified, the study of rare variants, using next-generation sequencing, is just beginning. In this study, we employ whole-exome sequencing to search for rare functional coding variants in 200-300 bipolar cases and 100-150 controls and in 10-15 families with multiplex bipolar disorder. Exome sequencing is performed using NimbleGen solution-based capture and paired-end sequencing on the Illumina GA II or HiSeq. Annotation is done with SIFT, PolyPhen2, and VEST. Data is analyzed using CAST, WSS, KBAC, SKAT, and VAAST. Mean coverage for the targeted exome sequence was 80% at 20X coverage and 89% at 10X. About 14,000 variants were identified per individual, including ~3,600 missense or nonsense. About 1,600 per subject were bioinformatically predicted to be damaging. Analyses of the case-control comparison and of segregation of variants with the phenotype in families will be presented. Conclusions: We are continuing to expand our case-control sample with a goal of 1,800 cases and 1,800 controls. The power of this work will be greatly enhanced by collaborative work within a newly formed bipolar disorder sequencing consortium.

## SS 9.3 The Bipolar Research in Deep Genome and Epigenome Sequencing (BRIDGES) Study

M. Burmeister<sup>1,2,3</sup>, S. Levy<sup>5</sup>, M. Flickinger<sup>4</sup>, A. E. Locke<sup>4</sup>, J. Li<sup>3</sup>, D. Absher, J. Huyghe<sup>4</sup>, B. Iocano<sup>6</sup>, M. McGue<sup>6</sup>, M. McInnis<sup>2</sup>, J. Vincent<sup>7</sup>, H. Akil<sup>1,2</sup>, S. Watson<sup>1,2</sup>, L. J. Scott<sup>4</sup>, G. Abecasis<sup>4</sup>, R. M. Myers<sup>5</sup>, M. Boehnke<sup>4</sup>

<sup>1</sup>Molecular & Behavioral Neuroscience Inst., <sup>2</sup>Psychiatry, <sup>3</sup>Human Genetics, <sup>4</sup>Biostatistics, University of Michigan, <sup>5</sup>Hudson-Alpha Institute, <sup>6</sup>Psychiatry, University of Minnesota, <sup>7</sup>Centre for Addiction & Mental Health, University of Toronto.

Although Bipolar Disorder is highly heritable, genetic studies to date have identified only a handful of confirmed genetic risk variants, and those with modest relative risks. This missing heritability has many possible explanations. The BRIDGES study seeks to determine whether less common and rare variants, not captured by a typical genome-wide association study, play a significant role in determining bipolar disorder risk. We are carrying out medium-depth (~10x) whole genome sequencing of 2000 European-origin bipolar cases and 2000 psychiatrically normal controls. Cases have a diagnosis of Bipolar I disorder (DSM IV) based on the DIGS or the SCAN. Controls are chosen for absence of psychopathology (no diagnosis of major depression or other major psychiatric illness, absence of alcohol/substance abuse diagnosis) and are matched to cases based on principal component analysis of available SNP genotype data. We are sequencing DNAs from blood to avoid genetic variation that may arise during cell culture. Our analysis will focus on burden tests of sets of variants within genes and other functional units, as well as on single-variant tests of association. At the time of abstract submission, ~500 individuals have been sequenced on Illumina HiSeq machines using paired end reads; sequencing continues at a current rate of ~120 individuals per month. We will present preliminary results of a first data freeze of ~800 individuals.

## SS 9.4 Family Genome Sequencing of Bipolar Disorder

Seth Ament<sup>1</sup>

<sup>1</sup>*Institute for Systems Biology*

The genetic basis for bipolar disorder and other psychiatric diseases is likely both highly multigenic and heterogeneous between individuals. The Family Genomics Group at the Institute for Systems Biology has pioneered applications of whole genome sequencing to identifying genes predisposing to simple and complex genetic disorders. In collaboration with NIMH, UCSD and the Bipolar Genome Study (BiGS) consortium, we hypothesized that some families with apparently inherited risk for bipolar disorder would have identifiable detrimental genetic variation. An advantage of family-based sequencing is that disease cases within a given family are likely genetically homogeneous and unlikely to be associated with variants that have arisen de novo and confined to a single individual. We selected 150 individuals for whole genome sequencing from 40 pedigrees of European ancestry with segregating bipolar disorder. These pedigrees have previously been analyzed by linkage analysis with 6090 genome-wide SNPs. We selected two to twelve individuals from each pedigree: i) all individuals from five three-generation pedigrees, ii) a single case and a haplotype control from pedigrees with a locus with a high LOD score and apparently dominant disease inheritance; iii) family quartets from pedigrees with apparently recessive disease inheritance; and iv) multiple affected cases from pedigrees with more complex inheritance. We describe regions of shared inheritance across disease cases within and between families, and rare variants within these regions that may contribute to disease susceptibility. We present strategies to validate our results with additional cases and controls and to elucidate disease-related gene networks in the brain using computational and experimental approaches. Genetic susceptibility to psychiatric disease involves a combination of common and rare alleles, as well as de novo mutations. These factors may vary in different families. Family-based genome sequencing is a compelling approach to discover disease-causing variants.

## Symposium 10

### Comorbidities and Cross-disorder Analyses in Psychiatric Disorders

**Chair: Manuel Mattheisen**

**Co-Chair: Preben Bo Mortensen**

**Wednesday, 17 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** Recent genome-wide association analysis for psychiatric disorders provided strong evidence for the presence of common and rare (structural) variants, which are underlying psychiatric disorders, as well as a number of somatic disorders. Irrespective of the unquestionable importance of such findings, little is still known about the shared genetic risk architecture for these disorders. Classical epidemiological studies provided strong evidence for overlapping (genetic) risk factors among psychiatric (among others bipolar disorder and schizophrenia) and between psychiatric and somatic disorders (among others autoimmune diseases and schizophrenia / major depressive disorder). Therefore, it is no surprise that some genetic loci became promising candidates for more than one disease entity, respectively (e.g. genes that code for calcium channels and the MHC region). In addition, the application of polygenic modeling approaches provided first insights into shared genetic risk profiles on the basis of genome-wide data. To our opinion studies that aim to identify shared genetic risk components between disorders could not only help to identify new risk factors for the disorders under study, but also lead to advances in the classification of disease. This symposium will therefore try to shed light on some of the factors that are important while studying the (genetic) overlap of two or more disorders. In particular, we will focus on four different aspects. The source of information (i.e., national disease registries) and the biostatistical approaches that help to leverage this data (i.e., causal inference approaches). We will give examples for both, the genetic overlap in psychiatric disorders (schizophrenia, bipolar and major depressive disorder, autism and ADHD), as well as with somatic disorders (asthma and major depressive disorder).

## SS 10.1 Somatic Comorbidities as Clues for the Etiology of Schizophrenia

Preben Bo Mortensen<sup>1</sup>

<sup>1</sup>National Centre for Register-based Research, Aarhus University

Recent genome-wide association analysis provided strong evidence for the presence of common and rare (structural) genetic variants, which contribute to the disease risk in psychiatric and somatic disorders. Although classical epidemiological studies provided strong evidence for overlapping (genetic) risk factors for psychiatric (e.g. bipolar disorder and schizophrenia) and between psychiatric and somatic disorders (e.g. immune diseases and schizophrenia / major depressive disorder), little is still known about the shared genetic risk architecture for these disorders. Ideally large cohorts need to be available, ascertained without bias and recorded for at least two disorders, in order to study the genetic overlap between these disorders. Therefore, national disease registries like the Danish Psychiatric Central Research Register have been identified as valuable sources of information. They allow for the identification of adequate study samples, not only in the context of less prevalent disorders. In addition, they also help to identify reasonable phenotypes of interest for an efficient design in comorbidity studies. We will demonstrate how linking nationwide population-based registers may help to identify specific combinations of disorders for which subsequent genetic studies might be able to identify shared genetic risk variants. Using the Danish Psychiatric Central Research Register and the National Hospital Register we could show, that an association between atopic disorders and the risk of developing schizophrenia is stronger for asthma in contrast to other entities in the spectrum of atopic disorders. Hence, it is reasonable to assume, that studies into the shared (genetic) overlap of asthma and schizophrenia are more likely to be successful than studies including other atopic disorders. In addition, we will demonstrate the importance of disease registries in the planning and execution stage of comorbidity studies and describe some important properties of such registries (e.g. quality of diagnosis, coverage and others).

## SS 10.2 Estimation of Variance Explained by SNPS for 5 Disorders and Estimation of Genome-wide Pleiotropy between them

Naomi Wray<sup>1</sup>, S. Hong Lee<sup>1</sup>, Psychiatric Genomics Consortium-Cross Disorder Group

<sup>1</sup>Queensland Brain Institute, University of Queensland

Evidence for a shared genetic etiology between disorders could make important contributions to psychiatric nosology. However, to estimate the genetic correlation between two disorders from family data would require extremely large cohorts to be ascertained without bias and recorded for both disorders. Not surprisingly, such studies are difficult to achieve, and hence are limited. Data from genome-wide association studies (GWAS) provide perhaps our best hope of understanding the shared etiology of psychiatric disorders. Estimates of genome-wide pleiotropy can be achieved from independently collected cases and controls. Here, we apply methods, extended to bivariate models, for estimation of the variation within, and covariation between disorders, to data from the Psychiatric GWAS Consortium (PGC). The variance and covariance estimates are derived from the average genome-wide similarity between all pairs of individuals determined using all SNPs. Non-zero genetic (co)variation is estimated when case pairs and control-control pairs are on average more similar genome-wide than case-control pairs. PGC data comprises cases and controls for the disorders schizophrenia (SCZ), bipolar disorder (BIP), major depressive disorder (MDD), autism (AUT) and attention deficit hyperactivity disorder (ADHD), each imputed to a common reference panel (Hap Map 3, ~900,000 imputed SNPs). We analyzed each pair of disorders in turn, ensuring that the coefficient of the relationship of genome-wide similarity between individuals did not exceed 0.05. For all disorders, SNPS explained ~0.2 of the variance in liability, the SNP-chip heritability. Estimates of SNP-chip genetic correlations were high between SCZ and BIP, moderate between SCZ and MDD and between BIP and MDD and low between SCZ and AUT. All other correlations were not significantly different from zero.

### SS 10.3 Causation and Causal Inference in Genetic Epidemiology

Christoph Lange<sup>1</sup>

<sup>1</sup>*Department of Biostatistics, Harvard School of Public Health*

The research interest of studies for complex diseases in genetic associations, gene-gene interactions and gene-environment interactions is motivated by causal questions: Does this gene affect disease risk? Do these genes, or genes and environmental factors work in tandem to influence disease risk? Yet, notions of causality have hardly entered formal analyses of genetic association studies. In the statistical and epidemiological literature, formal thinking about causality is becoming more and more mainstream since pioneering work by Judea Pearl, James Robins and Donald Rubin. This contribution tries to make the reader aware of the developments in the causal inference literature that can be relevant for the analysis of genetic association studies. The basic concepts of causal inference will be introduced. In particular, it will be demonstrated how causal inference approaches can be used to shed light into causal dependencies in settings with two or more comorbid phenotypes. Here, the incorporation of prior knowledge, i.e., the functional relationship between two phenotypes of interest (e.g. in a causal diagram) will help to describe and analyze the shared genetic etiology between these two phenotypes. While this approach has been successfully applied in the context of lung cancer and nicotine addiction, little work has been done in other psychiatric phenotypes. The relationship between schizophrenia and alcohol (or nicotine) addiction for example, may serve as interesting study objects. Throughout this work, various examples from genetic epidemiology will be given. Together with pointers to the recent literature, they illustrate where the developments on causal inference have contributed to novel or improved insights and analysis possibilities.

### SS 10.4 Asthma as a Comorbidity in Psychiatric Disorders

Manuel Mattheisen<sup>1,2</sup>

<sup>1</sup>*Channing Laboratory, Brigham and Women's Hospital, <sup>2</sup>Harvard Medical School*

Classical epidemiological studies provide strong evidence for overlapping (genetic) risk factors between psychiatric and somatic disorders. Among others patients with asthma show an increased risk to develop major depressive disorder (MDD) and vice versa. In order to identify shared genetic risk factors for both disorders we therefore leveraged data from genome-wide association studies (GWAS) for asthma, as well as major depressive disorder and compared their results. In addition, we analyzed a dataset, for which data was not only available with respect to asthma, but also with respect to depression symptoms. The Childhood Asthma Management Program (CAMP) is a multi-center, double-masked, randomized, placebo-controlled study of children (aged 5-12 years at enrollment) with mild to moderate asthma during a 4- to 6-year period. Clinical assessments of a child's asthma began during the baseline period and continued at regular intervals during the course of the trial. Not only does CAMP provide information on the severity of asthma symptoms, but it also contains a rich variety of cognitive, psychological, and behavioral data collected on repeated occasions. We used the Childhood Asthma Management Program data to specifically address two questions. Our first aim was to identify shared genetic risk factors for asthma and major depressive disorder. We therefore used the severity of asthma and depression symptoms to identify underlying genetic variation for both. More generally we also aimed to identify genetic factors that might explain resilience in patients with asthma, which did not develop major depressive disorder (i.e., patients with a severe chronic disorder that substantially affects quality of life).

**Identification and Functional Consequence of Genetic Variants  
Conferring Risk of Psychiatric Disease in Outcome of NEWMEDS  
Collaboration**

**Chairperson: Michael Didriksen**

**Wednesday, 17 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** NEWMEDS - Novel Methods leading to New Medications in Depression and Schizophrenia - is an international consortium of scientists that has launched one of the largest ever research academic-industry collaboration projects to find new methods for the development of drugs for schizophrenia and depression. NEWMEDS brings together top scientists from academic institutions and most major players in the biopharmaceutical industry. Among others, NEWMEDS focuses on how genetic variants influence disease biology and drug responses. Through NEWMEDS deCODE has phenotyped (imaging and neuropsychological tests) over 1,300 subjects, including 346 subjects carrying Copy Number Variants (CNVs) under negative selection pressure and 470 non-carrying controls. Population controls, carrying the CNVs conferring risk of schizophrenia, but without a diagnosis, perform significantly worse than non-carrying controls on the neuropsychological tests. Structural brain imaging data has been analyzed at the Central Institute of Mental Health in Mannheim and the Institute of Psychiatry in London. For carriers of the 15q11.2 deletion and duplication, voxel-based morphometry the results demonstrate a pronounced dose-dependent reduction in white matter in cerebellum and superior temporal lobe, and of grey matter volume in the perigenual cingulate cortex and the angular gyrus. Duplication and deletion carriers showed opposite effects in key regions implicated in early psychosis. Correlations with neurocognitive phenotypes will be discussed. H. Lundbeck A/S has generated mouse models of the microdeletion syndromes. The 15q13.3 deletion syndrome model has been extensively characterized and the model recapitulates several aspects of the human condition. The identification of CNV's and the associated phenotypes in humans will be presented along with the characterization of the animal model of the schizophrenia-associated 15q13.3 microdeletion syndrome.

Hreinn Stefánsson<sup>1</sup>

*<sup>1</sup>deCODE Genetics Incorporated*

A set of 33 genomic regions containing large recurrent copy number variations (CNVs) was identified using a sample of 100,000 chip-typed individuals from Iceland. The number of distinct mutation events per CNV locus was assessed using long-range phased genotypes flanking the CNV regions. Genedrop simulations conditioned on a population-wide genealogy database were used to estimate the mutation rates of the CNVs under an assumption of neutral evolution. Forward-in-time simulation was then used to assess departures from neutral expectations in the allele frequency spectra of the CNVs, using the mutation rate estimates and a demographic model of Icelandic population from settlement (40 generations). The results indicate that many of the CNVs are subject to negative selection. One third of the recurrent CNVs under negative/purifying selection confer risk of psychiatric disease. Also, population controls carrying these CNVs perform worse on cognitive tests although the phenotypic stamp conferred by the different CNVs varies. CNVs conferring high-risk of schizophrenia affect in particular spatial working memory while CNVs conferring modest risk of schizophrenia contribute more to specific learning disabilities.

### SS 11.2 Gene-dosage Dependent Effects of a Copy Number Variant Associated with Schizophrenia Risk on Brain Structure

Andreas Meyer-Lindenberg<sup>1</sup>

<sup>1</sup>Central Institute of Mental Health

A microdeletion at 15q11.2 has been repeatedly found associated with schizophrenia risk, but the neural mechanisms mediating this observation were unknown. In a collaboration in the context of the NEWMEDs initiative, structural brain imaging data were analyzed from 178 control subjects (C), 45 carriers of the 15q11.2 microdeletion (M), and 45 subjects who carried a duplication at this site (D). Subjects were phenotyped and genotyped in Iceland. High-resolution structural brain imaging data were segmented into grey and white matter volumes and analyzed using voxel-based morphometry (VBM8, DARTEL template, volume modulation, smoothing with a 12 mm kernel). Multiple regression analyses with age, gender and CNV group affiliation as covariates were performed to analyze dosage effects. We hypothesized that microdeletion carriers would show abnormalities in regions implicated in first episode-psychosis and defined a-priori regions of interest in perigenual cingulate cortex and insula, performing family-wise error multiple comparison correction in these regions as well as the whole brain. Pronounced and significant gene-dosage dependent effects on grey matter were found in the left angular gyrus, the left insula and the perigenual cingulate cortex ( $D > C > M$ ) and in white matter in bilateral temporal lobe and cerebellar lobe ( $D > C > M$ ). Multivariate analyses support and extend these findings. Our results are consistent with previous evidence for associations of cingulate and insular abnormalities with psychosis (e.g. Fusar-Poli et al., World J Biol Psychiatry 2012). The affected regions are also implicated in networks supporting neurocognitive function associated with these genetic variants in the larger sample reported by Steffansson et al. in this session. To our knowledge, these data provide the first demonstration of dose-dependent effects of copy number variation in human brain in vivo and begin to define a circuit that mediates risk for schizophrenia in carriers of the 15q11.2 microdeletion.

### SS 11.3 A Mouse Model of 15Q13.3 Microdeletion Syndrome Recapitulates Several Phenotypes of the Human Syndrome

Jacob Nielsen<sup>1</sup>

<sup>1</sup>H. Lundbeck A/S

The 15q13.3 microdeletion syndrome is associated with a significantly increased risk of schizophrenia and epilepsy, but not all individuals with the deletion have obvious clinical findings. We have generated a mouse model of this condition with a deletion of the corresponding region from the mouse genome that is located on chromosome 7qC. The mice have been characterized by immunohistochemistry, microarray analysis and a broad battery of behavioral tests. The mice had no gross changes in brain morphology and were normal in most tested behavioral assays. However, they had schizophrenia relevant phenotypes in amphetamine disrupter PPI measurements and Morris Water maze. Furthermore, they exhibited a strong but complex phenotype in seizure assays, with a significantly increased seizure threshold in response to PTZ and other acute seizure induction paradigms, but increased interictal spiking after PTZ administration as measured by EEG. Thus, the mouse model recapitulates several aspects of the human condition including schizophrenia and epilepsy relevant phenotypes and provides a tool for investigating the biology underlying the 15q13.3 microdeletion syndrome and the associated conditions. The mouse model will be further profiled as part of the NEWMEDS project.

#### SS 11.4 A Mouse Model Of 15Q13.3 Microdeletion Syndrome Display Pre-attentive Processing Deficits and Eeg Phenotypes Seen in Schizophrenia

Michelle Rosgaard Birknow<sup>1</sup>

<sup>1</sup>H. Lundbeck A/S

Event-related magnetoencephalography (MEG) and electroencephalography (EEG) studies of pre-attentive processing of auditory-evoked potentials (AEP) have identified several characteristic phenotypes associated with schizophrenia. More specifically, reduced loudness dependence of AEPs, AEP amplitudes and sensory gating, as well as reduced mismatch negativity (MMN) amplitude has been repeatedly reported in schizophrenia. Moreover, abnormalities in cortical gamma-band (30–80 Hz) synchrony have been observed in schizophrenia with an increased or unchanged level of gamma during resting state, but a decreased capacity for evoked gamma oscillations e.g. when investigating auditory steady-state responses.

#### Symposium 12

##### The Challenge and Future of Depression Genetics

**Chair: Gerome Breen**

**Co-Chair: Douglas Levinson**

**Wednesday, 17 October, 2012 – 3:00 pm – 4:30 pm**

**Overall Abstract:** Major depressive disorder (MDD) is heritable but GWAS mega-analyses of 21,000 subjects have not identified risk loci. This is unique in biomedicine where samples this large have always identified at least one locus. What have we learnt and where do we go from here? Sullivan will summarize the Psychiatric GWAS Consortium (PGC) mega-analysis results (11,215 MDD cases and 9,761 controls) in the context of all other biomedical GWAS, discuss the central problem of heterogeneity, and discuss biomarker approaches and present new data from a large twin/MDD eQTL study. Power will discuss how current research in psychiatric genetics rests primarily on either common or rare deleterious mutations, though a perhaps more evolutionary plausible explanation is that common risk alleles may be beneficial under some circumstances. For depression there is both recent epidemiological evidence showing increased reproductive fitness in unaffected relatives of affected individuals but decrease it for affected individuals, and molecular evidence to show that some risk alleles are not always detrimental. This has implications on how we design studies to identify further risk alleles. Binder will discuss how exposure to stressful life events is one of the strongest established risk factors for the development of major depression and how it can be used to inform alternative approaches to GWAS analysis. Data from genome-wide gene expression before and after stimulation of the GR in peripheral blood of 202 male individuals will be presented and combined with genome-wide SNP and DNA methylation data for expression (eQTL) and DNA methylation quantitative trait locus (mQTL) analysis on GR-stimulated gene expression with the identification of many GR-eQTLs. Specific analyses of these eQTL SNPs in the PGC-MDD cohort will be presented. Breen will focus on what alternate genomic approaches can tell us about the biology of depression. Specifically, he will report on a combined GWAS and RNAseq analysis of 474 recurrent major depression cases and 475 controls using the Illumina Omni1-Quad GWAS arrays and multiplexed RNA sequencing using the Illumina HiSeq 2000 platform, (giving between 60-70 million 50 bp single-end reads per subject). He will report on the final analysis of gene expression and co-expression module QTL analysis finding for depression. Levinson will act as discussant and the overall aim of the discussion will be to crystallize some approaches to take the field of depression genetics beyond its current disappointments.

## SS 12.1 eQTL Analysis of Glucocorticoid Regulated Gene Expression: New Insights into the Genetics of Major Depression

Elisabeth Binder<sup>1</sup>

<sup>1</sup>Max-Planck Institute of Psychiatry

Exposure to stressful life events is one of the strongest established risk factors for the development of major depression. The stress hormone axis is one of the systems orchestrating the short and long-term effects of stress on the organism and a dysregulation of this system has been implicated in the pathophysiology of major depression. One of the main effectors of this system is the glucocorticoid receptor (GR), a nuclear receptor with transcription factor function. Genetic polymorphisms that alter the transcriptional effects of GR-activation might alter the short term as well as long-term effects of exposure to stress and thus the risk to suffer from major depression. Data from genome-wide gene expression before and after stimulation of the GR with 1.5 mg of dexamethasone in peripheral blood of 202 male individuals will be presented and combined with genome-wide SNP and DNA methylation data for expression (eQTL) and DNA methylation quantitative trait locus (mQTL) analysis on GR-stimulated gene expression. We identified 2,364 significant response eQTLs, i.e., loci that are only associated with variation in GR-stimulated gene expression changes, but not with baseline gene expression. These response eQTLs show a longer average SNP/probe distance of 406 kb than baseline eQTLs (136 kb) indicating a more long range regulation of gene expression by the GR. We not only observed an enrichment of GR response elements in response eQTL SNP sequences but also transcription factor (TF) binding sites that have been previously shown to be important for GR transcriptional effects, including AP1, HNF4 and OCT1. The response eQTL SNPs were enriched in loci previously been reported to be associated with depression (<http://www.genome.gov/gwastudies/> and <http://geneticassociationdb.nih.gov/>). Interestingly, the majority of these overlapping SNPs do not alter the gene expression of the closest gene but of more distant genes. For example, SNPs within the CLOCK gene moderate the gene expression of PAICS, which is 947 kb upstream of the CLOCK gene. The same SNP set was also enriched among SNPs associated at  $p \leq 0.05$  with major depression in the mega-analysis of the Psychiatric Genomics Consortium, both compared to random as well as baseline eQTL SNPs ( $p < 0.001$ ). Analysis of epistatic effects and effects of DNA methylation and mQTL SNPs on GR-stimulated gene expression are currently underway. In summary, using functional annotation of SNPs that may reflect differences in reactivity to stress, such as GR-stimulated gene expression appears to be a promising tool for a better understanding of the genetic risk factors of major depression.

## SS 12.2 Analysis of the Evolutionary Effects and Context of Depression

Robert Power<sup>1</sup>

<sup>1</sup>Institute of Psychiatry, London

The search for the causal genetic variants predisposing to psychiatric disorders has mainly focused on direct effects of either common or rare deleterious mutations. Considering the high prevalence of psychiatric disorders and apparent persistence despite reduced reproductive fitness presumably leading to purifying selection, a more evolutionarily plausible explanation is that risk variants may be beneficial under some circumstances. Belsky & Pleuss (2009) suggested that gene-environment interactions might not reflect just high risk genotype-environment combinations but plasticity genes i.e. variants that are deleterious in negative environments but allow an individual to thrive in positive environments. Our analysis of 5 psychiatric disorders a Swedish birth-cohort of ~2.3 million individuals from the general population showed that, unlike most other psychiatric disorders, depression does not appear to be under negative selection. Increased reproductive fitness in unaffected relatives of those with depression more than compensated for the decreased fitness of affected individuals. We have followed this up with analysis of molecular data in a subset of genotyped Swedish individuals (n~6200). Here, the Psychiatric GWAS Consortia's recent findings were used to construct polygenic scores for the risk alleles of both schizophrenia and depression. These were then tested for association with an individual's number of grandchildren. Again, while schizophrenia risk alleles were associated with significantly reduced reproductive fitness, there was no evidence of negative selection on depression. We now hope to test if depression risk alleles are associated with greater variance in reproductive fitness, rather than a mean difference. If indeed the main cause of depression is a mismatch between an individual's genotype and environment, it has implications on how we design studies to identify further risk alleles for depression. It would suggest the level of contamination of controls may be higher than previously thought, and that future etiological studies should put a greater emphasis on an individual's development in context and interactions of genetic disposition with the environment.

### SS 12.3 Depression Genes and Networks: Combining Genotype and Gene Expression Data to Unravel Regulatory Networks Contributing to the Risk of Major Depressive Disorder

Sara Mostafavi<sup>1</sup>, Douglas Levinson<sup>1</sup>  
<sup>1</sup>*Stanford University*

The goal of this project was to identify co-regulated gene modules (groups of co-expressed genes) whose expression levels differ between cases with recurrent major depressive disorder (MDD) and controls. All subjects were recruited from a national survey research panel that was representative of the US population. Panel members with self-reported European ancestry who responded to an emailed invitation were screened with an online CIDI-SF for (a) absence of current substance dependence, and (b) presence of either recurrent MDD or the absence of any two-week period with more than 3 of 9 MDD criteria. These individuals were then interviewed with the SCID (DSM-IV) after giving a blood sample for DNA and RNA (Paxgene collection system). The 474 fully eligible and 475 controls were assayed for common SNP genotypes (Illumina Omni1-Quad) and for genome-wide gene expression (multiplexed RNA sequencing using the Illumina HiSeq 2000 platform, 60-70 million 50 bp single-end reads per subject). We developed two sets of methods for data analysis: a pipeline for aligning sequenced reads, mapping them to genes, and correcting for latent variations due to numbers of reads per subject and differences in proportions of white blood cell types in these fresh blood samples; and a computational method that utilizes statistical learning to construct hierarchical regulatory networks from genotype and gene expression data. The method identifies expression and/or genetic variations in a set of key regulatory elements that affect expression levels of gene modules, and analyzes their association with MDD case status, after accounting for covariates that influenced expression independently of diagnosis. In addition, our method also analyzes the association of gene modules with other depression-related phenotypic variables, such as disease severity, family history, and age of onset. Our preliminary results identify several gene modules that significantly predict disease status and severity.

### SS 12.4 The Current State and Future of Depression Genetics

Gerome Breen<sup>1</sup>  
<sup>1</sup>*Institute of Psychiatry, King's College London*

I summarize the Psychiatric GWAS Consortium (PGC) mega-analysis results of >1.2 million autosomal and X chromosome single-nucleotide polymorphisms (SNPs) in 18 759 independent and unrelated subjects of recent European ancestry (9240 MDD cases and 9519 controls) and a large scale replication effort, discuss the central problem of heterogeneity, and discuss biomarker approaches and present new data from a large twin/MDD eQTL study.

# **INDEPENDENT ORAL PRESENTATIONS**

## Oral Presentations

### Oral Presentation Session 1

#### Schizophrenia

##### OS 1.1 Investigation of Pak7 Duplications as Risk Factors for Schizophrenia and Psychotic Disorder

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**Background:** Schizophrenia is a severe, heritable mental disorder which affects ~1% of the adult population worldwide. Common risk variants have been identified at 13 loci, although the effects on risk are modest (odds ratio (OR) 100kb), termed copy number variants (CNVs), have also been implicated. CNVs can confer substantial risk (OR= 2-30), but are rare.

**Methods:** Here we present further phenotypic studies of duplication carriers; analysis of the origin of the duplication event; and investigation of the role of the implicated gene in synaptic function.

**Results:** We confirmed association in a broader psychosis phenotype, including an additional 2,243 bipolar disorder cases analysis ( $p=0.00002$ , OR=4.77). All 15 cases and 3 of 5 controls from the UK/Ireland exhibit a 132-146.5 kb duplication involving the first two exons of the gene p21 Protein-Activated Kinase 7 (PAK7). We cannot exclude a single founder mutation, but investigation of the Irish cases suggests that this is not of very recent ancestry. Clinically, 11 of the 12 schizophrenia carriers had a chronic course and poor outcome. Duplication carriers showed a range of developmental phenotype and cognitive outcomes, including normal IQ. Finally, we demonstrate that the gene is over-expressed in response to NMDA-related synaptic plasticity.

**Discussion:** PAK7 is a brain specific gene and may have an important role in maintaining synaptic networks critical to psychosis susceptibility. However, the impact of the duplication on gene expression, the genetic mechanism involved and the consequences for cellular function require further investigation. Furtherwork is required to investigate the founder mutation event and to elucidate the molecular mechanisms involved.

##### OS 1.2 Psychopathological Characterization of Two Families with Brain Disorders and Segregating Mutations of Neurexin1

Linh Duong<sup>1</sup>, Thomas Werge<sup>1</sup>, Andrés Ingason<sup>1</sup>, Louise Hoeffding<sup>1</sup>  
<sup>1</sup>Institute of Biological Psychiatry, University of Oslo

**Background:** Neurexins are a family of highly polymorphic presynaptic adhesion molecules that bind to postsynaptic neuroligins and constitute scaffolding complexes essential for synapse formation and function. The Neurexin1-gene (NRXN1) is located on chromosome 2p16.3 and is 1,1 Mb in length. Recently, copy number variations (CNV) affecting the promoter and first exons of NRXN1 have been associated with schizophrenia, autism spectrum disorders and intellectual disability. To fully understand possible gene-specific psychopathological phenotype we carried out a study involving two families with segregating NRXN1 mutations and mental disorders.

**Methods:** The probands were referred to our institution due to severe clinical symptoms of epilepsy and schizophrenia, respectively. Both patients had relatives suffering from mental disorders. Members of the families were ascertained and prior to the enrollment all participants had given written informed consent. The Danish Scientific Committees (journal no. 2004-2-08) and the Danish Data Protection Agency (journal no. 2004-41-4222) have approved the study. The clinical characterization was assessed using The Schedules of Clinical Assessment in Neuropsychiatry (version 2.1, program version 1.0.3.5) and available medical records in order to obtain DMS-IV diagnoses. The DNA was extracted from peripheral blood lymphocytes and the genotyping was conducted using Human1M-Duo Beadchip and Affymetrix SNP 6.0 microarray. The deletions were subsequently confirmed by sequencing long range PCR products spanning the breakpoint junctions or using TagMan qPCR. In addition we performed sequencing of the coding exons 2-22 of the  $\alpha$ -NRXN1 isoform and their corresponding exon-intron boundaries with primer sequences suggested by Zweier et al. (Am J Hum Genet 85, 655-666, 2009).

**Results:** In the first family the proband was found to carry a compound heterozygous mutation in NRXN1 comprised of a large CNV of 451 kbp and a nonsense mutation in the same locus at exon 15. Screening of the family revealed that the deletion was transmitted from the mother to the proband, but not to his brother. The point mutation was subsequently identified in the proband's brother, but absent in the mother. This finding indicates paternal inheritance of the point mutation, as a de novo event in both brothers seems highly unlikely. The psychopathological assessment confirmed a diagnosis of mental retardation, autism and epilepsy in the proband, a psychotic disorder in his brother and subdiagnostic traits of autism in the mother. The proband's father was deceased, but according to the medical records he fulfilled a diagnosis of schizophrenia. The proband's uncle fulfilled the criteria of bipolar disorders but did not harbor NRXN1 alterations. The second family had two closely linked CNV spanning 26 kb and 33 kb, respectively, and they were located approximately 40 and 350 kb upstream of exon1 in the NRXN1-locus. The CNV's were inherited from the paternal grandmother, who had past the mutations to two of her sons, one of which subsequently passed the mutations to his son, the proband. The clinical evaluation confirmed that the proband carried the diagnosis of early onset of schizophrenia, but also uncovered that the father fulfilled a diagnosis of a schizophreniform disorder during a 3 month hospitalization at a mental hospital. In addition the grandmother had suffered from recurrent depression for many years. The proband's uncle who also carried the same mutations, was somatically and mentally healthy.

**Discussion:** The results suggest that mutations in the NRXN1-locus confer susceptibility to brain disorders. Additionally, we observe in the first family a novel gene-dose-effect of NRXN1 mutations

implying that NRXN1 is vital for normal brain function. The proband with a compound heterozygosity in the NRXN1 has a much more severe phenotype comparing to the other family members who are heterozygous. Together the study of two families with segregating mutations of NRXN1 demonstrates a striking pleiotropi. However, incomplete penetrance suggests that other factors are relevant to the phenotypic outcome. Together these findings highlight the importance of identifying other genetic variants and possible environmental factors affecting penetrance and specific clinical manifestation of NRXN1 deletions.

### OS 1.3 Additive Genetic Variation in Risk to Schizophrenia Shared between African American and European American Populations

#### ECIP

Teresa de Candia<sup>1</sup>, Hong Lee<sup>2</sup>, Jian Yang<sup>2</sup>, Brian Browning<sup>3</sup>, Pablo Gejman<sup>4</sup>, Douglas Levinson<sup>5</sup>, John Hewitt<sup>1</sup>, Peter Visscher<sup>2</sup>, Naomi Wray<sup>2</sup>, Matthew Keller<sup>1</sup>

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**Background:** Schizophrenia is a highly heritable disorder (~.7 to .8) with a prevalence rate of ~1%, but with prominent variation between populations (McGrath, 2006). However, to date, few studies have specifically examined whether SNPs associated with schizophrenia are shared across populations or, rather, are population specific. While Purcell et al. (2009) confirmed that SNPs associated with schizophrenia in a European American (EA) discovery sample replicated more highly in an independent EA target sample than they did in an African American (AA) target sample, so far there have been no direct estimates of the overlap in genetic variance contributed across ethnically distinct populations.

**Methods:** We used a recently developed method (Lee et al., 2011) to determine the extent to which additive genetic variance in liability to schizophrenia tagged by common SNPs is shared across African American (AA) and European American (EA) populations (AA n=2006; EA n=4796) in the Molecular Genetics of Schizophrenia (MGS) samples. Using 771112 genome-wide SNPs that passed rigorous quality control procedures, genetic similarities were derived for all pairs of individuals. Genetic variation underlying schizophrenia was then estimated as (1) that shared between ethnicities and (2) that which is shared only within ethnicities.

**Results:** After controlling for plate, we estimate the genetic variance in liability to schizophrenia associated with these SNPs to be .32 (SE .12) in AAs and .35 (SE .04) in EAs. For the combined samples, we estimate the genetic variance in liability to schizophrenia associated with these SNPs that is shared across the two populations (n=6802) to be .20 (SE .06) and the variance that is unique within each population to be .21 (SE .07). Constraining all of the genetic variance to be shared between populations results in a model with a significantly worse fit. However, AAs are known to be highly admixed. By limiting the analysis to EAs and only low-admixture AAs (n=5893), we find no evidence for shared genetic variance between the two populations (point estimate of 0, SE .08) and unique genetic variance within ethnicities to be .39 (SE .09). Because ethnicity and plate are completely confounded, plate effects could not be controlled for in the joint analysis of two ethnicities. Therefore, it is possible that plate effects contribute to the high unique genetic variance estimates. To test whether this was a plausible alternative explanation of our results, EAs were randomly divided into two groups such that group and plate were perfectly confounded. We repeated this 100 times and estimated genetic variance between and within these groups each time. This analysis showed no evidence of genetic variance unique within groups (average point estimate of 0, empirical SE .08), indicating that plate effects are unlikely to be the reason for the low genetic overlap observed in our results.

**Discussion:** These results suggest that common causal variants are likely to be important factors in the etiology of schizophrenia in both African and European descent populations, but also suggest that GWAS results of schizophrenia from EA populations cannot

typically be extrapolated to populations of African descent. These findings are novel and would not have been possible without molecular information, using only traditional family or twin study designs. Further work is needed to disambiguate whether the lack of shared genetic variance tagged by SNPs is a result of unique causal variants or different LD structures in the two populations. To the degree that SNPs associated with schizophrenia differ between EA and AA populations, continued investment in GWA studies using only European samples may yield results with a strong Eurocentric bias and may be inconsistent with the National Institutes of Health inclusiveness requirements.

#### **OS 1.4 Analysis of Recessive and Compound Heterozygous Variants in a Schizophrenia Exome Sequencing Sample of 5,000 Individuals**

Douglas Ruderfer<sup>1</sup>, Menachem Fromer<sup>1</sup>, Elaine Lim<sup>2</sup>, Jennier Moran<sup>3</sup>, Kim Chambert<sup>3</sup>, Christina Hultman<sup>4</sup>, Mark Daly<sup>2</sup>, Steven McC Carroll<sup>3</sup>, Patrick Sullivan<sup>5</sup>, Pamela Sklar<sup>1</sup>, Shaun Purcell<sup>1</sup>

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**Background:** Schizophrenia is a common, complex disease with a strong genetic basis. Although recent genome-wide studies have indicated important roles for both common single nucleotide polymorphisms (SNPs) and large, rare genic deletions and duplications, most of the heritability remains unexplained. Next-generation sequencing technologies can interrogate genetic variation that has previously eluded detection in genome-wide studies, namely rare SNPs, short indels and smaller copy number variations (CNVs). Here, we aimed to assess the role of rare recessive and compound heterozygote variants found from sequencing, to investigate the extent to which individuals who have genes with “multiple hits” are at an increased risk for disease.

**Methods:** Exome sequences were obtained using Agilent Sure Select hybrid selection arrays and a combination of Illumina GAI and HiSeq machines. Reads were processed using the Picard/BWA/GATK pipeline resulting in SNP/Indel calls. CNVs were called using XHMM. Analyses were performed using the Plink/Seq package and additional internal software.

**Results:** Using a preliminary dataset we analyzed over 1,000 Swedish schizophrenia cases and 1,000 matched controls looking for genes enriched for homozygous or compound heterozygous variants. We found that loss of function (LOF) variants at lower frequencies (below 5%) of these classes are present at over 30% higher rates in cases compared to controls. Additionally, we identify genes enriched for situations where both copies have been knocked out due to multiple LOF SNPs/indels or an overlap of a large deletion and a single LOF SNP/indel event.

**Discussion:** From previous work on rare CNVs and de novo mutation, there is strong reason to suspect that rare variants of moderately large effect contribute to psychiatric disease risk in disorders such as autism and schizophrenia. Here, we focused on recessive and “multi-hit” events that are likely to affect protein structure and function. Our results suggest that rare LOF recessive and compound heterozygous events are one facet of the genetic architecture of schizophrenia risk. Such knowledge will inform the search to identify specific risk genes in this and other datasets.

## OS 1.5 An Assessment of Tandem Repeat Variation in Schizophrenia Exomes

Colm O'Dushlaine<sup>1</sup>, Menachem Fromer<sup>2</sup>, Jennifer Moran<sup>1</sup>, Kimberly Chambert<sup>1</sup>, Pamela Sklar<sup>2</sup>, Shaun Purcell<sup>3</sup>, Christina Hultman<sup>4</sup>, Steven McCarroll<sup>1</sup>, Patrick Sullivan<sup>5</sup>

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**Background:** Schizophrenia is a genetically complex and clinically heterogeneous disorder. Several large-scale genome-wide association studies have been conducted which have focused on variations such as copy-number variation and single nucleotide polymorphisms. However, tandem repeats have been largely neglected in this paradigm of large-scale genetic surveys, with the exception of a few candidate analyses (e.g. DRD4). This is somewhat surprising given the higher mutation rates of tandem repeats and putative functional consequences of this.

**Methods:** We used software optimized to align repetitive regions (LobSTR) to scan for repeat sequence length variation in human exomes. We report here an initial survey of tandem repeat variation arising in the coding sequences of schizophrenia and control exomes (N=2,109). Focusing on 2-6mers, we assess global and local patterns of variation.

**Results:** Consistent with selective constraints on coding sequence, we find the vast majority of repeat indels do not represent frameshifting events, with 99.5% of 46,651 indel events being in-frame. Globally, we find little evidence of enrichment of repeat variants in cases, refuting the notion that a burden of repeat mutation across the exome might contribute to schizophrenia susceptibility. We detected about 7% more frameshifting repeat variants in cases but this was not significant (p=0.66), most likely due to the small number of events (124 vs. 115). Locally, we report a number of repeats significantly enriched in schizophrenia cases, highlighting possible new candidates in schizophrenia susceptibility. We also compare the spectrum of repeat variability, per locus, in cases versus controls and find a number of loci with increased diversity in cases. Thus, a number of different mutations at these loci might increase risk, in accordance with the concept of events dynamic repeat mutational processes leading to pathologically expanded repeat arrays, but also with more simple single and multi-copy repeat slippage events.

**Discussion:** The rate at which repeats mutate and the potential functional consequences of those mutations in relation to schizophrenia risk remain largely understudied. We present the first full-exome survey of such variation in over two thousand individuals. Our results detect a large number of events, the majority being non-frameshifting. While cases and controls show very little differences in rates of repeat variability globally, we do report a number of highly divergent loci and explore the potential ramifications of this.

## OS 1.6 Analysis of Copy Number Variants (CNV) in Genes Reported to Carry De Novo Point Mutations in Schizophrenia

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**Background:** Schizophrenia is a severe neuropsychiatric disorder with an estimated heritability of 60-80. Recent studies have shown that rare, highly penetrant variants account for a fraction of the overall genetic risk. Some of these disease-associated rare variants have been identified in sporadic cases as de novo mutations and are subject to negative selection. The chromosomal loci of those genes affected by de novo mutations in patients might be promising candidate regions for the identification of additional risk variants, such as rare copy number variants (CNVs). Two studies by Xu et al. (2011) and Girard et al. (2011) identified via exome sequencing a total of 49 genes that carried de novo mutations in patients with schizophrenia.

**Methods:** We tested whether CNVs affecting these genes might contribute to the allelic spectrum in schizophrenia. SNP array data of 1,637 clinically well characterized patients with schizophrenia or schizoaffective disorder and 1,627 population-based controls were analyzed. All individuals were of German descent and genotyped on Illumina's HumanHap550, Human610 or Human660W arrays. The CNV calling algorithms QuantiSNP and PennCNV were applied to identify potential CNVs. Only those CNVs that were found at least in two patients were further analyzed. The detected CNVs were subject to technical verification using TaqMan® qRT-PCR.

**Results:** Microduplications on 8q11.23 affecting the gene RB1CC1 were detected in five patients (0.3%) and one control (0.06%). All of the CNVs in this region had different putative breakpoints and spanned 12 – 66 consecutive SNPs. Additionally, microduplications on 11p11.2 affecting the gene OR4C46 were overrepresented in patients (five patients versus two controls). These CNVs had the same putative breakpoints and spanned 18 consecutive SNPs with the exception of one larger microduplication in one control (spanning 258 consecutive SNPs). Currently, we are extending our analyses to large, independent case-control datasets.

**Discussion:** The overrepresentation of CNVs in RB1CC1 and OR4C46 in schizophrenia patients provides further tentative support for a possible involvement of RB1CC1 and OR4C46 in schizophrenia, two genes that were previously reported to carry de novo point mutations in sporadic schizophrenia cases. Probably as a result of the limited power of our German schizophrenia sample, the p-values for the two genes were not robust to correction for the 49 tested gene regions. Follow-up in extended samples is clearly necessary and we are currently following this road. From a functional point of view, it is noteworthy that RB1CC1 insufficiency causes neuronal atrophy. This may provide a link to pathophysiological concepts in the development of schizophrenia. From an analysis point of view, our study shows that there can be a benefit from testing CNV datasets for smaller CNVs. Usually, the applied CNV QC filter criteria are a trade-off between false-positive and false-negative CNV calls, and we prefer more stringent criteria (≥30 consecutive SNPs) in systematic, genome-wide CNV analyses. In studies testing a selection of previously reported

candidate regions, these criteria may be relaxed in order to decrease the false-negative rate. This requires careful manual evaluation of the identified CNVs which normally is too labor-intensive for all CNV calls in systematic screens. The application of more stringent CNV filter criteria is also the reason why we did not notice RB1CC1 and OR4C46 in a previous CNV screen of the same German case control sample.

## Oral Presentation Session 2

### Bipolar

#### OS 2.1 Replication of Bipolar Disorder Susceptibility Alleles and Identification of 2 Novel Genome-wide Significant Associations in a New Bipolar Disorder Case-control Sample

Elaine Green<sup>1</sup>, Marian Hamshere<sup>1</sup>, Liz Forty<sup>1</sup>, Peter Holmans<sup>1</sup>, Shaun Purcell<sup>2</sup>, Pamela Sklar<sup>2</sup>, Michael Owen<sup>1</sup>, Michael O'Donovan<sup>1</sup>, Wellcome Trust Case-Control Consortium (WTCCC), Nick Craddock<sup>1</sup>  
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**Background:** We have conducted a genotyping study using a custom Illumina Infinium HD genotyping array, the ImmunoChip, in a new UK sample of 1,218 bipolar disorder cases and 2,913 controls that have not been used in any studies previously reported independently or in meta-analyses.

**Methods:** The ImmunoChip was designed prior to the publication of the Psychiatric GWAS Consortium Bipolar Disorder Working Group (PGC-BD) meta-analysis data. As such 3106 SNPs with a P value less than  $1 \times 10^{-3}$  from the bipolar disorder meta-analysis by Ferreira et al., 2008 were genotyped.

**Results:** We report replication for two of the three most strongly associated chromosomal regions in the Ferreira study, CACNA1C (rs1006737,  $p=4.09 \times 10^{-4}$ ) and 15q14 (rs2172835,  $p=0.043$ ) but not ANK3 (rs10994336,  $p=0.912$ ). We have combined our ImmunoChip independent data with the recently published PGC-BD meta-analysis data. Our data provide support for two regions at ODZ4 and CACNA1C with prior evidence for genome-wide significant association in PGC-BD meta-analysis. In addition, the combined analysis shows two novel genome-wide significant associations. First, rs7296288 ( $P = 8.97 \times 10^{-9}$ , OR = 0.9), an intergenic polymorphism on chromosome 12 located between RHEBL1 and DHH. Secondly, rs3818253 ( $P = 3.88 \times 10^{-8}$ , OR = 1.16), an intronic SNP on chromosome 20q11.2 in the gene TRPC4AP which lies in a high linkage disequilibrium region along with the genes GSS and MYH7B.

**Discussion:** In summary, we have provided additional support for prior association findings in CACNA1C, ODZ4, and at chromosome 15q14. In addition combined analysis with the PGC-BD data shows 2 novel genome-wide significant associations. Firstly, in a region of high LD at TRPC4AP on chromosome 20q11.2 which has not been highlighted previously and secondly at a region on chromosome 12q13.1 with a SNP located between RHEBL1 and DHH.

## OS 2.2 De Novo CNVs in Bipolar Affective Disorder

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**Background:** An increased rate of de novo copy number variants (CNVs) has been found in schizophrenia, autism and developmental delay, but fewer studies have reported this rate in bipolar affective disorder (BD). In this study we wanted to identify de novo CNVs in BD.

**Methods:** We used Illumina OmniExpress microarrays to genotype a total of 302 BD patients: 186 from Bulgaria and 116 from the UK, with all their parents genotyped as well, and who passed strict QC criteria. CNVs were called by PennCNV. We excluded CNVs <10kb, covered by <10 probes, overlapping segmental duplications and with a frequency >1%. Putative de novo CNVs called by PennCNV were validated by a Z-Score calling algorithm, manual inspection of the logRratios of the trios, and subsequently with qPCR probes.

**Results:** We found 11 de novo CNVs, (5 deletions, 6 duplications), a rate of 3.6%. This is higher than the reported ~1-2% rate in controls, but smaller than in schizophrenia and autism. The median size of de novo CNVs was 160kb, again smaller than the 321 kb that we reported in a previous study on schizophrenia (Kirov et al, 2011). One de novo deletion intersected an exon of DLG2, and one large duplication intersected 27 genes at 16p11.2, both regions having been implicated in de novo CNV studies of schizophrenia and BD. Two other de novo duplications are very large, at >3Mb, and have not been implicated before. Three of the de novo CNVs did not intersect genes.

**Discussion:** De novo CNVs in BD are found at increased rates compared to controls, but possibly at lower rates than in schizophrenia, although our sample size is too small to establish this rate more definitively. Overall they tend to be smaller than the ones reported in schizophrenia, and a smaller proportion of them are found at loci that have been shown to be pathogenic for neurodevelopmental disorders.

## OS 2.3 Massively Parallel Sequencing of the Brain Transcriptome Reveals Differential Expression of Novel Genes in Bipolar Disorder

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**Background:** Massively-parallel sequencing of mRNA (RNA-seq) is a novel approach to gene expression studies. Sequence data provides a direct estimate of transcript abundance, with counts proportional to the absolute abundance of target transcripts. For this reason, RNA-seq is a highly sensitive method to detect alternative splicing, low abundance transcripts, novel transcripts, allele-specific expression, and post-transcriptional modifications, such as RNA-editing. Here we used RNA-seq to characterize differential expression of brain transcripts in bipolar disorder.

**Methods:** We performed deep-sequencing (~110M-300M paired-end reads) of high quality total RNA (RNA-integrity number  $\geq 7$ ) extracted from dorsolateral prefrontal cortex (DLPFC) obtained post-mortem from 10 cases diagnosed with bipolar I disorder and 11 age- and sex-matched, psychiatrically-healthy controls, provided by the Stanley Medical Research Institute and the Clinical Brain Disorders Branch at the NIMH. Library preparation, fragmentation and PCR enrichment of the target RNA was followed by paired-end sequencing on an Illumina GA-IIx (9 samples) or on the HiSeq 2000 sequencing platform (12 samples). The resulting reads were subjected to quality control and then mapped and aligned to the reference genome. Principal Component Analysis (PCA) was performed with JMP, differential expression was analyzed with DESeq, and the results were combined across sequencing platforms by meta-analysis. Gene-set enrichment analysis (GSEA) was performed with DAVID. Enrichment for genome-wide association (GWAS) signals was tested by permutation of results from a published GWAS meta-analysis comprising over 15,000 cases and controls (Chen et al 2011).

**Results:** The first 3 principal components explained 67% of the variance in the first set of 9 samples, and detected significant separation between cases and controls. The meta-analysis identified 10 differentially expressed transcripts at a false discovery rate (FDR) of <5%. These comprised 3 unique genes, PROM1, LINC00173, and CD34. A set of 2,144 transcripts in 1,309 unique genes differentially expressed at a nominal p-value <5% were subjected to GSEA. This analysis detected functional enrichment of 10 gene ontology (GO) categories at FDR < 5%. Genes in 4 of these categories ("homophilic cell adhesion," "ion homeostasis," "passive membrane transporter activity," and "channel activity") showed significant enrichment of markers with small p-values (p-value < 0.05) in the GWAS data. A total of 62 differentially expressed genes replicated by microarray in an independent sample of 30 cases and 30 controls (hypergeometric test p-value =  $1.37 \times 10^{-7}$ ).

**Discussion:** Our results demonstrate that many genes are differentially expressed in DLPFC from individuals with bipolar disorder. Several known isoforms of PROM1, which encodes prominin, were

significantly down regulated in the bipolar disorder cases we studied. Prominin is strongly expressed in neural and hematopoietic stem cells, suggesting a role in neuroplasticity. The gene CD34, 2 transcripts of which were significantly under expressed in the cases we studied, appears to be an important cell adhesion molecule, is involved in morphogenesis and cell migration. LINC00173 is a non-coding RNA. Some of the differentially-expressed genes we measured are probably a consequence of bipolar disorder or its treatment, while others may reflect genes involved in causal pathways. Enrichment of GWAS signals in differentially expressed genes involved in certain pathways suggests that these pathways may play an etiologic role in bipolar disorder. These results demonstrate that RNA-seq may reveal differential expression of genes in biologically-relevant pathways that were not detected consistently in previous microarray-based gene expression studies. Further analyses to identify novel alternate transcripts, RNA-editing, and allele-specific expression are underway.

## OS 2.4 Genetic and Functional Abnormalities of the Melatonin Biosynthesis Pathway in Patients with Bipolar Disorder

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**Background:** Bipolar disorders (BD) are among the most common and devastating psychiatric disorders. Patients affected by BD frequently report abnormalities in sleep/wake cycles. Moreover, they have abnormal oscillating melatonin secretion, a key regulator of circadian rhythms and sleep patterns. Among the genes that could affect circadian regulation, the acetylserotonin O-methyltransferase (ASMT), a key enzyme of the melatonin biosynthesis, was recently associated with psychiatric disorders such as autism spectrum disorders (ASD) and depression.

**Methods:** We sequenced the coding and the regulatory regions of ASMT in a discovery sample of 345 patients with BD and 220 controls. We performed an association study on this discovery sample using common variants located in the promoter region and a replication study using 480 additional patients with BD and 672 controls. For rare variants, functional consequences were analysed by assaying the ASMT activity in B-lymphoblastoid cell lines (BLCL).

**Results:** We identified that the same allele of rs4446909, a common promoter polymorphism previously associated with ASD and depression, was significantly associated with BD ( $p=0.01$ ) and associated with a lower mRNA level ( $p<10^{-4}$ ) and a lower enzymatic activity ( $p<0.05$ ) of ASMT in BLCL. The replication study and meta-analysis showed a significant association for rs4446909 ( $p=0.002$ ). These results correlate with the general lower ASMT enzymatic activity observed in patients with BD ( $p=0.001$ ) compared with controls. Finally, several deleterious ASMT mutations identified in patients were associated with low ASMT activity ( $p=0.01$ ).

**Discussion:** In this study, we determined how rare and common variations in ASMT might play a role in susceptibility to BD and suggest the role of the melatonin biosynthesis as susceptibility factor for BD.

## OS 2.5 Market Research Tool Approach Detects Significant Genotype-phenotype Correlations in Bipolar Disorder

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**Background:** Genome-wide association studies (GWAS) have identified the first robust genetic findings in bipolar disorder (BD). However, results of polygenic approaches have shown that a large number of true associations still await identification. Approaches that not only test single SNPs with categorical diagnoses but combinations of SNPs with phenotypes that underlie the diagnoses may be more powerful. We have developed and implemented a method based on frequent item-set mining, a method which has been successfully used for market basket analysis in the retail business, to systematically test for associations between combinations of SNPs and multiple phenotypic traits.

**Methods:** The main goal was to identify frequent multilocus genotype patterns strongly associated with specific phenotypes. At present, this is computationally unfeasible for whole genome data without constraining e.g. the number of SNPs or the length of the analyzed combinations. Thus, we used only those SNPs which had passed the predefined p-value threshold of  $p < 0.001$  in our previous GWAS of BD (McMahon et al., 2010); this set was subsequently LD-pruned to remove redundant SNPs. We performed a two-stage process: 1) discovery of candidate rules, and 2) validating these rules in an independent BD GWAS sample. To perform these analyses we developed a novel software tool, termed RUDI (RULe DIScovery), that provides methods for data preparation, as well as the discovery of candidate rules and their validation. Here we used 3 independent BD data sets comprising 2835 cases. 1000 cases (Smith et al., 2009) were used for the discovery step, and the remaining 1835 cases (Cichon et al., 2011; Smith et al., 2011) were used for validation. Traits were selected based on their evidence for familiarity and/or heritability and completeness from the phenotype databases related to the 3 samples (Fangerau et al., 2004; Potash et al., 2007). A total of 1581 SNPs and 23 traits were analyzed.

**Results:** Around 21,000 candidate rules were extracted in the discovery step. In the validation step two multilocus genotype patterns were identified that were significantly associated after correction for multiple testing. Each consists of 3 markers. The first genotype pattern is overrepresented in a subgroup of patients with a comorbid eating disorder (combined:  $p = 3.012 \times 10^{-13}$ , OR=4.009 [95 CI: 2.655-5.924]), and the second in a subgroup with a comorbid simple phobia (combined:  $p = 3.476 \times 10^{-13}$ , OR=3.551 [2.453-5.063]). Further analyses involving around 2700 controls revealed: (a) no differences between cases and controls in the combined sample when cases from one of the two subgroups were removed; (b) no differences between all BD cases and controls; and (c) significant differences when each subgroup was compared with controls ( $p_{\text{eating}} = 2.192 \times 10^{-13}$ ;  $p_{\text{phobia}} = 1.686 \times 10^{-11}$ ).

**Discussion:** With this novel approach we were able to identify associations between multilocus genotype patterns and subgroups of BD with distinct comorbidities. One gene implicated by the comorbid eating disorder SNPs we detected has previously been reported to be associated with socially transmitted food preferences in mice (Munger et al. 2010). This gene is expressed in the mouse brain and is reported to influence synaptic plasticity and behaviour (Michalakis et al., 2011). Frequent item-set mining may be a useful strategy to identify additional genetic susceptibility variants underlying BD and other complex disorders and to help dissect the phenotype of heterogeneous conditions. Our results also stress the importance of careful phenotyping of patients, especially with regard to common comorbid conditions.

## OS 2.6 Psychiatric Genomic Consortium (PGC) Report on an Expanded GWAS of over 25,000 Samples in Bipolar Disorder

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**Background:** The purpose of the Psychiatric Genomics Consortium (PGC) is to conduct meta-analyses of genome-wide genetic data for psychiatric disease. Recognizing that individual GWAS studies are too small to have adequate power for gene discovery, an international PGC Working Group has focused on extending their meta-analysis of bipolar disorder. Recently, we reported a combined GWAS of bipolar disorder in a sample of 16,731 individuals that identified two genome wide-significant loci (Nature Genetics, 2011).

**Methods:** We now provide an update on a sample of approximately 25,000. At the time of the abstract submission 10988 cases and 14139 controls have been analyzed. New samples (n~8400) have been received from collaborators in France, Norway, Sweden and the USA. Data were prepared by the PGC central analytic pipeline as described previously. The data were imputed with HapMap3 and 1000 Genomes Project data and analyzed using standard logistic regression with MDS components as covariates.

**Results:** We will report on analyses of the entire dataset and demonstrate that there are additional genome-wide significant findings (n~5), as well as strong support for prior loci. We will also report on subphenotype and pathway exploration of the dataset.

**Discussion:** In conclusion, we provide support for the importance and utility of continued GWAS exploration in bipolar disorder in efforts to increase the number of genetic loci with compelling association to bipolar disorder.

## Oral Presentation Session 3 Autism

### OS 3.1 A Genomic Instability Model of a Neurodevelopmental Disorder: Global Copy Number Burden Associated with Autism

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**Background:** Recent work has demonstrated an elevated frequency of large, rare copy number variants (CNVs) in a number of neurodevelopmental disorders, including autism spectrum disorder (ASD). However, the global load of deletions or duplications per se, and the relationship between total copy number burden and the range of behavioral deficits associated with ASD has not been documented.

**Methods:** We examined copy number variation in 553 individuals with ASD or typically developing (TD) controls from the population-based Childhood Autism Risks from Genetics and Environment (CHARGE) study[1]. We interrogated 107 genomic regions flanked by segmental duplications (genomic hotspots) for events >50Kb and the entire genomic backbone for variants >300 Kb using a microarray specifically targeted to regions prone to recurrent rearrangements flanked by segmental duplications. This analysis was complemented by a separate study of five dynamic, repeat-rich, segmentally duplicated regions using a finely-tiled array platform that provided a high resolution (CNVs >1 Kb) assessment in 142 gender and ethnicity-matched ASD and typically developing children.

**Results:** We examined copy number variation in 553 individuals with ASD or typically developing (TD) controls from the population-based Childhood Autism Risks from Genetics and Environment (CHARGE) study[1]. We interrogated 107 genomic regions flanked by segmental duplications (genomic hotspots) for events >50Kb and the entire genomic backbone for variants >300 Kb using a microarray specifically targeted to regions prone to recurrent rearrangements flanked by segmental duplications. This analysis was complemented by a separate study of five dynamic, repeat-rich, segmentally duplicated regions using a finely-tiled array platform that provided a high resolution (CNVs >1 Kb) assessment in 142 gender and ethnicity-matched ASD and typically developing children.

**Discussion:** These findings were compared to data derived from previously published work[2] on copy number measured for a number of neurodevelopmental disorders, including a different autism cohort (Simons Simplex Cohort, SSC) using the same aCGH platform. This analysis revealed a replication of the elevated level of duplication burden in the SSC autism cohort. In addition, more severe neurodevelopmental phenotypes (intellectual disability) were associated with greater overall copy number burden, and an increase in the frequency of both large deletions and duplications. Our results suggest a mechanistic bias toward duplications in the etiology of autism spectrum disorder and emphasize the importance of genomic instability in neurodevelopmental disorders. References 1. Hertz-Picciotto I., Croen L. A., Hansen R., Jones C. R., van de Water J., et al. (2006) The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect* 114: 1119-1125. 2. Girirajan S., Brkanac Z., Coe B. P., Baker C., Vives L., et al. (2011) Relative Burden of Large CNVs on a Range of Neurodevelopmental Phenotypes. *PLoS Genet* 7: e1002334.

### OS 3.2 Identical by Descent Filtering Reveals Asd Genes Detected by Exome Sequencing in Extended Families

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**Background:** There is a strong genetic component to autism spectrum disorders (ASDs), but ASD etiology is complex. To help overcome the genetic complexity of ASD, we utilized 40 extended ASD families for whole exome sequencing to identify risk factors and ASD mutations. These families have segregation and identity by descent (IBD) information to confirm relationships and show segregation of rare variants with ASD. By studying these unique pedigrees and employing IBD filtering, we establish that extended families can be used to identify potentially damaging ASD specific alterations in known and novel candidate genes.

**Methods:** A total of 164 individuals including 100 ASD patients, 5 relatives with ASD features, and 59 unaffected relatives from 40 ASD extended families were included in this study. DNA samples were captured using Agilent's SureSelect Human All Exon system and prepared using standard Agilent and Illumina protocols for exome sequencing. Paired end 2x100 sequencing was performed on the Illumina HiSeq 2000. Sequencing data was processed and aligned to the hg19 human reference genome using the Burrows-Wheeler Aligner. Variant calling and annotation were performed with the Genome Analysis Toolkit (GATK) and SeattleSeq programs. Exome sequencing samples were evaluated on Illumina genome genotyping arrays, required to have a call rate of 98% or higher to pass quality control, and was used to delineate identical by descent (IBD) regions in each family. Only regions shared across all ASD individuals within a family were used as IBD shared segments. Priority was given to novel and rare variants, defined as a minor allele frequency (MAF) less than 5%. Variants were evaluated for conservation with the Genomic Evolutionary Rate Profiling and alterations measured for damaging consequence on protein function using PolyPhen-2 and Sorting Intolerant From Tolerant (SIFT) programs. For validation, 107 of the 164 samples were also run on the Infinium HumanExome BeadChip (Illumina). Variant calls were compared between the exome sequencing and genotyping with the PLINK program. A subset of variants was also validated by Sanger sequencing.

**Results:** Average depth of coverage for samples run on the HiSeq 2000 was 54.5x with 74.1% of reads falling within an exon target region. Variant calls between the whole exome sequencing and genotyping averaged a concordance of 98.4%. Following variant calling, each family typically had approximately 90,000 changes. We tested both heterozygous and homozygous inheritance models. Variants were filtered to include only alterations shared amongst IBD regions. Variants were further parsed to include only novel or rare variants designated as likely damaging by either the PolyPhen or SIFT programs. This filtering method typically reduced the number of heterozygous alterations of interest in each family to less than 25 and homozygous to 10. Results were overlaid with genes previously connected with ASD and other neurological disorders. A number of IBD alterations identified fell within known or suspected ASD genes, including AGAP1, CADPS2, CDH9, FBXO40, MBD4, NF1, NRCAM, RELN, and VPS13B. Additionally, a number of genes related to other neuropsychiatric or neurodevelopmental disorders were found to carry alterations in our ASD families. These include genes related to epilepsy (CLCN2), mental retardation (AP4M1, CEP290), and schizophrenia (CNTN5, CSMD1). To isolate genetic variants that

affect multiple families, we evaluated changes across families that fell within the same genes. Of the 680 genes that were found to carry a potentially damaging, heterozygous alteration, seventy-three (10.7%) were found to have variants in a minimum of two or more families. In contrast, 27 of 32 homozygous alterations were identified in more than one family (84.3%). Three of these genes have been previously recognized to play a role in neurological disorders: CEP290, GJA8, and HLA-A.

**Discussion:** To date, autism studies have recognized a considerable amount of genetic heterogeneity, with no locus contributing to more than 1% of ASD cases. The inescapable conclusion is that ASD is caused by multiple variants, each having a small attributable risk at the population level. Extended families can be used to further investigate and identify rare, ASD specific variants that segregate and may have variable penetrance.

### OS 3.3 Identification of Autism Spectrum Disorder Variants through Targeted Next Generation Sequencing in a Case and Control Cohort

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**Background:** Autism Spectrum Disorder (ASD) is a highly heritable disorder, yet recent genome-wide association studies (GWAS) and copy number variation screens have found no single common variant accounting for an appreciable percentage of genetic risk. These studies have led to the hypothesis that alterations in one or a combination of genes confer ASD susceptibility with many genetic variants in potentially hundreds of genes each having a small effect size in the overall population. Since disease causing variants are unlikely to be genotyped in GWAS, sequencing of associated regions to identify rare or low frequency variants with potential functional significance to ASD is essential.

**Methods:** We have sequenced autism candidate regions in 919 unrelated cases with ASD and 854 controls. Candidate regions were chosen from analyses of two autism datasets that employed the GWAS noise reduction method in conjunction with prioritization of haplotype blocks based on the Truncated Product Method (TPM) (Hussman et al., 2011). We designed an Agilent SureSelect probe set covering 17Mb corresponding to: 1) exons of 681 genes overlapping blocks with TPM p-values < 0.05; 2) evolutionarily conserved regions in those genes plus 5kb from their transcriptional starts and ends; 3) evolutionarily conserved regions within non-genic haplotype blocks that showed significant association; and 4) entire haplotype blocks with TPM p-values < 0.01. Sample preparation and capture hybridization were performed on the Caliper Sciclone G3 NGS and sequencing on the Illumina HiSeq2000. A fully developed next-generation sequencing data pipeline was used to align sequencing reads to the hg19 human genome build with BWA and call variants with the GATK Universal Genotype Caller. Annotation of variants was accomplished with a combination of SeattleSeq Annotation, PolyPhen2, SIFT, and ANNOVAR.

**Results:** Sequencing and alignment revealed 78.9% ± 9.1 of sequenced bases on target with 87.9% ± 6.3 of our targeted bases covered at least 10X, allowing for robust variant identification. Genotype calling quality and sample integrity showed 98.6% ± 1.2 concordance with approximately 5,000 markers previously genotyped on the Illumina-1M beadchip. Across all individuals, a total of 603,124 single nucleotide variants (SNVs) were identified. Of this total, 257,900 (42.8%) were not found in dbSNP 134. All SNVs were called in at least one individual and passed sequencing (VQSLOD > -3) and genotyping (minimum PL-score for 2nd most likely variant genotype > 100) quality controls. In total, 48,891 (8.1%) SNVs occur within exons or at splice junctions of one of our targeted genes, 15,099 (2.5%) induce nonsynonymous amino acid changes, and 8,829 (1.5%) are predicted by PolyPhen2 and/or SIFT to induce a damaging amino acid change. To identify individual variants potentially contributing to ASD, we first identified a subset of 39 genes from among our 681 targeted genes that have been previously associated with neurodevelopmental disorders. There were 112,617 SNVs within targeted regions of these genes: 4,941 exonic, 1,425 nonsynonymous, and 808 predicted damaging. Of the 808 damaging variants, 329 were unique to cases and several are found in well-established ASD candidate genes including 10 SNVs in CNTNAP2, 6 SNVs in MACROD2, 8 SNVs in NRXN1, and 12 SNVs in SEMA5A.

Moreover, we find 72 genes in which only cases have more than one damaging alteration in a single individual, including 4 cases with more than one damaging SNVs in the cadherin associated protein CTNNA3.

**Discussion:** These studies have yielded important findings regarding individual variants found uniquely in ASD cases in previously identified candidate genes. This approach will also identify new candidate genes by burden and association tests and extend analysis beyond the exons of the targeted genes to identify potential regulatory variants within introns, promoters, and intergenic regions. Targeted sequencing of ASD candidate regions in a large set of cases and controls will provide a compendium of variation and allow for discovery of new variants in genes and genetic networks contributing to ASD risk.

### OS 3.4 Functional Polymorphisms in the Cntnap2 Gene Promoter in Context of Autism Spectrum Disorder

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**Background:** Autism spectrum disorders (ASD) are predominantly genetically determined with an onset in early childhood and a high heritability of around 80 (Hallmayer et al., Arch Gen Psychiatry. 2011;68:1095-1102). A core symptom of these genetically complex neurodevelopmental disorders is a marked deficit in language development. Previous association analyses suggested the involvement of CNTNAP2 as a candidate gene (Arking et al., Am J Hum Genet. 2008;82:160-164; Alarcon et al., Am J Hum Genet. 2008;82:150-159), located in one of the best replicated linkage regions for ASD on 7q35. Despite the putatively high relevance of promoter variants no study focused on their functional characterization so far. Several single nucleotide polymorphisms (SNPs) and a short tandem repeat (STR) are described for the promoter region of CNTNAP2, but to our knowledge none of them has been assessed as a risk factor for ASD. The aim of the present study was i) to screen for unknown promoter variants within the CNTNAP2 gene, ii) to test SNPs for association with ASD and iii) to analyse the transcription efficiency of the associated alleles.

**Methods:** The genomic region of the CNTNAP2 promoter was analysed in more than 200 families by direct sequencing using blood-derived DNA. ASD-associated variants were genotyped by RFLP in further 400 families. Family-based association tests (TDT) were performed using UNPHASED and quantitative analyses of phenotype/genotype correlation was done using SPSS and R. Functional analysis of promising promoter SNPs was performed by Dual-Luciferase<sup>®</sup> Reporter Assay (Promega).

**Results:** Of the ten annotated SNPs in the CNTNAP2 promoter region, we identified three in our preliminary sample (N=200). Furthermore, we revealed seven novel un-described SNPs. A preliminary association analysis in these 200 families showed a nominal association with ASD of the minor alleles of SNP rs34712024 and the trimeric STR rs71781329 (NG\_007092.2:g.4865insGCGGCG). Nominal association of rs150447075 was observed with age of first sentence in months (ADI-R) in the subsample of ASD children with the respective data (N=200). In the combined sample of 600 families, we could confirm the nominal association of the minor allele of rs34712024 as being putatively protective (OR = 0.478; CI = 0.233-0.981; Chi<sup>2</sup>-p-value = 0.038). Online databases (USCS, dbSNP, EMBOSS) show that the variants screened in the whole set are all three localized in the binding site of the transcriptional repressor NRSF and its cofactor SYN3A. The luminescent reporter gene assay showed a significant increase (~ 1.7 fold) in the CNTNAP2 promoter function of rs34712024 and rs71781329 but not of rs150447075. Furthermore, for STR rs71781329 we could show that the insertion of one GCG triplet did not alter the transcriptional efficiency, whereas insertion of GCGGCG induced a 1.6 fold significant increase of luciferase expression, putatively by affecting the transcription factor binding affinity.

**Discussion:** Our findings suggest a functional association of CNTNAP2 promoter variants rs34712024 and rs71781329 with ASD. We propose that the changes in the transcription factor binding site of

NRSF and its cofactor SYN3A act as a protective factor by altering the expression level of CNTNAP2. This is supported by the recent findings that several ASD implicated genes (with CNTNAP2 among them) are differentially regulated during embryogenesis and early childhood neuronal development (Kang et al., Nature. 2011;478:483-489). In summary, our study provides a further piece for solving the autism puzzle, by supporting the functional relevance of rare alleles in psychiatric disorders.

### OS 3.5 Impact of a Rare Rpl10 Mutation on the Molecular Phenotype of Autism in a Patient Specific Cell Model

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**Background:** Autism spectrum disorders (ASD) are severe neurodevelopmental disorders with early onset in childhood. Despite ASD's first description in 1940, and innumerable studies of genetic research, little is known about the molecular phenotype. Recently we have identified two rare missense mutations in the ribosomal protein L10 in three independent families. A first functional characterization at the translational level showed that these mutations alter the translational capacity. Therefore, we were further interested whether this affects the protein expression pattern in patient specific lymphoblastoid cell lines (LCL), and how this alteration is related to the molecular phenotype of ASD in these cell lines.

**Methods:** We used a 2D-differential in gel electrophoresis (2D-DIGE) approach to compare the whole proteome of family members (N=5) harboring the RPL10 [H213Q] mutation to non-mutated members (N=1) and unrelated controls (N=2). To investigate, whether these patterns are specific for the families, for the mutations or whether a similar pattern can be observed in the overall molecular expression pattern of selected ASD patients not inheriting RPL10 mutations we performed a similar approach to compare ASD patients (N=10) to healthy controls (N=10). In this setup we screened for proteins with an altered overall protein expression, and an altered variance in the abundance between the two groups. Spots of interest were picked from preparative Coomassie gels and identified via tandem mass-spectrometry. Bioinformatic analyses of the identified candidates and the putatively overlapping sets of RPL10-specific alterations and ASD-specific candidates was performed using the R program. Validation of differentially regulated proteins was performed at mRNA and overall protein expression level applying RT-PCR and Western Blot methods.

**Results:** We discovered alterations (t-test p-value > 0.05) in the expression of 5 protein isoforms relevant for gluconeogenesis (TPI1; 3 isoforms), oxidative stress (ECH1) and mRNA regulation (HNRNPK) in the RPL10 mutation analysis. Furthermore, we identified 19 candidates in the ASD-specific set implicated in the processes of translation regulation (EEF1D), oxidative stress (ECHS1, PRDX2), mRNA and protein turnover (HNRNPK; HNRPA2B1; HNRPDL; HSPD1; PCBP1; ERP29; PSMA1, PSME2), gluconeogenesis (ALDOC; GAPDH, PGK1), mitochondrial processes (ATP5B; ATP5H; GLUD1) and cell-morphology (ACTB; TAGLN2). Five candidates (ATP5B; EEF1D; HNRNPK; PRDX2; PSME2) were significantly (Mann-Whitney U-test) altered at the mRNA level. Furthermore, we found evidence that candidates altered in their protein expression variance (N=5) were more likely to present with an overall altered mRNA expression. Cross comparison of the RPL10-dependent alterations and the ASD-specific alterations showed that the discovered candidates of the RPL10-mutated probands are distinct from that of the non-RPL10-mutated ASD patients, but overlap in their molecular function, notably in energy metabolism and oxidative stress response. Candidates of both sets have been associated to other mental disorders like

schizophrenia and over 80% of the overall identified candidates are regulated by the same transcription factors. Network and GO-term enrichment analyses showed that both sets of proteins are part of the energy metabolism network and are likely (p-value < 10E-4) to converge on the synthesis of ATP.

**Discussion:** While lymphoblastoid cell lines are not a direct neuronal model for psychiatric disorders they present a person's specific genetic make up and thus reflect the genetic diversity present in each of us. We think that the observed alterations may have a more deleterious (not observable in LCL) effect in neural cells which depend on a rapid regulation of energy metabolism. Therefore we propose that these alterations are on the basis of the ASD etiology, and many other proteomic alterations (putatively at the level of posttranslational modifications) will be found when directly analyzing neuronal patient specific cells. In summary, i) we show that lymphoblastoid cell lines are a useful model to investigate the functional impact of single genetic variations on protein expression, ii) we were able to describe a general proteomic phenotype for ASD and iii) we demonstrate that ASD related mutations in RPL10 contribute to this phenotype by altering a distinct but similar mechanism. Therefore we would like to emphasize the need for more functional analyses of rare genetic variations especially in patient specific models to understand the underlying mechanisms in ASD.

### OS 3.6 Excess of Rare Novel Loss-of-Function Variants Identified in Putative Susceptibility Genes for Schizophrenia and Autism Spectrum Disorders

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**Background:** Schizophrenia (SZ) and autism spectrum disorders (ASD) are complex genetic neurodevelopmental disorders that share certain phenotypes (e.g. cognitive deficits), and may share an underlying pathology due to shared genetic risk variants (e.g. already-identified NRXN1). This study involves next-generation sequencing of the exonic regions of 215 putative susceptibility genes in an Irish sample of 151 cases of ASD, 273 cases of SZ and 287 controls, to identify rare mutations contributing to one or both disorders. Genes were primarily selected for their function in the synapse.

**Methods:** A multiplex target enrichment method combined DNA samples using indexes/barcodes followed by enrichment of exonic regions using Agilent SureSelect and paired-end sequencing on an Illumina GAI. Selected genes were categorised as: 1) NRXN1 and interactors, 2) Postsynaptic Glutamate Receptor Complexes (NMDA, mGluR5 and AMPA), 3) Neural cell adhesion molecules, 4) DISC1 and interactors, and 5) Functional and Positional Candidates. Our primary category of variants to study was rare (MAF <0.01) Loss-of-Function (LoF) variants that are predicted to severely disrupt protein-coding sequence: nonsense single nucleotide variants (SNVs) that introduce stop codons, SNVs that disrupt splice sites and indels that disrupt a transcript's open reading frame or a splice site. LoF variants were confirmed by capillary sequencing. Parental DNA was available for ASD samples to test if variants were de novo.

**Results:** Thirty-one of 33 rare LoF variants detected amongst >2,000 rare variants were confirmed by capillary sequencing. All of these variants were novel. We detected an excess of singleton LoF variants in the combined cases (n=21) compared to controls (n=3; p=0.005). This excess was present in both SZ (n=13; p=0.01) and ASD (n=8; p=0.01). Two genes carried multiple singleton LoF variants in cases: GRIP1 (1xSZ, 1xASD) and INADL (2xSZ, 1xASD). Analysis of parental samples for an ASD case revealed that a LoF variant in GRIN2B, a nonsense SNV in exon 10, is de novo.

**Discussion:** These data support a role for rare LoF variants in synaptic genes in the susceptibility of SZ and ASD. We provide new information for known ASD risk genes (e.g. GRIP1) and identify putative new susceptibility genes for both disorders. The discovery of a new ASD de novo mutation in GRIN2B adds to the growing evidence that this is an important risk gene for the disorder.

### Oral Presentation Session 4 Biostatistics/Bioinformatics

#### OS 4.1 Using Identity-by-Descent Information to Detect De Novo and Recent Mutations in Population-based Exome-sequencing Studies

##### ECIP

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**Background:** De novo copy number variations and point mutations have been implicated in a number of psychiatric disorders, including schizophrenia and autism. To detect de novo variation, a trio design is typically used in which the proband is sequenced with both parents, although trios are more difficult to collect in large numbers for adult-onset diseases such as schizophrenia. We developed a method that utilizes identity by descent (IBD) information from exome sequencing in large population-based samples of unrelated samples to identify mutations that are more likely to be relatively recent, "private" mutations. These prioritized mutations will be enriched for de novo events in patients, as well as mutations arising within the past several generations.

**Methods:** For each singleton mutation (a variant seen only once) in a population-based sequencing study, we search for other subjects who share the surrounding region IBD, by use of a hidden Markov model. If we observe data consistent with the index subject's maternal and paternal haplotypes each being shared by at least one other person (none of whom also share the index's singleton mutation), we can conclude that the mutation must post-date their most recent common ancestor. By focusing only on sharing of multi-megabase segments (indicative of more recent co-ancestry), we can effectively reduce the large number of rare variants each individual carries to a shortlist of truly rare alleles, more likely to contain large effect disease alleles. De novo variants represent a special case of this class.

**Results:** To evaluate this approach, we applied the method to a set of Bulgarian schizophrenia trios from an ongoing exome sequencing study, part of a larger prior GWAS, to generate a "true positive" control dataset. For validated de novo mutations, we asked whether we could successfully flag these mutations without using any parental data, but instead using genome-wide IBD data from ~2000 unrelated, Bulgarian individuals. We were able to recover approximately 10% of the validated de novo mutations with high confidence: this proportion would be expected to increase with larger sample sizes (and also reflects population genetic and demographic factors). For each proband, our approach flagged only a small fraction of the hundreds of neutral, novel singleton variants each case carries. We posit that this fraction will be enriched for private mutations of large effect on disease as well as de novo variants and we are currently testing this hypothesis in a large cohort of ~12,000 unrelated Swedish samples (schizophrenia cases and controls), of which 5,000 have exome sequence data. We

predict that singletons flagged by our approach will be more likely to be novel, loss-of-function and enriched in cases, compared to the much larger class of all singleton variants.

**Discussion:** We developed a novel approach to identify from population-based exome sequence data those rare variants that are more likely to be private, recent mutations. Gene-based association tests can use this information, in assigning weights to different variants, over and above information on sample frequency and functional class.

## OS 4.2 A Polygenic Analysis of Schizophrenia and Depression Risk Alleles Effect on Reproductive Fitness in the General Population

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**Background:** The so called schizophrenia paradox has long existed in psychiatric research: how does a disorder remain highly prevalent if it is also highly heritable and substantially reduces an individual's reproductive fitness. Several hypotheses have been put forward to try to explain how risk alleles might survive in the face of strong negative selection but as yet there is still no consensus. With the recent progress in genome-wide association studies, it is now possible to examine how risk alleles affect reproductive fitness. While few specific causal loci have been replicated for psychiatric disorders, methods using aggregate scores of risk alleles across the genome have had been reasonably successful in explaining a significant proportion of variance in affection status. In this analysis we tested for an association between these polygenic scores and number of grandchildren in individuals from the general population.

**Methods:** The polygenic scores were constructed using the data from the Psychiatric GWAS Consortia (PGC) from their analysis of schizophrenia and depression. A set of SNPs pruned for linkage disequilibrium was used, using batches based on a p value threshold (less than or equal to 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 or 1). These were used to create polygenic scores was constructed within a Swedish dataset where fecundity data and genetic data were available. These were 12,000 twins born between 1910 and 1943 in Sweden, with one individual from each twin pair included. The Swedish Multi-Generation Register was used to identify their children and then grandchildren. A linear regression model was then used to test for association between polygenic scores for the disorders and number of grandchildren. Analysis was corrected for sex and the first two ancestry informative principal components.

**Results:** Depression scores were not associated with reproductive fitness at any threshold for significance. As it has been suggested depression risk variants may reflect 'plasticity alleles', we also tested for a difference in mean variance across SNPs thresholds for the top 25% and bottom 25% of depression scores. The results trended towards increased variance with increased depression score but were not significant (p=0.11). Greater schizophrenia scores were associated with fewer grandchildren in half the threshold categories and trending in this direction in all. The greatest significance was found at a cut-off for the top 40% of schizophrenia associated SNPs (p=0.008) and explained 0.11% of the variance in number of grandchildren. The effect size remained when the top and bottom 10% extremes of the polygenic score were removed, suggesting it was not driven by the extremes. No sex specific effect was found.

**Discussion:** Interpretation of these results is unexpected, as it seems to exacerbate the schizophrenia paradox by increasing the negative selection against schizophrenia risk alleles. Especially if one considers that only a small fraction of the heritability of schizophrenia is currently captured by the polygenic score. It is possible that this result reflects deleterious mutations that lead both to schizophrenia and also behavioural deviations that are sub-clinical but severe enough to

reduce fitness. This might be evidence for the need to better screen controls for sub-clinical features. Overall it is clear that there is no evidence for balancing selection and the risk alleles for schizophrenia experience negative selection. We now hope in to incorporate the results from the PGC autism study into the analysis.

### OS 4.3 Partitioning Genome-wide Autozygosity to Target Polygenic Signals: Methods and Application to Schizophrenia GWAS Data

#### ECIP

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**Background:** Inbreeding, even from distally related kin, increases the probability of deleterious recessive alleles pairing together in homozygous form. Autozygosity, measured using Runs of Homozygosity (ROHs), is a genomic signature of inbreeding at the molecular genetic level, and can be examined using genome-wide SNP data. The two usual approaches to analysis of ROHs involve either a region-by-region approach (akin to an association test) or an overall “load” approach. However, potential effects of autozygosity on a given phenotype may be too polygenic to implicate any particular genomic region, but also may not be under the influence of the entire genome. Under this scenario, no specific region may reach genome-wide significance, while a whole-genome “load” analysis may lose power by incorporating regions that have no impact on the phenotype. In the current study, our goal was to detect regions of the genome that have an effect when autozygous, and use only these regions in an overall “load” analysis.

**Methods:** Our general approach was to rank each ROH region by its statistical significance, accounting for the direction of effect. Regions were then aggregated step-by-step according to rank and tested for an excess of positive or negative effects, with maximum deviations from the baseline proportions treated as the most likely set of ROH regions influencing the phenotype. Permutation testing was used to account for linkage disequilibrium between regions, and cross-validation procedures were implemented to derive an unbiased estimate of ROH in recommended regions.

**Results:** Our study investigated various types of significance ranking under a number of simulated ROH effects, and found that controlling for an existing genome-wide effect is important in reducing inflation in the direction of effects at higher significance. In addition, we were able to determine the specific patterns expected from rank-ordered testing when reduced proportions of the genome are driving an effect, as well as when the whole genome is driving an effect. We illustrate the application of these methods using a case/control GWAS of Schizophrenia from data provided by the Molecular Genetics of Schizophrenia (N=5,163). Results of the analysis show that ROH predict Schizophrenia across the majority of the genome, and are not confined to a smaller subset of genomic regions.

**Discussion:** The proposed method and results from simulated ROH data provide a statistical framework for targeting and validating a specific set of genomic regions that may affect the phenotype of interest when autozygous. Furthermore, this method provides a complementary measure to test if whole-genome “load” analysis are truly genome-wide, as is the case with Schizophrenia data investigated here. Finally, this framework can be applied to other polygenic approaches of GWAS data, such as whole-genome SNP heritability estimates.

#### OS 4.4 Pleiotropy and Power: Methods for Improving Gene Discovery in Psychiatric GWAS

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**Background:** Schizophrenia (SCZ) and bipolar disorder (BD) share several clinical characteristics, including impairment of cognitive functions. Both disorders also have a high heritability, estimated to 0.7-0.8, but are regarded as complex disorders with a polygenic architecture. However, in published GWAS few SNPs have been discovered in either SCZ or BD that meet GWAS significance of 5 x 10<sup>-8</sup>.

**Methods:** We propose a new methodology based on the local false discovery rate of Efron (locfdr; 2010). Computed from GWAS summary statistics, the locfdr for a given SNP is its posterior probability of null status conditional on its z-score. Low locfdr indicates high probability of being non-null. Our extension of this methodology, termed “covariate modulated local false discovery rate” (cmlocfdr), incorporates pleiotropic relationships of one phenotype with another, potentially leveraging information from multiple GWAS. Here we used summary statistics from Psychiatric GWAS Consortium (PGC) BD (n=16731) and SCZ (n=21856).

**Results:** We found a high level of enrichment of SNP effects for BD when taking into consideration pleiotropy of effects with SCZ. Q-Q plots of effects (Figure 1), showing the potential for large increases in power to detect true effects below the traditional GWAS threshold using the cmlocfdr methodology we have developed.

**Discussion:** Our results demonstrate the potentially sizeable gains in power for gene discovery available from incorporating pleiotropic relationships into hypothesis testing. Crucially, this enables us to borrow power from large GWAS for detection and replication of SNPs in smaller GWAS.

#### OS 4.5 Network and Structural Properties of Monogenic and Complex Disease-related Genes

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<sup>1</sup>Vrije Universiteit

**Background:** The advent of genome-wide association (GWA) analysis as the pre-eminent tool for gene-discovery in complex traits has resulted in the identification of a large number of human disease genes. The parallel developments in systems biology based approaches of studying human genetic diseases have shifted focus from the effects of single genes to the effect of molecular and interaction networks. Early work on cancers and revealed that up-regulated genes in cancerous tissues were central in the interactome and highly connected (often referred to as hubs), suggesting differential network properties of disease genes compared to non-disease genes. In contrast, later studies including complex diseases showed that disease related genes were not likely to encode hubs in molecular networks. In addition, it has been suggested that structural properties of genes, such as the number of tissues in which it is expressed or the number of isoforms, might be informative for a role in disease. In this study we derive network and structural properties of genes and investigate whether these properties are predictive of monogenic and complex diseases.

**Methods:** We obtained the list of complex human disease associated genes identified by GWAS from ‘A catalog of published genome-wide association studies’. Genes associated with monogenic disorders were obtained from the Online Mendelian Inheritance in Man (OMIM) database. We obtained PPI data from 10 source databases and 11 studies (that had not been covered by other databases yet) and constructed a molecular interaction network. Based on this network we derived five network properties for each protein coding gene: degree, closeness, eccentricity, betweenness centrality, and clustering coefficient. In addition, we obtained gene information from NCBI and for each gene extracted four structural gene properties: number of exons, number of isoforms, number of expressed tissues, and gene size. The nine network and structural properties of genes were binned and differential distributions between genes associated with complex disorders, monogenic disorders and genes not associated with disease were evaluated using multidimensional Fisher exact tests.

**Results:** We observed that the distributions of degrees, closeness and eccentricity (all  $P < 3.7 \times 10^{-9}$ ) of disease genes were significantly different from those of non-disease genes. In addition, we found highly significant differences (all  $P < 5.3 \times 10^{-12}$ ) between the distributions of structural properties of genes related to complex disorders and genes not related to diseases. For example, we observed that genes with a higher number of exons and multiple isoforms were more likely to be related to complex disease. In addition, there were highly significant differences in the distribution of the number of expressed tissues between monogenic disease related genes and complex disease related genes ( $P < 2.2 \times 10^{-16}$ ).

**Discussion:** Network and structural properties of genes currently associated with disease are quantitatively different than genes not associated with disease. This knowledge might aid in understanding genetic mechanisms underlying diseases and may provide insight in the role of single genes in their molecular context.

## OS 4.6 Evaluation of Algorithms for In Silico Prediction of Deleterious Mutations in a Large Whole-exome Sequencing Study

### ECIP

Laramie Duncan<sup>1</sup>, Douglas Ruderfer<sup>2</sup>, Menachem Fromer<sup>2</sup>, Jennifer Moran<sup>2</sup>, Kimberly Chambert<sup>2</sup>, Paul Lichtenstein<sup>3</sup>, Christina Hultman<sup>3</sup>, Patrick Sullivan<sup>4</sup>, Steven McCarroll<sup>5</sup>, Pamela Sklar<sup>6</sup>, Shaun Purcell<sup>6</sup>  
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**Background:** Computational tools that predict the likely deleteriousness of specific genetic variants have been available for over a decade and have proven useful in the identification of true risk variants. These deleteriousness-prediction programs (e.g. PolyPhen2 and SIFT) will become even more valuable as more large-scale genetic sequencing studies become available.

**Methods:** We applied PolyPhen2 and SIFT to a large genetic sequence dataset, comprising over 5,000 Swedish individuals (schizophrenia cases and controls). We aimed to investigate four related questions: a) the extent of concordance between the approaches, b) the relationship between allele frequency and annotation (on the supposition that damaging alleles will have lower population frequencies, due to negative selection), c) the information content of continuous versus categorical PolyPhen2 predictions, and d) the extent to which different weighting schemes substantively impact gene-based rare-variant association test results.

**Results:** Based on an interim dataset (~1000 individuals), of the 478,861 total variants that passed quality control, 154,972 were non-synonymous coding variants that yielded both PolyPhen2 and SIFT predictions. Concordance between predictions from PolyPhen2 and SIFT was generally high: 80% of variants predicted 'benign' by PolyPhen2 were predicted 'tolerated' by SIFT, and 73% of PolyPhen2 'probably damaging' variants were predicted 'deleterious' by SIFT. Correlation of continuous scores was moderate ( $r=0.45$ ,  $p < 1e-16$ ). Consistent with evolutionary theory, minor allele frequency decreased as predicted deleteriousness increased. Missense variants rated as "benign" had frequency profiles similar to silent mutations, whereas missense rated "probably damaging" had frequency profiles similar to loss-of-function mutations (essential splice sites and nonsense variants). The relationship with allele frequency was also true for continuous scores from both PolyPhen2 and SIFT, but more variance in minor allele frequency was explained by PolyPhen compared to SIFT and when continuous scores were used instead of categorical scores.

**Discussion:** Deleteriousness prediction programs provide an empirical way of prioritizing the tremendous number of variants identified in sequencing studies. Our preliminary results suggest that their use may be maximized - and power in studies increased - by using the continuous (vs. categorical) scores.

## Oral Presentation Session 5 Schizophrenia

### OS 5.1 Genome-wide Association Study of 32,143 Individuals Reveals Several Novel Associations in Schizophrenia

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**Background:** Schizophrenia is a genetically complex and clinically heterogeneous disorder. Several large-scale genome-wide association meta-analyses have discovered 14 independent loci with genome-wide significant association. Here, we present results from the latest and largest such study, representing an increase in sample size of 59 over previous studies. Our primary study consisted of 5,001 cases and 6,243 controls ascertained from a Swedish national sampling frame. The genetic data were imputed using HapMap3 and 1000 Genomes data and analyzed using logistic regression with MDS components as covariates.

**Methods:** We used two methods to compare the results to already published data from the collection from the Psychiatric GWAS Consortium (excluding the Swedish cohorts, 8,832 cases and 12,067 controls of European ancestry). We first compared directionality in SNPs with high-significance ( $p < 10^{-4}$ ) via a sign test. Second, we performed polygene testing (Purcell, 2009) to evaluate information content in SNPs with lower significance levels. After showing excellent concordance in both directions, we meta-analyzed these two result-sets in a complete sample size of 13,833 cases and 18,310 controls.

**Results:** With this unprecedented sample size we found increased evidence of association for many previously implicated loci in addition to evidence for many novel loci: in total we find 14 LD-independent SNPs with genome-wide significance. An additional 31 LD-independent SNPs with  $p < 10^{-6}$  are currently under investigation with a reasonable sized replication dataset. Finally, we conducted gene-based and pathway analyses to test for aggregate-level associations, possibly in the presence of allelic and/or locus heterogeneity. Our gene-based tests recapitulate many of the top GWAS hits but also highlight additional loci of interest (containing multiple LD-independent hits of modest effect). Pathway analysis using KEGG, Gene Ontology and TargetScan datasets, revealed several highly significant associations, most notably relating to postsynaptic density in addition to the previously-reported Cell Adhesion Molecule Pathway. Intriguingly, of 87 microRNA families tested (range of 2-200 genes), 26 had a pathway  $p$ -value  $< 10^{-4}$ , potentially implicating a number of additional microRNAs in schizophrenia. We confirm enrichment of association among targets of mir137 ( $P=1.8e-9$ ) and find that 45 of 299 targets with at least one SNP have a gene  $p$ -value  $\leq 0.05$ .

**Discussion:** Our results confirm a number of previous associations in schizophrenia and yield a number of new insights at both the gene and pathway level, together representing major steps forward in our understanding of the molecular pathophysiology of schizophrenia.

## OS 5.2 Dissection of Genetic Architecture of Bipolar Disorder and Schizophrenia: Results from a Combined Dataset of Nearly 40,000 Individuals

### ECIP

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**Background:** Bipolar disorder (BP) and schizophrenia (SCZ) are two chronic debilitating psychiatric illnesses that together affect ~3 of the world's population. They have been dichotomized since the early part of the century and this distinction has been maintained in research and epidemiology until very recently. A major aim of the Psychiatric Genomics Consortium is to quantify the extent of common genetic variation across distinct psychiatric diseases. Here, we present the insights generated by the SCZ / BP working group in better understanding the specific loci shared by both diseases as well as those that discern the two.

**Methods:** Genotypes were present for all individuals and were obtained from a variety of Illumina and Affymetrix arrays. Imputation was performed using the Beagle software. All analyses were done using dosage data in Plink.

**Results:** We analyzed a dataset of 39,202 independent individuals by combining the PGC SCZ sample (9,369 cases and 8,723 controls) and the PGC BP sample

(10,410 cases and 10,700 controls). All subjects were distinct and independent. As reported in previous literature, we find highly significant polygenic overlap across disorders and many shared associated loci. Several follow-up analyses were performed in order to dissect the signal

at regions/genes of interest for disease specificity. Furthermore we investigated the presence of genetic loci with significantly different effects by comparing odds ratios as well as creating a disease only dataset and performing GWAS with SCZ as case and BP as control. Finally, we look to assess the role of these loci and overall polygenic burden to a set of sub-phenotypes collected for these samples.

**Discussion:** Our data show that large cross-disorder datasets can be a powerful resource for understanding the shared genetic architecture of these disorders and ultimately will help in defining the differences in molecular function and treatment.

## OS 5.3 Genetic Pleiotropy between Schizophrenia and Multiple Cardiovascular Disease Risk Factors

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**Background:** Epidemiological and clinical studies suggest an association between schizophrenia (SCZ) and cardiovascular disease (CVD) risk factors. Though the nature of this relationship is not well understood, lifestyle factors and antipsychotic medication have primarily been implicated. We investigated whether this phenotypic association arises from shared genetic factors (pleiotropy).

**Methods:** Using summary statistics from recent genome-wide association studies of over 230,000 individuals, we investigated overlap in single nucleotide polymorphisms (SNPs) associated with SCZ and CVD risk factors (cigarettes per day (CPD), systolic (SBP) and diastolic blood pressure, triglycerides (TG), total cholesterol (TC), body mass index (BMI), waist-hip-ratio (WHR), and type 2 diabetes).

**Results:** We discovered significant, joint association between individual SNPs associated with SCZ and TG (6 loci), TC (10 loci), SBP (2 loci), BMI (1 locus), WHR (3 loci), and CPD (1 locus), of which 15 were new SCZ loci. The most significant combined association was between SCZ and TG (rs2272417, IFT172, 2p23.3  $p = 8.4 \times 10^{-33}$ ), TC (rs983309, AK055863, 8p23.1,  $p = 1.5 \times 10^{-24}$ ), and CPD (rs8042059, CHRNA3, 15q25,  $p = 5.7 \times 10^{-25}$ ). Q-Q plots showed consistent enrichment of SNP association with SCZ as a function of significance of association with several CVD risk factors, especially plasma lipid levels.

**Discussion:** We demonstrate a shared genetic basis between SCZ and several CVD risk factors suggesting an etiological relationship between CVD and SCZ independent of lifestyle factors or pharmacological treatment. Genetic pleiotropy was strongest for plasma lipid levels indicating a possible mechanistic relationship between lipid biology and SCZ pathogenesis.

## OS 5.4 Genome-wide Study of Association and Interaction with Maternal Cytomegalovirus Infection Suggests New Schizophrenia Loci

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**Background:** Genetic and environmental components as well as their interaction contribute to the risk of schizophrenia, making it highly relevant to include environmental factors in genetic studies of schizophrenia.

**Methods:** The study comprised genome-wide association (GWA) and follow-up analyses of all individuals born in Denmark since 1981 and diagnosed with schizophrenia as well as controls from the same birth cohort. Furthermore, we present the first genome-wide interaction study of SNPs and maternal cytomegalovirus (CMV) infection (maternal anti-CMV IgG antibody titer). The GWA analysis included 888 cases and 882 controls, and the follow-up investigation of the top GWA results was performed in independent Danish (1,396 cases and 1,803 controls) and German-Dutch (1,169 cases, 3,714 controls) samples.

**Results:** The SNPs most strongly associated in the single-marker analysis of the combined Danish samples were rs4757144 in ARNTL ( $P = 3.78 \times 10^{-6}$ ) and rs8057927 in CDH13 ( $P = 1.39 \times 10^{-5}$ ). Both genes have previously been linked to schizophrenia or other psychiatric disorders. The strongest associated SNP in the combined analysis including Danish and German-Dutch samples was rs12922317 in RUNDC2A ( $P = 9.04 \times 10^{-7}$ ). A region-based analysis summarizing independent signals in segments of 100kb identified a new genome-wide significant locus overlapping the gene ZEB1 ( $P = 7.0 \times 10^{-7}$ ). This signal was replicated in the follow-up analysis ( $P = 2.3 \times 10^{-2}$ ). Genome-wide significant interaction with maternal CMV infection was found for rs7902091 (PSNP  $\times$  CMV =  $7.3 \times 10^{-7}$ ) in CTNNA3, a gene not previously implicated in schizophrenia.

**Discussion:** ZEB1 encodes an E-box binding zinc finger transcription factor, which is widely expressed in the central nervous system and plays an important role in development of the brain and neuronal differentiation. It has been demonstrated that the expression of ZEB1 is partly regulated by TCF4 (repeatedly associated with schizophrenia in GWA studies), and that ZEB1 is involved in the regulation of CDH13 expression, indicating that ZEB1, TCF4 and CDH13 are elements of a common pathway involved in schizophrenia susceptibility. In the interaction analysis of SNPs with maternal CMV infection, which to our knowledge is the first genome-wide survey of G $\times$ E in schizophrenia, we found a genome-wide significant interaction signal for rs7902091 located in an intron of CTNNA3, just upstream the gene LRR13, which is nested within CTNNA3. CTNNA3 and LRR13 have both previously been found associated with Alzheimer's disease as well as autism spectrum disorder. The finding warrants future replications and highlights the potential of genome-wide G $\times$ E studies.

## OS 5.5 Genome-wide Significant Associations in Schizophrenia to ITIH3/4, CACNA1C and SDCCAG8, and Extensive Replication of Associations Reported by the Schizophrenia PGC

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**Background:** The Schizophrenia Psychiatric Genome-Wide Association Consortium (PGC) highlighted 81 single nucleotide polymorphisms (SNPs) with moderate evidence for association to schizophrenia. After follow up in independent samples, 7 loci attained genome wide significance (GWS), but multi-locus tests suggested some SNPs that did not do so represented true associations.

**Methods:** We tested 78 of the 81 SNPs in 2,640 individuals with a clinical diagnosis of schizophrenia attending a clozapine clinic (CLOZUK), 2,504 cases with a research diagnosis of bipolar disorder, and 2,878 controls.

**Results:** In CLOZUK, we obtained significant replication to the PGC-associated allele for no fewer than 37 (47) of the SNPs, including many prior GWS MHC SNPs as well as 3/6 non-MHC SNPs for which we had data that were reported as GWS by the PGC. After combining the new schizophrenia data with those of the PGC, variants at three loci (ITIH3/4, CACNA1C and SDCCAG8) that had not previously been GWS in schizophrenia attained that level of support. In bipolar disorder, we also obtained significant evidence for association for 21 of the alleles that had been associated with schizophrenia in the PGC. Comparison of odds ratios from samples with clinical (CLOZUK) and research diagnosis (Irish PGC stage 2) schizophrenia showed borderline significant evidence for a difference ( $p=0.039$ ), but this was not the case when restricted to the non-MHC SNPs ( $p=0.31$ ).

**Discussion:** Our study independently confirms association to 3 loci previously reported to be GWS in schizophrenia and identifies the first GWS evidence in schizophrenia for a further 3 loci. Given the number of independent replications and the power of our sample, we estimate 98 (C.I. 78-100) of the original set of 78 SNPs represent true associations. We also provide strong evidence for overlap in genetic risk between schizophrenia and bipolar disorder. Our findings suggest some samples based upon clinician reported diagnosis may make an important contribution to the large samples required for genetic studies of schizophrenia. To inform the results and discussion, we will also present results for a further 4,000 individuals.

## OS 5.6 Analysis of Low-frequency, Protein Altering Variation in 13,000 Individuals from a Swedish Schizophrenia Cohort on the Exome Array

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**Background:** The recently developed exome chip array enables the assessment of rare coding variation. Containing approximately 250,000 variants drawn from whole genome and exome sequencing of 12,000 individuals, this array enables assessment of ~200,000 missense, ~5,500 nonsense, and ~10,000 splice site mutations. Consequently, we can now assess the role of coding variation in a large population derived sample of schizophrenia.

**Methods:** We genotyped over 13,000 individuals drawn from the Swedish Schizophrenia Study on the Illumina Infinium HumanExome BeadChip. Using a subset of samples for whom exome sequencing and exome chip genotype data are available, we designed new genotype caller, zCall, that improves accuracy of rare variant genotyping. We also examined the nature of rare variation in the Swedish population, showing that a significant subset of the Swedish sample has Finnish ancestry.

**Results:** Technical Results: Based on comparisons with sequencing, ~99% of variants with a singleton (i.e. only one heterozygote observed) in 947 individuals were called with a single heterozygote in the correct individual. The overwhelming majority of the variation on the assay is rare, with ~46% of the sites invariant and another ~25% variable sites having fewer than 10 copies. We identified a significant subset of the sample of apparent Finnish ancestry enabling the identification of rare functional variation significantly increased in Finland due to the bottleneck. Disease Association Results: We will present single SNP association analysis using logistic regression with principal components as well as using a mixed model incorporating genome-wide similarity in the analysis. Beyond single locus association, we also describe regional association analyses, combining evidence for association across a gene, testing for recessive disease models, and critically evaluating loss of function alleles. While our analysis is provisional as of the date of submission of this abstract, the results to date suggests that at most a few (and possibly no) protein-altering alleles influence schizophrenia in Sweden with an effect size that makes them detectable at genome-wide significance in a cohort of 13,000.

**Discussion:** The exome array allows large scale analysis of functional variation. The data generated here demonstrate that this assay performs well and captures most of an individual's variation. Furthermore, these results bound how much standing coding variation influences schizophrenia in Sweden and provide context for previously reported genome-wide association results, partly addressing the question of synthetic association.

## Oral Presentation Session 6 Functional Genomics and Model Organisms

### OS 6.1 MIR137, A Candidate Gene for Schizophrenia Risk: Identification of Targets and Downstream Effects

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**Background:** Recent genetic evidence strongly suggests that schizophrenia has a highly polygenic genetic basis, consistent with the hypothesis that many hits to the same pathway increases risk for schizophrenia. Findings from the Psychiatric GWAS Consortium (PGC) have identified significant and replicated association for a region on chromosome 1 near MIR137 (rs1625579;  $p=1.6 \times 10^{-11}$ ; the second top hit in the PGC GWAS). Unpublished studies increase the significance of this finding to  $p=1.3 \times 10^{-12}$ . MIR137 has also been implicated through identification of genome-wide significant associations for genes with confirmed miR-137 target sites (CACNA1C, TCF4, CSMD1, and C10orf26). microRNAs bind mRNA's with the appropriate seed sequence and target the transcript for degradation or inhibition of translation. The ability of microRNAs to regulate hundreds of genes yields the hypothesis that miR-137 regulates one or more pathways involved in schizophrenia etiology. Moreover, miR-137 has been implicated in other psychiatric disorders, including deletions in individuals with intellectual disability and autism spectrum disorders, and altered levels in Alzheimer's patients and Rett syndrome mouse models. Combined with indications that miR-137 plays an important role in neuronal differentiation and proliferation, it is imperative that we understand miR-137 function. Therefore, we evaluated potential targets for direct or indirect regulation by miR-137, in order to elucidate a pathway altered in schizophrenia.

**Methods:** We used human neural stem cells (ReNcell-VM). These cells were transduced with lenti-viral vectors containing the pre-miR-137 (to overexpress miR-137), a construct which will sequester miR-137 (functionally inhibiting its regulation), or the respective control. Control vectors consist of the same vector backbone with a random miRNA-like sequence instead of miR-137. Transduction efficiencies are estimated by fluorescent microscopy of mCherry (inhibition) or eGFP (overexpression) expressed from the lenti-viral vectors. miR-137 levels are measured by Taqman miRNA expression assays. RNA was extracted from cells transduced with either the miR-137 overexpression or control vector. Sequencing libraries were generated and sequenced with single end 100bp reads on Illumina's HiSeq 2000.

**Results:** ReNcell-VM cells have successfully been transduced with lenti-viral vectors for overexpressing and inhibiting miR-137 and the respective controls. Transduction efficiencies have approached 100% for all vectors. For overexpression, miR-137 levels are increased by  $\geq 40X$ . For inhibition, miR-137 is sequestered not degraded, therefore testing of established targets of miR-137 (e.g., MIB1 and MTF) is in progress to verify the functionality of the inhibition. Our major interest is to evaluate the downstream effects miR-137 overexpression or inhibition in a human neuronal cell line. We are now testing candidate genes (known and predicted targets of miR-137 plus genes implicated by schizophrenia GWAS) via qPCR of transcripts and western blot for proteins to determine the impact of miR-137 dysregulation. To identify novel downstream effects, we have completed RNA-seq on the overexpressing cells and controls (analysis in progress) with remaining work to be completed shortly. Comparison of predicted binding sites and direction of alteration will yield a candidate list of direct versus indirect targets.

**Discussion:** miRNAs are intriguing candidates in complex disease, as the ability to regulate hundreds of genes may allow identification of many other contributors to disease pathogenesis. Moreover, given the robust and replicable evidence supporting MIR137 in schizophrenia, identification of members of the network regulated by miR-137 could identify critical participants in schizophrenia pathogenesis, even in the absence of genetic variants that affect risk. Additional work including constitutively overexpressing/inhibited cell lines, differentiation of the neuronal stem cells into neurons, and experiments, such as PARCLIP, that can identify translationally inhibited targets will also be needed to further our understanding of this micro-RNA. Nonetheless, elucidation of this pathway could provide a myriad of potential novel targets for pharmaceutical intervention. Therefore, the need to identify miR-137's targets and effects of dysregulation is imperative to schizophrenia research.

## OS 6.2 Expression QTL Analysis of Glucocorticoid Regulated Gene Expression: New Insights into the Genetics of Mood and Anxiety Disorders

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**Background:** Abnormal regulation of the hypothalamic-pituitary-adrenal axis, the main stress-responsesystems, is a key neurobiological characteristic of major depression. Glucocorticoid receptor (GR) function has been shown to be disturbed in depression, hence polymorphisms altering the transcriptional effects of GR-activation might be interesting candidates for this disorder. The aim of this study was to identify SNPs associated with glucocorticoid (GC)-induced gene expression changes of nearby genes (cis-eQTL analysis) in peripheral blood.

**Methods:** In total, 160 male Caucasian individuals (69 cases, 91 controls) were genotyped using Human610-Quad and Human660W-Quad BeadChips (Illumina Inc.). Approximately 2 million loci at high quality were imputed using IMPUTE-v2 from HapMap Phase 3 and 1,000 Genomes Project data. A Dexamethasone stimulation test was performed in all subjects and gene expression (baseline and following GC-stimulation with 1.5 mg dexamethasone) was analyzed using Human HT-12v3. After extensive quality control and removal of batch effects we performed linear regression analysis conducting permutation testing in PLINK.

**Results:** In a first step, we identified a total of 4,395 significant cis-eQTLs. Of those, 2,364 were significant response eQTLs, i.e., loci that were only associated with variation in GC-stimulated gene expression changes, but not with baseline gene expression. Over 67% of these response eQTLs were located in a distance of >200 kb from the probe (mean distance=406 kb). By contrast, baseline eQTLs showed only 19% cis-association in a distance of >200 kb (mean distance=136 kb), thus indicating a more long range regulation of gene expression by the GR. In a second step, we tested for an enrichment of transcription factor (TF) binding sites nearby eQTL SNPs (eSNPs). We identified the GC response element as one of the most enriched TF binding sites (TFBSs) as well as TFBSs that moderate the GR signaling, including AP1, HNF4 and OCT1. We further observed differences in the affinity of GREs between opposite SNP alleles. In a last step we integrated our data with results from GWAS for depression originating from publically available data sets (<http://www.genome.gov/gwastudies/> and <http://geneticassociationdb.nih.gov/>). We found an overlap of significant response eSNPs and SNPs associated with traits related to depression. Interestingly, the majority of these overlapping SNPs does not alter the gene expression of the closest gene but of more distant genes (mean distance=687 kb). For example, SNPs within the CLOCK gene moderate the gene expression of PAICS, which is 947 kb upstream of the CLOCK gene. Finally, we combined depression susceptibility loci from the recent PGC mega-analysis of GWAS for major depressive disorder and found that response eSNPs were significantly more likely to be associated with the disease as random and baseline eSNPs ( $p < 0.001$ ).

**Discussion:** In summary, our data suggest that GC-stimulated eQTLs could expand our understanding of the genetic basis of stress-related disorders, including major depression, in which GR-function plays an important pathophysiologic role.

### OS 6.3 Mapping Genetic and Epigenetic Factors Influencing Human Hippocampal Gene Expression

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**Background:** Several studies have investigated the effects of genetic variation on gene expression (expression quantitative trait loci, eQTLs) in peripheral tissue, cell lines, or post-mortem brain tissue. eQTL studies from pre-mortem, fresh-frozen brain samples would be highly interesting but are hampered by the restricted accessibility of such samples.

**Methods:** At the University of Bonn, we have access to a unique sample of pre-mortem human hippocampus samples originating from surgery of treatment-resistant epilepsy patients. To systematically determine eQTLs in a total of 148 hippocampus samples, we generated whole-genome SNP (Illumina Human660W) and gene expression data (Illumina HumanHT-12v3). Genome-wide methylation measurement was performed using Illumina's new HumanMethylation450 array which interrogates more than 485,000 methylation sites. In addition to the conventional data analysis, we applied a new "hidden factor" analysis that identifies and corrects for unknown confounding factors in the data and thus diminishes the false-positive and false-negative eQTL rate (PEER, <https://github.com/PMBio/peer/wiki>). Fifteen hidden factors were identified and used as co-variables for expression analysis.

**Results:** We detected 78 trans-regulating (>1Mb between SNP and probe) eQTLs that withstood Bonferroni correction for multiple testing. Moreover, 1,925 cis-regulating (≤1Mb distance) eQTLs remained significant after permutation-based Westfall-Young correction. In an additional step, we extended our analysis to the systematic investigation of the influence of DNA methylation on gene expression.

**Discussion:** To our knowledge, our study is the first to integrate genotype, expression and methylation data from pre-mortem brain tissue and will provide a valuable resource for the functional interpretation of genetic and epigenetic sites, in particular those associated with brain diseases.

### OS 6.4 Using Measures of Allelic Expression to Elucidate Regional and Temporal Risk Mechanisms for Psychiatric Disorders

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**Background:** Genetic effects on gene expression are considered to be a major determinant of human phenotypic diversity, including susceptibility to complex disease. This is borne out by genome-wide association studies of psychiatric disorders such as schizophrenia, where risk alleles are, en masse, enriched for cis-effects on gene expression. These cis-acting effects on gene regulation can be specifically detected using measures of relative allelic expression, where RNA transcript expression from one chromosomal copy of a gene is compared with that from the other. Studies using this method have indicated that cis-regulatory effects can be both tissue- and temporally- specific. As such, it should be possible to determine not only if and how identified genetic risk variants for psychiatric disorders impact on the cis-regulation of a gene, but also where and when they are active.

**Methods:** We have measured allelic expression of several psychiatric candidate genes across multiple regions of the adult human brain and between cell populations of the human hippocampus, isolated through laser capture microdissection. We have also tested cis-effects of a genome-wide significant risk variant for schizophrenia / psychosis on expression of the ZNF804A gene in both adult and foetal human brain.

**Results:** Within subjects, we found significant differences between brain regions in the allelic expression of all genes examined. For ZNF804A, we also found significant differences in the extent of allelic expression imbalance between cells of the CA1 and dentate gyrus of the hippocampus. The schizophrenia risk variant rs1344706 had no significant effect on the cis-regulation of ZNF804A in any of the adult brain regions, but was associated with altered allelic expression in foetal brain tissue from the second trimester of gestation, with significantly reduced expression of RNA transcribed from the risk allele.

**Discussion:** Effects of cis-regulatory variation can differ across regions, cell types and development of the human brain. As well as highlighting an important caveat for studies of regulatory polymorphism in brain tissue, our findings indicate that it is possible to delineate brain areas and developmental stages in which cis-regulatory variants are active. This may provide important insights into the fundamental biology of neuropsychiatric phenotypes with which such variants are associated.

## OS 6.5 Whole-genome Sequencing Analysis of Human Induced Pluripotent Stem Cell Lines Uncovers Lineage-manifested Copy Number Variation

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**Background:** The ability to reprogram human somatic cells into induced Pluripotent Stem Cells (iPSC) and from there differentiate them into various cell types, including neurons, is opening up new avenues to study the effects of genetic and genomic sequence variation that is implicated in neurodevelopmental, neuropsychiatric diseases, in a physiologically relevant model system. However, there are reports that there might be an increased rate of de novo CNV formation in iPSC, perhaps as a consequence of the reprogramming process, which - depending on the extent of such a phenomenon - might make it more challenging to unlock the full potential of iPSC as a model system for the study of complex disorders.

**Methods:** We have performed whole-genome sequencing based CNV analysis (read-depth analysis, frequently confirmed by paired-end mapping) in 7 fibroblast samples and 20 corresponding iPSC lines obtained from two families [Abyzov et al., in revision]. Each family had one child with autism spectrum disorders - however, here we present the aspects of our findings that we believe are of utility and importance for iPSC model based research in general. We defined the term LM-CNV to describe CNVs detected by genome-wide analyses in an iPSC line but not in the fibroblast culture from which the given iPSC line was derived (and typically also not found in other iPSC lines derived from the same fibroblast culture) but without making a statement as to the nature of the CNV-forming event (i.e. whether the CNV arose de novo during reprogramming from fibroblast to iPSC or whether it was present as a somatic variant in mosaic fashion in the fibroblast culture).

**Results:** We found that on average an iPSC line has two LM-CNVs (lineage-manifested CNVs). After detecting LM-CNVs by sequencing based analysis in the iPSC lines we were able to design PCR primers that would cross the CNVs' breakpoints and using those PCR primers we investigated the masked, mosaic presence of the same CNVs in the fibroblast tissue of origin. We determined that more than half of the LM-CNVs detected in iPSC lines were already present as low allele frequency, mosaic somatic CNVs in the fibroblasts and that approximately 40% of fibroblast cells carry such medium-sized to large somatic CNVs. We also carried out correlative analyses between the LM-CNVs and gene expression determined by RNA-Seq from the same iPSC lines. When analyzing expression levels of genes intersecting LM-CNVs in iPSCs we found a clear tendency (p-value of 0.02 by Fischer's exact test) of increase in expression for genes in duplications and decrease in expression for genes in deletions, respectively. However there are also genes in deletion CNVs with increased expression and genes in duplication CNVs with decreased expression, an observation that requires further analysis on the molecular level.

**Discussion:** Based on our findings de novo CNVs in iPSCs may not be an obligate consequence of reprogramming into iPSCs. Our analysis unexpectedly revealed extensive somatic copy number variability in fibroblasts carrying over into iPSC and becoming unmasked in the process. This study underlines the necessity of carrying out high-resolution genome analysis during iPSC-model based studies and demonstrates that whole-genome sequencing would allow to detect and subsequently manage the potential confounds introduced by emerging genomic variation such as LM-CNVs.

## OS 6.6 Induced Pluripotent Stem Cell (iPSC) Models for Bipolar Disorder

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**Background:** An integrated approach to the development of cellular models for complex neuropsychiatric disorders such as Bipolar Disorder (BP) will maximize the use of additional clinical, biological and environmental data. It offers opportunities to emulate variable in vivo exposures, e.g. to lithium treatment, in vitro, in the background of detailed knowledge of the originating subject. Study of iPSC-derived cells is the base for the specific study affected tissues from a cellular and developmental biology perspective. Since BP is a heterogeneous disorder, selection of subjects for establishment of iPSC lines may benefit from dimension phenotypic assessment. The goal of this study is to develop and characterize iPSC lines from BP individuals and controls with deep phenotypic assessments.

**Methods:** BP individuals from the Prechter BP longitudinal study were selected for dermal biopsy based on the presence of BP high vs low measures of Neuroticism on the personality inventory NEO-PI. Fibroblast cell lines were developed from 2 BP and 4 control subjects, and early passage freezer stock banked. Dermal fibroblasts at passage 1-2 were then transduced with individual retroviral constructs expressing pluripotency factors, resulting in more than 50 iPSC lines. For neuronal differentiation, iPSC were grown in suspension culture in N2 medium + retinoic acid, nodal and BMP inhibitors, followed by plating on polyornithine coated coverslips. Neural differentiation was assessed using antibodies to: nestin, GFAP, bIII tubulin, MBP, and Tau followed by appropriate secondary antibodies and in quantitative PCR.

**Results:** As of submission 51 iPSC lines have been derived, at least two per individual; 17 BP and 34 control. With reprogramming, the pluripotency genes Nanog, Oct4, SSEA3,4, and alkaline phosphatase are induced, while levels of fibroblast-restricted genes e.g., Te-7, are down-regulated. Both control and iPSC derived from BP individuals are capable of widespread neuronal differentiation, forming highly branched networks of neurites. Studies of neurotransmitter phenotype, pre- and post-synaptic characteristics, and electrophysiology are in progress, to be followed by karyotype, derivation and characterization of additional cell lines.

**Discussion:** The selection of individuals to develop iPSC lines was determined by the base clinical phenotype as well as a dimensional assessment of personality and temperament (high vs low neuroticism). This was selected because neuroticism appears to associate with longitudinal course and outcome of the illness. The initial appearance of these lines was similar with no differences in pluripotency gene expression, morphology or cell cycle characteristics, although there was a tendency of the iPSC from BP to differentiate prematurely. The derivation of iPSC lines from dermal fibroblasts provides an opportunity to develop models of complex neuropsychiatric illness. We describe the derivation and characterization of iPSC lines from patients with Bipolar Disorder, and demonstrate their ability to undergo widespread neuronal differentiation, setting the stage for future studies of their gene expression profile and response to medication.

## Oral Presentation Session 7 Endophenotypes

### OS 7.1 Quantitative Trait Loci Identified for Working and Spatial Memory: Identifying Endophenotypes for Psychosis using Realistic Phenotypic Models

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**Background:** While the genetic influence on intelligence is well established, attempts to isolate genetic loci or specific genes have been met with difficulty; this lack of progress is a reflection, in part, of the phenotypic complexity intelligence. The aim of this study was to characterise the genetic architecture of cognition using phenotypically detailed models rather than relying on general IQ or individual neuropsychological measures.

**Methods:** The sample comprised 1,269 Mexican American individuals from extended pedigrees for whom comprehensive neuropsychological and genetic data were available. Participants were genotyped for 1M SNPs on Illumina microarrays. Three-tier hierarchical models of genetically clustered cognitive traits were subjected to linkage and gene identification analyses.

**Results:** Two significant QTLs were identified for working memory on chromosome 8 (8q24.22 LOD = 4.13 and 8q21.11 LOD = 4.03) and for spatial memory on chromosome 17 (17q23.2 LOD = 3.96 and 17q25.1 LOD = 3.50). No significant association was found for general cognitive ability. Post-hoc analyses revealed genetic variants that contribute to memory ability.

**Discussion:** Using a new phenotypic approach we identified a number of QTLs for working and spatial memory performance. The creation of detailed and realistic phenotypic models seemingly enhanced the power to detect genetic effects. Spatial and working memory are well-established endophenotypes for psychosis – identifying the genes that influence these phenotypes will provide empirically nominated candidate genes for psychosis.

### OS 7.2 Reduced Inferior Frontal Gyrus Activation during Response Inhibition to Emotional Stimuli in Youth at High Genetic Risk of Bipolar Disorder: Genetic Associations

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**Background:** Functional brain imaging of young people at increased genetic risk for bipolar disorder provides a means of identifying potential endophenotypes for this condition. Dysfunctional neural mechanisms for the cognitive control of emotion are postulated in the genetic predisposition to bipolar disorder, with aberrant activity in fronto-cortical, striatal and limbic networks having previously been reported in subjects with established bipolar disorder during inhibitory and emotion processing tasks.

**Methods:** Functional brain activity during inhibition of emotional material in young people at increased genetic risk for bipolar disorder was investigated using a facial-emotion Go/No-Go task during functional magnetic resonance imaging. Data from 47 genetically high-risk individuals aged 18-30 years with at least one first-degree relative with bipolar disorder were compared with 49 controls (within the same age range but without a family history of bipolar disorder or other severe mental illness). Genetic associations of peak beta values were examined.

**Results:** Whole brain corrected analyses revealed a highly specific and significant lack of recruitment of the inferior frontal gyrus when inhibiting responses to fearful faces in the high-risk participants compared to controls ( $p=0.011$ , FWE, peak voxel). This finding remained significant after exclusion of subjects who were currently depressed or receiving psychotropic medications. There was a significant effect of genotype on imaging peak beta values with the HTR2A gene (rs6311 SNP;  $P=0.005$ ) but no genotype x group interaction.

**Discussion:** Impaired inhibitory function of the inferior frontal cortex may represent a trait marker of vulnerability to bipolar disorder. These findings further implicate dysregulated cortical and sub-cortical brain networks as a potential neurocognitive endophenotype for bipolar disorder and add to the growing evidence for pre-existing functional and structural disturbances in those at high genetic risk for bipolar disorder.

### OS 7.3 Identification of Convergent Molecular Pathways of Human Working Memory Performance: Evidence from Genome-wide Pathway Analyses and Brain Imaging Studies

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**Background:** Genome-wide association studies (GWAS) are a potent tool for the unbiased search for single marker associations with a specific trait. By reporting the best single p-values beyond the genome-wide level of significance, GWAS ignore medium-sized effects possibly acting in combination. Prior biological knowledge is of special value and can inform pathway analyses that are used to identify combinations of genes implicated in the trait of interest. Statistical methods originating from gene expression analysis have been modified to identify genetic pathways in GWAS data. In general, these methods test the enrichment of functionally connected association signals against the distribution of all GWAS association signals.

**Methods:** In this study we performed gene set enrichment analyses for working memory performance (n-back task) in three independent GWAS samples of healthy Swiss individuals (n(combined)=1'876). Additionally, brain imaging during n-back performance was conducted in a subset of 510 individuals.

**Results:** In the first GWAS data set, we identified 23 significant pathways ( $p(\text{corrected}) < 0.0001$ ). In a functional brain imaging study (n=510), we observed score-dependent moderation of brain activity in the right precuneus ( $p=0.00005$ ).

**Discussion:** Taken together, our study provides first evidence for a role of the gene ontology category GO:0022843 in working memory performance.

### OS 7.4 Sparse Reduced-rank Regression as a Multivariate Technique for Genome-wide Association Studies: Application to Identify Genetic Variants Associated to Neuro-cognition and Brain-imaging Traits

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**Background:** Recently, genome-wide association studies (GWASs) have become more widespread. Traditional GWASs are based on multiple univariate linear models (MULM), in which each genetic marker, primarily single nucleotide polymorphisms (SNPs), is individually tested for association with one phenotypic trait at a time. Thus, traditional GWASs do not account for correlations in genotype and phenotype data. To overcome these limitations, and especially to perform analyses of large data sets with multivariate phenotypes, such as those of brain imaging, we have developed a multivariate technique known as sparse reduced-rank regression (sRRR) model. This approach has been validated on simulated data and on the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset, identifying SNPs in the known Alzheimer's genes, APOE and TOMM40. Here, we applied the sRRR model to neuro-cognitive and brain-imaging traits relevant to schizophrenia. These included scores for the Digit Symbol Substitution (DSS) test, at which patients with schizophrenia have been shown to perform poorly, and measures of brain cortical thickness and surface area from a healthy sample of 440 Norwegian individuals.

**Methods:** The sRRR model is a multivariate regression technique which accounts for the multivariate nature of GWAS data by modelling all genotype markers and phenotypic traits simultaneously. Thus, unlike the MULM approach, which only identifies single polymorphisms with relatively large effects on the phenotype, the sRRR model enables the identification of genetic markers, each with small effects, working together. It is assumed that only a handful of genetic markers are associated with the phenotype, thus the Lasso penalty is introduced into the sRRR model to achieve variable selection in the genotypic domain. To enable ranking the genetic markers according to their association strength, the sRRR model is combined with a data resampling technique, whereby the model is fitted repeatedly in data subsamples. The genetic markers are then ranked according to their frequency of selection. The sRRR model was applied to the genotype and phenotype data of 440 healthy volunteers (34.8% male, median age 58 years) from the Norwegian Cognitive NeuroGenetics (NCNG) sample. After imputation and quality control, the final genotype dataset consisted of 636,668 SNPs. In the first round of analysis, the ~600K brain cortical thickness and surface area measures were reduced to the ~11K most correlated with DSS test performance. In the second round, the set of SNPs the most associated with these brain measures was selected by the sRRR model.

**Results:** The sRRR model identified a set of 29 SNPs highly associated with the imaging traits. LD-based gene-binning with LDsnpr assigned 14 of those SNPs to 13 Ensembl 54-defined genes, some of which have already been shown to play important roles in neuronal development. About half of the SNPs remained un-annotated or within genes that appear not to be relevant to the traits studies. Hence, further annotation is warranted.

**Discussion:** We used the sparse reduced-rank regression model, which is a multivariate technique for GWASs, in the analysis of high-dimensional genotype markers and brain-imaging traits of healthy Norwegian subjects. Further work will include a thorough investigation of the identified candidate genes, further annotation of SNPs and replication in an independent sample.

### **OS 7.5 Genetic Variation in the Atrial Natriuretic Peptide Transcription Factor Gata4 Modulates Amygdala Responsiveness to Alcohol Cues and Relapse Risk in Alcohol-dependent Subjects**

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**Background:** Converging evidence from both preclinical and clinical studies suggests an involvement of atrial natriuretic peptide (ANP) in the symptomatology of alcohol withdrawal and dependence. Moreover, a single-nucleotide polymorphism (rs 1327367) located in the gene encoding a transcription factor of ANP (GATA4) with alcohol dependence, was among the top-findings in two independent GWAS. AIM: We now examined whether amygdala-reactivity to alcohol cues, a biomarker associated with addictive behaviour, differs depending on GATA4-genotype and whether these differential activations influence relapse behaviour.

**Methods:** 81 alcohol-dependent patients completed an fMRI cue-reactivity task in a 3T scanner, during which they had to passively view alcohol-associated and neutral pictures. Follow-up data was available from 48 patients.

**Results:** Significantly lower alcohol cue-induced activations were found in the “high-risk” (AG/ GG) compared to the “low-risk” (AA) group in the bilateral amygdalae (ROI:  $p < .05$  [FWE-corrected];  $kE \geq 10$ ). Moreover, a subsequent survival analysis revealed that in the AA-group, high amygdalae activation significantly predicted a lowered risk for a relapse to heavy drinking (significant genotype x activation effect:  $p = 0.0183$ ).

**Discussion:** These results suggest a GATA4-genotype dependent protective effect of increased amygdala activation in response to alcohol relative to neutral pictures. They further illuminate potential underlying mechanisms of involvement of the GATA4 gene in the aetiology of alcohol addiction.

## OS 7.6 GABA and NMDAR-Agonists in Human Cerebrospinal Fluid: From Hypothesis-driven to Genome-wide Association Studies

### ECIP

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**Background:** Genetic and pharmacological approaches in psychiatric patients furthermore converge on alterations in NMDAR and GABA trafficking. NMDAR co-agonists play crucial roles in NMDAR-mediated neurotransmission. Although several amino acids (AAs) have been subjected to GWAS, to our knowledge proline, serine and glycine have not been included in such studies. Moreover, cerebrospinal fluid (CSF) - the body fluid compartment most proximate to the CNS- has not been used in GWAS of AAs and there are no known genetic variants influencing GABA levels in human CSF. Furthermore, while knowledge regarding the differential effects of AA L- and D-isomers on the CNS has been accumulating over the past decade, genetic mechanisms underlying enantiomer concentration variations and correlations remain largely elusive. Understanding the genetic background of NMDAR co-agonist concentration variations may enhance the understanding of NMDAR physiology. Such insight in turn may ultimately open avenues for clinical applications, e.g. improvements in co-agonist add-on approaches in schizophrenia and disease prediction.

**Methods:** The study population consisted of 414 healthy subjects undergoing spinal anesthesia. HPLC was used to measure GABA, glycine and the two isomers of serine, proline and alanine in plasma and CSF. Genotype data were collected using the Illumina HumanOmniExpress Beadchip. Imputed genotypes were generated using the HapMap2 and 1000Genomes projects. For the GWAS of the NMDAR agonists, a linear additive genome-wide association model using age, sex and storage time was run in Plink. For GABA, one variant in ERBB4 (rs7598440) influencing GABA levels measured by H1-MRS was considered in a subset of this sample (N=151) and all covariates known to possibly influence CSF GABA in humans were included (age, sex, storage time, the rostrocaudal gradient, time elapsed prior to storage, and amount of CSF drawn).

**Results:** A significant dose-dependent association of the rs7598440 genotype with CSF GABA levels was detected ( $\beta=-0.23$ ;  $p=0.0066$ ). Regarding the NMDAR-agonists, two genome-wide significant loci were detected: one for plasma L-Proline in a region surrounding PRODH and one for CSF L-Proline in SLC6A20. With regard to serine, the strongest association signal was found for a common intronic variant in DAO ( $P = 8.98 \times 10^{-8}$ ).

**Discussion:** Both hypothesis-based and hypothesis-generating quantitative trait locus analyses of GABA and NMDAR-agonists in plasma and CSF resulted in associations of both biologically plausible and novel variants. The directionality of our GABA finding agrees with previous H1-MRS and postmortem studies and constitutes the first proof that CSF can be used to study genotype-phenotype associations of GABA in human CSF. Transporter mechanisms seem to influence CSF variation in proline, whereas possibly regulatory regions surrounding PRODH impact on plasma proline. A variant in DAO previously associated with schizophrenia was the main determinant of the plasma/CSF ratio of D-serine. Although the collection of CSF in this study population has been enterprising, a major limitation is the absence of a replication sample.

## Oral Presentation Session 8 Childhood Psychiatric Disorders

### OS 8.1 WTCCC3 and GCAN: A Genome-wide Scan for Anorexia Nervosa

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**Background:** Anorexia nervosa (AN) is a debilitating and potentially lethal mental illness (prevalence ~1 in women; sex ratio ~9:1; heritability estimates 22-88). The Genetic Consortium for Anorexia Nervosa (GCAN) is a 20-country consortium dedicated to identifying genes that influence AN. As part of the WTCCC3, we conducted largest GWAS for any eating disorder ever performed.

**Methods:** In collaboration with the third wave of the Wellcome Trust Case Control Consortium (WTCCC3), we conducted a genome-wide association study (GWAS) to identify unequivocal evidence of associations between DNA sequence variations and AN. All cases met DSM IV diagnostic criteria for AN (excluding amenorrhea) and were genotyped using the Illumina 660W-Quad array. Controls were carefully selected to match for ancestry of each AN site.

**Results:** Genotyping was completed on 2,907 AN cases and 14,860 geographically matched controls. Individual analyses were carried out across 15 strata. Eleven association analyses were conducted per stratum: one with no principal components (PC) adjustment and the others adjusted up to 10 PCs. On the basis of QQ plots, lambda values and 54 population stratification SNPs, we selected the best analysis per each stratum for meta-analysis. The uncorrected lambda was 1.024. One SNP on chr5 exceeded genome-wide significance. Ninety-four SNPs with p-values < 10<sup>-4</sup> derived from the fixed effects meta-analysis. Most of these SNPs are in the frequency and effect size classes expected for common diseases. We are currently following up promising signals in up to 2,500 independent replication cases.

**Discussion:** For strong/replicated findings, GCAN is well-positioned immediately to pursue genomic, transcriptomic, molecular biological, and epidemiological studies in humans. Cross disorder analyses with psychiatric disorders and obesity are planned. Furthermore, functional analysis of human anorexia genes in mice will provide novel insights in the neurobiological mechanisms underlying this perplexing psychiatric disorder.

## OS 8.2 Genome-wide Meta-analysis of Internalizing Problems at Age 3

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**Background:** Internalizing problems measured at age 3 are highly heritable. In a large sample of Dutch twins (N = 10,708), 59% of the variance of maternal internalizing problem ratings was explained by genetic factors. Gene-finding studies focusing on preschool internalizing problems, and other psychiatric symptoms in young children, are still rare.

**Methods:** Internalizing problems were assessed with the internalizing problem subscale of the Child Behavior Check List (CBCL). Genome wide association (GWA) data were available for 1,594 twins from the Netherlands Twin Register (NTR), 2,037 Dutch unrelated individuals from Generation R (GenR) and 1,084 individuals from Raine, Australia. GWA analyses were performed in PLINK, with sex and principal components included as covariates and corrected for family structure if required. The Meta-analysis was carried out in METAL and followed by analyses in VEGAS and Ingenuity to obtain significantly associated pathways.

**Results:** No single SNP reached genome wide significance. The most promising results appeared on chromosome 9 and 20, with several SNPs with a p-value below  $1 \times 10^{-4}$  lying in a gene. The pathway-analysis showed a significant overrepresentation of genes that were associated with "psychological disorders" in Ingenuity. Looking into this result in further detail showed that the genes found in the current study were also associated with adult psychiatric symptoms such as psychosis.

**Discussion:** Similar to GWA studies in adult anxiety and depression, a GWA meta-analysis of internalizing problems at age 3 did not yield genome wide significant results, but there were some promising hits. However, the sample is small by current standards for adult GWAs of psychiatric disorders. Moreover, genes associated with adult psychiatric symptoms appeared to be associated with internalizing problems at age 3. It seems that genes influencing internalizing problems at age 3 have an effect on a broad range of adult psychiatric symptoms.

## OS 8.3 Childhood Adversities Affect Adult Age Leukocyte Telomere Length of the Finnish Population

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**Background:** Leukocyte telomere length (LTL) has been suggested as a biomarker of aging based on its putative capacity to function as a cumulative index of oxidative stress and inflammation. Childhood adversities are well-known risk factors for psychiatric morbidity later in life, and psychiatric disorders have been associated with increased incidence of somatic illness, indicative of accelerated aging. Several recent studies, but not all, have shown that childhood stress is associated with shorter LTL later in life. However, it is unknown whether childhood stress and consequently shorter LTL are important on a population level. We hypothesized that the number of childhood adverse life events predicts shorter LTL at adult age and investigated this hypothesis in an epidemiological Health 2000 Cohort that represents the entire Finnish population.

**Methods:** This cohort was collected during 2000 and 2001 to assess the major public health problems, functioning and their determinants of the adult Finns, aged 30 years or older. Childhood adversities were assessed with a questionnaire containing a series of 11 questions regarding the childhood social environment before age of 16. The total number of reported adversities per subject was recorded from 0 to 11, and was categorized into four groups: 0 (N=2462), 1 (N=1508), 2 (N=902), or >2 (N=927) adversities. Relative LTL was determined from genomic DNA extracted from peripheral blood by a quantitative real-time PCR-based method. After quality control, the sample consisted of 5799 individuals with information available on childhood adversities and LTL.

**Results:** LTL was significantly affected by age (F=758.99, df=1,  $P < 10^{-6}$ ) and sex (F=73.79, df=1,  $P < 10^{-6}$ ), with younger individuals and females having longer LTL, as expected. Having experienced more than two childhood adversities predicted shorter LTL (F=5.42, df=3,  $P=0.001$ ; adjusted by age and sex; figure). This finding remained significant when adjusting for factors previously reported to affect LTL (F=5.49, df=3,  $P=0.0009$ ; adjusted for age, sex, body mass index, smoking, physical activity). We also adjusted for past 12 month or lifetime alcohol use disorder, depression or dysthymia and anxiety disorder diagnoses, and the effect of childhood adversities on TL remained significant (F=5.08, df=3,  $P=0.002$ ).

**Discussion:** Relatively common childhood adversities were associated with shorter LTL at adult age, and this effect can be detected in a nationally representative population-based cohort. These results imply that childhood adversities may cause accelerated telomere shortening. Our finding has potentially important implications as it supports the view that childhood adversities may have a considerable impact on psychological and somatic well being later in life. Prospective evidence would be urgently needed to support the public health conclusions.

## OS 8.4 Genome-wide Analysis of Rare Copy Number Variations Reveals Park2 as a Candidate Gene for Attention-Deficit / Hyperactivity Disorder

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**Background:** Attention-deficit/hyperactivity disorder (ADHD) is a common, highly heritable neurodevelopmental disorder. Genome-wide significant genetic loci have not yet been identified. Rare copy number variations (CNVs), such as chromosomal deletions or duplications have been implicated in ADHD and other neurodevelopmental disorders.

**Methods:** To identify rare (frequency  $\leq 1\%$ ) CNVs that increase the risk of ADHD, we performed a whole-genome CNV analysis based on 489 young ADHD patients and 1,285 adult population-based controls. Locus-specific tests of association were used to assess if there is an increased number of rare CNVs in cases compared with controls. Detected CNVs in the ADHD group were validated by qPCR. Findings were replicated in an independent sample of 386 young patients with ADHD and 781 young population-based healthy controls.

**Results:** We identified one rare CNV within the parkinson protein 2 gene (PARK2 with a significantly higher prevalence in ADHD patients than in controls (p-value =  $2.8 \times 10^{-4}$  after correction for genome-wide testing). In total, the PARK2 locus harboured 3 deletions and 9 duplications in the ADHD patients and 2 deletions and 2 duplications in the controls. By qPCR analysis, we validated 11 of the 12 CNVs in ADHD patients (p-value =  $1.2 \times 10^{-3}$  after correction for genome-wide testing). In the replication sample, CNVs at the PARK2 locus were found in 4 additional ADHD patients and 1 additional control (p-value =  $4.3 \times 10^{-2}$ ).

**Discussion:** Our results suggest that copy number variants at the PARK2 locus contribute to the genetic susceptibility of ADHD. Mutations and CNVs in PARK2 are known to be associated with Parkinson disease.

## OS 8.5 A Shared Polygenic Contribution between ADHD in Childhood and Schizophrenia

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**Background:** Previously, we found overlap of structural genomic variation (copy number variants; CNVs) between schizophrenia and childhood attention deficit hyperactivity disorder (ADHD). This is evidence of some degree of shared genetic susceptibility between schizophrenia and childhood ADHD, at least for rare variants. In the present study, we have investigated another possible source of genetic overlap, namely, common polymorphisms and we have extended the analysis to consider bipolar disorder as well as schizophrenia.

**Methods:** Specifically, we have examined multiple schizophrenia and bipolar disorder common risk alleles when considered en masse using polygenic score analysis. We used the recently published Psychiatric Genome-wide Association study (GWAS) Consortium analyses for schizophrenia and for bipolar disorder as the discovery sets on which to define polygenic scores that were assigned to each individual in our UK ADHD GWAS data set (727 cases, 2067 controls).

**Results:** Schizophrenia risk alleles were able to discriminate ADHD case individuals from controls (p= $1.04 \times 10^{-4}$ , R<sup>2</sup>=0.45%). We observed borderline evidence for discrimination between ADHD and controls using bipolar disorder risk alleles (p=0.0519, R<sup>2</sup>=0.11%). Strongest discrimination of ADHD cases from controls was provided by alleles that were risk alleles for both schizophrenia and bipolar disorder (p= $9.98 \times 10^{-6}$ , R<sup>2</sup>=0.59%). We also investigated the possibility that the signal is driven by SNPs in genes that are expressed in the brain, either in foetal development, or through to early adulthood.

**Discussion:** Our findings suggest the genetic relationship of ADHD may be closer to schizophrenia than it is to bipolar disorder and indicate the need for further studies of the genetic architecture of psychiatric disorders across traditional diagnostic boundaries.

## OS 8.6 Exome Sequencing in Adults with a Family History of ADHD

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**Background:** Recent exome sequencing studies have shown that certain rare genetic variants confer an increased predisposition for neuropsychiatric disorders, including autism and intellectual disability [1] However, it is not known to which extent rare variants contribute to the aetiology of attention-deficit/hyperactivity disorder (ADHD). We have therefore initiated an exome sequencing study, combined with exome chip genotyping in families with high load of ADHD and related disabilities.

**Methods:** The exomes of 24 ADHD probands (9 males and 15 females) in families with multiple affected individuals were captured using Roche-Nimblegen's SeqCap EZ Exome v2 and sequencing on the Illumina HiSeq. The paired-end 100nt reads were analyzed with CASAVA v1.8 (Illumina Inc) followed by alignment with BWA. PCR duplicates were removed with PICARD and the Genome analysis toolkit (GATK) was used for base quality recalibration and variant calling. Annovar was used for variant annotation. Genotyping of all probands and 200 family members was performed using the Infinium Human Exome Bead Chip (Illumina), containing ~250 000 exonic markers. Sanger sequencing was used for verifying selected variants.

**Results:** Median coverage of the exome was 125X in the 24 probands. We found an average of 19530 variants per sample in the exome, with 9334 predicted as coding or splicing variants. Filtering against an in-house database of variants found using the same exome sequencing pipeline in other Norwegian samples and 1000 Genomes data (MAF 0.5%) resulted in ~228 very rare coding variants per individual. We compared our results with a list of 112 genes implicated in autism spectrum disorder (ASD) and 196 genes conferring risk for intellectual disability (ID) described elsewhere [1] and found on average 1.4 (ASD) and 2.5 (ID) rare variants per individual in these genes. Results will be compared against non-ADHD samples and exome chip genotyping data in the entire family sample.

**Discussion:** The combination of exome sequencing and imputation using exome chip genotyping provides efficient means of studying rare variants in large pedigrees. Using this strategy in selected ADHD patients with strong family history of the disorder, we aim to search for rare coding variants with potentially stronger effects on ADHD risk. 1. Neale B. M., Kou Y., Liu L., Ma'ayan A., Samocha K. E., et al. (2012) Patterns and Rates of Exonic de Novo Mutations in Autism Spectrum Disorders. *Nature*.

## Oral Presentation Session 9 Epigenetics/Schizophrenia

### OS 9.1 Pre-, Peri- and Postnatal Stress in Human and Non-human Off-spring: A Translational Approach to Study Epigenetic Impact on Depression

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**Background:** The genetic code as well as the environment contribute to human health or disease. With regard to psychiatric disorders, early life adversities may play a role for depression and other disorders. It is assumed that early life stress (ELS) contributes to "epigenetic" modifications of the genome that do not change the genetic code, but the probability that a gene is used. The length of time until these mechanisms take effect in humans is only one reason, why an integrated cross-species approach is necessary to disentangle these gene-environment interaction effects on human disorders.

**Methods:** The POSEIDON study will experimentally investigate the effect of different early life stressors at different time points (pre-, peri-, postnatal) on the "epigenome" in rodents and non-human primates. Different tissues (neuron, immune cells, buccal cells) will be studied in order to test if peripheral cells reflect central nervous modifications. In animals it can be tested, whether these effects persist in adulthood and, thus, could help to explain human disease. In parallel a large cohort of children will be carefully characterized with regard to ELS. Using non-invasively available tissues (cord blood at birth; buccal swabs at birth and 6 months), we can immediately test whether the epigenetic modifications identified in rodents and non-human primates are relevant for humans.

**Results:** Out of a cohort of so far 220 mothers-to-be we selected 10 highly stressed and 10 non-stressed subjects. We phenotyped for psychosocial, perceived stress as well as psychopathology. We performed MeDIP analysis on T cells derived from cord blood. We found 3400 differentially methylated genes. For validation we used pre-, and postnatal Rhesus monkey stressed models and found 23 genes overlapping between the human sample and T cells of blood from monkeys aged 14 days and 2 years.

**Discussion:** Our findings show that a translational approach using animal models is a powerful method to identify differentially methylated genes after early life stress. The pathophysiological significance of the genes identified will be discussed.

## OS 9.2 The Role of Genetic and Epigenetic Factors in Future Risk of Bipolar Disorder: A Longitudinal Study in a High-risk Cohort

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**Background:** Bipolar disorder is a severe mood disorder with complex etiology. The molecular, biological and neuropsychological factors that are associated with conversion to bipolar disorder in young individuals who are at increased risk of illness are largely unknown, and can only be dissected through longitudinal studies of high-risk individuals. We are collecting a cohort of young individuals (12-30 year olds) who are at increased risk of bipolar disorder, with at least one first degree relative with BPI disorder, and controls in the same age range but with no family history of mental illness. These individuals will be reviewed annually for 5-10 years, with structured clinical assessments, and neuropsychological assessments, particularly focusing on executive function and emotional regulation. Structural and functional brain imaging data will be collected biannually and peripheral blood samples collected at baseline. Genetic and epigenetic analysis will be conducted to identify molecular risk factors which are associated with later conversion to bipolar disorder, or modulating neuropsychological endophenotypes which may precede clinical diagnosis.

**Methods:** For genetic association analysis, we selected 39 genes (49 SNPs) on the basis of prior evidence of association or differential mRNA expression in bipolar disorder, and compared allele frequencies between at-risk and control groups using PLINK. For epigenetic analysis, we selected 47 at-risk individuals and 47 controls to analyse for methylation differences across the genome via the Illumina Methylation 450K chip. Statistical analysis was conducted using PARTEK genomics suite (v6.6).

**Results:** We found the G allele at SNP rs2283265 in DRD2 to be over-represented in the at-risk cohort (n=113) compared to controls (n=75) (Freq(G)= 0.888 vs 0.756; Genotypic: X<sup>2</sup>=9.39, p=0.002; Allelic: X<sup>2</sup>=11.18, p=0.0008; corrected p =0.04). The G allele of this SNP leads to increased DRD2-short splice isoform, which mediates the behavioural effects of antipsychotics, and is increased in post-mortem brains from schizophrenia patients (F(1,64)=9.10, p=0.004). We found 179 methylation sites which differed significantly between at-risk and control groups (p< 2.4×10<sup>-5</sup>; FDR<0.05), including CpG sites 480bp upstream of the CACNB2 gene on chromosome 10p12, the 5'UTR of CSNK1D on chromosome 17, and intron 1 of OPCML on chromosome 11.

**Discussion:** Our results indicate that dysregulation of dopamine and epigenetic modification of a number of genes may contribute to increased risk of bipolar disorder. Further analysis will be conducted longitudinally, as participants who were at-risk of developing bipolar disorder at baseline convert to bipolar disorder in subsequent years. Identification of biomarkers which predict conversion of at-risk individuals before attaining a clinical diagnosis will be conducted as the study progresses. Additionally, the influence of genetic and epigenetic factors on endophenotypes for bipolar disorder will be analysed, as these become apparent.

## OS 9.3 A Role for DNA Methylation in Neurological Phenotypes Associated with Declarative Memory

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**Background:** Although a genetic component to psychiatric disorders is well established, very little of disease liability has been explained by current genetic studies. Epigenetic analysis has begun to reveal a role for DNA methylation in psychiatric disorders, but such studies have been limited in their overall scope.

**Methods:** In a prospective study, we used previously collected methylation profiles (1,505 CpG sites in 807 genes) obtained from blood cells of Mexican Americans to investigate the potential role of DNA methylation in neurocognitive (n=35) and neuroanatomical (n=50) phenotypes, assessed up to 14 years later in 95 individuals.

**Results:** We identified 22 associations that withstood Bonferroni correction for testing of 807 genes. Several interesting associations pertaining to declarative memory were identified, including methylation within: GAS7 and visual declarative memory (p=2.40×10<sup>-5</sup>); DAPK and entorhinal cortical thickness (p=3.39×10<sup>-5</sup>); EGF and ventral diencephalon volume (p=5.11×10<sup>-5</sup>); and SOX1 with inferior parietal cortical thickness (p=6.18×10<sup>-5</sup>) and visual declarative memory (trend-level p=1.09×10<sup>-4</sup>). ELK1, involved in synaptic plasticity and memory formation, was suggestively associated with several memory-related measures (p=2.5×10<sup>-3</sup>-1.49×10<sup>-4</sup>); and BDNF, which has a convincing role in synaptic plasticity and memory retention, was suggestively associated with a measure of the California Verbal Learning Test (p=6.19×10<sup>-4</sup>). Additional suggestively significant associations were also seen between several brain-related genes and our neurological traits. We have recently started DNA methylation profiling of >450,000 CpG sites in ~1,000 individuals using the Illumina Infinium assay. Preliminary results of 108 individuals lend additional support to a role for DNA methylation in memory and show association of methylation within the neuropeptide CCK (p=2.4×10<sup>-8</sup>), a well established player in consolidation of memories, with amygdala volume. Given the involvement of memory in several psychiatric disorders, we performed gene enrichment analysis of methylated genes nominally associated with ventral diencephalon volume (p<5×10<sup>-3</sup>), a strong neuroanatomical marker for recurrent major depressive disorder (rMDD) and a region comprising largely of the hypothalamus. Analysis revealed an overrepresentation of immune and inflammatory markers, as well as further implicating the CREBBP memory-related signaling pathway.

**Discussion:** We have identified several candidate genes whose methylation may play a role in neurological traits that influence psychiatric disorders, particularly those involved in memory and learning. We are continuing to assess additional CpG sites in a larger sample population to further clarify the role of DNA methylation in memory and learning. Additional analyses of such genes in patients with psychiatric disorders may help to identify genetic pathways that could be targeted for therapeutic intervention.

## OS 9.4 Psychiatric Genomics Consortium (PGC) Doubles Schizophrenia GWAS Sample-size to an Estimated 40,000 Individuals

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**Background:** The PGC (Psychiatric Genomics Consortium) is an international group of researchers whose chief aim is to maximize the utility of extant psychiatric GWAS through mega-analysis. The project is on-going with the intention of including researchers from all continents as GWAS data become available. In a previous study, the PGC schizophrenia working group identified multiple loci involved in this genetically complex and clinically heterogeneous disorder (Nature Genetics, Sept 2011). While around 20,000 individuals were necessary to achieve this result, detailed analysis of the data suggested that there are many more genes to discover, and that this should be possible by further increase of sample size.

**Methods:** Here we present an update of this international endeavor, which we anticipate will be based on approximately twice the GWAS sample size of our published paper by incorporating new data from the UK, Sweden, and the US (all samples have been genotyped and quality control and analyses are in progress). The presented data will be imputed into HapMap3 and 1000 Genomes Project data and analyzed using standard logistic regression with MDS components as covariates.

**Results:** We will present the results of a new combined meta-analysis. Preliminary analyses show that the new data are highly consistent with the first wave of the analysis, and that the full analysis will increase the number of genome-wide significant loci to at least 20 regions. We will also present preliminary follow-up analyses, e.g. replication, pathway exploration, subphenotype analysis.

**Discussion:** The current GW dataset of unprecedented sample size for a single psychiatric disease provides demonstrates an increasing number of insights into the genetic structure and consequently into the biologic mechanisms of this debilitating psychiatric illness.

## OS 9.5 Integrating the Spectrum of Genetic Variation and Protein Domain Annotation in Schizophrenia Sequencing

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**Background:** In recent decades, scientists have investigated the role of various classes of inherited genetic variation in schizophrenia. Researchers are now also focusing on genetic variation that is not inherited from the parent, i.e., de novo mutations. Importantly, DNA mutations can be at varying scales, ranging from a single nucleotide variant (SNV), the deletion or insertion of up to hundreds of bases (short indels), deletion or duplication of large sections of a chromosome of hundreds, thousands, or even millions of bases (copy number variation, CNV), and other structural variations. The arrival of next-generation sequencing technologies permits us to simultaneously detect rare and de novo SNVs, indels, and CNVs in individual patients. However, due to the large numbers of genes affected by de novo mutations that are emerging from such screens, new methods are needed to interpret how these various events together play a role in increasing risk for schizophrenia. It has recently been observed that that SNPs that fall on protein domains are highly statistically enriched among SNPs linked to hereditary disorders and complex diseases (Liu and Tozeren, 2010). In light of this, we developed a method that combines analysis of both gene-based biological pathways and functional protein signatures in genes bearing mutations, motivated by the fact that risk-increasing variants in genes ultimately mediate phenotype through the modular protein signatures that interact and carry out protein function in the cellular milieu.

**Methods:** We used exome sequencing to analyze the coding DNA sequences of all genes from two independent schizophrenia exome-sequencing studies: 600 trios (offspring with schizophrenia and their parents) from Bulgaria, as well as 2,500 Swedish schizophrenia cases and 2,500 matched controls. Exome sequences were obtained using a combination of Agilent SureSelect, Agilent 50 MB, and Nimblegen exome capture arrays and a combination of Illumina HiSeq 2000 and GAII machines. Reads were processed using the BWA/Picard/GATK pipeline resulting in SNP and indel calls, and CNVs were called using XHMM. We applied InterProScan to comprehensively annotate all RefSeq gene transcripts with the location of their functional protein signatures (domains, motifs, and repeats). We also annotated each gene with its functional pathways using Gene Ontology (GO) hierarchical categories. Analyses were performed using Plink/Seq software.

**Results:** We analyzed exome sequences from over 600 schizophrenia patients and their parents in order to find de novo mutations that seem damaging and may be involved in schizophrenia. To combine (de novo or rare) variants, including SNVs, indels, and CNVs, we annotate each variant as belonging to a particular gene and the pathways in which that gene partakes as well as the protein signatures overlapping the amino acids affected by the variants. We will then quantify the rate of de novo mutations and search for a pileup of de novo mutations for particular genes and biological pathways. Moreover, we will quantify the recurrence of mutations in particular protein signatures and domains within those genes, by aggregating genes either within or across different GO pathways. In the large set of Swedish case-controls, we will focus on the rare variants and attempt to replicate any enrichment of the protein pathway and domain annotations that we discovered in the trios, thus searching for similar biological commonalities and validating this approach. We will also use the

5,000 case-controls as an independent starting point to look for enrichment of case-specific deleterious variation in protein motifs, domains, and families.

**Discussion:** A number of recent works have provided increasing evidence that some fraction of risk can be attributed to de novo germline mutations. However, directly making inferences about the etiology of schizophrenia from these results has been complicated by a number of factors: 1) most mutations are unlikely to be fully penetrant, 2) the large size of the pool of genes that, if mutated, increase risk, and 3) the relatively high baseline rate of de novo mutations in the general population. Taken together, this implies that many, if not most, de novo mutations carried by individuals with schizophrenia will not be related to their disease. Nevertheless, by considering a very large set of over 600 trios and 5000 case-controls, integrating across multiple classes of variation, and considering protein pathways and conserved functional subsequences of proteins, our methods should be powered to yield a clearer view of the emerging etiology of schizophrenia by leveraging known genetic and sub-genic protein annotations, which reflect the mechanisms underlying the population effects of variation.

## OS 9.6 Gene Co-expression Network Analysis in Schizophrenia

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**Background:** Over the past decade, common and rare DNA sequence variants have been identified in genome-wide association studies (GWAS) as risk factors for schizophrenia (SCZ). We have learned that: 1) genetic risk for schizophrenia is highly polygenic and will necessitate the study of multiple genes; 2) effect sizes, even for rare or de novo events, are modest; 3) broad brain and synapse pathways rather than single linear biological pathways are being implicated. Thus, SCZ most likely involves hundreds of genetic loci that perturb molecular and cellular networks underlying disease pathophysiology. In this study we examined whether gene subnetworks, identified through gene coexpression analysis are enriched for SCZ associated genetic risk variants.

**Methods:** Gene coexpression networks were constructed using transcriptome profiling in the dorsolateral prefrontal cortex (DLPFC) of human postmortem non-disease samples (N=269) reported recently as part of the BrainCloud database (<http://braincloud.jhmi.edu>). The relationship of these modules was explored with respect to those genetic risk factors likely to carry strongest risk for SCZ. Initially we conducted an expression quantitative trait loci (eQTL) analysis and examined whether common SCZ GWAS genetic risk variants are enriched for eQTLs. We also compiled a list of large CNVs from the literature that have been well established as risk factors and used de novo missense, nonsense and splice site mutations obtained from our exome sequencing study of over 600 schizophrenia patients and their parents. Finally, we examined whether there was gain or loss in the module connectivity in SCZ using two independent microarray datasets from the DLPFC of controls and cases with SCZ.

**Results:** Forty-six modules of sizes between 52 and 1792 nodes were detected. GWAS SCZ genetic risk variants were enriched for eQTL, supporting the notion that disease variants affect gene expression. Connectivity of modules related to transmission of nerve impulse, retrograde vesicle-mediated transport, immune response and axon ensheathment, was lost in SCZ. Overlap analysis demonstrated that these modules were enriched for SCZ genetic risk variants (eQTL, de novo mutations and CNVs) that primarily disturbed critical hub genes in the modules.

**Discussion:** Gene coexpression analysis was used to elucidate the organization of gene expression in a large cohort of non-disease samples and two independent microarray datasets of cases with SCZ and controls. The connectivity of modules related to cellular and molecular functions previously associated with SCZ was lost in the disease. These modules were significantly enriched for common and rare SCZ risk genetic variants. These results support the existence of convergent genetic abnormalities in SCZ that could potentially drive the disease leading to molecular and cellular alterations.

## Oral Presentation Session 10 Schizophrenia

### OS 10.1 MIR137, A Candidate Gene for Schizophrenia Risk: Genetic Follow-up

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**Background:** Recent genetic evidence strongly suggests that schizophrenia has a highly polygenic genetic basis. Findings from the Psychiatric GWAS Consortium (PGC) have identified significant and replicated association for a region on chromosome 1 near MIR137 (rs1625579;  $p=1.6 \times 10^{-11}$ ). Unpublished studies increase the significance of this finding to  $p=1.3 \times 10^{-12}$ . MIR137 has also been implicated through identification of genome-wide significant associations for genes with confirmed miR-137 target sites (CACNA1C, TCF4, CSMD1, and C10orf26). The ability of microRNAs to regulate hundreds of genes with evidence the miR-137 influences neural differentiation and proliferation, yields the hypothesis that miR-137 regulates one or more pathways involved in schizophrenia etiology. We have attempted to further elucidate the genetic findings in schizophrenia and to identify genes regulated by miR-137.

**Methods:** To localize the genetic signal in the MIR137 region, we performed haplotype analysis using schizophrenia cases and controls a subset of PGC samples genotyped with Affymetrix 6.0 chips (N=12,670). We evaluated the haplotype structure of the region (haplots) and constructed evolutionary trees (using Phylip). A variable number tandem repeat (VNTR), reported to affect levels of mature miR-137 was also genotyped in cases and controls.

**Results:** High linkage disequilibrium exists across this locus, and indicating a region of high linkage disequilibrium (LD) made of 3 Haploview predicted LD blocks, each in  $r^2 > 0.8$  with each other. This region includes MIR137 and the markers carrying significant association with schizophrenia. Haplotype association across the locus yielded a minimum omnibus  $p$ -value =  $3.2 \times 10^{-4}$  for a 5 SNP window including rs1198588. Haplotype analysis yielded a smaller  $p$ -value for association than single marker analysis in the same samples, suggesting that the causative variant may fall on a specific common haplotype. Two haplotypes with frequencies ~12% and ~4% had significant protective effects compared to the major haplotype. A haplotype tree indicates that these two haplotypes are close to each other evolutionarily, and more distant from the other common haplotypes. Additional analysis of other markers has included a potentially functional 15bp VNTR within the exon of MIR137HG that encodes pre-miR-137. There are ten alleles in our population with frequencies of at least 0.5%, ranging from 3 repeats to 12 repeats.

**Discussion:** Our results indicate a region of high LD across MIR137, within which the significant association to schizophrenia consistently falls. There are two protective haplotypes, which are similar to each other. Therefore, it is likely a protective variant arose on a common haplotype within the region of high LD prior to the divergence of these two haplotypes. A candidate for the protective variant is a VNTR which affects levels of mature miR-137. This VNTR does show variability within our population. Additional analyses will indicate whether these alleles segregate with associated haplotypes, and whether specific alleles of the VNTR are associated with schizophrenia.

### OS 10.2 Clinical and Genetic Validation of a New Sample (CLOZUK) and Sampling Methodology for Genetic Studies of Schizophrenia

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**Background:** Significant advances have been made in identifying genetic variants associated with schizophrenia through genome-wide association studies (GWAS) and copy number variant analyses. Despite these advances the majority of the heritability of the disorder remains unidentified, though a substantial proportion is due to common variants. Studies of other complex disorders and traits indicate that extremely large samples will be required to identify the missing heritability. In schizophrenia, the collection of such large samples has been hampered by the resource implications of existing recruitment and research diagnostic procedures. This study outlines the validation of a novel approach that we have used to recruit a large sample (n=6500) of those with schizophrenia (CLOZUK). After gaining ethical approval and ensuring compliance with the UK Human Tissue Act, genetic samples for CLOZUK were collected anonymously from those with a clinical diagnosis of schizophrenia who were receiving treatment with clozapine, an antipsychotic for those with treatment-resistant schizophrenia. The efficiency of this sample collection relied on the samples being available from central national labs of companies monitoring for adverse haematological effects of clozapine.

**Methods:** Clinical Validation: In order to mirror the attainment of the CLOZUK samples, we approached all those with a clinical diagnosis of schizophrenia who were taking clozapine within a large geographical area of the UK. 203 of these individuals underwent a research diagnostic interview (SCAN) and clinical note review before being assigned a consensus research diagnosis. This diagnosis was used in order to calculate the positive predictive value of recruitment based on a clinical diagnosis of schizophrenia in those taking clozapine. Genetic Validation: Genetic data is currently available in a sub-sample of the CLOZUK sample (n=2640), although the full sample is currently being analysed. Using SNPs with moderate evidence of association with schizophrenia from The Schizophrenia Psychiatric Genome-Wide Association Consortium (PGC) we tested for replication in the CLOZUK sample, with the aim of demonstrating association at a level greater than that expected by chance.

**Results:** Clinical Validation: Having a clinical diagnosis of schizophrenia and being prescribed clozapine was associated with a positive predictive value of 92% of having a research diagnosis of schizophrenia and 98% of having a schizophrenia, schizophreniform or schizoaffective disorder research diagnosis. Genetic Validation: We demonstrated significant replication in CLOZUK to the PGC-associated allele for 37 out of 78 (47%) of the Stage 1 PGC moderately associated SNPs for which we had data. Among the replicated SNPs were those identified as genome-wide significant in the MHC region as well as 3/6 non-MHC genome-wide significant SNPs. Of the alleles associated with schizophrenia in PGC Stage 1, 85% (66/78) showed the same direction of effect in CLOZUK, an excess that is highly significant (sign test  $p=1.7 \times 10^{-10}$ ). A highly significant excess ( $p=6.6 \times 10^{-6}$ ) was also seen in an analysis restricted to the non-MHC SNPs (79%, 45/57). There was also evidence that effect sizes were higher in the clozapine sample, although further analysis is required to determine if this is due to the more severe phenotype of the CLOZUK sample or due to its greater genetic homogeneity compared to the PGC.

**Discussion:** This study provides clinical and genetic validation for a new, large sample of those with schizophrenia, based on a recruitment strategy of acquiring anonymous genetic samples from those on clozapine with a clinical diagnosis of schizophrenia. Rare but serious adverse effects of clozapine necessitate regular blood monitoring for those taking the drug. This unfortunate requirement for patients could be turned into an advantage for those with schizophrenia, in providing a unique genetic resource of the magnitude that is required to elucidate the genetics of schizophrenia.

### OS 10.3 Complete Genome Sequence Based Genetic Analysis of Monozygotic Twins Discordant for Schizophrenia

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**Background:** The reality of individual genome sequencing now offers a new hope in search of the cause(s) of complex diseases. When combined with genetic relationships, individual sequences add an unrivaled proficiency. Given the near identical genetic structure of monozygotic twins, any difference between monozygotic twins discordant (MZD) for a disease will have a high likelihood of being causal. With this in mind we have sequenced the DNA of six individuals, which includes two pairs of MZ twins discordant for schizophrenia and one set of parents.

**Methods:** Sequencing was carried out using the Complete Genomics Analysis system (Drmanac et al, 2010). The Complete Genomics platform has a 99.999% sequencing accuracy, 47.27 to 50.25-fold coverage and >99% of the reference genome is called. The sequences were further assessed for accuracy in relation to Affymetrix SNP Array 6.0 results. Genome wide variations including SNPs, indels, CNVs and microRNAs were assessed. It has allowed us to evaluate the similarities and differences across unrelated individuals, parents and children, as well as between MZ twins.

**Results:** The results show that an individual carries approximately 3.7 million SNPs, 150 CNVs, 400,000 indels and 220 microRNA variants. Also, two unrelated individuals differed for 1.5-1.8 million SNPs (~45%), a parent and child differed for 0.9-1.0 million SNPs (30%) and a pair of MZ twins differed for 100,000 (~3%) SNPs. Differences in the identity of CNVs for the three comparisons were 45%, 30% and 4%, respectively. It should be noted however that a number of these differences will be the result of sequencing errors and therefore will require stringent confirmation. Interestingly, CNV and SNP differences between MZD twins affect a set of genes enriched in neurodevelopmental genes, as well as genes that have been already implicated in Schizophrenia. Further, potentially deleterious mutations (nonsense mutation in PDE4DIP and nonstop mutation in LOC339742) were found in both affected patients but not in their unaffected co-twins or parental samples.

**Discussion:** The results support our strategy and identify patient specific genetic changes that may lead to schizophrenia. The novel results re-enforce that individual genomes harbor extensive variability, some inherited and others acquired during parental meiosis and/or mitosis. There is no single human genome sequence. Even monozygotic twins are not identical and each individual may be a mosaic, potentially carrying different sequence variations in different cells. This is supported by a high mutation rate and the persistence of genetic diseases with a severely reduced fecundity in all human populations.

## OS 10.4 A Population Isolate Reveals a Recessively Inherited Deletion in Schizophrenia and Cognitive Disability

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**Background:** Studies of low frequency variants in complex traits are an emerging field of interest, but are challenged by high level of allelic heterogeneity. Population isolates demonstrating extended allelic sharing could provide a useful setting for their study. Previously isolate populations have been successfully used in identifying gene variants for many Mendelian diseases that have shown enrichment to these populations. Exploiting this hypothesis, we utilized an internal isolate of Finland, highly enriched for schizophrenia and investigated rare deletions that would have become enriched in this isolate due to multiple recent bottlenecks and rapid population expansion, and that would in part explain the high incidence of schizophrenia observed in the population. The role of large deletions in schizophrenia having been clearly established and these being easy to detect using GWAS data further motivated the study.

**Methods:** The deletions were called from GWAS data using QuantiSNP. In the first stage the deletion frequencies were compared between general populations from the high risk isolate (N=173) and south of Finland (N=1586) using PLINK. Only deletions of over 20 kb in size and frequency below 5% were included to the analysis. Deletions that were significantly more frequent in the isolate were tested for association with schizophrenia in a Northern Finnish sample of 265 cases and 5140 controls and further replicated in 9,539 schizophrenia cases and 15,677 controls. Analysis of additional neurodevelopmental phenotypes among non-schizophrenic deletion carriers were done in the Northern Finnish 1966 birth cohort (N=4872).

**Results:** A comparison between the high risk isolate (N=173) and south of Finland with low regional schizophrenia risk (N=1,586) revealed 3 enriched deletions to the isolate ( $p < 6 \times 10^{-8}$ ). Analysis against schizophrenia in Northern Finland (265 patients and 5140 controls) revealed nominal association with one deletion on 22q11.22. ( $p = 0.02$ , OR: 1.9) and was further replicated in 9,539 patients and 15,677 controls ( $p=0.03$ , OR2.1). The well documented co-morbidities of schizophrenia, the descriptive nature of psychiatric diagnoses and the limited knowledge on relevant biology, stimulated us to investigate the overrepresentation of relevant neurodevelopmental phenotypes among non-schizophrenic carriers of the 22q11.22 deletion in Northern Finnish population cohort (N=4872). Among eight co-morbid phenotypes studied, the deletion carriers were significantly more likely to have intellectual deficit and/or milder learning difficulties compared to non-carriers ( $p = 0.003$ , OR: 3.99). Four individuals were identified as homozygous for the deletion, all presenting with intellectual deficit and/or schizophrenia. The deletion disrupts one gene encoding for TOP3B and was found to additively down regulate its expression in peripheral lymphocytes ( $p = 8 \times 10^{-11}$ ).

**Discussion:** Our results suggest the involvement of large inherited deletion on 22q11.22 in the etiopathogenesis of familial schizophrenia and intellectual deficit. Furthermore our results highlight the usefulness of population isolates in the studies of rare variation in complex traits.

## OS 10.5 Mapping the Human Genome's Missing Pieces and Investigating their Relationship to Schizophrenia Structural Variants

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**Background:** Almost 30 megabase pairs of euchromatic human genome sequence, including many protein-coding genes, have no known location in the human genome. In principle, part of this sequence could be located in the genome assembly gaps within and abutting the recurring copy number variants (CNVs) associated with schizophrenia and autism. These CNVs could therefore be larger than currently annotated and contain potentially important genes. Among this sequence, over half a million bases fall within a region at 1q21.1 prone to recurrent deletions in schizophrenia and other psychiatric disorders. Due to sequence missing from the human genome reference, the size of this deletion is underestimated and potential explanatory genes overlooked.

**Methods:** We describe an approach for localizing the human genome's missing pieces by utilizing the patterns of genome sequence variation created by population admixture. We first leverage the data available from the 1000 Genomes project to identify variation in assembled sequence currently not mapped to the canonical human genome reference. Then we leverage the long-range "admixture linkage disequilibrium" present in the genomes of African-American individuals to map novel sequence missing from the human genome reference and to map cryptic segmental duplications. We then investigate if these missing sequences are copy number variable in patients affected by deletions and duplications at 1q21.1 and other loci.

**Results:** We mapped several unlocalized genomic scaffolds spanning over five million base pairs of the human genome's unplaced euchromatic sequence, including about a dozen protein-coding genes, and identified eight large and novel inter-chromosomal segmental duplications. We find that most of these sequences are hidden in the genome's heterochromatic regions, particularly its pericentromeric regions and the short arms of the acrocentric chromosomes. We also find that cryptic, centromeric genes are expressed at an RNA level, though their expression patterns often diverged from those of their known paralogs and that these missing pieces are more prone to CNVs than the rest of the euchromatic genome. In particular, we find that CNVs affecting the 1q21.1 distal region affect genome sequence missing from the human genome reference.

**Discussion:** Given the contribution of CNVs to psychiatric diseases, it will be important to identify the additional sequence missing from the human genome reference and catalog additional CNVs affecting this sequence. While only about 1% of human euchromatic sequence is missing from the reference genome, this can account for up to 15% of the segmentally duplicated part of the genome, which by its own nature is expected to be prone to acquire novel deletions and duplications, and as such is likely to play an important role in diseases with already known associations to CNVs. Single base-pair specificity provided by next-generation sequencing will play a key role in the future to understand the relation between these regions and disease.

## OS 10.6 Analysis of Gene Expression Patterns in Foetal Brains with Schizophrenia and Bipolar GWAS Data

### ECIP

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**Background:** Schizophrenia is postulated by many to be a neurodevelopmental disorder, and there is increasing interest in the possibility that the same may be true for bipolar disorder. Moreover, family studies have suggested genetic overlap between the two disorders, an idea that has been strongly supported by the results of GWAS studies. Here we investigate a possible relationship between the regional patterns of gene expression in foetal brain and both schizophrenia and bipolar GWAS association signals.

**Methods:** We used global transcriptome data for 13 brain regions sampled from 4 foetal (18-23 weeks gestation) brains. From these data, we scored genes by the extent to which their expression was relatively consistent across brain regions or relatively specific to particular brain regions. These sets of genes were tested for enrichment with either schizophrenia or bipolar signal using summarized gene-wide statistic (derived from publically available P values) from the largest association study of common SNPs to date from the Psychiatric GWAS Consortium (PGC). All methods were repeated in a second independent transcriptome dataset, taken from foetal brains at the same gestation time-points.

**Results:** Sets of genes with high and consistent expression across brain regions were strongly enriched for lower gene-wide P values in schizophrenia signals, and were also enriched in bipolar signals, a result which replicated in the second expression dataset. In schizophrenia, we also found an excess of association signals in genes whose expression was relatively low in hippocampus.

**Discussion:** In schizophrenia and bipolar disorder, GWAS association signals are enriched among genes that are highly and widely expressed across the foetal brain rather than genes that show patterns of region specific expression. Disruptions in the expression of these genes could have fairly global rather than specific effects on foetal neurodevelopment.

## Oral Presentation Session 11 Functional Genomics and Model Organisms

### OS11.1 Comparing Genome-wide Association Results for Fear Conditioning in Two Advanced Intercross Mouse Lines: Implications for Gene Identification in Posttraumatic Stress Disorder in Humans

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**Background:** Fear conditioning may provide a useful model for some components of post-traumatic stress disorder (PTSD). Traditionally, genetic mapping in mice studies have used recombinant inbred lines (RI), backcrosses (BC), F2 intercrosses or similar populations to identify quantitative trait loci (QTLs) for FC, yet gene identification has proven difficult. More recently, QTL mapping is being performed using highly recombinant populations such as advanced intercross lines (AILs) and outbred mice. Unlike traditional mapping populations, AILs and outbred mice show a rapid breakdown of linkage disequilibrium that allows for increasingly high resolution mapping. However, AILs possess distinct advantages and disadvantages. Because AILs are derived from two inbred founders, they maintain the simplicity of more traditional crosses while allowing GWAS to be performed in a situation where all alleles are common and where every marker that differs between the parental strains is perfectly informative. However, the mapping resolution of an AIL is dictated by the number of mixing generations and it remains unclear how many are needed to provide single gene resolution for a complex trait such as fear conditioning.

**Methods:** Here, we used two genetically distinct AIL populations (an integrated analysis of 625 F2 (308 male, 317 female) and 567 F8 (284 male, 283 female) AILs derived from C57BL/6J x DBA/2J mice, along with 490 F2 (249 male, 241 female) and 687 F34 (353 male, 334 female) AILs derived from LG/J x SM/J mice) to fine-map QTL associated with fear conditioning.

**Results:** We conducted an integrated genome-wide association analysis in QTLRel and identified four highly significant QTL affecting freezing to cue in the C57BL/6J x DBA/2J AIL (on Chr 1, 2, 5 & 13), and six highly significant QTL associated with freezing to cue in the LG/J x SM/J AIL (on Chr 2, 4, 8, 10, 11 & 17). The average percent decrease in the 1.5-LOD support interval between the F2 and the F8 integrated analysis was 59.2%, as compared to an 88.6% reduction between the F2 and the F34 integrated analysis. Interestingly, none of the QTLs between the two AILs overlapped, most likely due to different segregating alleles between the two populations and incomplete power to detect all relevant alleles. We have also exploited bioinformatic sequence data available for all founder strains to identify candidate genes based on the existence of non-synonymous coding polymorphisms. In addition, we utilized expression data from the C57BL/6J and DBA/2J founder strains to identify candidate genes based on the existence of gene expression data in amygdala, hippocampus, and whole brain.

**Discussion:** We identified multiple candidate genes that may be relevant to fear learning in animal models (Bcl2, Btg2, Dbi, Gabr1b, Lypd1, Pam and Rgs14) or PTSD in humans (Gabra2, Oprm1 and Trkb). These results will be compared to preliminary fear conditioning data obtained from outbred mice (CFW, n=720), which possess even greater amounts of recombination than AILs. Our results demonstrate that the integration of F2 and AIL data maintains the advantages of studying endophenotypes for complex psychiatric traits in model organisms while significantly improving resolution over previous approaches.

## OS 11.2 Effect of Paternal Age on Mutational Burden and Behavior in Mice

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**Background:** The paternal age effect (PAE) refers to an increased risk for a particular phenotype or mutation with increasing age of the father. This is of major relevance in psychiatry where three of the most severe disorders (autism, schizophrenia, and bipolar disorder) all show PAE with 2-3 fold increased risk in older fathers. Demonstrations of increased burden of de novo copy number variants (CNVs) in autism and schizophrenia could potentially explain the PAE for these disorders. In humans, it is difficult to achieve a highly-powered, genome-wide measure of the PAE on CNV and point mutation burden, so we are using the laboratory mouse to quantitate this effect.

**Methods:** First, the same 25 F1 male mice (reciprocal hybrids of WSB/EiJ, PWK/PhJ and CAST/EiJ) were mated with highly fertile, young FVB inbred females when the males were young (3 months) and in very old age (2 years). This breeding design, incorporating an F1 male and a highly divergent female, was carefully chosen to facilitate CNV detection and to identify the paternal or maternal mutational origin. There were a total of 441 offspring of young males and 435 offspring of very old males. As our first step in examining CNVs at high resolution, we have performed whole genome sequencing (25X coverage) on 3 offspring of very old males. Second, to measure the effect of paternal age on behavioral phenotypes relevant to autism and other neurodevelopmental disorders, old and young F1 males (PWK x CAST and the reciprocal) were crossed with FVB females to generate a testing population of 98 mice. Behavioral phenotypes included social approach, locomotor activity, light/dark test (anxiety), marble burying (restricted interest) and acoustic startle (sensorimotor gating).

**Results:** Whole genome sequencing has been completed and is now in analysis. The results will be presented and serve as pilot data for optimal sequencing coverage for CNV, SNP, and indel calling in a much larger dataset (96 offspring). Analysis of the behavioral data revealed multiple paternal age effects that were often sex-specific. For example, male offspring of old sires (both PWK x CAST and the reciprocal) showed decreased exploratory behavior in a novel environment ( $P=0.008$ ). Female offspring of old CAST x PWK sires failed to show significant social preference in a three-chamber approach task ( $P=0.003$ ), and had less response to novelty in a test for narrow, restricted interests ( $P=0.035$ ). These female offspring also had decreased startle amplitudes following an acoustic stimulus ( $P=0.033$ ), akin to a model for fragile X syndrome (Fmr1-null mice).

**Discussion:** Our mouse behavioral testing results are consistent with human data showing an increased risk for neurodevelopmental disorders with advanced paternal age. If our forthcoming genome sequencing data show that paternal age truly increases the mutational burden of offspring, it would suggest one possible mechanism for the PAE seen in psychiatric disorders.

## OS 11.3 Genome-wide Copy Number Variation in 162 Strains of Laboratory Mice: An Invaluable Tool for Investigators in Psychiatric Genetics

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**Background:** Copy number variation (CNV) is an important source of genetic diversity in vertebrates. CNVs play an important role in the etiology of schizophrenia and autism as well as in developmental delay, changes in brain size, and epilepsy. Laboratory mice are important tools for understanding mutational mechanisms of CNVs and their effects on behavior. However, the existing mouse CNV catalog is incomplete with evaluation limited to a few strains and with insufficient validation. Thus, relatively little is understood about the extent and consequences of CNVs in mouse. In this study, our objective is to improve the mouse CNV catalog to provide insight into the genetics of CNVs and complex traits.

**Methods:** We conducted a genome-wide survey of CNVs in 162 strains of laboratory mice using the Affymetrix Mouse Diversity Array (MDA), comprising > 1 million probes. The 162 strains were selected to canvass four major subspecies of *Mus musculus* (*M. m. domesticus*, *musculus*, *castaneus*, and *molossinus*) and have previously been well-characterized for other forms of variation. We developed an analysis protocol that combined microarray data with computational algorithms and genomic resources to predict CNVs. For high-throughput experimental validation, we used targeted capture with Agilent SureSelect enrichment followed by massively parallel sequencing with Illumina HiSeq2000. CNV status is verified by comparing read-depths. Additional validation was conducted using PCR, breakpoint sequencing, and gene expression data. To identify enriched functional annotation gene categories, we used DAVID and GO biological process, KEGG, and Biocarta pathways.

**Results:** We identified 1,499 copy number variant regions (CNVRs), spanning 1.5% of the mouse genome and ~67% are duplications and ~84% have minor allele frequency > 0.01. Glyoxalase 1 (Glo1) duplication has been implicated in anxiety-like behavior in mice (William et al 2009), and we have established the copy numbers of Glo1 for 162 strains of inbred mouse. We identified rare CNV deletions that created natural knockouts covering eighteen genes whose deletion in other inbred strains are embryonic lethal, suggesting a strain background effect. Pathway analysis suggests that deletions in laboratory mice were enriched for olfaction, olfactory receptor, cognition, and GPCRs and duplications for ATP binding, cytoskeleton, tRNAs, ncRNAs. There was 100% concordance between our predictions and the results of small-scale validation using gene expression data and PCR. A total of 399 of these CNVRs in 92 strains were captured and high-throughput sequencing is being completed at the time of this writing.

**Discussion:** This is the most comprehensive survey of CNVs yet reported in mouse. Our CNV catalog is complementary to existing genetic resources for laboratory mice and an invaluable tool for investigators in psychiatric genetics. It will accelerate studies using mouse models of how CNVs impact gene expression during development and how CNVs and pathways associate with behavioral and pharmacogenomic phenotypes.

## OS 11.4 Studies in Humans and Mice Implicate Neurocan in the Etiology of Mania

### ECIP

Sandra Meier<sup>1</sup>, Xavier Miró<sup>2</sup>, Jana Strohmaier<sup>1</sup>, René Breuer<sup>1</sup>, Thomas Mühleisen<sup>3</sup>, Andras Bilkei-Gorzo<sup>1</sup>, Sven Cichon<sup>1</sup>, Marcella Rietschel<sup>1</sup>, Markus M. Nöthen<sup>3,4</sup>, Andreas Zimmer<sup>5</sup>

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**Background:** A recent study reported genome-wide association between the NCAN gene and bipolar disorder. The aims of the present study were to characterize the clinical symptomatology most strongly influenced by NCAN, and to explore the behavioral phenotype of Ncan knock-out (Ncan<sup>-/-</sup>) mice.

**Methods:** Genotype/phenotype correlations were investigated in patients with bipolar disorder (n=641) and the genetically related disorders major depression (n=597) and schizophrenia (n= 480). In a first step, principal component and genotype association analyses were used to derive main clinical factors from 69 lifetime symptoms, and to determine which of these factors were associated with the NCAN risk allele. In a second step, these analyses were repeated using the associated factor(s) only in order to identify the more specific clinical subdimensions that drive the association. Ncan<sup>-/-</sup> mice were tested using diverse paradigms. These assess a range of behavioral traits, including paradigms corresponding to bipolar symptoms in humans.

**Results:** In the combined patient sample, the NCAN risk allele was significantly associated with the mania factor, in particular the subdimension overactivity. Ncan<sup>-/-</sup> mice were hyperactive, and showed more frequent risk-taking and repetitive behaviors, less depression-like conduct, impaired pre-pulse inhibition, amphetamine hypersensitivity, and increased saccharin preference. These aberrant behavioral responses normalized following the administration of lithium.

**Discussion:** NCAN preferentially impacted on mania symptoms in humans. Ncan<sup>-/-</sup> mice showed behavioral abnormalities that were strikingly similar to those of the human mania phenotype, and may thus serve as a valid mouse model.

## OS 11.5 Biochemical and Genetic Evidence for a Critical Role of the AKT1 Serine-threonine Kinase in Cognition, Depression and Suicide

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**Background:** The AKT family of serine/threonine kinases plays a key role in neuronal morphogenesis, cell growth, metabolism and survival. While all AKT isoforms (AKT1, AKT2, AKT3) are expressed in the brain, genetic evidence has confirmed the involvement of mutations in schizophrenia and other neuropsychiatric disorders.

**Methods:** To define the biochemical mechanisms mediating the involvement of AKT signaling in neuropsychiatric disorders, we investigated the expression and regulation of AKT and a panel of other intracellular molecules (p44/42 MAPK, PTEN, BAX, BCL2, GSK3beta, actin, GAPDH) in postmortem prefrontal cortex specimens from sex-/age-/PMI/pH-matched subjects. 16 triplets were investigated each consisting of one control and two suicides (depressed and schizophrenic subjects). Biochemical measures were assessed in Brodmann areas (BA) 9, 24 and 47/45. To model the impact of altered AKT signaling on normal and depression-related processing of emotional stimuli, we used single knockout mouse strains for individual mouse Akt genes (Akt1, Akt2, Akt3) to investigate behavioral, synaptic plasticity and biochemical phenotypes.

**Results:** Human postmortem studies: In BA9, we detected a significant change in AKT Ser-473 (pAKT) phosphorylation levels in both suicide groups. The changes in AKT phosphorylation were highly significant both in subjects with a diagnosis of Major Depressive Disorder (p=0.0002) and also in non-depressed suicides who included schizophrenics (p=0.0001). Relative effect sizes for pAKT as percent of control were 74.1±5.65 and 139.0±10.2 in depressed and non-depressed suicides, respectively. An analysis of AKT phosphorylation in BA24 and BA47/45 revealed no significant changes in depressed (p=0.125 and p=0.236, respectively) or non-depressed suicide victims (p=0.206 and p=0.836, respectively). Further analysis revealed a consistent pattern that depression had a different effect on pAKT in BA47/45 when compared to BA24 (p=0.0133) or BA9 (p=0.0370). In contrast to changed AKT phosphorylation, levels of total AKT, ERK1/ERK2, phospho-ERK1/ERK2, PTEN, BAX, BCL2 and GSK3beta did not differ significantly between depressed and non-depressed suicides when compared to controls in any of the areas studied (p>0.05). Mouse models: Only Akt1-deficient mice were significantly impaired in the acquisition of fear memories. We also examined synaptic plasticity in the hippocampus by inducing long-term potentiation (LTP). Consistent with deficits in fear memory acquisition, only Akt1 KO mice but not Akt2 KO or Akt3 KO showed impaired LTP in the hippocampus. To complement our results with a pharmacological approach, we inhibited Akt with an allosteric inhibitor prior to behavioral testing or electrophysiological recordings. Baseline anxiety levels in naive Akt-mutant mice or wt mice with pharmacologically inhibited Akt signaling were not altered. Following chronic stress exposure, Akt1-deficient mice exhibited pronounced depression-like behaviors. Similarly, acute stress exposure resulted in a depression-like phenotype in inhibitor-treated mice when compared to vehicle-treated mice. Most importantly, while treatment of chronically-stressed mice with antidepressants reversed the depression-like phenotype in wild-type and vehicle-treated mice, antidepressant treatment failed to ameliorate the depression-like phenotype in Akt1-mutant mice or mice injected with Akt inhibitor.

**Discussion:** Our biochemical and genetic results from human subjects and mutant mice converge to support a conserved and critical requirement for AKT1 signaling in normal cognition and the regulation of mood. Specific mechanisms of downstream substrate phosphorylation suggest a direct involvement in neuronal plasticity and carry great promise for the development of diagnostic markers and novel therapeutics in neuropsychiatric disorders.

## OS 11.6 ZNF804A Knockdown in Human Neurons Derived from iPSCs

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**Background:** ZNF804A has been identified as a candidate gene in schizophrenia (SZ) and bipolar disorder (BD) in several large-scale genome wide association studies (GWAS) (1-3). It codes for a protein containing a C2H2-type zinc-finger domain, suggesting that it has DNA binding properties and can act as a transcription factor. Recent gene knockdown (KD) and transfection experiments, and chromatin immunoprecipitation based assays, carried out in neural progenitor cells derived from human cortical neuroepithelium and rat neural progenitor cells supports this idea (4,5). However, these studies were limited in scope; real time PCR focusing specific candidate genes and microarray expression assays were used to identify ZNF804A target genes.

**Methods:** In order to obtain a more comprehensive representation of ZNF804A targets in neuronal cells, we subjected differentiating human neurons to transcriptome analysis by next generation sequencing (RNA-Seq). Human neural progenitor cells (NPCs) were cultivated in vitro from induced pluripotent stem cells (iPSCs). The NPCs were transduced with shRNA expression vectors targeting ZNF804A RNA. A scrambled shRNA was used as a control. The cells were induced to differentiate into neurons and transcriptomes were assessed in duplicate samples by RNA-Seq.

### Results:

The correlation coefficients were high for both shRNA KD and control duplicates (0.52-0.97, respectively). With the two KD data sets combined, we identified a total of 426 genes that were differentially expressed (1.5-fold or greater); 306 were down-regulated, 120 increased. Since heterogeneity in the differentiation could account for some of these changes, we repeated the KD in another set of NPCs, as well as in differentiated neurons. We are in the process of carrying out RNA-Seq on these samples. However, quantitative real-time PCR on several genes of interest show consistent changes in all 4 KD experiments, notably, significant changes in the expression of CCK (cholecystokinin) and SST (somatostatin) RNA. These genes code for neuropeptides expressed in specific subsets of GABAergic interneurons.

### Discussion:

These findings demonstrate the utility of using a gene knockdown approach in NPCs derived from iPSCs, and RNA-Seq, as methods for identifying pathways and networks involved in neuropsychiatric disorders. The preliminary findings suggest that ZNF804A may affect GABAergic differentiation.

### OS 12.1 Identifying the Genetic Contribution to Age at Onset in Major Depressive Disorder

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**Background:** Genome-wide association studies have to date failed to identify many replicable risk alleles for major depressive disorder (MDD), with the analysis of over 9000 cases in the Psychiatric GWAS Consortium MDD study identifying no SNPs at genome-wide evidence of association. Heterogeneity within the MDD cases may be adding sufficient noise to reduce the power to detect association. Several features of depression have been postulated as sources of heterogeneity, and including these features in the analysis plan may increase power to detect replicable associations. Here we analyse age-at-onset (AAO) of depression in the PGC-MDD data. Several studies suggest that age at onset can distinguish sub-types of depression with separate heritable components; however there is little consistency across studies. In the PGC-MDD study, AAO (available for 8920 MDD cases) has previously been considered in a case control analysis restricting cases to early-onset ( $\leq 30$  years), and by testing for association to the quantitative trait of AAO in a case-only analysis. Neither approach identified evidence for association; here we present a more detailed genetic analysis of AAO.

**Methods:** We assumed that much of the heterogeneity in the onset of MDD between studies arises from study-specific differences in design and questionnaire response, rather than underlying differences in depression cases. We therefore defined an analysis strategy using age-cut offs within each study. Octiles of the age of onset distribution within each study were determined, and MDD case subsets comprising the youngest 12.5%, youngest 25%, etc ... were constructed. All case-subsets were then analysed in a case control study. Similarly, we also defined AAO thresholds for older-onset MDD to identify genetic variants conferring risk only later in life (e.g. after menopause). Analyses were stratified by sex. Additionally, the proportion of heritability of AAO captured by common variants was assessed by GCTA using AAO as a continuous trait and for youngest v. oldest case subsets.

**Results:** The median AAO in PGC-MDD cases was 25 years. AAO differed significantly by study, with median AAO ranging from 20 years in RADIANT-UK to 37 years in the Max Planck Institute of Psychiatry study. GenRED restricted recruitment to recurrent cases onset by age 30, and was considered separately (median AAO 16 years). Younger age of onset was seen in recurrent cases (median 22, excluding GenRED), and slightly younger onset in females (median 24) than males (median 26). Full genetic results will be presented, correcting for multiple testing across the number of case subsets analysed.

**Discussion:** The extensive resource of the PGC MDD Consortium provides an unparalleled opportunity to dissect the genetics effects in MDD using age at onset as a covariate to reduce the substantial heterogeneity of depression, but analysis of such data across studies is challenging due to cross-study differences.

### OS 12.2 Genetic Predictors of Antidepressant Side Effects

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**Background:** The unwanted effects associated with antidepressant medications are key determinants of treatment adherence in depression. Genetic variation may play a role in influencing individual propensity for such side effects, however previous work attempting to ascertain the variants involved has had limited success. In this study, adverse drug reactions were categorised based upon their likely pharmacological basis, and genetic predictors of susceptibility to each group of side effects were evaluated.

**Methods:** Adverse drug reactions detected using the Antidepressant Side-Effect Checklist (ASEC) were placed into four groups; adrenergic, cholinergic, serotonergic and histaminergic. Using a large cohort (n=811) of moderately to severely depressed patients, a candidate gene analysis was performed to identify genetic predictors of susceptibility to each group of side effects.

**Results:** Putative serotonergic side effects (i.e. nausea, diarrhoea, insomnia, sexual problems and decreased appetite) were significantly associated with variation in HTR2C (rs6644093, OR=1.72, 95% CI=1.31-2.25, p=7.43 x 10<sup>-5</sup>), amongst those patients with no prior contraindications to the two antidepressants used in this study. This finding supports the hypothesis of serotonin receptor-mediated mechanisms underlie such effects. However, we found no significant predictors of presumed adrenergic, cholinergic and histaminergic adverse drug reactions.

**Discussion:** This study demonstrates the occurrence of serotonergic antidepressant side effects is associated with genetic variation in the HTR2C gene. Further work is needed, not only to verify these findings and consider their translational value, but also to explore how genetic variability in HTR2C leads to the observed differences in liability to serotonergic side effects.

### OS 12.3 GWAS Meta-analysis Targeting Shared Anxiety Disorder Susceptibility

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**Background:** Anxiety disorders, despite differing on diagnostic definitions based upon clinical presentation, might represent various expressions of an underlying common diathesis of abnormal regulation of basic threat-response systems. Twin studies find correlation in genetic factors underlying different anxiety syndromes, suggesting a partially shared genetic basis. We use these findings to integrate data across multiple human anxiety disorders for input to GWAS.

**Methods:** Data from panic, generalized anxiety, and several categories of phobic disorders were collated and standardized for six large European ancestry samples (total N= 24,000). GWAS were conducted in each sample using two phenotypic definitions: case-control status and common factor-derived quantitative scores. These were combined across the six datasets using standard meta-analysis.

**Results:** Preliminary GWAS from individual samples do not identify genome-wide significant signals, although several prior candidate genes show nominal evidence of association. We are currently integrating data from the six datasets in on-going meta-analyses and should have full results by end of June.

**Discussion:** We will discuss and compare the anxiety susceptibility genes identified using these two phenotypic approaches in GWAS.

### OS 12.4 TMEM132D Gene: Functional Validation Studies of the New Candidate Gene for Anxiety-related Phenotypes

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**Background:** In a genome-wide association study (GWAS) in panic disorder (PD), we identified the TMEM132D locus on chromosome 12 as a novel candidate gene for PD (Erhardt et al. 2010). A haplotype containing two intronic single nucleotide polymorphisms (SNPs), rs7309727 and rs11060369, was associated with PD in three independent German samples. In addition, three independent SNPs were associated with the severity of anxiety symptoms in patients with a number of different primary mental disorders. Further support for TMEM132D to be involved in the pathogenesis of anxiety is provided by studies showing higher *tmem132d* expression in the anterior cingulate cortex in high versus low anxiety animals (Landgraf et al. 2007) and association of TMEM132D risk alleles with higher TMEM132D mRNA expression in human postmortem cortex. Additionally, the initial association finding has been replicated by the International Panic Disorder Consortium (PanIC), with genome-wide significance for rs7309727 in European cohorts of patients with PD. Finally, rs11060369 was among the top hits in a study testing for genome-wide association with anxiety-related clinical subscales of the Child Behavior Checklist in children with attention-deficit/hyperactivity disorder (ADHD) (Mick et al. 2011).

**Methods:** We used next-generation sequencing to investigate additional functional polymorphisms within the TMEM132D gene. Furthermore, we investigated TMEM132D DNA methylation status in a highly traumatized population. DNA methylation at 116 CpG sites within the TMEM132 locus was measured in DNA from peripheral blood in 425 individuals using the Illumina 450k DNA methylation array.

**Results:** Using next-generation sequencing we observed a significant over-representation of rare functional variants in TMEM132D in healthy controls as compared to patients with PD, suggesting that both rare and common variants in this gene contribute to anxiety disorders. The fact that rare functional variants were more common in controls than in patients with panic disorder suggests that rare variants decreasing the functionality of TMEM132D have a protective effect. This is consistent with *tmem132d* mRNA expression being decreased in the cingulate cortex in low-anxiety mice and the protective allele conferring lower TMEM132D expression in the frontal cortex in human postmortem cortex as detailed above. Data on correlations of TMEM132D DNA methylation with anxiety sensitivity measures as well as analyses stratified by risk genotypes will be presented. In addition, association of putatively functional TMEM132D SNPs significantly impacting TMEM132D DNA methylation with anxiety sensitivity and categorical anxiety disorders in 2,500 individuals will be shown.

**Discussion:** The possible candidate gene for anxiety disorders TMEM132D encodes a single-pass type 1 membrane protein belonging to the TMEM132 protein family. The function of the gene product is still unknown. It has been proposed that the protein may serve as a cell surface marker for oligodendrocyte differentiation (Nomoto et al. 2003), but other data show that it may be most prominently expressed in neurons and colocalized with actin filaments (Walser et al. 2011). This finding suggests that TMEM132D is implicated in the neuronal sprouting and connectivity in brain regions important for anxiety-related behaviour. This hypothesis is currently tested by fMRI measuring genotype-dependent connectivity in healthy individuals.

## OS 12.5 Large Repeat Expansions in the C9ORF72 Gene Contribute to a Spectrum of Neurodegenerative Disorders including Alzheimer's Disease in Caucasians, but not African-Americans

### ECIP

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**Background:** Progressive neurodegenerative diseases are characterized by the gradual loss of neurons. Despite clinical and genetic heterogeneity, a number of molecular commonalities have long been recognized for disorders as diverse as Alzheimer's disease (AD), Parkinson's disease (PD), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS). Shared molecular mechanisms are usually the result of overlapping genetic factors. Thus, characterization of the phenotypic spectrum of genes involved in neurodegeneration improves clinical and molecular disease models. Recently, an intronic hexanucleotide (GGGGCC) repeat expansion in the C9ORF72 gene has been identified to account for up to 50 of FTD/ALS families, 7 - 12 of FTD families, and 2 - 5 of sporadic FTD cases in Caucasians. Given the clinical, pathological and genetic characteristics of FTD, we hypothesized that C9ORF72 expansions may also confer risk to AD, the most common neurodegenerative disease.

**Methods:** We applied repeat-primed PCR to type the C9ORF72 gene hexanucleotide (GGGGCC)<sub>n</sub> repeat expansion in a total of 1,561 AD cases and 1,659 elderly dementia controls of Caucasian and African-American ethnicity.

**Results:** In Caucasians, we found C9ORF72 expansions in the pathogenic range of FTD/ALS (>30 repeats) at a rate of 0.9 in AD cases versus zero in controls (p=0.0015, 1269 cases, 1039 controls). In contrast, no large expansions were detected in individuals of African-American ethnicity (N=292/620). Moreover, in the range of normal variation of C9ORF72 expansions (0-20 repeats), we detected significant differences in distribution and mean repeat count between Caucasians and African-Americans. However, C9ORF72 expansions in the normal range did not confer risk to AD in either ethnicity when compared to matched controls. Most relevant, evaluation of AD cases with large C9ORF72 repeat expansions revealed a phenotypic spectrum in affected families that includes ALS, FTD and classic AD, with autopsy confirmed diagnoses.

**Discussion:** Thus, our study supports the hypothesis that the expansion contributes to a broad phenotypic spectrum of neurodegenerative diseases and will be a key factor to further decipher the underlying molecular pathology. The observed repeat length differences between Caucasian and African-American samples are unexpected, but could reflect different haplotype distributions, including haplotypes at risk for a repeat expansion.

## OS 12.6 Mis-sense Mutations in CACNG5 are Associated with Schizophrenia and Bipolar Disorder

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**Background:** Schizophrenia (SCZ) and bipolar disorder (BPD) are severe psychiatric disorders, which are each estimated to affect about 1% of the population worldwide with high rates of heritability. This high heritability has encouraged research into the genetic aetiology of these diseases and there is evidence of shared genetic susceptibility to both diseases. For example genome-wide association studies have identified a number of genes have significant associations with both diseases, the best example of which is CACNA1C, a calcium channel gene which encodes the  $\alpha_1$  subunit of the L-type voltage-gated calcium channel. A number of other subunits of calcium channel genes have also been implicated in BPD and/or SCZ, including the  $\beta$  subunit (CACNA1B) and the  $\gamma$  subunits (CACNG2, CACNG4 and CACNG5). More evidences of this subunit have shown the genetic defect induces epileptic seizures in stargazer mice. Studies have indicated that all eight members of calcium channel  $\gamma$  subunits are a functionally diverse protein family, even though they share a common topology consisting of four transmembrane domains and intracellular N- and C-termini. Recently, the functional studies have classified these eight members into two clusters:  $\gamma_1$  and  $\gamma_6$  act primarily as subunits of calcium channels expressed in muscle, the second cluster function as regulators of AMPA receptor localisation and function in the brain and are collectively known as TARPs ( $\gamma_2$ ,  $\gamma_3$ ,  $\gamma_4$ ,  $\gamma_5$ ,  $\gamma_7$  and  $\gamma_8$ ). The research to date has focussed on the discovery of new genetic variation in  $\gamma_5$  subunits (CACNG5), which seems to be the regulation of trafficking and gating of AMPA receptors (AMPA) and in particular the AMPAR subunit 2 (AMPA2). This project aims to identify disease relevant genetic variation in CACNG5 and to study the biological implications of these variants in vitro. The identification of new disease susceptibility variants for BPD and/or SCZ should identify new drug targets and therapeutic pathways.

**Methods:** The exons and promoter region of CACNG5 were screened for new variants in 1,098 BPD, 618 SCZ and 1,087 control samples using high resolution melting analysis (HRMA). All mutations detected by HRMA were further confirmed by direct sequencing and/or Kaspar genotyping. Bioinformatic analysis was used to investigate the location and possible functional effects of each variant. Standard chi-square tests were used to assess the evidence for association of these variants with BPD and/or SCZ.

**Results:** A total 18 single nucleotide change were found by HRMA, including 6 SNPs in promoter regions, 4 synonymous SNPs and 8 non-synonymous SNPs. These four non-synonymous SNPs are not previously observed in the global 1000 Genomes project data and only two variants were found in the European populations from 1000 Genomes project. Pooled analysis of all non synonymous CACNG5 SNPs in BPD and SCZ versus the controls found evidence for association (p=0.0022). This association was strengthened by the addition of the European samples from the 1000 Genomes project (p=0.00082). Subdivision of the nonsynonymous SNPs (nsSNPs) by bioinformatic analysis based on their predicted deleterious effect on protein function using PolyPhen2 and SIFT did not strengthen the association (p=0.0014). The location of the nsSNPs in CACNG5 does not indicate a common domain that is affected by these changes. We are therefore interested to explore the biological significance of these changes in vitro.

**Discussion:** We have already created CACNG5 cDNA expression constructs containing these nsSNPs using site-directed mutagenesis. These constructs will be cotransfected with an AMPAR2 cDNA construct into human embryonic kidney (HEK293) cells. The effects of these mutations on the levels of CACNG5 and AMPAR2 will be monitored by Western blotting and the trafficking of AMPAR2 to the cell surface will be assayed using cell surface biotinylation followed by Western blotting. These experiments have the potential to begin to understand the pathogenic importance of TARP/AMPA interaction in BPD and SCZ and to pave the way for further investigations into the disruption of postsynaptic AMPA signaling.

# **POSTER SESSION I**

## **ABSTRACTS**

## Poster Presentations

### ADHD

#### Poster 1

##### Childhood ADHD And Obesity: Evidence for a Common Genetic Link

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**Background:** Epidemiological and clinical studies show that children and adolescents with ADHD have higher rates of obesity than children without ADHD. Several case reports of children with severe obesity and co-morbid ADHD indicate a role for genetic mutations in the melanocortin-receptor 4 (MC4R) (Albayrak et al., 2011) and in the brain-derived neurotrophic factor gene (BDNF) (Shinawi et al., 2011) gene. Gene variants predisposing to obesity potentially overlap with those relevant for ADHD.

**Methods:** We screened 32 known obesity risk alleles of single nucleotide polymorphisms (SNP) (Speliotes et al., 2010) in a genome-wide association study. (GWAS) for ADHD based on 495 young German patients (Illumina; Human660W-QuadV1 BeadArrays) and 1,300 population-based controls (Illumina; HumanHap550v3 BeadArray). We also performed in silico analyses of the SNPs in a large ADHD meta-analysis comprising 2,064 trios, 896 cases, and 2,455 controls (Neale et al., 2010). In addition, we explored the obesity risk alleles with regard to their quantitative effects on inattention and hyperactivity/impulsivity in both samples. Psychiatric GWAS Consortium: ADHD subgroup.

**Results:** In the German sample rs206936 in the NUDT3 gene (nucleoside diphosphate linked moiety X-type motif 3) was associated with ADHD risk (OR: 1.39;  $p=3.4 \times 10^{-4}$ ;  $p=0.01$  upon correction for 32 tests). In silico analysis of the meta-analysis data revealed the major allele of rs6497416 in the intronic region of the GPRC5B gene (G protein-coupled receptor, family C, group 5, member B;  $p=7.2 \times 10^{-4}$ ;  $p_{\text{corr}}=0.02$ ) as risk allele for ADHD. The risk allele is in linkage disequilibrium with the obesity risk allele at rs12444979. Exploratorily, BMI SNPs in NUDT3 and in the glucosamine-6-phosphate deaminase 2 gene (GNPDA2) were nominally associated with inattention ( $p<0.05$ ) in the German sample, whereas markers in mitogen-activated protein kinase kinase 5 (MAP2K5) and cell adhesion molecule 2 (CADM2) were nominally associated with hyperactivity and GPRC5B with the combined phenotype.

**Discussion:** Our results suggest that BMI risk alleles at the NUDT3 and at the GPRC5B locus confer an increased risk for ADHD. GPRC5B is homologous to genes of the metabotropic glutamate receptor family, which have recently been implicated in the etiology of ADHD (Elia et al., 2010, 2012).

## Poster 2

### Genetics of Preparation in ADHD

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**Background:** Performance and electrophysiological parameters of cognitive and response preparation, probably related to dopaminergic functioning are familiarly driven in ADHD and may represent endophenotypes of the disorder (McLoughlin, Asherson et al. 2011; Albrecht, Brandeis et al. under review). The aim of the current study is to test whether polymorphisms of three candidate genes associated with ADHD and dopaminergic functioning (Brookes, Xu et al. 2006) have an impact on performance and electrophysiological parameters of preparation.

**Methods:** Data were based on 94 boys aged 8 to 16 years with an estimated WISK-IQ above 80 and an ADHD combined type diagnosis. The sample was subdivided regarding the presence of the 7-repeat allele of an exon 3 variable number tandem repeat (VNTR) in the dopamine D4 receptor gene (DRD4 7rep, present in 26% of the sample), homozygosity of the 10-repeat allele VNTR in the 3UTR of the dopamine transporter gene (DAT1-3UTR 10/10, 48%) and the DAT1 intron 8 VNTR homozygotic for the 6-repeat allele (DAT1-INT8 6/6, 61%). Behavioral ratings were obtained from teachers using the SDQ. Response speed and Cue-CNV mean amplitude from a Continuous Performance Test with additional incongruent flanker stimuli were analysed using topographic t-maps.

**Results:** No effects of genotype were found for Age and IQ (all  $F(1, 89)<1.4$ ,  $p>.24$ ). SDQ teacher ratings did also not differ, with the exception that lower peer problems were reported for boys carrying the DAT1-INT8 6/6 repeat allele ( $F(1, 89)=8.4$ ,  $p<.01$ ). Reaction times were slower in boys with ADHD carrying the DRD4 7 rep. polymorphism ( $F(1, 89)=9.9$ ,  $p<.01$ ); effects for DAT1 were not significant (both  $F(1, 89)<2.1$ ,  $p>.15$ ). The mean CNV amplitude was assessed as the mean amplitude 1200 to 1650 ms following the cue onset where it was maximal over centro-parietal sites (see Figure a). T-map comparisons revealed diminished CNV in boys with ADHD carrying the DRD4 7repeat polymorphism over fronto-central sites (see Figure b);  $p<.05$  is retained for  $T(92)>2.0$ ). Regarding the dopamine transporter DAT1 gene, presence of the DAT1 intron 8 VNTR 6/6 and as a tendency also for DAT1 VNTR in the 3UTR 10/10 polymorphism was associated with elevated CNV over left-central electrodes.

**Discussion:** The current results highlight the role of candidate genes associated with preparatory and time processing in childhood ADHD. While the effect of DRD4 polymorphism may follow reduced D4 receptor sensitivity to dopaminergic stimulation particularly in prefrontal and anterior cingulate cortex, the functions of DAT1 polymorphisms are currently unclear and may be modulated by developmental and environmental effects.

### Poster 3

#### AD/HD Subtyping and Genetic Influences on the Occurrence of Comorbid Conditions

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**Background:** Individuals with Attention-Deficit/Hyperactivity Disorder (AD/HD) have increased risk for comorbid psychiatric conditions. Up to 60% of children with AD/HD will carry at least one comorbid diagnosis (August et al, 1996); this rate increases to 80% among adults with AD/HD (Barkley, Murphy, & Fischer, 2010). Although this increased comorbidity risk is relatively clear, the mechanisms by which this risk is conferred are not well understood. The role of subtyping and genetic influences in particular has been understudied. Thus, we conducted an exploratory analysis of these factors on the occurrence of comorbid conditions within a cohort of children with AD/HD.

**Methods:** The participants were drawn from a larger sample of families participating in a multisite genetic investigation of AD/HD. Proband and siblings were 5 to 12 years of age and met Diagnostic and Statistical Manual of Mental Disorders (4th Ed) criteria for a diagnosis of AD/HD, determined on the basis of parental interview responses, accompanied by elevated T-scores on parent- and teacher-completed AD/HD rating scales. Final determination of AD/HD status was established by a panel of three senior investigators. The same criteria and panel-review process were used for determining comorbid diagnoses. The final sample was comprised of 223 affected probands and siblings, including 150 boys and 73 girls (mean age 8.5 years); 22.3% was racially diverse. The role of AD/HD subtyping on the presence of comorbidity was examined. To reduce overlap of the Combined (C) and Inattentive (IN) subtypes, an IN-Pure group was formed, defined by having 2 or less Hyperactive-Impulsive (HI) symptoms. To determine whether or not genetic factors influenced the occurrence of AD/HD subtype and comorbid conditions, we examined 147 haplotype tagging SNPs in DAT1, DBH, SNAP-25, DRD1, DRD2, DRD3, DRD4, NET, HTR1B, and SLC6A4. Generalized estimating equations were used to assess the association between SNP and AD/HD subtype, as well as the presence of any comorbidity, while controlling for age, gender and family correlation. To reduce population stratification, we restricted our genetic analysis to non-Hispanic, white children.

**Results:** In terms of subtyping, 58.8% displayed the C type, 7.6% the IN type, 26% the IN-Pure type, and 7.6% the HI type. Children who were younger (5-8 years) and male were, respectively, 1.9 times and 2.8 times more likely to display the C type. 56.5% met DSM-IV criteria for one or more comorbid diagnoses, including ODD (34.9%), CD (7.2%), separation anxiety (9.9%), social phobia (6.3%), generalized anxiety (3.6%), obsessive compulsive disorder (3.62%), major depression/dysthymic disorder (4.0%), and tic disorders (5.4%). For children with the C type, 54.2% displayed comorbid externalizing disorders and 21.4% internalizing disorders; for the IN type, these comorbid rates were 25.3% externalizing and 22.7% internalizing; for the IN-Pure type, (29.3% externalizing, 31.7% internalizing; for HI, 23.5% externalizing, 11.8% internalizing. Significant subtype by gender interactions emerged; girls with the C type were more likely than girls with the IN-Pure type to display both externalizing (7.9 x) and internalizing (3.7 x) comorbidities, while there was no difference observed among boys. Although none of the genetic associations met a Bonferroni multiple testing correction, we did observe nominal associations. SNPs in DAT1 (n=3), DBH (n=3), SNAP-25 (n=1), DRD2 (n=1), NET (n=1) and HTR1B (n=1) were nominally associated

with AD/HD subtype. The most significant of these associations was rs2283124 (p=0.004) in DBH, whereby the T allele was associated with an increased risk for C type. SNPs in SNAP-25 (n=1), NET (n=1) and SLC6A4 (n=1) were nominally associated with the occurrence of a comorbid condition. The most significant of these associations was rs36016 (p=0.02) in NET, whereby the C allele was associated with increased risk for a comorbid condition.

**Discussion:** These data add to the body of literature describing the complex relationships between AD/HD and comorbid conditions. Our analyses suggest that AD/HD subtype is associated with differential risk for comorbidity and that genetic liabilities may influence the occurrence of specific AD/HD subtype as well as the occurrence of comorbid conditions. Due to the limited sample sizes in this analysis, replication of these associations in a larger data set is warranted.

## Poster 4

### Evidence from Polygenic Analysis that Conduct Disorder is Enriched for ADHD Risk Alleles

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**Background:** Attention Deficit Hyperactivity Disorder (ADHD), like all complex disorders, is typified by heterogeneity in its clinical manifestations. Comorbidity with Conduct Disorder (CD) has been considered an especially important index of both clinical and aetiological heterogeneity. We have investigated whether individuals affected with ADHD and CD have a higher loading of ADHD risk alleles.

**Methods:** We have examined multiple ADHD common risk alleles en masse using polygenic score analysis. We used the published ADHD vs. controls meta-analysis genome-wide association study (GWAS) results data as the discovery set on which to define polygenic scores that were assigned to each individual in our UK ADHD GWAS data set (538 cases, 5,081 controls). Analyses were performed to compare polygenic scores between our cases and controls. Further analyses were performed on the Conduct Disorder phenotype, specifically focusing on the 77 individuals with a diagnosis of CD (CD+), and 354 with a definite diagnosis of no CD (CD-).

**Results:** Independent ADHD risk alleles were able to discriminate our ADHD case individuals from controls ( $p=0.0010$ ,  $R^2=0.19\%$ ). Much of the effect was contributed by the individuals with ADHD and CD (ADHD CD+ vs. controls;  $p=0.00078$ ,  $R^2=0.22\%$ ). A significant difference was found when comparing the case individuals CD+ and CD- ( $p=0.011$ ,  $R^2=1.5\%$ ). Supporting evidence was also observed when analysing the number of conduct symptoms as a quantitative phenotype, with individuals with more symptoms having higher polygenic scores.

**Discussion:** Individuals with higher levels of CD (by diagnosis and number of symptoms) have more ADHD risk alleles than the other ADHD cases, and both have more than controls. This has implications for future studies of ADHD and further highlights the need to consider CD as a marker of aetiological heterogeneity within ADHD samples. The findings also highlight that polygenic risk scores for psychiatric disorder can be usefully used to examine phenotypic heterogeneity and that common risk alleles contribute to ADHD.

## Poster 5

### Genetic Variation in Genes Encoding 14-3-3 Proteins in ADHD

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**Background:** The 14-3-3 proteins constitute a family of seven regulatory proteins that are highly expressed in brain tissue and may interact with hundreds of different cellular phosphoproteins. The 14-3-3s are involved in a diversity of cellular functions, including signal transduction, trafficking and secretion. The 14-3-3s are highly conserved between mammalian species, consistent with their important functions. The first biological activity assigned to 14-3-3s was the activation and stabilization of tyrosine and tryptophan hydroxylases (1). These hydroxylases are rate-limiting in the biosynthesis of catecholamines and serotonin, neurotransmitters implied in neuropsychiatric disorders such as ADHD (2). Several 14-3-3 isoforms have previously been associated with other neuropsychiatric disorders, such as schizophrenia and bipolar disorder and have also been implied in rare Mendelian disorders affecting brain development (3).

**Methods:** We used genotype-tagging methods to cover common genetic variation for all seven genes encoding members of the 14-3-3 protein family. Additional variants were chosen from preliminary exome sequencing data from our biobank, which consists of nearly 800 ADHD cases and 1000 controls. A total of 37 SNPs were successfully genotyped and passed quality control. 641 cases and 668 controls were genotyped using SNP multiplex technology (MassARRAY iPLEX system, Sequenom, San Diego, CA, USA). Statistical analysis was performed using PLINK software.

**Results:** Two SNPs, rs16867073 and rs17462921, had nominally significant p-values, but did not pass Bonferroni correction (p-value 0.03 and 0.04, respectively).

**Discussion:** 14-3-3 protein encoding genes have been implicated in several neuropsychiatric disorders, and interact with other candidate genes in a phosphorylation dependent manner. In recent exome sequencing studies, genes interacting with 14-3-3 proteins were implicated in autism, through rare coding mutations (4-6). 14-3-3 proteins are highly conserved, and protein function may be vulnerable to mutations. We therefore aim to test whether rare coding variants might confer stronger effect on ADHD in a subset of patients using exome sequencing and exome chip genotyping data. In conclusion, we did not find any strong evidence for an association between common SNPs in the 14-3-3 protein encoding genes and ADHD. Further studies focusing on rare coding variants are being performed. References 1, Aitken A. Post-translational modification of 14-3-3 isoforms and regulation of cellular function. *Seminars in Cell and Developmental Biology*. Elsevier Ltd; 2011 Sep. 1;22(7):673–80. 2, Kleppe R., Martinez A., Døskeland S. O., Haavik J. The 14-3-3 proteins in regulation of cellular metabolism. *Seminars in Cell and Developmental Biology*. Elsevier Ltd; 2011 Sep. 1;22(7):713–9. 3, Grover D., Verma R., Goes F. S., Mahon P. L., B, Gershon E. S., McMahon F. J., et al. Family-based association of YWHAH in psychotic bipolar disorder. *Am. J. Med. Genet.* 2009 Oct. 5;150B(7):977–83. 4, O'Roak B. J., Vives L., Girirajan S., Karakoc E., Krumm N., Coe B. P., et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature*. Nature Publishing Group; 2012 Apr. 4;:1–7. 5, Sanders S. J., Murtha M. T., Gupta A. R., Murdoch J. D., Raubeson M. J., Willsey A. J., et al. De novo mutations revealed by whole-exome sequencing are strongly

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## Poster 6

### **Methylphenidate Improves Some but Not All Measures of Attention, as Measured by the Test of Everyday Attention in Children (TEA-CH) in Medication Naïve Children with Attention-Deficit Hyperactivity Disorder (ADHD)**

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**Background:** Clinical tools have been developed to provide reliable neuropsychological assessments of attentional control in children. One such task, the Test of Everyday Attention for Children (TEA-Ch) is based on the Posner model of attention, and measures three forms of attention. Methylphenidate (MPH) is a widely used and effective treatment to improve attentional difficulties in children with attention deficit hyperactivity disorder (ADHD). MPH acts on the catecholaminergic system and may modulate the fronto-striatal and fronto-parietal circuits underpinning attention. Previous studies investigating the effects of MPH on attention performance of children with ADHD have produced mixed results and prior MPH usage may have confounded these results. No previous study has tested the effects of MPH on the entire TEA-Ch battery.

**Methods:** This study investigated the effects of MPH on attention performance using the entire TEA-Ch in 52 medication-naïve children with ADHD compared with 36 non-medicated typically developing children. All children were tested at baseline and after six weeks: the children with ADHD were medication-naïve at baseline, received MPH for six weeks and were tested whilst on medication at the second testing session..

**Results:** A beneficial effect of MPH administration was found on at least one subtest of each of the three forms of attention (selective, sustained and attentional control) assessed by the TEA-Ch, independent of practice effects.

**Discussion:** MPH aided performance on the TEA-Ch tasks that were inherently non-arousing and that might require top-down control of attention. It is recommended that the TEA-Ch measures - Sky Search Count (selective attention), Score! (sustained attention), Creature Counting Time Taken (for older children, attentional control) and Same Worlds (attentional control) be prioritised for use in future pharmacological studies using MPH.

### Studies of DIRAS2, A Candidate Gene in Adult Attention-Deficit Hyperactivity Disorder (AADHD)

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**Background:** Attention-deficit/hyperactivity disorder (ADHD) is a highly prevalent neurodevelopmental disorder. It was first recognised in children, but it is now well-known that it frequently persists, and the prevalence is estimated as 3-4 in adults. As the heritability of ADHD is high, many genetic studies have been performed, although most studies have been conducted in children. Linkage studies have implicated the chromosome 9q22 region, which contains the brain-expressed GTP-binding RAS-like 2 gene (DIRAS2), in ADHD. We wished to examine whether nucleotide variants in DIRAS2 are associated with adult ADHD (aADHD) and if putative associated variants have an impact on gene expression. Finally, we aimed at studying the expression pattern of DIRAS2 in mouse brain.

**Methods:** The promoter, exons and untranslated regions of DIRAS2 were tagged with 14 single nucleotide polymorphisms (SNPs), and 600 German ADHD patients (18-65 years) and 420 healthy controls were genotyped. Haplotypes were defined with Haploview and analysed with Unphased (Dudbridge, 2008). Replication attempts and further meta-analyses were performed with 1035 aADHD patients and 1381 controls from Norway, Spain and The Netherlands. To further study the functional effect of an associated promoter SNP, rs1412005, we used the luciferase reporter system. Sequences with the reported risk and protective alleles of the SNP were subcloned into the pGL4.23 minimal promoter luciferase vector (Promega). After transformation into SH-SY5Y cells, gene expression was analysed using the GloMax luminometer (Promega). In order to examine the exons of DIRAS2 for rare variants, DNA from 300 patients and 300 healthy controls from Germany was prepared for next generation sequencing. The preparation involved long range polymerase chain reactions, gel purification and pool assembly steps of equimolar amounts of DNA from different amplicons and individuals. The sequencing was performed with the 5500xl SOLiD System (Applied Biosystems). DIRAS2 expression patterns in mouse brain were investigated using in situ hybridisation.

**Results:** A p-value < 0.05 was observed for four SNPs and for both linkage disequilibrium blocks defined by the haplotype testing. No significant associations were observed in the replication samples, but for two SNPs and a haplotype, consistent effect size estimates were observed. Meta-analyses resulted in significant associations for the SNP rs1412005 (OR = 1.23; p = 0.04) and the haplotype [ACGCTT] (OR = 1.45; p = 0.0003). Results from the analysis of rs1412005's effect on DIRAS2 promoter activity and rare variant data from the sequencing of exons will be presented. DIRAS2 showed marked expression in cortex, anterior cingulate, hippocampus and amygdala.

**Discussion:** DIRAS2 is expressed almost specifically in the brain, and we observed consistent expression in the cortex, anterior cingulate and amygdala, suggesting an influence on cognitive functions that are commonly impaired in ADHD. Effector mechanisms of Di-Ras2, the protein encoded by DIRAS2, is to date unknown. Nevertheless, roles in cell morphogenesis and neurogenesis have been found for other members of the small GTPase Ras family; as DIRAS2 is expressed almost specifically in brain, one could speculate that the protein might have effects on similar processes. Our risk haplotype, including rs1412005, is located within the DIRAS2 regulatory region. We therefore hypothesise that the observed association with ADHD is caused by gene expression changes due to DIRAS2 promoter variation. We are currently examining the functional effect of rs1412005 on DIRAS2 expression. Furthermore, we are searching for unknown genetic, potentially causative, variants in the coding region of DIRAS2.

## Poster 8

### Examining the Genetic Overlap of Attention-Deficit Hyperactivity Disorder with Autism Spectrum Disorder Traits

Joanna Martin<sup>1</sup>, Marian Hamshere<sup>1</sup>, Michael O'Donovan<sup>1</sup>, Anita Thapar<sup>1</sup>

<sup>1</sup>Cardiff University

**Background:** Attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) are both highly heritable neurodevelopmental disorders. Evidence for a genetic overlap across these conditions (as well as other neurodevelopmental problems, including schizophrenia and intellectual disability) is emerging from family and twin studies and genome-wide analyses of rare copy number variants. Both ADHD and ASD are associated with deficits in a number of executive functioning domains as well as communication and social cognitive problems. To understand the shared genetic susceptibility across ADHD and ASD, novel approaches exploring the shared phenotypic characteristics of these two conditions are required. Our aim was to examine the relationship between ADHD polygenic risk scores and intermediate cognitive phenotypes related to both ADHD and ASD.

**Methods:** Data from a large ADHD genome-wide association study were utilised as a discovery sample to calculate polygenic scores in the target sample: an independent, well-characterised group of UK children (who met research criteria for DSM-IV or DSM-III-R ADHD) ascertained from clinics. These scores were analysed in terms of association with intermediate phenotypic traits, including social-communication traits, verbal working memory (a cognitive sub-domain of executive functioning) and linguistic abilities.

**Results:** Preliminary analyses within the clinical sample (N=456) showed no association of polygenic risk with Social Communication Questionnaire scores (p=0.3), verbal working memory (p=0.6), reading and spelling abilities (p=0.6), or the other cognitive and developmental variables analysed.

**Discussion:** Preliminary analysis of the ADHD and ASD candidate intermediate phenotypes within the clinical sample showed no evidence for association with ADHD risk through polygenic analysis. Future work will focus on a more detailed analysis of candidate intermediate phenotypes within a general population study, as clinical samples are likely to not show sufficient phenotypic variability to detect associations.

## Poster 9

### Investigating Biological, Familial and Early Environmental Factors in Children with Attention-Deficit Hyperactivity Disorder with and without Mild Intellectual Disability

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**Background:** Children with attention-deficit hyperactivity disorder (ADHD) often have a lower IQ than their peers and many of them have mild intellectual disability (ID; IQ 50-69). It is often assumed that children with ADHD+ID need to be excluded from research studies of ADHD as the risk factors and correlates for this sub-group may differ. This study examined such an assumption.

**Methods:** Children with a lifetime history of ADHD without ID (N=909) were compared with those who had mild ID (N=98), in terms of pre-/peri-natal, social and familial factors previously implicated in ADHD. 561 cases (49 with ID) were used to examine whether copy number variant (CNV) related biological pathways previously found for ADHD without ID were also relevant to children with ADHD+ID.

**Results:** No significant differences between the groups were found in any of the pre-/peri-natal factors assessed. Children with ADHD+ID were significantly more likely to have low familial socioeconomic status than children with ADHD without ID. There was also a trend for greater rates of parental ADHD problems in children with ADHD+ID. CNV pathway analyses showed that ADHD-related biological pathways were also found to be enriched in the ADHD+ID group.

**Discussion:** These results suggest that children with ADHD and mild ID are more socially disadvantaged, have a strong parental history of ADHD problems and show a similar pattern of pre-/peri-natal and biological correlates to children with ADHD without ID. Consequently, the systematic exclusion of children with mild ID from ADHD research needs to be re-considered and investigated further.

## Poster 10

### What is the Total L SNP-Associated Heritability for Alcohol and Nicotine Dependence?

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**Background:** Much has been written about the so-called “missing heritability” for complex traits. Nowhere is this more pertinent than for alcohol and nicotine dependence (AD, ND) for which there are estimates of heritability of up to 65% from twin studies, yet few causal variants have been replicated from GWAS studies, despite large sample sizes, suggesting that individual effect sizes of SNPs must be very small. Recently new statistical genetic techniques have been developed which allow estimation of the total variance associated with all SNPs on a GWAS chip, but this has yet to be applied to AD and ND.

**Methods:** The current analysis is based on AD and ND symptom count data from over 8000 participants in our population-based twin-family studies who have used either alcohol or cigarettes at some stage of their lives. They were individually genotyped with Illumina 370K or 660K chips and 7.034M genotypes were imputed from HapMap 3 and 1000-Genomes data. The GCTA program of Yang, Visscher et al is used first to detect the degree of relatedness between apparently unrelated subjects, based on a set of about 300,000 SNPs pruned for LD. Phenotypic similarity is then regressed on IBS sharing for all possible relative pairs to estimate the total amount of variance due to SNPs on the chip. However, these estimates are highly sensitive to population stratification so great care will be taken to remove all traces of population stratification during the analysis.

**Results:** Based on GCTA analysis for other complex traits we expected to find SNP associated variance accounting for about half the heritability estimated from conventional genetic epidemiology designs. In fact the SNP-associated heritability for alcohol dependence is up to 80% of the total twin-based heritability suggesting that most of the variants affecting AD liability are common and that only a minority is due to rare variants.

**Discussion:** The gap between the SNP-associated variance estimated by GCTA and twin and family estimates of heritability is most likely due to several factors. First, the tag SNPs on the chip are not in perfect LD with the causal SNPs; for other traits, simulation has shown that correcting for imperfect LD raises the SNP “heritability” by about 10. Another major factor is that commercial chips only interrogate common SNPs so large effects of rare SNPs are simply not captured. Reasonable estimates from simulations suggest that this could account for another 20 of variance. Finally, we recognize that there are large sections of the genome containing highly repetitive DNA which are very poorly tagged by current chips, and where substantial proportions of genetic variance may be hidden.

## Affective Disorders

### Poster 11

### For Whom the Clock Ticks: Clock and PER3 Genetic Variants Interact with Stressful Life Events to Influence Patterns of Sleep

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**Background:** Disturbances in circadian rhythms are very prominent in mood disorders. The role of several genes implicated in the endogenous clock system has been investigated in mood disorders in order to uncover genetic vulnerability. Evidence is mixed: negative direct associations with mood disorders prevail; however, there is accumulating evidence of specific associations with symptoms involving circadian disruptions (mainly sleep problems) in healthy participants and patients with mood disorders. Furthermore, environmental influences may moderate associations between genes and phenotype and have not been previously considered together with clock-related genes.

**Methods:** We examined the CLOCK 3111T/C polymorphism and variants within the PER3 gene (rs228729, rs228642, rs228666, rs10462020, rs228697, rs2859388) and their possible interaction with stressful life events in a group of female participants (n=415) recruited from primary care. Gene-environment (GxE) interactions and gene main effects were investigated on depressive symptoms using the Beck Depression Inventory and on change of sleep patterns using this measure.

**Results:** Results showed a GxE interaction on alteration of sleeping pattern: the 3111C homozygous genotype reported greater disruption in sleep pattern after the experience of stressful life events. Within the PER3 gene, one GxE interaction was observed with rs228642 on sleep change. No associations were observed between the genes examined and total depressive symptomatology. Haplotype analysis with the PER3 variants did not show any significant results.

**Discussion:** Our findings show that the 3111T/C polymorphism is not associated with depressive symptoms, but only with symptoms of sleep disruptions in the case of prior stressful life experiences. Findings are discussed in the context of the relationship between circadian rhythm disruptions and stress, and how genes implicated in the CLOCK machinery may promote vulnerability or resilience. This is the first study to show that the combination of a sensitive genotype (3111C/C) and environmental stress increases vulnerability to sleep disruption.

## Poster 12

### Genetics of Bipolar Disorder

Susanne Bengesser<sup>1</sup>, Susanne Bengesser<sup>1</sup>, Bernd Reininghaus<sup>1</sup>, Hans-Peter Kapfhammer<sup>1</sup>, Stefanie Leopold<sup>1</sup>, Armin Birner<sup>1</sup>, Nina Lackner<sup>1</sup>, Erwin Petek<sup>1</sup>, Anna-Maria Painold<sup>1</sup>, Eva Reininghaus<sup>1</sup>

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**Background:** Bipolar disorder is a widespread and devastating disease, thus elucidation of its genetics and disease mechanisms is highly important, because it might help to develop new medication, prevention strategies and laboratory markers. This is an approach to review the literature on the genetic findings in bipolar disorder.

**Methods:** We performed a Pubmed search combining the catchphrases “bipolar disorder” and “GWAS”, genes analysed in GWAS, genes plausible in synopsis with the current model of bipolar disorder (e.g. neurotransmitter systems). All papers until the 8th of April 2012 have been included.

**Results:** Although molecular psychiatry is still in its infancy a huge amount of possible susceptibility genes have been discovered yet. Top candidates belong to the ion channel group, the growth hormones, the clock genes, the neurotransmitter systems, as well as genes involved in Lithium signalling pathways, cell adhesion, signal transduction and mitosis. Detailed results are discussed in the poster session.

**Discussion:** Even though many studies show significant associations, some results are still conflicting. Larger study designs, a closer look on ethical differences and subtypes of bipolar disorder might help to elucidate the genetics of bipolar disorder. Knowledge of predisposing genes can improve diagnostic means, individual pharmacological therapies and primary prevention.

## Poster 13

### Associations between Genome-wide Homozygosity and Neuroticism, Anxiety and Depression

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**Background:** Depression and anxiety are among the most prevalent psychiatric disorders. Heritability estimates from twin and from SNP-based approaches (e.g. Lubke et al. 2012) tend to be lower than for other psychiatric disorders. Here we explore whether evidence of increased homozygosity – estimated from genome-wide SNP data- is a risk factor for depression and associated traits.

**Methods:** In a large homogeneous sample from the Netherlands, we obtained inbreeding coefficients (F) and looked for a relation with complex phenotypes collected longitudinally in participants who were registered with the Netherlands Twin Register (NTR, N=5,506; 40 males and 60 females from 2795 families). Participants were randomly sampled from twin families across the Netherlands. Genotyping was performed on the Affymetrix Human Genome-Wide SNP 6.0 Array. Autosomal SNPs were analyzed; quality control was carried out in Plink by removing SNPs with MAF < 5, missing rate > 5, and HWE deviation with a p-value < 0.001. Individuals were removed if they had a missing SNP rate > 5. F was obtained from PLINK, based on a SNP panel of ~130.000 SNPs that were pruned for LD; there were no individuals with excess genome-wide F-values.

**Results:** Associations among F and personality traits and indices of psychopathology were present for Neuroticism and symptom (sum) scores of Anxiety and Depression. However, the associations were negative (e.g. a lower depression score was associated with higher values of F).

**Discussion:** We need to replicate these results in additional cohorts. A cohort of MDD cases from the NESDA (Netherlands Study of Depression and Anxiety; N=2,038; 32 males, and 68 females) study was genotyped with the NTR cohort and QC and estimation of F were carried out simultaneously. We will explore in the combined NESDA – NTR data whether the association seen in the NTR cohort for symptom scores also is seen for clinical diagnoses.

### Hint for Gender-specific Association of Creb1 and a History of Suicide Attempts in MDD: Results from a European Multicenter Study on Treatment Resistant Depression

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**Background:** Mental disorders and in particular mood disorders are present in more than 90% of suicides, and a genetic vulnerability to suicidality is well established. Numerous lines of evidence relate the transcription factor cyclic adenosine monophosphate (cAMP) Response Element Binding Protein (CREB) to suicide, as well as to the aetiology and pharmacotherapy of major depressive disorder (MDD). Our aim was to test for association between single nucleotide polymorphisms (SNPs) in CREB1 and both suicide risk and suicide attempts in MDD patients.

**Methods:** A sample of two hundred fifty MDD patients collected in the context of a European multicenter resistant depression study and treated with antidepressants for at least 4 weeks were genotyped for five CREB1 SNPs (rs2709376, rs2253206, rs7569963, rs7594560, and rs4675690). To assess suicidality, the Mini International Neuropsychiatric Interview (MINI) and Hamilton Rating Scale for Depression (HAM-D) were performed.

**Results:** Neither single-marker nor haplotypic association was found for suicide risk and/or personal history of suicide attempts with any SNP after multiple testing correction. Among the female subsample we found significant ( $p=0.016$ ) single-marker associations for rs2709376 with a personal history of suicide attempts as well as haplotypic association (individual  $p=0.022$ ), however the latter not resisting multiple testing correction. No significant associations were detected for treatment response phenotypes.

**Discussion:** Although we found significant CREB1 single marker association with a personal history of suicide attempts in female MDD patients, this could not be confirmed in haplotypic analyses after multiple testing correction. Larger well-defined cohorts are required to confirm or refute a possible association of CREB1 and suicide attempts in female MDD patients.

### Genome-wide Association Signals in Bipolar Disorder are Enriched for Genetic Variants Within Transcription Factor Binding Sites and Expression Quantitative Trait Loci

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**Background:** Genome-wide association studies (GWAS) have uncovered a number of loci associated with bipolar disorder (BD), but functional alleles have not been identified. Here we test the hypothesis that some GWAS signals reflect genetic variation in sequences regulating gene expression.

**Methods:** Genetic association data was extracted from a meta-analysis of worldwide BD GWAS, comprising ~14,000 cases and controls (Chen DT et al. Mol. Psychiatry 2011). This set of over 700,000 SNPs was mapped onto published transcription factor binding sites (TFBS) identified in human lymphoblastoid cells (Pique-Regi et al. 2011). Enrichment was tested with the Kolmogorov-Smirnov rank-sum statistic and empirical p-values were determined by permutation. For exploration of suggested candidate genes and functional pathways, INRICH ([atgu.mgh.harvard.edu/inrich/](http://atgu.mgh.harvard.edu/inrich/)) was used.

**Results:** We observed significant enrichment of GWAS signals among SNPs near TFBS (K-S  $p<7 \times 10^{-4}$ ; Empirical  $p<0.049$ ). Within the enrichment set, genes nearest these TFBS were significantly associated with the Gene Ontology terms “protein amino acid phosphorylation,” “transforming growth factor beta receptor activity,” and “protein serine-threonine kinase activity” (INRICH  $p<8 \times 10^{-4}$ ,  $p<1.2 \times 10^{-3}$ ,  $p<1.4 \times 10^{-3}$ , respectively). Overall ~32 pathways identified by INRICH were significant at the  $p<0.05$  level, representing a significant enrichment of pathways (Empirical  $p<2 \times 10^{-3}$ ).

**Discussion:** These results suggest that common alleles that contribute to risk for BD reflect, in part, genetic variation in TFBS that regulate gene expression. Further studies of gene expression and its genetic regulation are warranted in BD.

### Association of SORT1 with Bipolar Disorder

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**Background:** Accumulating genetic and functional evidence implicate brain-derived neurotrophic factor (BDNF) and its precursor proBDNF with psychiatric disorders. Both proBDNF and BDNF are potent regulators of long-term depression and potentiation in hippocampal synapses. Previously, we have reported that sortilin, a member of the Vps10p-domain family of type-1 receptors, is a high-affinity receptor for proBDNF abundantly expressed in a variety of neurons including those of the hippocampus. Moreover, we have recently generated a mouse model where lack or overexpression of sortilin results in behavioral abnormalities related to psychiatric disorders. On this basis we hypothesized that genetic variants in the sortilin encoding gene SORT1 might influence susceptibility to psychiatric diseases, e.g. by affecting receptor expression or functionality. We have investigated this further by performing a candidate gene study evaluating the association of SORT1 with bipolar disorder.

**Methods:** The sample initially investigated consisted of 165 cases diagnosed with bipolar disorder according to the ICD-10-DCR and DSM-IV criteria and 1,100 controls. All individuals were ethnic Danes. Eleven tag SNPs and two non-synonymous SNPs in SORT1 were genotyped. After quality control nine SNPs, 165 cases and 1086 controls remained for further analyses. Additional five markers in SORT1 were imputed with high quality and included in the association analyses. Furthermore, a combined analysis of our Danish sample and the bipolar cases-control sample from the WTCCC1 (1,863 cases and 14,305 controls) were performed. In order to identify variants in SORT1 with functional effect associated with bipolar disorder we performed a follow-up study including 500 cases from Norway and Denmark diagnosed with bipolar disorder (ICD-10DCR and DSM-IV criteria) and 2000 Danish controls from the LuCamp project. The cases and controls were whole-exome sequenced (WES) with a coverage of 30x and 50x, respectively.

**Results:** Two genotyped and four imputed SNPs were nominal significantly associated with bipolar disorder in the Danish sample (lowest P=0.006). There was an overlap of eight SNPs between the Danish and the WTCCC sample. In the combined analysis five of the eight SNPs were significantly associated with bipolar disorder after Bonferroni correction (lowest P=0.00005). The results of the association analyses of variants in SORT1 identified by WES will be presented at XXth WCPG.

**Discussion:** We found five SNPs in SORT1 to be significantly associated with bipolar disorder in the combined analysis of the Danish and the WTCCC1 samples. This result together with our functional and behavioral findings in the transgenic mouse models suggests the involvement of sortilin in bipolar disorder. The associated SNPs had no identified functional effect however they could be linked to unidentified causal genetic variants affecting the expression or function of sortilin. These genetic variants will hopefully be identified in the ongoing analyses of the WES data and presented at XXth WCPG.

### Alterations in Brain Somatostatin Receptors in Rats Subjected to Chronic Mild Stress, Responding or Not Responding to Imipramine

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**Background:** The neuropeptide somatostatin (SST) is produced by neuroendocrine, inflammatory, and immune cells in response to ions, nutrients, neuropeptides, neurotransmitters, thyroid and steroid hormones, growth factors, and cytokines. Substantial evidence demonstrates that central SST systems are altered in various neuropsychiatric illnesses. For example, SST cerebrospinal fluid concentrations are decreased in patients with depression dementia, Alzheimer and Parkinson's diseases [1-4]. SST was an early target of investigation for depression as the major neuropeptide that inhibits the hypothalamic-pituitary-adrenal (HPA) axis, inhibiting corticotrophin releasing hormone (CRH) and the stress response [5]. Furthermore, somatostatin receptor 2 knockout studies have showed that sst2 knockout mice exposed to stress display a behavioral profile that is consistent with increased anxiety [6]. This suggests that sst2 activation may be anxiolytic and perhaps also have antidepressant effects. An antidepressant-like effect was observed following infusions of either sst2 or sst3 agonists [7]. In the context of data indicating the involvement of SST in the depression, we examined the levels of this peptide in the brains of animals responding or not responding to administration of imipramine in Chronic Mild Stress (CMS), which is well established animal model of depression.

**Methods:** Rats, male Wistar HAN (Charles-River), at weight 270-300g at the beginning of experiments were used. After two weeks of 1sucrose water solution intake training, the animals were divided into two groups: control and subjected to Chronic Mild Stress procedure [8]. The procedure consists of several mild stressors applied to animals for several weeks. During the experiment animals develop behavioral response that is considered as an anhedonia-like model of depression. The response was measured once a week by the means of 1 sucrose water solution consumption test. On that basis stressed animals after 2 weeks of CMS, could be easily divided into two groups: reactive one in which animals drank significantly less sucrose solution, and non-reactive in which animals drank the same amount of the solution as at the beginning of the procedure. In the reactive group, additionally to CMS, the imipramine started to be administered (10mg/kg i.p., once a day, for 5 weeks). Imipramine was also administered to not stressed, control group. The autoradiography's of somatostatin receptors were performed, using [125I]SST. The autoradiograms were obtained by the means of phosphorimager (Fuji BAS5000 system). The measurements of autoradiograms were performed with Fuji Image Gauge software. Differences and/or interactions assessed with two way ANOVA (with stress as factor).

**Results:** The highest concentrations of the [125I]SST binding sites were observed in following brain structures: cingulate cortex (Cg), medial habenula nucleus (MhB), molecular layer (MoL) or dentate gyrus (DG) of hippocampus. The level of SST receptors in the Cg was affected by IMI and stress, but it was significantly reduced only in the group non-reactive to the drug (interaction F(1,67)=15.78; F1(1,67)=15.42; F2(1,67)=16.88). Moreover, the Bonferroni posttest significantly differentiated stress group vs control (p<0,001). In MhB in the group responding on the IMI the interaction between factors was observed (interaction F(1,67)=6,65), while in the group non responding

on the drug the stress factor had statistically significant impact ( $F(1,57)=4.84$ ). The levels of SST receptors in the hippocampus structures (DG and MoL) both group response and non-response on drug administration) was affected by IMI (for DG  $F(1,58)=14.72$ ;  $F(1,64)=8.87$ ; for MoL  $F(1,68)=9.07$ ;  $F(1,65)=15.17$ ).

**Discussion:** Our results indicate that the levels of SST receptors may be influenced by both chronic stress and antidepressant treatment. However, only in the MhB we observed the increase of binding sites in the group of animals non-responding to IMI treatment. The MhB is a key link between the limbic forebrain and the midbrain [9]. Various studies have suggested that it plays a role in organizing or regulating many behaviors, including pain or stress responses. Since the prolonged exposure to pain and stress in humans has been associated with major depression, the results obtained in our studies indicate that somatostatin – which differentiate animals subjected to CMS responding and resistant to IMI administration – can be a good endogenous marker of depression.

## Poster 18

### Mutation Screening and Tests of Association in the Glutamate Transporter 1 (SLC1A2) Gene in Bipolar Disorder

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**Background:** SLC1A2 encodes the excitatory amino acid transporter2 (EAAT2) protein. Under normal conditions it is predominantly expressed in astrocytes in brain and spinal cord. SLC1A2 mediates rapid removal of the excitatory neurotransmitter glutamate from the synaptic cleft and is responsible for more than 90% of total glutamate uptake. This process modulates the termination of glutamatergic synaptic signalling and prevents excitotoxic effects of glutamate on post-synaptic neurones. Reduced SLC1A2 protein and mRNA expression has been found in the cortex of post mortem brains from bipolar disorder patients. Allelic association between SLC1A2 and bipolar disorder was detected, by us, in the UCL-STEPBD genome wide association study. In the UCL sample 10 markers within the gene showed p-values ranging from 0.00105 to 0.0376. Furthermore the SLC1A2 SNP rs4755404 has been found to be associated with attempted suicide.

**Methods:** In order to find aetiological changes responsible for bipolar disorder, SLC1A2 was screened for mutations by a combination of sequencing in 32 cases selected for having inherited a susceptibility haplotype and by High Resolution Melting (HRM) curve DNA strand separation analysis in 1000 cases.

**Results:** The HRM and sequencing data detected 38 SNPs including 12 non SNPs not previously identified. 11 of these SNPs were intronic and 15 of them had an allele frequency of less than 0.01 or had not been reported before. Of these 15, seven are in the coding exons and eight are in regulatory regions.

**Discussion:** Full genotyping of the of the potential disease mutations will be carried out in our sample of 1000 cases and 1000 controls. In addition, subsequent functional experiments will be done to understand how these changes may affect the SLC1A2 protein transport and its pathway.

## Poster 19

### Copy Number Variants in Major Depression Disorder: Looking at Concordance within Affected Sibling Pairs

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**Background:** Major depressive disorder is a complex psychiatric disease with a substantial genetic component, with recurrent, severe and early-onset subsets showing the highest heritability estimates. However, the specific genes involved still await to be identified. Copy number variants (CNVs) have been found to be associated with several neuropsychiatric disorders. The three genome-wide association studies (GWAS) looking at CNVs in depression performed to date have shown inconclusive results. The aim of our study is to examine whether CNVs involved in other psychiatric disorders have an impact on the pathogenesis of depression.

**Methods:** The sample consists of 71 affected sibling pairs from the Depression Network (DeNT) Study. DSM-IV/ICD-10 recurrent unipolar depression of moderate or severe degree was ascertained using the SCAN interview. Subjects were genotyped using Agilent CGH microarray platform. We studied selected genomic regions located on chromosomes 3, 6, 10, 12, 15, 20 and 22, which have already been implicated in other psychiatric disorders. CNV data is being analysed using a sib-pair design to test for familiarity and associations with depression.

**Results:** We expect some of the CNVs to be inheritable and segregate with the disease.

**Discussion:** We examined CNVs that were associated with other psychiatric disorders to find whether they were involved in depression. To our knowledge, this is the first study that investigates the role of CNVs using a sib-pair design.

## Poster 20

### Genetics of Suicidal Behavior and Intermediate Phenotypes

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**Background:** Suicidal behavior is a major health problem worldwide. The risk of suicide-related behavior is supposed to be determined by a complex interplay of sociocultural factors, psychiatric history, personality traits, and genetic vulnerability. This view is supported by adoption and family studies indicating that suicidal acts have a genetic contribution that is independent of the heritability of Axis I and II psychopathology. There is strong evidence for a heritability of suicidal behaviour as shown by family, twin- and adoption studies. Several studies suggest heritability between 45 and 55.

**Methods:** In a sample of 3000 individuals a genome wide association study was performed on intermediate phenotypes related to the diathesis to suicidal behavior, namely aggressive impulsive traits.

**Results:** Top hits were investigated for association with suicidal behavior in a sample of 4200 individuals. These were then replicated in a new sample of over 4000 cases and controls.

**Discussion:** These new results will be presented in the context of already existing GWA studies on suicidal behavior and personality traits.

## Poster 21

### A Novel CIS-regulating Polymorphism of the Brain-derived Neurotrophic Factor Gene Expression Moderates the Susceptibility to Depressive Disorders

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**Background:** Empirical evidence strongly suggests that adverse psychosocial experiences like childhood abuse and neglect as well as stressful life conditions in adulthood are strongly associated with an increased risk of depressive disorders. The research of the interaction between environmental and genetic factors (G x E) offers new insights into the vulnerability to MDD. One major candidate gene for GxE in depressive disorders is the brain-derived neurotrophic factor (BDNF). BDNF was found to promote neuronal survival, neurogenesis and synaptic plasticity. It has been shown that acute and chronic experimental stress can decrease BDNF expression in the brain of animals. Numerous studies have investigated the BDNF Val66Met polymorphism (rs6265). The Met allele of the BDNF Val66Met polymorphism was found to decrease the activity dependent secretion of BDNF by altering the intracellular trafficking and was associated with lower hippocampal N-acetyl-aspartate levels and with impaired episodic memory. Despite of the well documented neurobiological importance of BDNF in the adaptation to stressful events the known genetic risk markers of BDNF are less promising. Many genetic association studies on rs6265 have yielded inconsistent results.

**Methods:** Whole blood mRNA concentrations of BDNF transcripts were associated with 188 SNPs spanning a 100kb distance upstream and downstream the BDNF gene in 976 subjects (general population) from the SHIP-TREND study. 21 eQTL top markers were tested in a gene-environment (G x E) interaction model investigating n=2143 Caucasian subjects from an independent study (SHIP-LEGEND) who completed the Beck Depression Inventory (BDI-II) and the Childhood Trauma Questionnaire.

**Results:** Several highly linked top markers significantly moderated the risk of depressive symptoms. Abused subjects (n=6) carrying rare Met/Met genotype of rs6265 were fully captured by the CC genotype of rs7949590 (n=33). The presence of the CC genotype in abused subjects was associated with a BDI-II mean score of 14.3 (95% CI 11.4-17.2) compared to abused TT/CT carriers with a BDI-II mean score of 8.8 (95 % CI 7.5 – 10.1; p=0.001).

**Discussion:** Our results point to the clinical relevance of highly linked, BDNF mRNA expression modifying SNPs in the moderation of depressive symptoms in subjects with childhood abuse. Those SNPs exceed by far the G x E effect of the frequently investigated Val/Met rs6265 in our study.

## Poster 22

### Heritability and Linkage Analysis of Temperament in Bipolar Disorder

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**Background:** The many attempts that have been made to identify genes for bipolar disorder have met with limited success, which has generally been attributed to genetic heterogeneity and small gene effects. However, it is also possible that the categorical phenotypes used in genetic studies of bipolar disorder are not the most informative or biologically relevant.

**Methods:** We have explored aspects of temperament as quantitative phenotypes for bipolar disorder through the use of the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Autoquestionnaire (TEMPS-A), which is designed to assess lifelong, milder aspects of bipolar symptomatology and defines five temperaments: hyperthymic, dysthymic, cyclothymic, irritable, and anxious. We first assessed the heritability of these five temperaments in 101 families collected for genetic studies of bipolar disorder and subsequently performed a genome-wide linkage study in the subset of 51 families for which genetic data was available using the regression algorithm in Merlin.

**Results:** All five temperaments were found to be significantly heritable in this sample with heritabilities ranging from 20% for the hyperthymic temperament to 62% for the irritable temperament. Four temperaments showed suggestive evidence for linkage with LOD scores >2.2 to at least one chromosomal region: chromosomes 5p15 and 6q27 for hyperthymic, chromosomes 3p22 and 16q32 for dysthymic, chromosome 7q32 for cyclothymic, and chromosome 2p24 for irritable.

**Discussion:** While not genome-wide significant, these results suggest that aspects of temperament may define subtypes of bipolar disorder that are more genetically homogenous, which may aid in the identification of predisposing genetic variants.

### Validity of Two and Three Onset Age Groups in Bipolar Disorder in Three Independent European Samples: Clinical Significance

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**Background:** Age of onset (AO) is a phenotypic trait with a strong genetic component and potentially useful in the molecular analysis of bipolar I disorder (BPI). AO was proposed as a clinical course specifier for the future DSM-V (Colom & Vieta, 2009). In this context the debate about the component number of an empirical AO distribution and about the cut-off point for defining the early onset (EO)/ late onset (LO) of BPI revives. Some studies revealed a three AO group model as the best fit for the BPI onset (Bellivier et al, 2003; Hamshere et al, 2009), while other studies supported a two AO group model (Kennedy et al, 2005; Ortiz et al, 2010, Javaid et al, 2011). Similar to our previous results (Grigoriou-Serbanescu et al, 2010), Tozzi et al (2011) found the three AO group model fitting marginally better than the two AO group model and addressed the question of the spurious admixture given by the marked skewness of the AO distribution (MacLean et al, 1976). AO groups seem not to have distinct clinical features. In a sample of 1665 BPI patients Baldessarini et al (2012) found that psychiatric family history had similar frequency over the AO range 12-40 years and there was no significant difference in terms of psychosis, rapid cycling, hospitalization, suicide attempts, and any comorbidity between juvenile onset ( $AO \leq 18$ ) and adult onset ( $AO 19-55$ ). Other studies could also not prove sharp clinical differences among EO, middle onset, and LO and especially between the middle onset and the LO (Hamshere et al, 2009). When comparing various traits, even studies finding the best fit for three AO groups contrast only the EO and LO groups. Our objective was to see whether a three group AO distribution is suitable for any BPI sample of acceptable size and whether a significant difference exists between a three AO group and a two AO group distribution.

**Methods:** We analyzed three samples of BPI patients recruited from consecutive hospital admissions in Germany (DE) (N = 1231), Poland (PL) (N = 354), and Romania (RO) (N = 581) (total = 2166 patients). We applied commingling (admixture) analysis as implemented in the SEGREG program of S.A.G.E.v6.1 software (2009). The commingling analysis was performed using the Box-Cox power transformation of the raw data since the AO distribution was skewed in all three samples and in the combined RO-DE sample. The PL sample was not combined with the others because of having a much higher AO mean. Mixtures of power-normal distributions were fitted by maximum likelihood. The model best fitting the data was chosen based on the smallest value of the Akaike's A information criterion (AIC).

**Results:** In all three samples the AIC-scores for the two-mean and the three-mean models were very close and the  $\chi^2$  for the difference between  $-2\ln$  (Likelihood) of the models indicated that they were not significantly different under power transformation of AO. In the combined RO-DE sample (N = 1812) the best models fitting the data were obtained under log transformation of AO (the log transformation is a particular case of power transformation.) The three-mean model was marginally better than the two-mean model ( $\chi^2 = 6.06$ ,  $df = 2$ ,  $p = 0.05$ ) (Fig. 1). Both in the three independent samples and in the

combined RO-DE sample the means and SDs of the EO group were not significantly different in the two-mean model and the three-mean model. For the RO-DE sample: mean EO = 20.10 years, SD = 5.83, case proportion 0.64 under the two-mean model; mean EO = 19.27 years, SD = 5.37, case proportion 0.54 under the three mean model. The three AO mean model fitted better the empirical AO distribution in a sample with younger global mean AO like the RO sample, while the two AO mean model was better supported in samples with older global AO means like the DE and the PL samples.

**Discussion:** Our data show that a three AO mean and a two AO mean model fit equally well the empirical AO distribution of BPI. Since the mean and the case proportion of the EO group may largely vary by sample and the AO groups revealed by admixture analysis overlap yielding a high percent of missclassification, perhaps less sophisticated methods would be helpful for selecting the EO group using sample independent landmarks. The age limit of adolescence (18 years) might be the cut off for the very early onset and the age by which 50% of the patients fall ill (age 25) (Merikangas et al, 2011 - world sample of 61,000 cases) might be a sample independent cut off for EO definition. In the RO-DE and the PL samples the mean + 1 SD of the EO group approached 25 years. Similar to Baldessarini et al (2012), 54% of the patients had onset by age 25 in our total sample of 2166 cases. Although the existence of three AO groups in BPI is certain, their clinical usefulness is doubtful.

### Replication of Functional Serotonin Receptor Type 3A and B in Bipolar Affective Disorder: A European Multicenter Study

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**Background:** Serotonin type 3 receptors (5-HT<sub>3</sub>) are involved in learning, cognition, and emotion, and have been implicated in various psychiatric phenotypes. However, their contribution to pathomechanisms remains elusive. Three SNPs in the HTR3A and HTR3B genes have been associated with bipolar affective disorder (BPAD) in pilot studies, and all of them are of functional relevance.

**Methods:** We performed a European multicenter study to confirm previous results and provide further evidence for the relevance of these SNPs for neuropsychiatric disorders. This involved analysis of the distribution of the three SNPs among 1804 BPAD cases and 2407 healthy controls.

**Results:** A meta-analysis revealed a pooled odds ratio of 0.881 ( $P = 0.009$ , 95% CI = 0.802 – 0.968) for the non-synonymous functional SNP HTR3B p.Y129S (rs1176744), thereby confirming previous findings. In line with this, the three GWAS samples BOMA-BD, WTCCC-BD and GAIN-BD, including more than 3500 patients and 5200 controls in total, showed an over-representation of p.Y129 in patients. Remarkably, meta-analysis revealed a P-value of 0.048 (OR = 0.934, fixed model). Expression analyses to gain further insights into the distribution of HTR3A and HTR3B mRNA in the human brain detected HTR3A and HTR3B in all investigated brain tissues with the exception of the cerebellum, and large differences in the A:B subunit ratio were observed. Interestingly, expression of the B subunit was most prominent in the brain stem, amygdalae, and frontal cortex, regions of relevance to psychiatric disorders.

**Discussion:** In conclusion, the present study provides further evidence for the presence of impaired 5-HT<sub>3</sub> receptor function in BPAD.

### The Risk Variant in ODZ4 for Bipolar Disorder Impacts on Amygdala Activation during Reward Processing

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**Background:** The largest GWAS analysis of bipolar disorder (BD) recently identified a new risk variant in ODZ4 (rs12576775). This gene is located on chromosome 11 and encodes the teneurins. The major functions of these genes are expected to be cell surface signaling and neuronal pathfinding. We investigated whether this gene influences altered brain functions described in BD such as increased BOLD responses in the amygdala in response to reward (Linke, King et al. 2012) and dysfunctions in a ventral-limbic brain network during emotional stimuli (Wessa and Linke 2009).

**Methods:** With an imaging genetics approach we analyzed the impact of ODZ4 rs12576775 (G / A alleles) on processing emotion and reward in the amygdala and the striatum in 485 healthy adolescents (aged 14 years) from the Imaging Genetics study (IMAGEN; (Schumann, Loth et al. 2010)). Three-hundred-and-forty-seven subjects were homozygous A-allele carriers, 126 were heterozygous, and 12 were homozygous G-allele carriers (Hardy-Weinberg Equilibrium:  $p = 1.00$ ). Given the small number of GG individuals, GG and GA carriers were treated as one group. Both groups were comparable with respect to age, sex and intelligence. For the analysis of processing emotion and reward we utilized a Face-task (Grosbras and Paus 2006) and a modification of the monetary incentive delay (MID) task (Knutson, Adams et al. 2001). Scanning was performed with 3T whole body MRI systems and the data were analyzed with SPM8.

**Results:** Carriers of the risk variant in ODZ4 showed a significantly increased BOLD response in the amygdala during both reward sensitivity (win versus no win in the feedback phase) and reward expectation (win versus missed time criterion in the feedback phase) components of the MID task ( $p_{FWEcorrected} \leq 0.05$ , see Figure 1). No significant differences between the two groups were found in the amygdala and the striatum during the Face-task.

**Discussion:** We could show that the ODZ4 rs12576775 risk variant contributes to an increased BOLD response in the amygdala in a reward sensitivity and reward expectation paradigm, with carriers of the risk allele more strongly involving the amygdala in reward processing. The absence of significant group differences in emotion processing (Face task) suggests that the risk variant does not affect this capacity. Our results are in line with the findings of (Yacubian, Glascher et al. 2006) which suggest that an imbalance of amygdala and striatum activation contributes to mood disorders.

**Association Study of DRD2 Polymorphisms and Affective Disorders in Case-control and Family Based Study of Patients with Bulgarian and Roma Origin**

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**Background:** The dopamine D2 receptor gene (DRD2), located on chromosome 11q, is encoding the D2 subtype of the dopamine receptor and has a crucial role for dopamine signaling and in the mechanism of action of antipsychotic medications. Genetic variants in DRD2 gene have been widely investigated in relation of personality traits and psychiatric illnesses, with the presumption of involvement of dopamine neurotransmission in the regulation of reward processing, cognition, addiction behavior and response to different life stressors. However, findings from linkage and association analyses are mixed and inconsistent across various studies.

**Methods:** The aim of this study is to investigate the association of DRD2 gene variations and the risk for affective disorder (AD). We analyzed four single nucleotide polymorphisms in DRD2 gene in case-control sample of 191 cases with BPI and BPII affective disorder, 174 cases with Major Depressive Disorder (MDD) and 139 healthy controls of Bulgarian origin. A family-based association study in 175 nuclear families of Bulgarian and Roma origin was also done. All subjects were diagnosed according to DSM-IV criteria. Genotyping of the four selected SNPs, rs6277, rs12800853, rs7350522, rs6589377 was performed using TaqMan assay (Applied Biosystems, Foster City, California). The selected variants are sparse across DRD2 gene, rs6277 is situated in exon 7, leading to a synonymous substitution, and affecting mRNA stability, and the others are in intronic regions. Statistical analyses were conducted using PLINK (Purcell et al. 2007). Association of each SNP with AD was tested under two distinct affection models, within the narrow model individuals were considered affected when diagnosed with BPI, BPII and BP-NOS. Broad model consisted of all subjects from the narrow model and patients with MDD, all other participants were classified as unknown. The allele frequencies of the investigated polymorphisms did not differ significantly between subjects from Bulgarian and Roma origin, and the family sample was analyzed together. Haplotype analyses were performed using the sliding window approach in PLINK.

**Results:** No deviation from Hardy–Weinberg equilibrium was detected for all four variants. Comparing the allele and genotype frequencies between the cases and controls in our sample no statistical significant difference was detected under both affection statuses. Haplotype analysis showed borderline significance only for haplotype rs6277|rs128008|rs735052|rs658937 – GCGG (p= 0.052, CHISQ= 3.76). This haplotype is more rare among the patients from narrow affection status, implying protective effect. In our family sample no preferential transmission of the alleles of rs6277, rs12800853, rs7350522, has been detected. For rs6589377 we observed preferential transmission of allele A in affected offspring both for narrow and broad affection statuses (p= 0.039, p= 0.044 respectfully). Haplotype analysis of the families showed that rs6277|rs128008|rs735052 - GCG haplotype is associated (p=0.040) and more common among families from the broad affection status model.

**Discussion:** The effect of association of allele A of rs6589377 is mainly driven by families of Roma origin, since separate TDT analysis of Bulgarian families didn't show preferential transmission of any of the alleles of this variant. Replication of the finding in larger group with Roma origin is necessary to validate this association. Recently rs6589377 has been associated with nicotine dependence in Han Chinese population (Wei et al. 2012), and it was among the variants showing globally significant associations with alcohol dependence in large case-control and family based association study (Yang et al. 2007). The molecular mechanisms underlying substance dependence and affective disorder might be similar. Despite the limited power of the sample, the results show that variations in the DRD2 gene do not have a major contribution as predisposing factor for AD. However influence on specific traits and endophenotypes, especially in the Roma population is possible and further investigations are warranted.

**A Functional Kozak Sequence Mutation in the GRM3 Glutamate Receptor Gene is Associated with Bipolar Disorder**

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**Background:** The GRM3 gene encodes the mGluR3 protein which is a G protein-coupled receptor and acts by inhibition of adenylate cyclase and reducing cyclic AMP production. Group II metabotropic glutamate receptors are involved in learning, memory, anxiety and the perception of pain. The GRM3 gene has been investigated in bipolar affective disorder as part of several GWA studies. In a collaborative study the SNP rs2237563 was the most significantly associated marker ( $P = 3.85 \times 10^{-5}$ ). In the Psychiatric Genome wide association Consortium bipolar study the most associated marker was rs17161018 ( $P = 0.00093$ ). Four alternatively spliced transcripts of GRM3 have been previously reported in human brain. Clinical studies of the effect of receptor agonists and antagonists of mGluR2/mGluR3 have been carried out for the treatment of anxiety, schizophrenia, depression and withdrawal from morphine and nicotine.

**Methods:** DNA sequencing and high resolution melting curve analysis followed by genotyping of the GRM3 gene was carried out in 1099 bipolar affective disorder cases and 1152 normal comparison subjects. Electrophoretic mobility shift assays (EMSAs) were performed with rs148754219 G/A allele probes and SH-SY5Y cell nuclear extract. The ability of the rs148754219 variant to modulate transcription and translation of GRM3 was tested using luciferase reporter vector constructs in HEK293 and SHSY5Y cells. Quantitative RT-PCR was used for mRNA expression analysis of the wild-type and mutant constructs in HEK293 and SHSY5Y cells.

**Results:** Mutation detection and Association analysis in GRM3: A base pair mutation (rs148754219) was found in exon 1 of the GRM3 gene, two bases before the translation start codon of a GRM3 receptor isoform (forming part of its Kozak sequence). The mutation was associated with bipolar disorder ( $P = 0.0046$ ,  $OR = 4.2$ ). Functional characterization of the rs148754219 G/A variant The rs148754219 G/A variant impacts transcription factor binding. EMSAs of the rs148754219G/A variant on transcription factor binding showed that the mutant allele caused gel shifts compared to the wild-type G sequence. This effect was completely abolished by the addition of a 200-fold excess of unlabelled A probe but not by unlabelled competitor wild-type G probe, indicating that the DNA-protein binding was specific for the mutant allele. The effect of rs148754219 G/A variant on promoter activity A 61% reduction in luciferase expression with the mutant clone was observed in SH-SY5Y cells ( $P < 0.001$ ). No difference was observed in HEK293 cells. Post-transcriptional or translational effects of the rs148754219 G/A variant. In both cell-lines, the mutant rs148754219 constructs led to complete elimination of luciferase activity. This effect was not caused by differences in the level of transcription of the mutant and wild-type constructs.

**Discussion:** The bipolar disorder associated variant rs148754219 is located in the first exon of GRM3, which is transcribed and not translated in the main isoform of the gene (NM\_000840). Based on bioinformatic analysis and the EMSA results we provide evidence that the mutation rs148754219 effects gene expression via the basal transcription apparatus. The reporter gene experiments indicated that this mutation may have independent effects both at the level of transcription and at the level of post-transcription or translation. For one of the GRM3 isoforms the SNP rs148754219 is located 2 base

pairs upstream of the translation initiation codon that forms part of the Kozak consensus motif. Mutations in Kozak sequences have been reported in Grave's disease and thalassaemia intermedia. These mutations have been shown to influence the translation efficiency of their respective genes. Confirmation of these findings could lead to the creation of a personalised treatment approach for a genetic subtype of bipolar disorder with GRM3 receptor agonists, antagonists and allosteric modulators.

## Poster 28

### Gene X Environment Interaction in Depressive Disorders: Which Environment of Risk?

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**Background:** Gene x environment interaction studies in depressive disorders have renewed interest in the investigation of the major environmental risk factors, particularly childhood traumas, that may increase the risk of a clinical episode of major depression in predisposed individuals. Many studies have been published, but to date, the effective role of childhood stressors is unclear.

**Methods:** To clarify the role childhood stressors in mood disorders, we performed a systematic review and meta-analysis of the literature focused on exposure to different childhood stressors, including sexual, physical, and emotional abuse, early significant loss and familiar adversity. Moreover, we preliminarily analyzed 250 patients affected by both mood and anxiety disorders evaluated for stressful life events in childhood, the year before the onset of the first episode and current episode. Previously published papers were retrieved in literature through common databases such as PubMed and ISI Web of Science using keywords such as childhood trauma, sexual, physical, emotional, psychological abuse and neglect. The reference lists of retrieved studies were employed to detect further studies relevant for the topic. Meta-analysis of studies was conducted by the RevMan software. Our sample was composed by 250 subjects satisfying criteria for a Mood disorder or Anxiety disorder consecutively admitted to our psychiatric inpatients and outpatients Units of the Institute of Psychiatry of Bologna, Italy. This sample was evaluated for stressful life events in childhood, the year preceding illness onset and current episode according to Brown & Harris interview. A number of other clinical and demographic variables were collected and considered in the analyses. Statistical analyses were conducted as appropriate employing the Statistica Software (StatSoft Italia), taking into account the distribution of variables and recurring to both linear and multivariate models.

**Results:** According to our preliminary meta-analysis, sexual abuse in childhood is a major risk factor for the development of a mood disorder in adulthood. Physical and emotional abuse, neglect, violence within the family also showed strong associations, while separation/divorce of parents or early losses showed only slight associations with the risk to develop a clinical episode of major depression. In our sample of patients suffering from depressive-anxiety disorders, we found strongest associations between childhood stressors and severity of disorders (in terms of hospitalization, illness duration, clinical picture, comorbidity for other axis I and II disorders) than stressful life events at onset and preceding current episode. However, stressful life event at onset had a significant role in the development of the first episode as well.

**Discussion:** These findings support the role of severe childhood trauma in the etiology of mood disorders. Therefore, gene x environment studies should focus more on severe childhood trauma instead of other mild or late in life stressful events. Moreover, it is likely that subjects having exposed to childhood stressors are more likely to develop clinical symptoms of depression and anxiety if exposed to stressful life events in adulthood as well.

## Poster 29

### Genetic Variation in Fkbp5 is Associated with the Extent of Stress Hormone System Dysregulation in Major Depression

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**Background:** The FK 506 binding protein 51 or FKBP5 has been implicated in the regulation of glucocorticoid receptor (GR) sensitivity, and genetic variants in this gene have been associated with mood and anxiety disorders. GR resistance is one of the most robust biological findings in major depression, but the extent of GR resistance may be moderated by FKBP5 polymorphisms.

**Methods:** FKBP5 mRNA expression in peripheral blood cells (baseline and following in vivo GR-stimulation with 1.5 mg dexamethasone p.o.) was analyzed together with plasma cortisol, ACTH, dexamethasone levels and the FKBP5 polymorphism rs1360780 in 68 depressed patients and 87 healthy controls. To further evaluate the function of the HPA-axis, we employed the combined dexamethasone/corticotropin-releasing hormone (dex/CRH) test in a subgroup (n=64/45).

**Results:** We observe a significant interaction between disease status and FKBP5 risk allele carriers status (minor allele T),  $p=0.02$  on GR-stimulated FKBP5 mRNA expression. Patients carrying the risk T allele, but not patients carrying the CC genotype showed a reduced induction of FKBP5 mRNA. This FKBP5 polymorphism by disease status interaction was paralleled in the cortisol and ACTH suppression following dexamethasone, with a reduced suppression only observed in depressed patients carrying the T allele.

**Discussion:** Only depressed patients carrying the FKBP5 rs1360780 risk allele show significant GR resistance compared to healthy controls, as measured by dexamethasone-induced FKBP5 mRNA induction and suppression of cortisol and ACTH. This finding suggests that endocrine alterations in depressed patients maybe more pronounced in specific genetic subgroups. Further epigenetic mechanisms are investigated and will be presented at the meeting.

### Genetics of Emergent Suicidality during Antidepressive Treatment: Data from a Naturalistic Study on a Large Sample of Inpatients with a Major Depressive Episode

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**Background:** Factors contributing to treatment-emergent suicidal ideation (TESI) using antidepressants have been in the focus of recent research strategies. We investigated previously established clinical predictors of TESI and combined these with several polymorphisms of candidate genes in patients with major depressive disorder.

**Methods:** Common polymorphisms involved in the tryptophan hydroxylase 1 (TPH1) and 2 (TPH2), serotonin transporter, monoamine oxidase A (MAOA) and brain-derived neurotrophic factor (BDNF) were investigated in a naturalistic inpatient study of the German research network on depression. We compared patients showing TESI with non-TESI suicidal patients and with non-suicidal patients using univariate tests to detect relevant factors, which were further tested in logistic regression and CART (Classification and Regression Trees) analyses.

**Results:** Of the 269 patients, TESI occurred in 22 patients (17 female), 117 patients were defined as non-TESI suicidal patients, and 130 patients were classified as non-suicidal. When comparing cases with both control groups we found the TPH2 rs1386494 (C/T) polymorphism to be moderately associated with TESI. Stronger associations were found when patients displaying TESI were compared with non-TESI suicidal patients ( $p = 0.086$  after correction for multiple testing). This polymorphism remained the only significant genetic factor in addition to clinical predictors in logistic regression and CART analyses. CART analyses suggested interactions with several clinical predictors. Haplotype analyses further supported a contribution of this polymorphism in TESI.

**Discussion:** The TPH2 rs1386494 (C/T) polymorphism might contribute to the genetic background of TESI. This polymorphism has been previously associated with committed suicide and major depressive disorder. The small number of cases warrants replication in larger patient samples. Lack of a placebo control group hampers definite conclusions on an association with antidepressive treatment.

### Kynurenines in Mood Disorders: Is there a Role for Genetics?

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**Background:** A consistent finding in major depressive disorder (MDD) research is dysfunction of the immune system. One of the relevant metabolic pathways in this regard is the kynurenine pathway. In patients with major depression, an imbalance between neuroprotective and neurotoxic arms of the pathway with lower plasma kynurenine acid concentration was demonstrated. Therefore, we investigated (1) Single Nucleotide Polymorphism (SNP) and haplotype association of candidate genes of kynurenine-3-monooxygenase (KMO) and kynurenine aminotransferase-2 KAT-III enzymes from this metabolism and (2) the functional role of KMO and KAT-III gene.

**Methods:** (1) KMO and KAT III SNPs and haplotype association analysis was performed in 338 (266 major depression and 72 bipolar depression) unrelated Caucasian patients with major depressive episodes and 310 age, gender and ethnicity matched controls. (2) KMO and KAT III SNPs analysis was performed and kynurenine metabolites were measured in 40 patients (up to now) treated with pro-inflammatory cytokines interferon- $\alpha$ . This part of the study is in progress.

**Results:** (1) In sliding window analyses using PLINK of the haplotypes of KAT III, all windows which include the first SNP (rs12729558), the overall haplotype distribution (OMNIBUS) was significantly different between patients with a major depressive episode and control for all windows, with p-values ranging between  $1.75 \times 10^{-5}$  and 0.006. This is due to the haplotype CGCTCT (referring to 6 SNP window analysis), which is found in about 5.7 of patients and 1.9 of healthy controls. It was due to CGCTCT haplotype and the frequencies of this haplotype in both bipolar patients and patients with major depression showed significantly higher than the control population ( $p=0.001$ ). Nonparametric correlations with risk-haplotype were significant for HRDS-subscale anxiety (psychological) ( $\tilde{r}=0.158$ ,  $p=0.01$ ) and anxiety somatic ( $\tilde{r}=0.191$ ,  $p=0.01$ ). A group comparison by Mann-Whitney-U between the risk and non-risk patients yielded the same results: Group differences were significant only for HRDS-subscale anxiety (psychological) ( $U=2,623,000$ ,  $p=0.007$ ) and anxiety somatic ( $U=2,395,000$ ,  $p=0.001$ ). (2) In association with the metabolites levels, one SNP from KMO gene and 3 SNPs from KAT III gene showed weak association with corresponding metabolites.

**Discussion:** These above findings indicate the possible role of genetics in immune-kynurenine pathways interaction in mood disorders.

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### High-resolution Melting Analysis of Regulatory Regions of Calcium Channel Genes

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**Background:** Genetic heritability for bipolar disorder is estimated at 60-80. However, Bipolar Disorder (BPD) genome wide association studies (GWAS) have yet to implicate specific mutations in risk genes. CACNA1C, a member of the voltage dependent calcium channel (VDCCs) family of genes, has been associated with BPD in a number of studies searching for risk genes to date. VDCCs are involved in a variety of biological pathways including brain circuits and cognitive processing, and mediate the influx of Ca<sup>2+</sup> into cells. They are comprised of the core  $\alpha$ -subunit and a number of auxiliary subunits, known as  $\beta$ ,  $\alpha 2$ ,  $\delta$  and  $\gamma$ , in a 1:1:1:1 ratio. CACNA1C, CACNB3 and CACNA1D genes encode for subunits of L-type calcium channels,  $\alpha 1C$ ,  $\beta 3$  and  $\alpha 1D$  respectively. CACNG4 encodes for a type 1 transmembrane AMPA receptor regulatory protein (TARP). TARPs are involved in the overall control of both the trafficking and channel gating of AMPA receptors. Current exome capture techniques do not generally incorporate regulatory regions such as the promoter and 5' or 3' UTR in their target regions. There is therefore the potential that bipolar disorder associated structural and non-coding variants in these regions are being overlooked using this method.

**Methods:** High Resolution Melting (HRM) curve analysis was employed to screen the promoter region and first exon of CACNA1C, CACNB3, CACNA1D and CACNG4 in 1000 BPD cases. KASPar<sup>®</sup> genotyping was used to determine SNP frequency in amongst 1000 cases and 1000 controls.

**Results:** Using HRM and sequencing analysis, 6 SNPs were detected in the promoter regions of these genes. Five of these SNPs have been previously reported in studies, with 1 SNP located in the promoter region of CACNA1C that had not been previously annotated on dbSNP or the 1000 genomes project. Genotyping data of this SNP in 1000 cases and 1000 controls showed that the SNP was present with high frequency in both cases and controls.

**Discussion:** Several calcium channel genes have been significantly associated with bipolar disorder, however to date none of the regulatory region SNPs that we have detected are associated with bipolar disorder.

## Poster 33

### USF1 Regulates Sleep and Depression in Humans

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**Background:** Epidemiological studies show association between sleep duration and lipid metabolism. Our aim was to characterize gene variants that regulate both metabolism and sleep. One such candidate is USF1 that regulates the expression of several lipid genes and has a similar structure as canonical circadian transcription factors. We studied the polymorphisms in USF1 gene region with sleep duration and coping with circadian stress in Finnish population based samples combined with gene expression and MRI analysis.

**Methods:** The known USF1 variants that had previously been associated with lipid traits were studied with sleep and coping with circadian stress. The analyses were performed in a Finnish population based sample with no sleep problems (N=1085) and Finnair workers (N=1415). RNA expression from mononuclear leucocytes was measured in additional 584 individuals. Finally the variants were studied in resting state fMRI data from 176 healthy individuals.

**Results:** The genetic analysis identified that the USF1 polymorphisms associated with sleep duration in the healthy individuals. The same variant associated with coping with circadian stress in Finnair workers. Gene expression analysis showed that those individuals carrying minor allele had higher USF1 expression. Finally, fMRI analysis identified brain regions that correlated with the USF1 genotypes.

**Discussion:** Our results show that the allelic variants of USF1 associate with sleep duration and coping with circadian stress. The finding may reflect the shared roots of sleep and metabolism. The shared genetic background and the effect of USF1 in functional connectivity of neuronal networks may at least partially explain the mechanism behind the well-established connection between diseases with disrupted metabolism, sleep and depression.

### P2RX7 Reveals Association to Alcoholism and Comorbid Psychiatric Disorders in a Population-based Sample

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**Background:** Several linkage and association studies have found a susceptibility locus on chromosome 12q24 for mood disorders related personality traits (Serretti and Mandelli, 2008). The genes in 12q24 include P2RX7 (purinergic receptor P2R, ligand-gated ion channel, 7), which codes for an ATP-gated non-selective cation channel. Previously, we found association of two non-synonymous allelic variants of P2RX7 gene, rs208294 (His155Tyr) and rs2230912 (Gln460Arg), consistently with mood disorders in three clinical cohorts (OR~1.3), in particular in cases with familial risk (OR~1.4) and comorbid anxiety and alcoholism (OR~1.5). Furthermore, the risk variants also predicted clinical course, the proportion of time that individuals with mood disorders spend ill in prospective follow-up (“time ill”), (Soronen et al., 2011), which was found to be mediated through neuroticism and anxiety in our subsequent study (Mantere et al, in press). Here, we hypothesized that allelic variants of P2RX7 could increase risk for depression anxiety disorder or alcoholism, as well as their comorbid states, also at population level.

**Methods:** In a Finnish population-based Health 2000 dataset, 5788 people (males N=2651, females N=3137) were interviewed for diagnosis of MDD, anxiety and alcoholism during the last 12 months based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria. We examined association of cases with MDD (males N=127, females N=272), anxiety disorder (males N=186, females N=371) or alcohol use disorder (males N=487, females N=113) to four single nucleotide polymorphisms (SNPs) in P2RX7. SNPs were genotyped with MassArray® iPLEX technology (Sequenom®, San Diego, CA). The genetic association analyses were performed using a logistic-regression model with max (T) permutation procedure as implemented in the PLINK software package, web-based version 1.07 (Purcell et al., 2007). The results were analyzed separately in males and females including age as covariates.

**Results:** Our results show association of an allelic variant of P2RX7 gene in males with alcoholism (P=0.0005, permutation-based corrected empirical P=0.0025; OR=1.42). The minor allele frequency of P2RX7 variant was higher in males with comorbid depression, anxiety disorder, and alcoholism (OR=1.69). However, no prominent associations were found among females with depression, anxiety or alcoholism (P>0.3).

**Discussion:** These results provide evidence that gene involved in glutamatergic neurotransmission, P2RX7, is one of the susceptibility factors behind psychiatric disorders. The results may indicate sex-dependent differences in the genetic background of alcoholism. Thus, the results have to be replicated.

### Analysis of the Intron 2 VNTR Polymorphism (STIN2) of the Serotonin Transporter Gene (SLC6A4) in a Sample of Bulgarian Outpatients with Recurrent Major Depressive Disorder

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**Background:** A positive association with major depressive disorder (MDD) has been reported for some SLC6A4 genetic variants among different ethnic populations. The VNTR polymorphism within the second intron of SLC6A4 gene (STin2) has been demonstrated to act as a transcriptional regulator of SLC6A4 expression in an allele-dependent manner, with higher enhancer-like properties supposed for the 12/12 allele than for the 10/10 allele. The aim of our study was to investigate genotype and allele frequencies of STin2 VNTR polymorphism in a sample of Bulgarian outpatients with recurrent depression compared to healthy controls.

**Methods:** A total of 237 unrelated ethnic Bulgarians (100 patients and 137 controls) who signed an informed consent for genetic analysis, were recruited in the study. The research protocol was approved by the Ethical Committee of Medical University-Pleven. Hamilton Rating Scale for Depression (HAM-D21) was applied by an experienced psychiatrist on first patient visit for severity assessment of the current MD episode. A semi-structured interview and outpatient protocols revision were used for depression history documentation. The matched controls were screened for psychiatric, neurological and other somatic illness and formulated inclusion and exclusion criteria were applied for the group selection. Genomic DNA was isolated from whole blood and PCR amplification was performed according to adapted protocol (Wendland, et al., 2006) for STin2 genotyping. The VNTR polymorphism was determined by 3.5% agarose gels electrophoresis and visualized by ethidium bromide stain. The sizes for the 9, 10 and 12 variant alleles were 250, 267 and 300-bp, respectively.

**Results:** Of all participants, 100 were outpatients with DSM-IV-TR diagnosis of recurrent MDD (male/female = 31/69; mean age ±SD = 45.58±11.8) and 137 were healthy subjects (male/female = 49/88; mean age ±SD = 47.91±11.9). Distributions of genotype frequencies were in accordance with Hardy-Weinberg equilibrium. Genotype distribution rates in the patient and control groups, respectively, were: 32% and 37.23% for the 12/12 genotype; 43% and 49.64% for the 10/12 genotype; 20% and 13.14% for the 10/10 genotype. The 9/10 (3%) and 9/12 (2%) genotypes were found only in the patient group (P=0.036). Allele 12 was determined in 62.04% of the controls, and in 54.5% of the patients. The 10 allele frequency was 43% (patients) and 37.96% (controls). No significant difference was found in the 12 and 10 allele distribution between patients and controls. The frequency of the 9 allele in the patient group only was 2.5%.

**Discussion:** Our preliminary data did not show significant association of STin2 VNTR polymorphism with the history of recurrent MDD. Slight, but not significant increase in 12/12 genotype distribution was found in the control group, as well as a slight increase in 10/10 genotype in the patient group. The 9 allele, which was detected only in the patient group and predominantly in females, may be associated with higher risk of recurrent MDD in a gender-dependent manner. Further detailed statistical and complete genetic analyses of SLC6A4 gene polymorphisms and variant interactions would permit generalization of the results.

### Homozygosity and Inbreeding as a Risk for Major Depression

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**Background:** Inbreeding within families (e.g. consanguineous marriages) has often been associated with risk of deleterious traits in the offspring. This is the result of homozygosity at loci for rare recessive mutations that are otherwise hidden, reflecting the strong selection that would occur against dominant deleterious variants. Using genome-wide molecular data it is possible to create more finely tuned measures of ancestral inbreeding for an individual and so remove reliance on pedigree information. This allows one to look at the effects of distant inbreeding in “outbred” populations. A recent study within the Psychiatric GWAS Consortium showed a significant association between increased inbreeding at a molecular level and increased risk for schizophrenia (Keller et al 2012, PLoS Genetics).

**Methods:** As the primary Psychiatric GWAS Consortium's (PGC) analysis of depression focussed on an additive model of depression, we first ran a secondary analysis of the sample (totalling over 9,000 for each of cases and controls) under a recessive model. We are now in the process of testing for evidence of increased inbreeding in the PGC major depression sample. This would include looking for evidence of increased homozygosity across the genome, specifically in the form of percentage of genome made up of runs of homozygosity. As well as looking at total inbreeding, we will be looking for associations between disease status and specific runs of homozygosity, in order to potentially identify the location of recessive risk alleles. To avoid sample specific differences in homozygosity, each of the 9 studies within the depression PGC sample will be analysed individually and a meta-analysis performed.

**Results:** The results of recessive model of depression produced no genome-wide significant hits and the QQ plot of associations suggests the analysis was underpowered. This was likely due to the recessive model focusing on the minor allele, for which the frequency of homozygotes was often very low. Our initial analysis of inbreeding suggests a potential association with depression. Three of the nine studies show nominal association, however the trend does not appear across all studies. We have yet to perform a meta-analysis across studies.

**Discussion:** A positive association with molecular homozygosity would identify a novel risk factor for depression. Association with specific runs of homozygosity might also provide the location of causal risk variants. Further, such associations would give insight into the genetic architecture of depression by showing an overabundance of recessive causal mutation. This would give insight into the level of historical selection against depression, with selection for dominance in beneficial variants expected.

### Genome-wide Gene-based Associations in Suicidal Behavior: A Cross-disorder Analysis

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**Background:** Suicide is one of the most devastating complications of psychiatric disorders, such as major depression and bipolar disorder. Predictors of suicidal ideation and behavior have been studied heavily, identifying several sociodemographic, psychological and neurobiological risk factors. Candidate gene studies and genome-wide scans implicated multiple candidate genes e.g., NGFR, BDNF, NTRK2, ABI3BP and SLC6A4. However, most of these studies analyzed single nucleotide associations, results derived from genomewide multimarker tests, such as haplotype-, gene- or pathway-based approaches are relatively scarce. The objective of our study was to investigate the shared genetic risk factors of suicidal behavior on a cross-disorder dataset of major depression, bipolar I and II disorders, by using a gene-based association approach.

**Methods:** We analyzed the GAIN Whole Genome Association Study of Bipolar Disorder version 3 (BIP, accession: phs000017.v3.p1, n=2802) and the GAIN Major Depression: Stage 1 Genomewide Association in Population-Based Samples (MDD, accession: phs000020.v2.p1, n=3541) whole-genome, case-control datasets accessed from the National Center of Biotechnology Information (NCBI) database of Genotypes and Phenotypes (dbGaP). Suicidal behavior was assessed by the Diagnostic Interview for Genetics Studies (DIGS) in the BIP sample and the Inventory of Depressive Symptoms (IDS) in the MDD dataset, n=606 subjects with suicidal behavior and n=1869 controls with MDD and bipolar disorder were identified. We performed a multipoint marker imputation by using IMPUTE2 and the 1000 Genomes Project's Phase I interim data as reference for the imputations. Annotations were converted to NCBI Build 37 by using liftOver. Population stratification was estimated by using ADMIXTURE. Gene coordinates were extracted from the hg19 RefSeq reFlat Gene Prediction Tracks accessed through the UCSC Genome Browser website. Quality control and dosage data analyses were carried out by using PLINK, whereas gene-based tests were conducted with VEGAS. All analyses were performed on pooled data and were controlled for populations stratification and dataset membership. False discovery rate (FDR) was calculated to correct the results for multiple comparison.

**Results:** From the 22,169 autosomal genes 1,158 achieved nominal significance ( $p < 0.05$ ), but none of those reached the genomewide significance threshold ( $FDR < 0.05$ ). Two genes, *dicer 1* (*DICER1*,  $p = 7.00e-06$ ) and *suppression of tumorigenicity 7* (*ST7*,  $p = 8.00e-06$ ) were identified with genomewide suggestive significance level ( $FDR < 0.1$ ). Surprisingly, *BDNF*, *NTRK2* or genes related to the serotonergic or dopaminergic transmissions failed to reach even nominal significance.

**Discussion:** Gene-based association methods differ from the classical SNP tests by more sensitively detecting genomic regions with multiple albeit weak association signals, but could also possibly “dilute up” single but strong signals from a large genomic area. Therefore, results from a gene-based scan are necessary different from those of a SNP based analysis. However, it is still puzzling that none of the genes implicated in the genetic architecture of impulsivity or suicidal behavior achieved nominal significance. One explanation is the nature of the pooled analysis that unavoidably increase the heterogeneity, and consequently reducing the power. While the function of *ST7* is not yet understood, *DICER1* is essential to neurodevelopment and was shown to be associated with executive functions in a recent study. In light

of recent reports suggesting the involvement of cognitive functions in suicide behavior among patients with affective disorders, we can speculate that DICER1 might be valid candidate gene and warrants further investigations. However, given the suggestive FDR and the multiple methodological differences of the analyzed two datasets, extra cautions are needed during the interpretations of this finding.

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### Identification of Rare Variants in the Susceptibility Gene for Depression SLC6A15 using Next-generation Sequencing

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**Background:** Major depression is one of the most prevalent psychiatric disorders with a lifetime prevalence of 17 % in the general population and one of the leading causes for loss in work productivity. The aetiology of depression is known to be influenced by a combination of genetic and environmental factors. A genome-wide association study from Kohli et al. identified the SLC6A15 gene, which encodes for a neutral amino acid transporter, as a susceptibility gene for major depression. SNPs in a gene desert of about 450 kb on 12q21.31 were associated with a decreased SLC6A15 gene expression in the hippocampus. This brain region is implicated in the pathophysiology of major depression. Environmental factors such as chronic stress were also associated with a downregulation of the SLC6A15 gene expression in a mouse model. To further characterize the novel susceptibility gene we re-sequenced the SLC6A15 locus to identify novel common and rare genetic variants which might be associated with changes in gene function.

**Methods:** DNA from 400 patients suffering from depressive disorder, especially recurrent depression (88.0 %) and 400 healthy controls, matched for age and gender, was screened for novel polymorphisms using the SOLID 4 next-generation sequencing platform (Life Technologies). Enrichment of the whole SLC6A15 locus on chromosome 12 and 10 kb upstream and downstream of the gene (70 kb in total) was achieved via long range PCR in pools of 50 individuals respectively. After amplification, 8 pools of PCR products consisting of equal amounts of DNA from 100 patients or controls were sequenced on the SOLID sequencer. For SNP identification we used VipR, a software specifically designed to detect genetic variants in pooled samples. To validate the putative variants detected in the re-sequencing experiment variants were genotyped in individual samples using the Sequenom platform.

**Results:** Using the pooled re-sequencing approach 403 genetic variants could be detected, 144 (37.5 %) previously identified SNPs reported in the dbSNP132 database could be confirmed. Fourteen coding variants were detected with ten leading to amino acid exchanges in the protein, of which seven are not reported in SNP databases. 50 % of the detected variants are low frequency SNPs showing a minor allele frequency (MAF) < 0.5 %. From 64 putative genetic variants that were detected in the next-generation sequencing experiment 45 (73.0 %) could be validated using Sequenom and yielded a correlation of allele frequencies of  $r^2 = 0.993$ . Four non-synonymous variants showed an odds ratio (OR)  $\geq 2$ , one variant could only be observed in one patient.

**Discussion:** The mutation screening using next-generation sequencing in pooled samples allowed a detection of 403 genetic variants in SLC6A15, 202 of them with frequencies lower than 0.5 %. Ten SNPs are non-synonymous coding variants, four of them with an OR  $\geq 2$ . We are currently extending this study by re-genotyping the newly identified non-synonymous and possible splice variants in a larger sample of over 1500 depressed patients and 1200 healthy controls using Sequenom. Additionally, the possible functional effects of the non-synonymous variants on the amino acid transporter activity are being characterized using site-directed mutagenesis and cellular expression systems.

### Genetic Relationship between Depression and Obesity: The FTO Gene Opens the Way

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**Background:** Depression and obesity are leading causes of disease burden and disability, as well as major public health concerns worldwide. Both conditions are highly prevalent and major risk factors for chronic physical diseases such as type II diabetes, cardiovascular disease and hypertension. The association between depression and obesity has repeatedly been reported in many studies, although the nature and direction of the association remains unclear. Genomic variants in the fat mass and obesity associated (FTO) gene have been consistently associated with obesity and increased body mass index (BMI) and this gene is now firmly established as a contributor to common human obesity. Recently, we have reported the first study implicating FTO in the mechanism underlying the association between depression and obesity. The aim of present study is attempt to replicate these findings by investigating the FTO rs9939609 polymorphism in an independent multiethnic sample of depressed cases and controls from the EpiDREAM international cohort study.

**Methods:** The sample consists of 18,243 individuals recruited from 21 different countries. The depression case sample comprises 3,406 individuals (834 men, 2,631 women; mean age  $\pm$  s.d.: 50.93 $\pm$ 10.51) who had a major depressive episode in the past 12 months. DSM-IV depression phenotypes were ascertained using a structured case report form. The control group consisted of 14,837 individuals (6,279 men, 8,697 women; mean age  $\pm$  s.d.: 53.04 $\pm$ 11.51) who were screened for depression. Participants were considered controls if they did not have depressive features according to DSM-IV criteria during the past 12 months. Height and weight data were collected by direct measures and BMI was defined as weight (kilograms) / height (metres)<sup>2</sup>. Linear regression models for quantitative traits assuming an additive genetic model were performed to test for association between BMI and rs9939609 polymorphism. We also explored the interaction between this variant and affected status for an effect on BMI. The statistical analyses were performed using the statistical package Statistical Analysis System (SAS) version 9 (SAS Institute Inc.). Table 1 summarizes the characteristics of the participants in the EpiDREAM cohort.

**Results:** We found a significant association between BMI and depression, after correcting for age, gender, ethnicity and medication (OR=0.982 [0.98-0.99],  $p < 0.0001$ ). Depressed individuals had significantly higher mean BMI in comparison with controls in both males and females (Table1). Linear regression analysis carried out with rs9939609 FTO polymorphism in the whole sample (depressive cases and controls), after correcting for depression, age, gender, ethnicity and medication yielded a significant association with BMI ( $\beta = -0.016$ ,  $p = 5.57 \times 10^{-17}$ ). We also found a significant interaction between rs9939609 and depression ( $\beta = -0.011$ ,  $p = 2.15 \times 10^{-2}$ ) in relationship to BMI taking gender, age, ethnicity and medication as covariates in the model.

**Discussion:** We have found a strong association between rs9939609 and BMI which replicate and confirm our previous findings and align with prior studies. Furthermore we again found a significant interaction between this variant and depression indicating a moderating effect of depression on the association between rs9939609 and BMI, in keeping with our previous findings. The results from this study demonstrate a strong and global effect of the interaction between rs9939609, depression and BMI as it is found in a sample of individuals from different ethnicities and countries around the world. Although the genetic mechanism underlying the association between obesity and mood disorders remains poorly understood, our study supports the involvement of FTO in the relationship between depression and obesity. In conclusion, our findings confirm a moderating effect of depression on the association between rs9939609 and BMI in a large independent cohort and implicate FTO in the mechanism underlying the relationship between depression and obesity.

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### Multi-candidate Association Analysis of Aggression

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**Background:** According to twin studies, the heritability of aggression is at least 50%. At the same time, the data available on the genes underlying this complex human trait are strikingly scarce and sometimes controversial. Here we present a multi-candidate gene association study using polymorphisms of the dopaminergic, serotonergic and other neurotransmitter system genes, and further candidates such as trophic and survival factors.

**Methods:** Valid genotype and phenotype data from 887 Caucasian adults (18-75 years old, median age 20.0 years, 54.2% female) with no psychiatric history were analyzed. Aggression total score was calculated from self-report items of the Buss-Perry Aggression Questionnaire. DNA was extracted from buccal swabs and genotyped with an OpenArray system for 55 SNPs (17 dopaminergic, 14 serotonergic, 7 other neurotransmission related, 17 trophic and cell survival related). Statistical analysis (Univariate ANOVA with age and sex covariates) was applied for a dominant model of the minor allele, using SPSS 19.0. The nominal level of significance (0.05) was changed to 0.00093 based on the Bonferroni correction for multiple testing.

**Results:** Significant association was shown between the Buss-Perry Aggression Questionnaire total score and the rs7322347 SNP of the HTR2A gene ( $p=0.00069$ ): carriers of the minor allele showed lower aggression as compared to major allele homozygotes. Linkage analysis demonstrated a strong linkage between the rs7322347 intronic SNP and an exonic missense variation (rs6314). Haplotype analysis of the two SNPs revealed that the two risk alleles did not occur together in our non-clinical sample and that the putative effect of the intronic rs7322347 seems to be independent from the exonic rs6314.

**Discussion:** Here we performed an association analysis between a set of neurotransmitter related SNPs and aggression in a non-clinical sample. Our results suggest that the serotonin receptors HTR2A is an important constituent of this endophenotype.

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### Regulation of TSPAN8 Gene Expression and Its Role in Bipolar Disorder

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**Background:** The single nucleotide polymorphism (SNP) rs4500567, located in the upstream region of Tetraspanin 8 (TSPAN8), was found to be associated with bipolar disorder (BPD) by Scholz et al. (Scholz, et al., 2010). The SNP's minor allele is part of a predicted binding site for the transcription factor Nuclear Factor, Interleukin 3 regulated (NFIL3), which is known for potentially activating or repressing effects on gene expression. In this work the effect of the putative NFIL3 binding to the minor variant of rs4500567 and the impact of the subsequent changes in TSPAN8 gene expression were investigated.

**Methods:** TSPAN8 gene expression in the presence of both rs4500567 alleles was examined with luciferase-based promoter assays. Therefore the SNP alleles and their flanking regions were synthetically produced and cloned in the pGL4.23 vector, containing a Firefly luciferase gene proximal to a minimal promoter. Luciferase assays were run in two human cell lines, which showed NFIL3 expression, validated with quantitative real-time PCR (qRT-PCR). To define the global effect of an altered TSPAN8 gene expression, a TSPAN8-knockdown experiment in the neuroblastoma cell line SH-SY5Y was combined with microarray analysis. Promising differentially expressed candidate genes that were previously associated with psychiatric disorders or involved in neuronal development were further validated with qRT-PCR.

**Results:** Luciferase assays revealed a significantly ( $p < 0.05$ ) higher expression in the presence of the more frequent (major) allele compared to the less frequent (minor) allele. The TSPAN8-knockdown induced the differential expression of 311 microarray probe sets, which map to 231 genes. Of 25 selected genes, 10 were validated with qRT-PCR. Of these NTRK2, TSPAN2 and ST3GAL5 showed the highest significance.

**Discussion:** The rs4500567 allele-specific expression of TSPAN8 can be explained by the repressing function of NFIL3 in neuronal cells. However, the definite repressive function of NFIL3 by binding to the minor allele needs to be validated in further experiments. The diversified downstream associated genes of TSPAN8, revealed in the knockdown experiments, point to a role of TSPAN8 in the development of neuronal cells and the etiology of BPD, but further work is required to provide more detailed insight in the molecular framework.

### Preliminary Results of a BICC1 and NLGN1 Association Study in MDD: An Attempt to Replicate Previous GWAS Findings

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**Background:** In an attempt to replicate the findings of a recently published genomewide association study (GWAS) (Lewis et al. 2010), we investigated the BICC1 (bicaudal C homolog 1, drosophila) and NLGN1 (neuroligin 1) genes for association with major depressive disorder (MDD), using a tagging SNP (single nucleotide polymorphisms) approach.

**Methods:** Applying the same diagnostic instruments as Lewis et al., we collected a sample of 236 Austrian subjects suffering from ICD-10 and/or DSM-IV-TR diagnosis of MDD and 332 screened healthy control subjects. Genotyping was performed using the Sequenom MassARRAY® iPLEX Gold assay and 104 SNPs were successfully genotyped. Four SNPs were out of HWE and thus excluded from statistical analyses. A total of 40 SNPs located in BICC1 and 58 SNPs located in NLGN1 were used for statistical analyses after quality control procedures. To test for genotypic association with each SNP, a standard chi-square ( $\chi^2$ ) statistic was calculated using SPSS Statistics version 20 for MAC. The computer program FINETTI (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>) was used to calculate the Cochran-Armitage trend statistic to test for allelic association. Multiple testing corrections were performed by application of the false discovery rate (FDR, Benjamini et al. 2001).

**Results:** Before multiple testing correction, two BICC1 SNPs (rs12776000 and rs7897958) and five NLGN1 SNPs (rs1948162, rs4499633, rs481359, rs7629797 and rs9877241) showed genotypic and/or allelic association, however, none of the significant p-values resisted multiple testing correction.

**Discussion:** Preliminary results show no single marker association of BICC1 and/or NLGN1 with MDD in our Austrian sample. However, non-replications of GWAS findings are quite common, and further investigations, including increase in sample size, are required to dissect this further.

### BDNF Haplotypes Including the Functional VAL66MET Polymorphism Associated with Suicide Risk in Male MDD Patients of a European Multicenter Treatment Resistant Depression Study

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**Background:** Associations between the brain-derived neurotrophic factor (BDNF) gene and psychiatric disorders including major depression (MDD) are well established. The BDNF gene has further been associated with suicidal behaviour, as well as with treatment response phenotypes, although with conflicting results. In the present study, we further elucidate the impact of BDNF in MDD patients with suicide risk and/or a personal history of suicide attempts.

**Methods:** Two hundred fifty MDD patients were collected in the context of a European multicentre resistant depression study and treated with antidepressants at adequate doses for at least 4 weeks. Suicidality was assessed using Mini International Neuropsychiatric Interview (MINI) and Hamilton Rating Scale for Depression (HAM-D). Treatment response was defined as HAM-D  $\leq$  17 and remission as HAM-D  $\leq$  7 after 4 weeks of treatment with antidepressants at adequate dose. Genotyping was performed for the functional Val66Met polymorphism and seven additional SNPs within the BDNF gene.

**Results:** With regard to suicide risk and personal history of suicide attempts, neither single marker nor haplotypic association was found with any SNP after multiple testing correction. However, in gender-specific analyses, we found haplotypic association with suicide risk in males, but not in females (rs925946-rs10501087-rs6265, rs10501087-rs6265-rs122733). The only single-marker association with suicide risk in males (rs908867) did not resist multiple testing correction. No significant associations were found in gender-specific analyses with regard to a personal history of suicide attempts.

**Discussion:** We found two BDNF haplotypes including the functional Val66Met polymorphism significantly associated with suicide risk in male MDD patients. However, replication in larger well-defined cohorts is required to dissect this further.

**Alopecia Areata: Genetic and Psychological Factors**

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**Background:** Alopecia areata (AA) is an autoimmune, inflammatory disease directed against the hair follicle with a life time risk of 1.7 in men and women (Cooper et al. 2009; Safavi et al. 1995). AA is characterized by sudden onset of patchy loss of the scalp and/or body hair. The disease course and extent of hair loss are unpredictable. It may progress to complete baldness (alopecia totalis) and loss of all body hair (alopecia universalis). To date, no cure or preventive treatment has been established. The etiology of AA is complex and still poorly understood. Genetic factors are contributing (Blaumeiser et al. 2006). A high comorbidity with psychiatric disorders has been reported, though the majority of these studies were small in sample size (N~30). The two largest ones reported higher rates of psychiatric disorders, particularly of major depression (MD), in AA patients (Chu et al. 2012; Koo et al. 1994). The aims of the present study were to assess the rate of life-time MD in a large sample of AA patients and a sample of the general population; (ii) to evaluate whether patients with MD consider AA as cause of their depression; and (iii) to compare the genetic risk for psychiatric disorders (i. e. family history (FH) of psychiatric disorders) in AA patients with and without MD and the general population.

**Methods:** A questionnaire assessing life-time occurrence of MD, depressivity, traumatic life events, chronic stress, stress reactivity, personality traits, FH of psychiatric disorders in first-degree relatives, and impact of AA on daily life was sent 1595 patients AA who had previously participated in a study indentifying the genetic factors of AA (John et al. 2011). A control sample for genetic studies (471 men, 587 women, mean age=46, range=18-71) was recruited in the area of Mannheim and assessed with a similar questionnaire.

**Results:** Five hundred and eighty-one patients (114 men, 467 women, mean age=46, range=18-86) responded to the questionnaire. 39 of the AA patients (25.2 males, 42.3 females) met the criteria of lifetime MD as compared to 18.5 of controls (14.2 males, 22 females). 53.6 of AA patients with lifetime MD consider AA as a cause of their depression. 43.8 of the AA patients with lifetime MDE have a positive FH of psychiatric disorders as compared to 22.7 of AA patients without lifetime MD. In the control group, the corresponding rates were 33 and 20, respectively.

**Discussion:** MD is highly prevalent in patients with AA. Patients with AA should therefore routinely be screened for depressive symptoms and in case of comorbid MD receive professional treatment of their depression. Patients with AA who report a FH of psychiatric disorders are particularly vulnerable to MD. Literature Blaumeiser B., van der Goot I., Fimmers R., Hanneken S., Ritzmann S., Seymons K. et al. 2006. Familial aggregation of alopecia areata. *J Am Acad Dermatol* 54:627-632. Chu S. Y., Chen Y.J., Tseng W. C., Lin MW, Chen T. J., Hwang C. Y. et al. 2012. Psychiatric comorbidities in patients with alopecia areata in Taiwan: a case-control study. *Br J Dermatol* 166:525-531. Cooper G. S., Bynum M. L., Somers E. C. 2009. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. *J Autoimmun* 33:197-207. John K. K., Brockschmidt F. F., Redler S., Herold C., Hanneken S., Eigelshoven S. et al. 2011. Genetic variants in CTLA4 are strongly associated with alopecia areata. *J*

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### Investigation of a Polymorphic Repeat in the Retinoic Acid Induced 1 Gene in Perinatal Depression

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**Background:** About 10% of women who undergo pregnancy and childbirth are affected by antenatal/postnatal depression. The incidence is higher for those with family history of depression and other mental illness, suggesting the contribution of genetic variants. A candidate gene is the Retinoic acid induced 1 (RAI1) gene which is highly active in nerve cells in the brain. The gene is dosage sensitive and has been implicated in Smith-Magenis syndrome and Potocki-Lupski syndrome. Variants in the gene have also been associated with spino-cerebellar ataxia, non-syndromic autism and schizophrenia. In this study, we investigated the distribution of the polymorphic repeat in this gene which had not been studied in depression.

**Methods:** Ninety-eight cases with confirmed diagnosis of depression related to pregnancy and 246 postpartum women who had scores of < 7 on the Edinburgh Postnatal Depression Scale (EPDS) were recruited into the study. Demographic information and saliva samples were collected from the participants. Genomic DNA extracted from saliva was sequenced to determine the number of CAG/CAA repeats that encodes the polyglutamine tract in the N-terminal of the protein. Difference between cases and controls was assessed by chi-square analysis.

**Results:** There was statistically significant association of perinatal depression with positive family history of mental illness. Thirty-nine cases (40%) have at least one family member with mental illness compared to 8 (3.3%) for controls. For the RAI1 gene CAG repeat, there was no statistically significant difference in genotype and allele distribution between cases and controls. The number of CAG/CAA repeats ranged from 10 to 15 with the 13-repeat allele the most common (0.862 in cases and 0.748 in controls).

**Discussion:** Although there was no statistically significant difference between controls and cases, we have determined the different population distribution for this polymorphic repeat in RAI1 which is different from Western populations for which the 13-and 14-repeats allele are equally common. Given the strong association with family history, we will continue to explore other genetic factors.

### CACNA1C Risk Variant and Amygdala Activity in Bipolar Disorder, Schizophrenia and Healthy Controls

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**Background:** Several studies have implicated CACNA1C polymorphism rs1006737 in bipolar disorder (BD) pathology. Increased amygdala activity has been reported in BD subjects undergoing emotional functional Magnetic Resonance Imaging (fMRI) paradigms. A recent study found this CACNA1C polymorphism to be associated with enhanced amygdala activity in a combined sample of healthy controls and patients with BD. In order to confirm the association between the CACNA1C risk allele and increased amygdala activity, we performed an fMRI study in a sample of BD and schizophrenia (SZ) cases and healthy controls. Subgroup analyses were undertaken to determine the potential diagnostic specificity of this effect.

**Methods:** We genotyped rs1006737 in 250 individuals (N = 66 BD, 61 SZ and 123 healthy controls), all of Norwegian ethnicity, who underwent an fMRI negative faces matching paradigm. Statistical tests were performed with SPM2, with an ANCOVA model using sex, age, diagnostic category and medication status as covariates in the total sample. Additional tests were undertaken for each diagnostic subgroup.

**Results:** Carriers of the risk allele had enhanced activation in the left amygdala ( $x = -24, y = -2, z = -14; Z = 3.47$ ), (Family-wise error (FWE)  $P = 0.026$ ) in the total sample. When analyzing group-wise, this effect was significant in the BD group ( $x = -24, y = 0, z = -14; Z = 3.35$ ) (FWE  $P = 0.041$ ), but not in the other subgroups.

**Discussion:** The current results are in line with previous findings, and strengthen the hypothesis that CACNA1C SNP rs1006737 is involved in amygdala activity during emotional processing. This effect seems most pronounced in BD, suggesting that the CACNA1C effect on amygdala activity is of importance for BD pathophysiology. Moreover, these findings support ion channelopathy as a putative underlying mechanism in BD, which might be of interest for future development of pharmacological agents.

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### The Role of the HLA System in Major Depression: A Microarray Study on Human Fibroblast Samples

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**Background:** Major depression is estimated to affect about 16% of the population. Finding possible susceptibility factors is a major issue to help improve the patients' quality of life and also the clinical treatment methods. The goal of our project was to examine the gene expression changes of depressed patients compared to healthy controls in order to identify possible susceptibility genes or groups of genes using microarrays.

**Methods:** Fibroblast samples from 16 depressed and control subject pairs (N=32) were used in this microarray study. After cell culturing from skin biopsies, RNA isolation and quality control the samples were hybridized to an Affymetrix HT HG-U133 chip and gene expression analysis of almost 55,000 transcripts has been carried out. During the statistical analysis differentially expressed genes between the cases and controls were identified and pathway analysis, WGCNA (Weighted Correlation Network Analysis), RNA sequencing and qPCR validation was also used to evaluate the data.

**Results:** In our comparative study of the two subject groups we examined the global expression differences of the depressed and control groups. 101 differentially expressed genes between the groups were identified with a 1.7 fold-change and a p-value < 0.05. Among the changes we found a group of up-regulated genes belongs to the HLA (human leukocyte antigen) system (e.g. HLA-DRA, HLA-DRB1, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, etc.). The role of the immune system in the context of major depression is a promising area based on previous clinical and research studies. The pathway analysis also revealed the possible role of the HLA system with the up-regulation in the depressed samples. Results were successfully validated by qPCR method.

**Discussion:** The present study focuses on the genome-wide expression analysis of depressed fibroblast samples and their possible association with depression and therapy. Our results indicate that significant differences can be detected between the expression patterns of the groups and the results suggest an increased importance of the immune system genes, especially the ones with functions like antigen-processing and antigen-presenting.

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### Microna Expression Profiling of a Genetic Animal Model of Depression-like States

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**Background:** MicroRNAs (miRNAs) are involved in the posttranscriptional regulation of messenger RNAs (mRNAs) and aberrations in miRNA expression have been associated with numerous human diseases. However, few miRNA studies have been conducted with regard to psychiatric disorders and especially depression. The Flinders Sensitive Line (FSL) is a genetic rat model of depression-like states that, compared to its controls (the Flinders Resistant Line; FRL), exhibits a number of behavioral and neurochemical features observed in human depression and has been successfully used for translational neuropsychopharmacological research.

**Methods:** Direct multiplexed measurement of miRNA expression was performed using the nCounter rat miRNA expression assay kit (Nanostring technologies). In total, 423 rat miRNAs from miRBase 17 were studied in the prefrontal cortex (PFC) of FSL and FRL rats. Statistical exploration of the data, generated by different normalization methods, was performed using R. The IPA software (Ingenuity) was used to analyze the candidate miRNAs from a biological system/pathway perspective. During abstract submission, validation of the differentially expressed miRNAs was being performed using quantitative real-time PCR (qRT-PCR). Following validation, miRNAs will be analyzed both in additional brain regions involved in depression (e.g. hippocampus) and also following different pharmacological and non-pharmacological interventions shown to alleviate depressive symptoms in the FSL model (e.g. treatment with a histone deacetylase inhibitor and physical exercise in the form of wheel-running).

**Results:** One of the miRNAs of the preliminary panel was miR-25 which was found to be up regulated in the FSL rats (P = 0.007). A putative target of miR-25 is the Grm2 gene which has been implicated in depression. The preliminary analysis of the data also generated a panel of approximately 10 candidate miRNAs that are differentially expressed in the PFC of FSL and FRL rats. As validation experiments and analyses following different interventions were still ongoing during abstract submission, additional results will be presented during the meeting.

**Discussion:** This work, conducted using an animal model of depression-like states, provides insights into which miRNAs may be involved in the pathogenesis of depression. Data require replication in human settings before firm conclusions can be made.

**DGKH: Candidate Gene for Bipolar Disorder**

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**Background:** Bipolar disorder (BPD) is a genetically complex mental disorder in which patients suffer from both depressive and manic episodes, occurring with a typical cyclical course. Linkage studies and genome wide association studies (GWAS) have revealed DGKH as a candidate gene in the etiology of BPD. In a recent study we were able to identify a risk haplotype for BPD, as well as for unipolar depression and adult attention-deficit/hyperactivity disorder, within the DGKH gene. Interestingly, there is also evidence that DGKH expression is significantly higher in the prefrontal cortex of BPD patients. DGKH codes for the eta ( $\eta$ ) isoform of diacylglycerol kinase (DGK), which is involved in the phosphoinositol pathway. Lithium is one of the most effective pharmacological treatments of BPD and it is thought to mediate its effect via this pathway, lending further evidence for the involvement of DGKH in BPD.

**Methods:** To screen for disease associated rare variants in the human DGKH gene, DNA from 300 patients suffering from bipolar disorder and 300 healthy control subjects was investigated using next generation sequencing. The expression pattern of the two known murine Dgkh transcripts in different tissues was investigated by semi-quantitative PCR. In addition, the expression levels of Dgkh during mouse brain development were examined using quantitative real-time PCR (qPCR). To investigate the Dgkh expression pattern in the brain, in situ hybridization (ISH) as well as immunofluorescent double staining on murine brain sections were conducted.

**Results:** The two Dgkh transcripts exhibited distinct occurrence in a variety of murine tissues and they also differed in their expression level. qPCR analyses revealed an increase in Dgkh expression during mouse brain development. ISH on murine brain slices showed a strong Dgkh expression in the hippocampus and the cerebellum. Immunohistochemistry confirmed these findings, but also showed staining for the protein Dgk $\eta$  throughout all regions of the brain. Immunofluorescent (IF) double staining showed occurrence of Dgk $\eta$  in neurons, but not in astrocytes, suggesting that Dgk $\eta$  is a neuron specific protein in the brain.

**Discussion:** The proteins encoded by the different Dgkh transcripts differ in some functional protein domains suggesting distinct biochemical and cell biological properties. Therefore, the two isoforms of this kinase may accomplish different functions in the tissues in which they are present. The expression patterns of Dgkh in the mouse brain provides further evidence for its role in BPD, as the hippocampus and the cerebellum have been implicated in the psychopathology of this disorder. This interesting candidate gene needs to be further examined in order to reveal its precise function for better understanding of the neurobiological basis of the disease.

**Association of PCLO with HPA Axis Activity and Clinical Symptoms in Patients with Depression**

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**Background:** A recent genome-wide association study (GWAS) demonstrated association of a cluster of single nucleotide polymorphisms (SNPs) in a region overlapping the gene piccolo (PCLO) which is important for monoaminergic neurotransmission in the brain<sup>1</sup>. In a subsequent study, we could show that healthy risk allele carriers of a functional SNP in this cluster, rs2522833, had a suppressed cortisol awakening response and higher neuroticism scores<sup>2</sup>. In this study, we now analyzed the association of this variant with basal cortisol levels and cortisol suppression after administration of dexamethasone in a sample of depressive patients. We furthermore tested the association of rs2715148 which in the original GWAS had been associated with a p-value of  $8.4 \times 10^{-8}$  after stratification for recurrent early onset depression as well as the influence of both SNPs on the course of clinical symptoms.

**Methods:** A sample of unmedicated subjects with major depression and/or dysthymia (n=61, mean age 47.8 years, 52.5% women, 45.9% with double depression, mean BDI-II score 25.6) underwent an extensive psychopathologic diagnostics. Basal cortisol was measured via night urine, and a dexamethasone suppression test was conducted the consecutive day (1 mg dexamethasone at 11 p.m., blood withdrawal the following day for dexamethasone test). Subjects were followed-up after 6 months with psychopathological assessment. Rs2715148 and rs2522833 were genotyped with TaqMan 5' nuclease assays.

**Results:** 39 subjects (63.9%) were carriers of the risk allele C for rs2522833, and 42 (68.9%) were carriers of the risk allele C for rs2715148. Risk allele carriers of both SNPs showed higher cortisol suppression after the dexamethasone suppression test ( $p < .01$  each). Basal cortisol levels showed no differences with respect to the comparison groups. Rs2715148 C-allele carriers displayed a significantly worse course of depression scores (BDI-II,  $p = .044$ ) at follow-up than A-allele homozygotes. There were no associations of rs2522833 allele status and course of depression.

**Discussion:** In a sample of depressive patients, we could find further evidence of a differential HPA-axis activity associated with SNPs in PCLO, a gene which before had been implicated in depression in a GWAS and down-regulation of the HPA-axis in a subsequent study. In detail, risk allele carriers of both SNPs showed higher cortisol suppression after the administration of dexamethasone. Furthermore, in the original GWAS, rs2715148 had displayed an improved p-value after stratification for severity of depression. Interestingly, in our study this SNP was associated with a worse course of depression. A possible pathomechanism of how variation in PCLO acts on the stress response could be based in altered monoaminergic modulation of the HPA-axis. Sullivan et al, Molecular Psychiatry 2009. 2 Kuehner et al, Translational Psychiatry, 2011.

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### Transcription Profiling and Pathway Analysis in Euthymic and Manic Bipolar Patients and Controls

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**Background:** Bipolar disorder (BPD) is a polygenic disorder with many genes contributing to its etiology. The aim of this investigation was to search for differentially expressed genes and molecular and cellular pathways dysregulated in this disorder. We did not only compare bipolar patients to control persons but also differentiated between manic and euthymic phases.

**Methods:** We conducted a blood-based microarray investigation using Human Exon 1.0 ST Arrays (Affymetrix) in a sample of inpatients with a diagnosis of bipolar disorder in their euthymic and manic phase (N=10). Diagnostic groups were compared to controls, and euthymic and manic phases were compared with each other. Analyses of covariance comparing mean expression levels on a gene-by-gene basis were conducted to generate the top significantly dysregulated gene lists for patients by each diagnostic group and controls (p-value < 2.95x10<sup>-3</sup>). A pathway-based analysis will be conducted for the top-differentially expressed genes by applying the global test analytical method (Manoli et al. Bioinformatics2006) to gene sets from the Molecular Signatures Database MSigDB (BioCarta, KEGG, Reactome, Gene Ontology, MIR, TFT, positional gene sets). Gene expression and pathway analysis data will be cross-matched with data from an independent genome-wide association study of single-nucleotide polymorphisms into bipolar disorder.

**Results:** Results show over 200 differentially expressed genes comparing patients in the manic phase with controls and over 200 differentially expressed genes comparing patients in the euthymic phase with controls. A limited number of genes was differentially expressed comparing the manic and euthymic phases. A subset of the significant hits were differentially expressed in both manic and euthymic phases vs. controls, and there was an overlap of genes which showed differences in expression comparing euthymic phase vs. controls and euthymic vs. manic phase. Pathway analyses and integration of SNP data are currently being conducted and results will be presented.

**Discussion:** Findings provide evidence of general and specific genetic regulation in manic and euthymic phases of BPD. Although the results are preliminary, they suggest that significantly more genes are differentially regulated in the manic and in euthymic phase compared to controls than in manic compared to euthymic phase with a partial but not complete overlap between differentially expressed genes in the two bipolar phases. The results of this approach could lead to the identification of new genes and molecular and cellular pathways that contribute to the development of BPD and thus support further investigation of for the identification of biomarkers for BPD.

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### Association between SNPs in the Promoter Region of the Tryptophan Hydroxylase 2 Gene (TPH2) and the Hypothalamic-Pituitary-Adrenocortical (HPA) Axis Dysregulation in Patients with Major Depression

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**Background:** Recent models of affective disorders propose an involvement of malfunctions of the serotonergic system, as well as a dysregulation of the activity of the HPA axis. Previous studies from our and other groups have shown that the neuronal TPH2, the rate limiting enzyme in the synthesis of the neurotransmitter serotonin (5-HT), is predominantly expressed in several regions of the human brain and that TPH2 polymorphisms are associated with major depression. Moreover, we could recently demonstrate that a functional SNP in the TPH2 promoter region (rs11178997) alters the transcriptional activity of the gene. Therefore we investigated the impact of this polymorphism and two additional promoter SNPs (rs11178998, rs4570625) in the TPH2 gene on HPA axis dysregulation, determined by the Dex/CRH test.

**Methods:** At admission the combined dexamethasone/corticotrophin-releasing hormone (Dex/CRH) test was performed in 206 drug-free patients suffering from major depression. The SNPs rs11178997, rs11178998 and rs4570625 in the TPH2 gene were genotyped applying the TaqMan® technology (Assay-on-Demand).

**Results:** Carriers of the A-Allele of rs11178997 showed a decreased cortisol and ACTH stimulation during the first Dex/CRH test after admission than the other genotypes (ANOVA (AUC): p < 0.01). Interestingly the A-allele of rs11178997 leads also to a decreased transcriptional activity. Similar results were obtained for carriers of the A-allele of rs11178998. SNP rs4570625 showed no relation to the HPA-axis activity.

**Discussion:** Our data suggest that polymorphisms in the promoter region of the TPH2 gene might be crucial factors for the HPA system hyperactivity in major depression.

### RNASeq Analysis using the Pipeline Graphical Workflow Environment in Neuropsychiatric Disorders

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**Background:** RNAseq is increasingly becoming the method of choice for investigating the transcriptional landscape of cells. This includes not only transcript abundance (i.e. differential expression), but also transcript diversity due to alternative splicing events, different promoter or transcription start sites or differential base pair editing of mRNA. An increasingly large number of workflows are available today to manage high-throughput genomics sequencing data, from basic data processing to high-quality visualization of results. Graphical workflow environments are emerging as useful tools for constructing, modifying, interconnecting and executing computational genomics protocols using data processing workflows, such as pipelines.

**Methods:** We have developed the Graphical Pipeline for Computational Genomics and Visual Informatics for RNAseq (GPCGR) with a flexible graphical infrastructure for efficient RNAseq analysis computing and distributed informatics research within the framework of a joint collaboration between LONI (Laboratory of Neuro Imaging) at UCLA and BIRN (Biomedical Informatics Research Network) at UCI, ISI and USC. We use this pipeline to analyze RNAseq data from 10 controls, 9 subjects with Bipolar Disorder (BD), and 8 Schizophrenics (SZ).

**Results:** Using our developed set of pipelines we performed, reference genome alignment and assembly of the reads into transcripts, estimation relative transcript abundance, identification of differentially expressed and spliced genes. We will report the possible diagnosis dependent differential expression and splicing of genes in pathways relevant to BD and SZ compared to controls.

**Discussion:** This study demonstrates the utility of collaboratively developed graphical pipelines to improve the efficiency of high-throughput RNAseq data analysis in investigations of the underlying pathophysiology of neuropsychiatric disorders.

### Genetic Risk Factors for Interferon-induced Depression

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**Background:** Chronic hepatitis C infection (CHC) represents a public health problem that affects around 3% of population worldwide. Pegylated Interferon-alpha and Ribavirin is the recommended treatment reaching about 40-80% of sustained virological response. However, common treatment side-effects include neuropsychiatric symptoms such as depression, which impairs patient's quality of life and treatment adherence. (1,2) Genes related with inflammation and neurotransmission systems may play an important role in the pathogenesis of IFN-induced neuropsychiatric symptoms. The GC/GG genotype at a polymorphism located in the IL-6 synthesizer gene (IL6) was has been reported to be associated to a higher production of IL-6 and consequently with "higher inflammation response". (3,4) A previous study with a relatively small sample showed that patients carrying the CC genotype on at IL6 had lower rates of IFN-induced depression. (2,4) Moreover, a polymorphism in the serotonin transporter gene (SLC6A4 or SERT) has been related with major depression and antidepressant response: subjects carrying the LL genotype might be at a lower risk of depression. (5) However, the association between SERT and IFN-induced neuropsychiatric symptoms is not clear and some studies have shown contradictory results. (4,6) The aim of the study was to assess the role of IL6 and SERT polymorphisms as predictive variables of IFN-induced depression, anxiety and fatigue in a cohort of 385 consecutive outpatients with CHC treated with PegIFN- $\alpha$ /RBV in a Liver Unit.

**Methods:** All subjects were Caucasian and euthymic before starting the treatment according a clinical evaluation using DSM-IV-R criteria (SCID). Exclusion criteria included additional positive serology, decompensated liver cirrhosis, and current drug/alcohol abuse. Patients were assessed at baseline, 4, 12, 24, and 48 weeks after antiviral treatment initiation using the Hospital anxiety and depression scale (HADS) and a visual analogue scale of fatigue. A blood sample was obtained at baseline, and genomic DNA was extracted from the peripheral blood leukocytes of all the participants using Flexi Gene DNA kit according to the manufacturer's instructions. The polymorphism located at the promoter region of SERT (5-HTTLPR) was genotyped using polymerase chain reaction (PCR) as previously described, and alleles were termed S (short allele with a higher basal transcription) and L (long allele with lower transcription). The polymorphism located at the promoter region of IL6 was genotyped using a custom Illumina Vera Code Golden Gate Genotyping Assay (Illumina San Diego, CA, USA) according to the manufacturer's protocols. Statistical analysis using a linear mixed-effects model was performed.

**Results:** Genotypic distribution was in the Hardy-Weinberg equilibrium for SERT (P=0.41) and for IL-6g (P=0.72). We did not found a significant effect of SERT polymorphism (S vs. LL) on depressive symptoms (P=0.21), anxiety (P=0.15), and fatigue (P=0.20). Regarding the IL6 polymorphism (G vs. CC), we found that patients carrying the G allele (GC or GG genotype) had more depressive (P=0.005) and more anxiety symptoms (P=0.004) during antiviral

treatment. Image 1. Moreover, we found that subjects carrying the G allele IL6 had more fatigue at baseline ( $P=0.04$ ). However, the increase in fatigue values during IFN treatment did not differ between IL6 genotypes. We did not detect any significant interaction between both genes.

**Discussion:** Our study showed that subjects with GC/GG genotype, that imply higher levels of IL6, had more anxiety and depressive symptoms during IFN-treatment. These results are in agreement with those of a previous study, and support the hypothesis that patients with “high rates of inflammation”, such increased serum levels of IL6 or IL10, presented a higher risk of developing INF-induced depression. Finally, despite subjects carrying LL genotype at SERT showed lower rates of anxiety and depressive symptoms during antiviral treatment, these differences were not significant according to our study.

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### Genes of the Serotonin System and Depression in Patients with Coronary Heart Disease

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**Background:** Depression is commonly present in patients with coronary heart disease (CHD). Identification of early predictors of depression in CHD patients is an important direction of current research in the field of psychosomatic medicine. Molecular-genetic studies suggest that genes can increase susceptibility to depression caused by a somatic disease. Serotonin transporter gene polymorphism (5-HTTLPR) has been previously reported to contribute to the development of depression comorbid to CHD. The present study aimed at investigating the association of the HTTLPR polymorphism and the -1438A/G polymorphism of the serotonin receptor type 2A (5-HTR2A) gene with depressive symptoms in CHD patients.

**Methods:** A study included 169 male patients, aged from 31 to 84 years, mean age 59 (8,8 years). The control group consisted of 314 healthy men, aged from 18 to 40 years, mean age 20,9 (7,4 years). Depression was diagnosed in 135 (79,9%) patients. Symptoms of depression were measured by the HAMD-21. Timing of depression was coded as either current (due to CHD), i.e. nosogenic, or lifetime that had developed before the disease. Current depression was recorded in 71 (42%) patients. Trait anxiety was assessed using STAI.

**Results:** The association study revealed the higher frequency of the SS 5-HTTLPR genotype in patients with depression compared to the control group ( $\chi^2=3,8$ ;  $df=1$ ;  $N\epsilon=0.048$ ; OR 1,7, 95%CI 1,0 -2,8). Effects of gene polymorphism and timing of depression on the depression severity (HAMD-21 scores) were calculated using ANCOVA adjusted for age and trait anxiety. The interaction effect between 5-HTTLPR and timing of depression was identified ( $F=8.1$ ;  $p=0.005$ ). Patients with one or two copies of an S allele who developed depression due to CHD had highest depression scores compared to those with other combinations of 5-HTTLPR alleles and timing of depression. Both the -1438A/G 5-HTR2A polymorphism and timing of depression predicted depression scores though no interaction between them was found. The highest scores had patients with a G allele in the presence of the nosogenic factor. Trait anxiety contributed significantly to the variance of depression scores but its effect was less significant than that of genetic variant and nosogenic factor.

**Discussion:** The approach suggested in the study may be useful for the prediction of depression and its severity in patients with CHD.

**Psychopathological Disorders (Depression) on Chronic Hemodialysis Patients at EMMS Nazareth: Identification and Assessment**

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**Background:** Psychopathological conditions such as depression illnesses are the commonest symptoms encounter hemodialysis (HD) patients, which can manipulate quality of life, morbidity and mortality of End Stage Renal Disease (ESRD) patients. The purpose of the present study was to assess the prevalence of depression and its types in the Arab population undergoing hemodialysis treatment in Nazareth metropolitan area, Israel.

**Methods:** We conducted a cross-sectional prospective study at Hemodialysis unit of the Nazareth Hospital from 1st May 2010 to 30th November 2011. We recruited patients [(n=71), mean age (61.9 ± 14.13)] who underwent Hemodialysis more than three months, and healthy control [(n=26), mean age (59.3 ± 9.3)]. Written consent was obtained from both control and patient. Beck's Depression Inventory (BDI) and Hamilton Depression Scale were administered. Blood sample were drawn for hematological, biochemical parameters and viral markers. Diagnosis was made by using the Statistical Manual of Mental Disorders, (DSM-IV) for correlation of psychological variables with clinical, hematological and biochemical parameters. The statistical analysis carried out by one-way analyses of variance (ANOVA) followed by Turkey post-hoc multiple comparison tests..

**Results:** The mean ages of the chronic depressed Hemodialysis patients (HP) and control subjects were 61.92 ±14.13 and 59.3± 7.3 years, (p>0.05), respectively. The prevalence of depression among (HP) was 43.7%. The frequency of depression was twofold high among young females patients (n=8, age < 27 years) undergoing Hemodialysis at least for twelve months of duration comparing to adulthood females (HP) patients (n=29, age > 27 years), p<0.0001. Depression was two folds frequent among unmarried (HP) patients (n=43) versus married (HP) patients (n=28), (p=0.003), and three fold frequent among illiterate (HP) patients (n=33) versus literate patients (n=38), (p=0.001). Depression also was highly notified among females (HP) patients 61% vs. 39% among (HP) males patients (p=0.003). The standard of living and the socioeconomic status for all our patients were stumpy, and almost all need financial supports, to hold up their disability after losing their employment. A covariant connection was observed between the joblessness (n=66: 93%) and depression. Indeed, in our study, when the socioeconomic status deteriorates, the tendency to depression increases accordingly (p<0.001). Hospitalizations rate were highly notified among depressed HD patients versus controls (61% vs 24%, P<0.004), respectively. Comparing Cortisol values between (HD) patients and control, the levels were statistically significant in favor of HP [(p<0.0001 (95% CI 2.416 to 6.825; Mean Cortisol ± SEM of (HP) patients (n=71) = (16.96 ± 0.5476); and for control (n=26) = (11.96 ± 1.116)]. Comparing cortisol values between depressed HP patients and control, the values were also statistically significant in favor of (HP) patients [(p=0.0013 (95% CI 1.868 to 7.184; Mean ± SEM of depressed patients (16.48 ± 0.72); and for control (11.96 ± 1.116)]. Regarding cortisol values between depressed HD patients versus undepressed HP patients, there were no statistically significant differences between the two groups (p>0.05). Nevertheless the levels of Norepinephrine between depressed (HP) patients and control were insignificant. C-Reactive protein (CRP), Albumin, Hemoglobin, Calcium and Phosphor values were compared between depressed (HP) patients versus undepressed

(HP) patients the results were statistically insignificant (p>0.05). In spite of the warmly endeavors and the strong familial embracement found in Arabic traditions have failed to decrease the depression among our patients.

**Discussion:** Majority of patients undergoing hemodialysis were depressed. Studies of Glucocorticoid turnover activity such as Cortisol - a potent chemical stress hormone in bloodstream- maybe manipulated as a model and marker for early diagnosis of depression among HP patients. In contrary Norepinephrine has been found futile. Notwithstanding, the warmly endeavors and the strong familial embracement found in Arabic traditions have failed to decrease the depression among these patients.

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**The BCL1 Polymorphism in the Glucocorticoid Receptor Gene is Associated with Emotional Memory Performance in Healthy Individuals**

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**Background:** Glucocorticoids, stress hormones released from the adrenal cortex, are important players in the regulation of emotional memory. Specifically, in animals and in humans, glucocorticoids enhance memory consolidation of emotionally arousing experiences, but impair memory retrieval. These glucocorticoid actions are partly mediated by glucocorticoid receptors in the hippocampus, amygdala and prefrontal cortex, key brain regions for emotional memory. In a recently published study in patients who underwent cardiac surgery, the BclI polymorphism of the glucocorticoid receptor gene (NR3C1) was associated with traumatic memories and posttraumatic stress disorder symptoms after intensive care therapy. Based on this finding, we investigated if the BclI polymorphism is also associated with emotional memory in healthy young subjects (N=841).

**Methods:** To assess memory performance, we used a picture-learning task consisting of learning and recalling emotional and neutral photographs on two consecutive days. Genotyping of the BclI polymorphism was done with Pyrosequencing.

**Results:** The BclI variant was associated with short-delay recall of emotional pictures on both days, with GG-carriers showing increased emotional memory performance as compared to CG- and CC-carriers. We did not find a genotype-dependent difference in recall performance for neutral pictures.

**Discussion:** These findings suggest that the BclI polymorphism contributes to inter-individual differences in emotional memory also in healthy humans.

**Psychological Treatment Response of Cognitive Behaviour Therapy for Social Anxiety Disorder and Genetic Polymorphisms in Three Candidate Genes**

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**Background:** The association between cognitive behaviour treatment response and genetic variation - therapygenetics, has showed some promising results in recent studies. Only once before has the relationship between polymorphisms and Cognitive behaviour therapy (CBT) for Social anxiety disorder (SAD) been addressed. Never before have the long-term effects of genetic influence for SAD been investigated. Further understanding of underlying biological mechanisms for treatment response might help contribute to a more specified therapy and higher treatment response for SAD. Top candidate genes as the serotonin transporter (5-HTTLPR)-, the catechol-o-methyltransferase (COMT) val158met- and the tryptophan hydroxylase-2 (TPH2) gene are earlier suggested as important in amygdala reactivity and fear extinction and might hereby be of relevance for treatment response. We wanted to examine whether some genotypes respond differently to CBT-treatment, and if so, whether this effect is sustained over time. Our overall goal was to identify genetic markers associated with CBT treatment outcome for SAD on a long-term basis in two independent samples.

**Methods:** Participants were recruited from two independent randomised controlled trials with cognitive behaviour therapy for social anxiety disorder (sad) in Stockholm (n=112) respectively in Uppsala (n=202). Participants were pooled and analysed pre treatment and at long-term follow up. The short-term (post) effect was analysed separately in the samples due to differences in time points of treatment. The main outcome measure in both samples was improvement on the Liebowitz Social Anxiety Scale- Self Report (LSAS- SR) after treatment compared to baseline.

**Results:** We found no effect of any of genotype and cognitive behaviour treatment response at long-term follow up for SAD-patients. Nor were there any significant effects of gene x gene interaction and treatment response. However, the ANOVA analysis of the subsamples displayed a significant effect (Stockholm-sample p= .031, df 1, F 4,79) of the TPH2-gene and response to CBT immediate after treatment.

**Discussion:** None of the polymorphisms in the three serotonergic candidate genes (5-HTTLPR, COMTval159met and TPH2) seems to have any long-term effect on treatment outcome for SAD. The TPH2-gene could have an effect on short-term CBT-response suggesting a potential placebo effect early in treatment and an extinction effect later in treatment. However, the results in the different samples are somewhat contradictory and the effect diminishes at long-term follow up. Altogether, the overall impression is that these three genes are not necessary predictors for cognitive behaviour therapy response for social anxiety disorder.

### The Interaction of the Catechol-O-Methyltransferase VAL158MET Polymorphism and Early Life Experiences Affects an Intermediate Endophenotype of Anxiety Disorders

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**Background:** The pathogenesis of anxiety disorders is considered to be multifactorial with a complex interaction of genetic factors and individual environmental factors. A functional polymorphism of the catechol-O-methyltransferase gene (COMT val158met) has been reported to be relevant for the etiology and pathophysiology of these disorders. The aim of the present study is to examine a gene-by-environment interaction of the COMT val158met polymorphism and life events with respect to anxiety sensitivity as an endophenotype of anxiety disorders.

**Methods:** A sample of healthy subjects (N = 772) was genotyped for the COMT val158met polymorphism and assessed for childhood adversities (Childhood Trauma Questionnaire CTQ) and anxiety sensitivity (Anxiety Sensitivity Index ASI). Main and interaction effects of genotype, environment and gender on anxiety sensitivity were conducted by means of hierarchical multiple regression analyses.

**Results:** Association analysis revealed no significant modulation of anxiety sensitivity by variations of the COMT gene. A significant interactive effect of childhood adversities and COMT genotype was observed: Homozygosity for the low active met allele and high CTQ scores conducted a significant increment of explained ASI variance ( $R^2 = .016$ ,  $\Delta R^2 = .008$ ,  $p = .014$ ).

**Discussion:** Our results indicate a gene-by-environment effect of the COMT val158met polymorphism and childhood adversities on the intermediate endophenotype anxiety sensitivity in healthy subjects. Homozygosity for the met allele might increase the vulnerability to anxiety disorders, if there is a supplementary exposure to aversive early life experiences.

### Are TMEM Genes Potential Candidate Genes for Panic Disorder?

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**Background:** A recent German genome wide association study (GWAS) has shown a possible role of the transmembrane protein 132D in the aetiology of panic disorder (PD). (1) Two transmembrane proteins - transmembrane protein 98 (TMEM98), and transmembrane protein 132E (TMEM132E) - are located in close proximity to a previously reported candidate gene on chromosome 17.2. This study aimed to determine the possible role of TMEM genes in the aetiology of PD by analysing single nucleotide polymorphisms (SNPs) in TMEM98 and TMEM132E, and by replicating the association between TMEM132D and PD. The SNPs were analysed in three samples: a Faroese, a Danish, and in the German GWAS sample.

**Methods:** Two SNPs located within TMEM132D, 11 SNPs within TMEM98 and 25 SNPs within TMEM132E were genotyped using the Sequenom® platform. The Faroese sample consisted of 36 cases and 162 control individuals. The Danish sample consisted of 243 cases and 649 control individuals. The German sample consisted of 236 cases and 222 controls. The Faroese and Danish patients were diagnosed with PD or agoraphobia with PD according to the ICD-10 Diagnostic Criteria for Research (WHO: 1993). The German patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria. Cochran-Armitage trend tests were performed as implemented in PLINK.

**Results:** The Faroese sample: Two SNPs within TMEM132E showed nominal significant association with PD (rs4795942,  $p=0.035$ ; rs12602358,  $p=0.029$ ). The Danish sample: Three SNPs within TMEM132E showed nominal significant association (rs887231,  $p=0.004$ ; rs887230,  $p=0.029$ ; rs4795942,  $p=0.006$ ). One SNP within TMEM132D showed nominal significant association (rs7309727,  $p=0.02151$ ). The German sample: Two SNPs within TMEM132D showed significant association (rs7309727,  $p=8.41E-06$ ; rs11060369,  $p=0.002$ ).

**Discussion:** We observed nominal association between PD and SNPs within TMEM132E in the Faroese and Danish samples. No association was seen between SNPs within TMEM98 and PD in any of the analysed samples. In the Danish sample we were able to replicate the significant association between TMEM132D and PD seen in the German GWAS. (1) However, when correcting for multiple testing the significant association disappears and therefore it is likely that TMEM genes only contribute a moderate effect in the aetiology of PD.

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### Evaluation of Anxiolytic Effect of the Essential Oil of *Myrtus Communis* in Mice

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**Background:** With the currently available agents, the majority of patients with anxiety disorders can be treated. Nevertheless, as many as one-third of patients in controlled studies are unresponsive to any one of these medications. Apart from this, clinical evaluation of these drugs has shown different side effects. Thus, there remains a need for new safe and effective anxiolytic agents, for which natural products are potential candidates. *Myrtus communis* is used in aromatherapy to treat oily skin, acne, coughs, anxiety, infections, insomnia, asthma and bronchitis.

**Methods:** The present study was undertaken to evaluate the anxiolytic effect of the essential oil of *M. communis* using different models of anxiety including, elevated plus maze model, stair case model and open field model. Control groups were given Tween 80 (5%, v/v) in distilled water (vehicle). Positive controls received diazepam (0.5 mg/kg, orally, suspended in the vehicle), test groups were given the essential oil suspended in the vehicle at doses of 50, 100, 200 and 400 mg/kg.

**Results:** At a dose of 50 mg/kg and 400 mg/kg no significant difference was noted on the measured EPM parameter compared to the control. The essential oil of *M. communis* at a dose of 200 mg/kg did not produce significant increase in percentage of open arm entry as compared to the control. In contrast, 100 mg/kg (54.1%) of the oil and 0.5 mg/kg diazepam (54.5%) ( $P < 0.05$  in both cases) did show a significant increase in percentage of open arm entry compared to the control (38.5%). The essential oil of *M. communis* showed a significant increase in percentage of open arm time in both test groups treated with 100 mg/kg (52.82%) ( $P < 0.01$ ) and 200 mg/kg (47.95%) ( $P < 0.05$ ) compared to the control (26.15%). The essential oil of *M. communis* at 100 mg/kg ( $P < 0.01$ ) and 200 mg/kg ( $P < 0.05$ ) doses resulted in a significant reduction in the number of rearing compared to the control. At a dose of 100 mg/kg, the oil resulted in a better reduction (57.52%) of the number of rearing compared to that of diazepam (37.25%). Mice treated with a 100 mg/kg dose of the oil did produce significant increase (73.85%) in the time spent in central squares ( $P < 0.05$ ) compared to the control. However, the essential oil at a dose of 50, 200 and 400 mg/kg failed to reach statistical significance compared to the control. The total number of square entries into open field was comparable in groups treated with the vehicle and different doses of the oil (50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg, p.o).

**Discussion:** In the present study, the essential oil of *M. communis* at a dose of 50 mg/kg lacks anxiolytic effect as it is a subthreshold dose. However, the oil at a dose 100 mg/kg demonstrated intriguing activity as evidenced by the significant increase in percentage of open arms entries as well as percentage of time spent in open arms. Although at a dose of 200 mg/kg, the oil was able to increase percentage of open arm time, it failed to increase significantly the percentage of open arm entry unlike the effect of the oil at a dose of 100 mg/kg implying 200 mg/kg is not as effective as 100 mg/kg for the treatment of anxiety. The oil at a dose of 100 and 200 mg/kg was able to reduce anxiety as evidenced by the significant reduction in the number of rearing. However, the level of significance at which the 100 mg/kg (\*\* $P < 0.01$ ) showed activity was obviously higher than that of 200 mg/kg (\* $P < 0.05$ ). Thus, the oil at a dose of 100 mg/kg has a much better anxiolytic effect than the one at 200 mg/kg. Also, the oil at a dose of 50 mg/kg and 400 mg/kg did not exhibit any anxiolytic activity as it did not significantly change the number of rearing. The effects produced by the essential oil

of *M. communis* at doses of 50, 100, 200 and 400 mg/kg and diazepam (0.5 mg/kg) in the open field test demonstrated that these products do not modify the spontaneous locomotor activity of mice as there was no significant change in the number of total squares crossed.

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### The Association of 5-HT3 Receptor Gene Polymorphisms with Obsessive-Compulsive Disorder

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**Background:** A role for the serotonin (5-HT) system in the pathophysiology of OCD has been supported in previous research, including studies on peripheral markers of 5-HT function and pharmacological challenge tests, as well as studies on the efficacy of selective serotonin reuptake inhibitors (SSRIs) on obsessive-compulsive (OC) symptoms. 5-HT acts on various subtypes of postsynaptic and presynaptic 5-HT receptors. Recently, an antagonist of the 5-HT3 receptor, ondansetron, has been reported to have therapeutic effects on OC symptoms. These findings suggest the potential involvement of 5-HT3 receptor in the pathophysiology of OCD. Therefore, we investigated the association between genetic variants of 5-HT3 receptor and OCD.

**Methods:** 262 subjects (male 176, female 86) with OCD were recruited from three university hospitals. 220 unrelated control individuals (male 110, female 110) were recruited by advertisement. Mean Y-BOCS of OCD subjects was 22.90±6.9. All subjects donated a blood sample through venipuncture, and DNA was isolated using standard techniques. Haploview 4.1 was used to generate a linkage disequilibrium map and to test for Hardy-Weinberg equilibrium. The "R" software employed to analyze haplotypes. SNPs across 5-HT3C, D, E genes in HapMap database were used to select tag SNPs. Based on the distance between adjacent tag SNPs, we selected two SNPs (rs6807362, rs6807670) of 5-HT3C, four SNPs (rs6443930, rs1000952, rs6799766, rs10937160) of 5-HT3D, and two SNPs (rs6765267, 7627615) of 5-HT3E genes.

**Results:** The allelic distribution of rs10937160 from 5-HT3D is significantly different between OCD and controls. ( $\chi^2=7.84$ ,  $p=0.005$ , and  $p=0.03$  after permutation of  $n=1000$ ). The allele of rs10937160 (3'UTR C/T of 5-HT3D) was more frequently found in OCD. The genotypes of rs10947160 was significantly associated with OCD in additive model ( $p=0.0067$ ). When we adjusted the potential effect of gender, it was still statistically significant ( $p=0.027$ ) even after Bonferroni correction. However, in haplotype analyses of those SNPs, we found no significant association between OCD and control.

**Discussion:** Our data suggest that a genetic variant of 5-HT3D might be related to the pathophysiology of OCD.

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### Investigating Telomere Length and Psychological Stress in South African Rape Victims

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**Background:** Women are at an increased risk of depression and other mental health problems following rape. Various aetiological factors for depression, including predisposing genetic factors, have been identified. Telomeres are repetitive nucleoprotein structures located at chromosomal ends that protect them from premature degradation. Telomeres reduce in length with each cell division, resulting in cellular senescence and apoptosis. Additional factors, such as oxidative and psychological stress, can further induce telomere shortening.

**Methods:** This study performed relative quantification of telomeric repeats with the use of real-time PCR methods to investigate whether shorter relative leukocyte telomere length (LTL) in a cohort of rape victims was associated with resilience, the development of trauma-related major depressive disorder (MDD), as well as the development of PTSD after three months.

**Results:** No significant associations were observed between relative LTL and resilience or the development of MDD at either baseline or after three months in this cohort. However, a significant association was evident between relative LTL and PTSD status.

**Discussion:** The significant association between relative LTL and PTSD suggests that shorter relative LTL might have acted as a predisposing factor to the development of PTSD after a severely traumatic event. Telomere shortening may be an important marker of PTSD risk, which has implications for early intervention and timely treatment.

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### The Role of Serotonergic Genes and Environmental Stress on the Development of Depressive Symptoms and Neuroticism

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**Background:** Depression is considered to be the result of a complicated synergy between genetic and environmental factors. Several genes of the serotonergic neurotransmission have been related to depression phenotypes, however results are inconsistent, possibly due to the oversight of the role of environmental stress.

**Methods:** We examined gene-environment (GxE) interactions with serotonergic genes on depressive symptoms and neuroticism in a homogeneous population-based sample of 415 females. We chose several genetic variants within candidate genes (SCL6A4, TPH2, HTR1A) that have been previously found to provide some evidence of association with depression outcomes.

**Results:** Single marker analyses showed a significant GxE interaction with several TPH2 variants, including rs4570625, on depressive symptoms. Significant GxE interactions were also observed with TPH2 haplotypes. No reliable associations were observed with SCL6A4 and HTR1A genes. We did not find any robust evidence of a direct impact of serotonergic genes on depressive symptoms or neuroticism.

**Discussion:** The present study indicates an association between TPH2 and depressive symptoms that is conditional on prior experience of stressful life events. Further evidence is provided about the role of the environment in genetic vulnerability to depression.

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### Genetic Studies of Oxidative Stress Reveal the Mechanism by which GLO1 Influences Behavior

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**Background:** Numerous mouse genetic studies have identified associations between the expression of Glyoxalase 1 (Glo1) and anxiety-like behavior; however, the underlying mechanism has been elusive. We have previously shown that a common genomic duplication that is present in about 1/3 of inbred mice leads to the duplication or triplication of Glo1, which is responsible for previously observed expression differences among inbred and outbred mice. GLO1 is an enzyme that detoxifies methylglyoxal (MG). We have recently established that Glo1 expression increases anxiety by reducing MG levels.

**Methods:** We produced mice with a transgenic bacterial artificial chromosome containing Glo1.

**Results:** Transgenic mice displayed increased anxiety-like behavior and reduced MG concentrations. Acute administration of MG reduced anxiety-like behavior and, at higher doses, caused locomotor depression, ataxia, and hypothermia; effects that are characteristic of GABA-A receptor activation. When applied to primary cerebellar granular neurons in culture, physiological concentrations of MG selectively activated GABA-A receptors with about 1/3 the potency of GABA. These effects could be blocked by the GABA-A selective antagonist SR-95531. Competition studies suggest that GABA and MG may compete for the same binding site.

**Discussion:** Taken together our data establish that Glo1 expression increases anxiety by reducing levels of MG, thereby altering GABA-A receptor activation. More broadly, they provide a link between metabolic state, neuronal inhibitory tone, and behavior. Finally, they point the way toward potentially novel pharmacological interventions.

**Individual Differences in Presentation of Anxiety and Affective Disorders Predict Genetically Determined Differences of Brain Limbic System: The Three Human Personality Type Model as a Guide for Clinicians and Researchers**

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**Background:** Cumulative evidence from genetic and genomic studies, and neurobiological, neuroimaging, and clinical research supports the notion that individual variability in emotion processing may be associated with genetic variation as well as with dispositional affect and personality styles, that are mirrored in our brains. In this study, we focus on the genetic origins of individual differences in emotion processing, as well as on identifying several phenotypes of anxiety and affective disorders, with different clinical presentations. The Three Human Personality Model, that researcher by the name of G. Paschalidis has described, demonstrates that individual differences in activation, volume and functional connectivity of the three brain structures of limbic system, (temporal cortex, amygdala and hippocampus) determine three human types that have common personality traits and are determined by the genetic make-up of one of the individual parent. This study has the following aims: 1) to present the Three human Personality Model, 2) to examine and to verify whether the knowledge of the three human types meets fully the research data and 3) to compliment the research data of review with a section of a large clinical study that examined specific parent-child transmission for distinct personality traits of the three types associated with heightened risk for subtypes of anxiety and affective disorders.

**Methods:** We conducted a systematic review and synthesize the recent scientific literature on research on the neurobiology and imaging genetics of emotion. Thorough research was performed using main literature databases, and web search engines such as Pubmed, Google, for relevant studies, by using appropriate keywords. In the clinical study, we studied 80 adolescents, 12-18 years old, with a diagnosis of Anxiety and Affective disorder and their mothers and fathers, and 80 matched controls. Adolescents and parents were given a detailed psychiatric assessment and were studied on measures of demographics, personality traits and psychopathology, using questionnaires and interviews. All completed the Three Human Personality Type Model Questionnaire.

**Results:** The Three Human Personality Type model can fully underpin the individual differences in affective processing, which are of great importance because they are central to conceptualizations of personality and temperament, and contribute to risk for different phenotypes and subtypes of Anxiety and Affective disorders. Persons with Human Type A behaviour pattern are characterized by action at the moment, rage, extraversion, high novelty seeking, risk taking which are associated with genetic structural and functional differences of temporal cortex. These persons presents Panic Disorder. People with Human Personality Type B show anxiety about future, fear and appear to have hyperactive amygdala. They exhibit high risk for Depression and Generalized Anxiety Disorder and Phobia. Persons with Type C are characterized by overconscientious, hard working and self-disciplined, mania with persistence and reliving the past with intrusive thoughts, traits that are linked to abnormal functioning of the hippocampus. They exhibit Bipolar Disorder, Obsessive-Compulsive disorder and Major Depressive Disorder with Psychotic features. The clinical study examined the distinct influences of parent personality traits, and anxiety and affective disorder symptoms in the transmission of the risk for psychopathology to their children. The study highlights the positive association of the specific type personality traits of the

three human type model to different subtypes of anxiety and mood disorders. Each participant in the study was identified to have personality characteristics that belong only to one of the three types of the presenting model, that were described stable with aging. The study support that the personality traits of the type of adolescents matches with personality traits of their father when the birth order is first, third and fifth and of their mother when the adolescent was second and fourth in birth order. All persons with type A had the diagnosis of panic disorder, all of type B had depression, generalized anxiety disorder and phobia, and with type C diagnosed with bipolar disorder and obsessive compulsive disorder.

**Discussion:** The three Human Personality Type Model shed new light on variability in neural networks of emotion. This model leads to the identification of the mechanisms that give rise to individual differences in emotional stability and vulnerability to stress and anxiety and determine genetically influenced neurobiological intermediate phenotypes that are associated with subtype, severity and the course of anxiety and affective disorders.

**Investigating the Effect of Early Postnatal Maternal Separation and Adult Restraint Stress on Gene Expression and DNA Methylation in the Rat Ventral Hippocampus**

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**Background:** Posttraumatic stress disorder (PTSD) is a debilitating anxiety disorder that may arise after exposure to a traumatic event. PTSD is characterized by an inability to efficiently regulate and inhibit fear responses. The majority of individuals may be exposed to a trauma throughout their lifetime, however only a minority will develop PTSD. Family and twin studies have indicated a genetic contribution to the development of the disorder. To identify genetic factors involved in PTSD aetiology, the present study focused on two stressors, namely early postnatal maternal separation (MS) and adult restraint stress (RS). The hippocampus is known to be important in PTSD pathophysiology, given that it is critical in regulating the stress response via the hypothalamus-pituitary-adrenal (HPA) axis. Synaptic plasticity plays an important role in hippocampal memory processes that are dysregulated during the stress response; therefore this pathway was the focus of the study. It is hypothesized that epigenetic mechanisms, which play a role in the regulation of genes, may explain the differences between individuals with resilience and those with vulnerability to stress-related disorders. The aim of this study is to identify the effect that early postnatal maternal separation (MS) and adult restraint stress (RS) have on expression and DNA methylation in genes encoding components of the synaptic plasticity pathway in the ventral hippocampus (VH) of the rat.

**Methods:** Fifty-one male, Sprague-Dawley rats were divided into four experimental groups: (1) MS (n = 12), (2) RS (n = 13), (3) MS + RS (n = 13) and (4) no MS + no RS (controls) (n = 13). MS was performed between postnatal day (PND) 2 and 14, while adult RS was performed from PND 65-70. Gene expression analysis of 84 genes encoding components of the synaptic plasticity pathway was performed using pathway-based gene expression profiling arrays. Differentially expressed genes identified with pathway-based gene expression analyses, were further investigated to assess DNA methylation patterns. DNA methylation analysis of the CpG dinucleotides of the promoter regions of the genes were assessed using custom methyl-profiler PCR arrays.

**Results:** Six genes encoding components within the synaptic plasticity pathway were differentially expressed by at least 2-fold between the experimental groups compared to the control group. Ionotropic glutamate receptor AMPA 1 (Gria1) was overexpressed in MS compared to control rats, while the pim-1 oncogene (Pim1) and tumor necrosis factor (Tnf) genes were overexpressed in the MS+RS group. Genes that were underexpressed in the MS group included the cannabinoid receptor 1 (Cnr1) and nerve growth factor receptor (Ngfr). The cannabinoid receptor 1 gene was also underexpressed in the MS+RS group, while, guanine nucleotide binding protein (G protein) alpha inhibiting 1 (Gnai1) was underexpressed in the RS group. Three of these genes met the criteria for the DNA methylation analyses (Cnr1, Ngfr, Pim1). The levels of hypermethylation, intermediate methylation and hypomethylation were assessed and compared between the experimental groups and the control group.

**Discussion:** Gene expression analysis of the 84 genes encoding components of the synaptic plasticity pathway revealed differential expression between the stressed groups compared to the control group. Of the 84 genes that were assessed, six genes were identified

for further study. Long-term potentiation (LTP) and long-term depression (LTD) are two of the most studied cellular models of synaptic plasticity, involved in activity-dependent strengthening and weakening of synaptic transmission, respectively. The lasting effects of these changes in the strength of the synaptic transmission enable the acquisition of new memories. The downregulated genes identified in this study include Cnr1, which modulates memory and is involved in glutamatergic synaptic transmission, emotional regulation, cognitive information processing, as well as LTP and LTD regulation and inhibition; Gnai1, which is associated with cognitive ability; and Ngfr, which is involved in cell survival and migration, synaptic transmission and plasticity. Upregulated genes include Gria1, which is involved in activity-induced regulation of synaptic transmission and neural plasticity; Pim1, which is an immediate-early response gene (IEG) that is rapidly induced and form the earliest genomic response to synaptic activity; and Tnf, which influences synaptic strength in the hippocampus and is involved in LTP and LTD.

### Allelic Variation in CRHR1 Predisposes to Panic Disorder

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**Background:** Corticotropin releasing hormone (CRH) is a major regulator of the hypothalamic-pituitary-adrenal (HPA) axis. Binding to its receptor CRHR1 triggers the downstream release of cortisol, a hormone needed for regulation of stress response. The latter is often dysregulated in mood and anxiety disorders and insight from biochemical, behavioral and genetical studies revealed CRHR1 as a possible candidate gene. Here, we aimed at extending our previous finding that variants in CRHR1 are associated with panic disorder.

**Methods:** Allelic variation throughout the CRHR1 gene was captured by 9 selected single nucleotide polymorphisms (SNPs); these were genotyped in matched discovery (n=478) and replication (n=584) samples of German descent. For the replication sample, also information about the dimensional anxiety trait sum scores Agoraphobic Cognitions Questionnaire (ACQ) and Anxiety Sensitivity Index (ASI) was available. Genetic associations with panic disorder were determined with  $\chi^2$  tests, quantitative data employed linear regressions.

**Results:** The SNPs rs7209436, rs12936181, rs17689918 and rs17689966 were found to be associated with panic disorder; the latter both also affect the ACQ and ASI scores. Analysis of the linkage disequilibrium structure revealed 88 perfect proxies for rs17689918 and rs17689966, which also hit neighboring genes C17orf69, IMP5 and MAPT. Bioinformatical prediction of SNP function revealed a high proportion of differential neuro-relevant transcription factor binding and implied allele-specific changes in transcript splicing. Also, polymorphisms with deleterious effects on the coding sequence were found in adjacent genes.

**Discussion:** Our results confirm the previously reported finding that allelic variation in the CRHR1 gene is associated with panic disorder. The discordant associations between both genders may point to the presence of gender-specific genotypic effects. Furthermore, the analysis of local linkage disequilibrium structure in conjunction with functional predictions revealed that - apart from CRHR1 variants - also polymorphisms in the neighboring genes C17orf69, IMP5 and MAPT (some of which are known for their influence on psychiatric disorders) may predispose to panic disorder. These results strengthen the role of CRHR1 and suggest possible new candidate genes for panic disorder. However, an independent replication to support their validity would be necessary.

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### Strong Genetic Evidence of Protocadherin-Alpha (PCDHA) as a Susceptibility Gene for Autism

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**Background:** Altered synaptic development and plasticity due to impaired synaptic protein synthesis has been proposed as a causative of autistic phenotypes. Neurons involved in synaptogenesis possess unique molecular cues that are crucial in establishing specific synaptic connections. The protocadherin alpha (PCDHA), owing to their localization to synaptic junctions, has been suggested to play an important role in this process. By a molecular mechanism involving differential promoter activation, alternative splicing and monoallelic expression, a great combinatorial diversity of individual PCDHA mRNAs are expressed at the pre- and post- synaptic neurons, providing diverse molecular signals that are vital in specifying neuronal identities and synaptic connections. PCDHA has also been found to be important for the serotonergic projections to appropriately innervate target brain areas. Given these, we hypothesize that PCDHA is a suitable candidate gene for autism.

**Methods:** 14 PCDHA SNPs were examined for genetic association with autism in DNA samples of 3211 individuals (841 families, including 574 multiplex families) obtained from Autism Genetic Resource Exchange (AGRE). TaqMan method was used to score the genotypes. The genetic association of SNPs with autism was examined by FBAT software in a family-based association test under an additive model. Pairwise linkage disequilibrium between SNPs was estimated using Haploview. Quantitative Transmission Disequilibrium Test (QTDT) was used to examine the association between quantitative ADI-R scores and SNPs selected from FBAT analysis ( $p < 0.05$ ). Furthermore, in autism samples, the variability in the distribution of ADI-R phenotypic data across the homozygous- and heterozygous-genotypes of the SNPs selected from FBAT analysis ( $p < 0.05$ ) was examined by one-way analysis of variance (One-way ANOVA).

**Results:** Five PCDHA SNPs (rs251379, rs1119032, rs17119271, rs155806, rs17119346) showed significant associations with autism. The strongest association ( $p = 0.0006$ ) was observed for rs1119032 (Z-score of risk allele G=3.415) in multiplex families. SNP associations were found to withstand multiple testing correction in multiplex families. Upon analyzing families with male and female autistic individuals separately, significant SNP associations were observed only in male samples. Haplotypes involving rs1119032 showed very strong association, withstanding multiple testing correction, with autism. In QTDT analysis, the G allele of rs1119032 showed a significant association ( $p = 0.033$ ) with ADI-R\_D scores (early developmental abnormalities). We also found a significant difference in the distribution of ADI-R\_A scores (social interaction) between the A/A, A/G and G/G genotypes of rs17119346 ( $p = 0.0025$ ).

**Discussion:** Loss-of-function mice have revealed that Pcdha plays pivotal roles in neuronal survival, synaptic connectivity, axonal convergence, and learning and memory. PCDHA has also been found to be essential for the serotonergic projections to appropriately innervate target brain regions during early development. In PCDHA knockout mutant mice, abnormal distribution of serotonergic fibers have been observed in various target regions, along with abnormal levels of serotonin in the hippocampus. Abberant serotonergic innervations have been implicated in several behavioral abnormalities related to autism. Previously, structural variations of protocadherins such as PCDH9 and PCDH10 have been observed in autism. Recently, a new microdeletion syndrome of 5q31.3, the locus of PCDHA, has been reported in patients with severe developmental delays. We report, for the first time, a strong association of PCDHA gene cluster with autism. The G allele of the SNP rs1119032 was found to be a risk allele in both single SNP- and haplotype- association tests. In QTDT analysis, the G allele of rs1119032 showed a significant association with ADI-R\_D scores. This is an interesting observation, since PCDHA has been reported to function during neurogenesis in early development, owing to its role in neuronal circuit maturation. In conclusion, our study provides strong genetic evidence of PCDHA as a potential candidate gene for autism.

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### Study of Single Nucleotide Polymorphism in Chromosomes 11 and 15 In Autism Spectrum Disorder

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**Background:** Several genetic loci in chromosomes 11 and 15 have recently been associated with non-syndromic autism spectrum disorder (ASD) in populations from North America and Europe. The aim of the present study was to investigate whether such an association exists in a North-Eastern European population.

**Methods:** Ninety-five patients with ASD in the age range 3–20 years (mean age 8 years, SD 3.18) participated in the study. The control group consisted of 161 healthy, non-related individuals without ASD randomly selected from the Latvian Genome Database. Four single nucleotide polymorphisms (SNPs) – rs11212733, SNP rs1394119, rs2421826, rs1454985 – were genotyped by the TaqMan method. Allele frequency differences between ASD patients and control subjects were compared for each SNP using a standard chi-square test with Bonferoni correction. The level of statistical significance was set at 0.05 for nominal association.

**Results:** Only the genetic marker rs11212733, localized on the long arm of chromosome 11 in locus 22.3, was found to be strongly associated with the ASD patient group (c2 6.982, padjusted 0.033, Odds ratio 1.625).

**Discussion:** Our data demonstrating a significant relationship between the SNP rs11212733 and the development of ASD in a North-Eastern European population suggest it is not a population-specific relationship. Thus, future studies focusing on the DDX10 gene and related genetic loci are indicated.

### The Brain and Body Genetic Resource Exchange (BB-GRE): A Recall by Genotype Bioresource for Translational Research into Neurodevelopmental Disorders

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**Background:** Genome imbalance (copy number variation; CNV) is a major source of human disability, especially brain disorders. Recently, association between rare pathogenic CNVs and common, complex disorders (autism, schizophrenia, epilepsy) has emerged. However information is lacking on detailed genotype-phenotype relationships, and the underlying basic biology. Since CNVs are rare, population referrals for clinical genetic testing using array competitive genome hybridisation (aCGH) are an important resource for research. BB-GRE describes such patients from the southeastern region of the UK, and combines phenotype information from these patients with neurodevelopmental, behavioural problems or other non-CNS indications with clinical genetic data, and displays this information on the human genome browser.

**Methods:** Testing was carried out at a regional cytogenetics CPA accredited laboratory, using an Agilent oligonucleotide array 60K platform (designs 028469 and 017457) with a 24% detection rate of a clinically significant imbalance. Genomic data and referral phenotype information was recorded in a clinical database, which at the time of analysis contained 10,397 clinical referrals (November 2011), including approximately 1,400 patients referred for ASD, 26% of whom were female. Records were anonymised and added to the BB-GRE database if they contained a potentially pathogenic imbalance (n = 2227). Consent for re-contact for research studies is being requested from new referrals.

**Results:** The BB-GRE database can be found at [bbgre.org](http://bbgre.org) in a searchable format. The data can be viewed as a custom track on the UCSC genome browser. Phenotypic data recorded in the referral includes data on cognitive development, specific developmental disorders, neurodevelopmental/behavioral problems, neurological disorders, growth abnormalities, congenital malformations/dysmorphism, heart disease (e.g. ASD, VSD) and endocrine and metabolic conditions. By mapping the BBGRE phenotypes to the NCBI MeSH headings using keywords and then manual curation, the inherent hierarchical structure can be used to regroup phenotypes and aid data analysis. Known pathogenic CNVs are represented in BB-GRE (e.g. del1q21.1; intragenic NRXN1 deletions; del15q13.3; del/dup16p11.2; del/dup16p13.11; del22q11) as well as novel imbalances. With consent, it is also possible to recall patients for further studies, such as phenotyping (cognition, imaging), biological sampling (gene expression, proteomics) and potential interventions such as clinical trials.

**Discussion:** BB-GRE has been developed as a resource for translational medicine aimed at understanding diseases caused by genome imbalance.

### Mutation Screen and Copy Number Detection of NLGN4 in a Chinese Population with Autism Spectrum Disorder

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**Background:** Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication, absence or delay in language development, and stereotyped or repetitive behaviors. NLGN4, a postsynaptic cell adhesion molecule, plays an important role in synaptic function and trans-synaptic signaling by interacting with presynaptic neuroligins. Missense/non-sense mutations of NLGN4 were identified in a small percentage of ASD cases. Genetic variants in the regulatory region and copy number of NLGN4 are deserved to be studied in ASD since they may influence the expression level of NLGN4.

**Methods:** In this study, we sequenced the regulatory region including the promoter region and 5'/3' untranslated regions (UTR) and the entire coding region of NLGN4 in a cohort of 285 ASD patients and 384 controls using the method of Sanger sequencing. We also detected the copy number variants of NLGN4 in 285 ASD cases using the AccuCopy technology. Moreover, six common SNPs within NLGN4 were analyzed for the association with ASD.

**Results:** No non-synonymous mutation in NLGN4 was detected in our cohort. No significant difference in 6 SNP allele frequencies was observed between male ASD patients and male controls. All female ASD cases showed two copy of NLGN4 and male ASD cases had one copy of NLGN4. No deletion or duplication of NLGN4 was identified in our cohort.

**Discussion:** These findings showed that NLGN4 was not a major disease gene in our ASD cohort. Other genes in the neuroligin-neurexin pathway should be investigated in ASD.

### Association between the Clock Gene and Autism Symptoms in a Swedish Twin Sample

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**Background:** Autism spectrum disorders (ASDs) are pervasive developmental disorders that include Autistic disorder, Asperger syndrome, and pervasive developmental disorder-not otherwise specified (PDD-NOS). Many patients with ASD have sleep impairments and timing problems, suggesting disturbances in the regulation of circadian rhythm as causative factors for these disorders. Indeed, low levels of melatonin are recurrent biological findings and we have previously found association between genes in the melatonin pathway and ASDs. Melatonin is closely related to the circadian rhythms, which is mainly regulated in the suprachiasmatic nucleus (SCN) by a set of clock genes. Genetic variation in the clock genes have previously been investigated in autism patients showing an association with the clock genes PER1 and NPAS2. In this study, we have investigated the possible association of five circadian clock genes on autism symptoms in a Swedish twin sample.

**Methods:** Single nucleotide polymorphisms in five circadian clock genes were genotyped in The Child and Adolescent Twin Study in Sweden (N=1771, 9-12 years old). The measured autism symptoms were restricted and repetitive behavior, language impairments and impairments in social interaction. In addition, the CLOCK gene was screened for mutations in patients with autism (N=90).

**Results:** Our results show a significant association in girls between rs1801260, in the 3'-UTR, of the CLOCK gene, and the symptom restricted and repetitive behavior (p=0.009), but not with the symptoms language impairments and impairments in social interaction. The mutation screening revealed five rare, previously not reported, variants in six different patients.

**Discussion:** In conclusion, our results support the hypothesis that clock genes may be involved in autism related disorders. Moreover, since all symptoms of autism did not show similar association with the investigated clock genes in this study, our findings also emphasizes that genetic research may benefit from taking a symptom-specific approach to finding genes associated with autism.

### The RBFOX1 Gene at 16P13 IS Strongly Associated with Autism Spectrum Disorders in Finnish Families

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**Background:** Autism spectrum disorders (ASD) are neuropsychiatric disorders characterized by restricted repetitive behavior and abnormalities in communication and social interaction (American Psychiatric Association 2000). The model of inheritance of ASD seems to be very complex and probably involves multiple interacting genes. Genome wide screens published to date have identified several regions of low/modest predictive value, and only a few studies have been able to replicate the findings (Autism Genome Project Consortium 2007; Wang 2009 et al.; Weiss et al. 2009; Anney et al. 2010). Most significant SNP association this far is in chromosome 5p14 which includes cadherin genes CDH9 and CDH10 (Wang et al 2010).

**Methods:** A total of 83 Finnish families with 257 members participated in the GWA study. Each family had 1-3 autistic probands. Control material consisted of 303 unrelated healthy Finnish individuals. All the samples were genotyped using the Illumina Infinium Human OmniExpress-12v1 beadchip with an approximate number of 730,000 markers per sample. We used PLINK for TDT analyses within the autism families, and PLINK for population based allele association tests with the case-control materials.

**Results:** The strongest association at TDT analysis was found at chromosomes 16p13.2 containing the gene RBFOX1 that is a neuron-specific splicing factor gene controlling neuronal excitation in the mammalian brain. The strongest association from case-control analyses was found at chromosome 2p12 (rs13404338) in CTNNA2, catenin (cadherin-associated protein), alpha 2 (p-value =  $4.6 \times 10^{-29}$ , Bonferroni-corrected). Interestingly, SNPs rs7099996 and rs12572585 in CTNNA3 (catenin, alpha 3) at 10q21.3 also showed strong association with autism.

**Discussion:** In this study the narrow association peak at chromosome 16p13.2 is at RBFOX1 alias A2BP1 gene which is one of the largest genes in human genome. RBFOX1 is strongly expressed in the brain and it regulates tissue-specific splicing (Fukumura et al. 2007). Transcriptional and splicing dysregulation are the underlying mechanisms of neuronal dysfunction in autism (Voineagu et al. 2011). Interestingly, in a different set of Finnish families, in EU Autism Molgen Consortium, the same area at 16p13 showed the strongest association to autism (Holt et al. 2010). Thus, this finding was replicated in the present study. Considering the catenin (cadherin-associated protein) alpha 2 (CTNNA2) detected in the case-control analysis, the protein is localized in central portion of the postsynaptic density and it controls the stability of dendritic spines and synaptic contacts (Abe et al. 2004). Association between its close homolog CTNNA3 and autism has previously been reported in autism studies (Wang et al. 2009; Weiss et al. 2009). CTNNA3 contributes to the formation of stretch-resistant cell-cell adhesion complexes (Janssens et al. 2001). Our results are in agreement with previous studies and strengthen the hypothesis that genes related to transcriptional and splicing regulation and synaptic functions have a significant role in autism.

### Detection and Characterization of Copy Number Variations in Jewish Israeli Autistic Patients

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**Background:** Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder with an estimated prevalence of 1:100. Evidence from twin studies strongly supports autism as being a genetic disorder. With concordance rates for monozygotic twins of 70–90, and 10 for dizygotic twins, ASD is the most heritable of all neuropsychiatric disorders. Recent advances in array-based technology have increased the resolution of detecting submicroscopic deletions and duplications, referred to as copy-number variations (CNVs). CNVs are more common in individuals with ASD, and unique ASD associated CNVs have been repeatedly reported. In this study our objective was to identify unique ASD associated CNVs in the genetically homogenous Jewish-Israeli population.

**Methods:** We performed a whole-genome copy number variation (CNV) study on 120 cases with autism from the Jewish-Israeli population and 7,767 controls using one million Single Nucleotides Polymorphisms (SNPs). Cases and controls were all genotyped on the Illumina Infinium 1M duo platform, and CNVs were called with PennCNV. A CNV segmentation algorithm was applied independently to deletions and duplications in order to define shared “core” CNV regions (CNVRs) to test for significance.

**Results:** In all, we analyzed over 47K CNVRs for significance. The top regions were dominated by deletions and included a total of 68 open reading frames (ORF’s). We examined these ORF’s in order to find candidate genes for autism by using diverse bioinformatic recourses. The main criteria for the examination were expression in the brain, involvement in neural activity, relevance to psychiatric or neurodevelopmental / neurodegenerative disorders and previous findings of CNVs studies in autism. Only 23 genes from the list met these criteria and therefore were regarded as strong candidate genes for autism. Some of these genes were already associated with autism. For example one such gene is syntaxin binding protein 5 (STXB5). This gene is thought to be involved in neurotransmitter release by stimulating the SNARE complex formation. Another previously reported gene associated with autism is neurobeachin (NBEA), which is required for dendritic spine formation and synaptic function. The rest of the genes will be presented as well as further results of this study.

**Discussion:** We identified in this Jewish sample a number of CNVs impacting genes biologically relevant to autism. Some are unique and some are in genes where rare mutations were found in the general population. However, the CNVs uncovered here harbor different CNVs that are much more common in this Jewish population. Also, we identified CNVs impacting other genes in pathways thought to be associated with ASD. If validated, these variants can potentially improve diagnosis of children who are predisposed to autism and can serve as novel targets for drug development.

### The Association between Autism Spectrum Conditions and Psychosis: Investigating the Importance of Copy Number Variants

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**Background:** The relationship between autism spectrum conditions and psychotic illness has long been apparent, both from a behavioural and a biological perspective. Parallel research in genetics of the two conditions separately revealing overlapping regions of interest, particularly focusing in recent years on copy number variants (CNVs). A key region of interest seemed to us to be chromosome 15q11-13, which is known to have importance in the aetiology of both autism spectrum conditions (ASCs) and psychosis, particularly in people with Prader-Willi Syndrome. However, no research group to our knowledge has examined CNVs in individuals with both an ASC and a co-morbid psychotic illness. We will present the preliminary findings of the first half of our project, including data from the genetic testing of approximately 40 individuals with ASC and psychosis.

**Methods:** Individuals were identified via a range of sources across the UK and represent a wide range of intellectual ability from learning disabled to FSIQs in excess of 130. Clinical data was collected from participants or an informant regarding the presence of psychiatric symptoms using either the Diagnostic Interview for Psychosis or the PAS-ADD in the case of people with learning disabilities. Information regarding autistic features was collected using the Autism Diagnostic Interview - Revised or the Autism Diagnostic Schedule, and family history data was collected using the Family Interview for Genetic Studies. A blood sample was taken for DNA and RNA isolation, and CNVs analysed using either the Affymetrix Cytogenetics 2.7M array or Affymetrix Cytoscan HD.

**Results:** Preliminary results indicate that our initial hypothesis about the importance of chromosome 15q11-13 are not strongly supported, although strikingly, one individual tested has a perfect duplication of the Prader-Willi Syndrome critical region (something that occurs approximately 1:5000 in people with psychosis). However, we have found that there are regions on a number of other chromosomes where CNVs cluster in individuals with ASCs and psychosis more frequently than in the general population of people with ASCs or the general population. The affected genes have all been implicated in neurotransmission and neural networks. The association between particular psychiatric symptom profiles and patterns of CNVs will be explored further in the talk - this analysis has yet to take place as the results are very new. Additionally, more data may be available at the time of the conference as the research is ongoing.

**Discussion:** The search for a single genetic cause for either ASCs or psychosis has been futile. This study has attempted to narrow the phenotype and thus give greater power to explore the importance of CNVs in the case of people who have both ASCs and psychosis. Using this unique sample, we have identified regions of interest. We would argue for the possibility of a network of sites that, when CNVs occur, predispose to ASCs and psychosis. It is our hope that this research would allow for the discovery of epigenetic mechanisms to further characterise the link between the two conditions.

### The Metabotropic Glutamate Receptor Theory in Fragile X Syndrome: Testing the Safety and Efficacy of AFQ056/Mavoglurant in Adults and Adolescents

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**Background:** Fragile X syndrome is the most common cause of inherited mental retardation and is associated with behavioral problems including hyperactivity, attention deficit disorder and autism. It is caused by the expansion of a CGG repeat in the FMR1 gene, leading to hypermethylation and transcriptional silencing of FMR1, and absent or reduced levels of the translational repressor FMR1 protein (FMRP). The metabotropic glutamate receptor (mGluR) theory hypothesizes that without FMRP, uncontrolled protein synthesis occurs in response to activation of synaptic mGluRs and may lead to the clinical symptoms of FXS.

**Methods:** Randomized controlled data suggest that the mGluR5-antagonist AFQ056/Mavoglurant might improve behavioral symptoms of FXS, especially in patients with fully-methylated FMR-1 promoter regions (30 points improvement on Aberrant Behavior Checklist-Community edition (ABC-C) vs. placebo, n=7).

**Results:** Novartis currently conducts the largest clinical development program in FXS, testing the efficacy and safety of AFQ056/Mavoglurant. It is the first program conducted in Europe (Denmark, France, Germany, Italy, Spain, Switzerland, Sweden, UK) and across multiple continents, languages and cultures. Adults (18-45 years) and adolescents (12-17 years) are randomized in two separate studies to up to 4 months treatment with AFQ056/Mavoglurant or placebo. ABC-C (primary outcome), other behavioral scales and safety parameters are measured. After completing these studies, patients can enroll into open-label, long-term studies with AFQ056/Mavoglurant for  $\geq 24$  months.

**Discussion:** In summary, the AFQ056/Mavoglurant program is testing the mGluR theory in FXS and attempts to replicate the promising results seen previously. It is actively recruiting patients worldwide. Studies in smaller children and over longer treatment periods are planned.

### Rare Variant Analyses Show Association with Autism Spectrum Disorder

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**Background:** Autism spectrum disorder (ASD) is a developmental disorder characterized by social, communicative and behavioral impairments leading to average yearly medical costs that are 4.1-6.2 times higher for those with an ASD compared to those without an ASD. It is currently estimated that one in eighty-eight children are affected, thus representing a significant public health burden. ASDs are known to be highly heritable, suggesting that ASD is genetically controlled. Despite ASDs demonstrating high heritabilities, the loci identified thus far explain only a small proportion of the risk for ASD. Previously, efforts have focused primarily on common variants (minor allele frequency (MAF) > 5), which generally have smaller effect sizes, or copy number variants (CNV). Rare variants are hypothesized to have larger effect sizes and may also contribute to ASD risk. Therefore, we analyzed rare variants data to identify loci associated with ASD.

**Methods:** Participants were drawn from a large, family-based study of ASD and included 983 unrelated cases and 493 controls that were genotyped using the Illumina HumanExome-12v1 Array. This array was designed to include coding region variants across the genome, with a majority of variants (88) being rare (MAF).

**Results:** After applying our quality control criteria, 944 cases and 467 controls remained for these analyses along with 240,130 SNPs. A majority of the included study participants were male (73.1%) and of self-reported White race (87.4% of cases and 82.4% of controls). Mean age between cases and controls was similar (16.0 years for cases and 15.4 years for controls). Of the 16,602 genes analyzed, ten genes showed p-values < 0.001. Two of these genes fall within two pathways associated with ASD in CNV analyses; PODN which falls within a cell motion pathway and GPSM3 which falls within a GTPase activator activity pathway. Validation of variants in top genes and additional analyses are ongoing.

**Discussion:** Autism spectrum disorders are complex traits, known to be influenced by several genes. Rare variants are thought to exert large influences on disease risk therefore making their study of great interest. One challenge with studying rare variants is the method by which rare variants data is obtained. Currently, sequencing is widely used, however, this method has notable caveats including being labor intensive and expensive. For our study we were able to utilize genotype data for rare variants using the Illumina HumanExome-12v1 Array in order to help overcome these caveats. In our analysis of this data, we identified several genes of interest. Two moderately associated genes fall within two pathways associated with ASD in CNV analyses; PODN which falls within a cell motion pathway (GO:0006928) and GPSM3 which falls within a GTPase activator activity pathway (GO:0005096). Additional analyses, including pathway analysis of previously implicated pathways are currently in progress. These results suggest that rare variants play a role in ASD.

### Rare De Novo Copy Number Variations in Japanese Autism Subjects

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**Background:** Although autism has a strong genetic basis, none of the known genetic causes of the disorder individually account for more than 1-2 of the cases. The agreement between published findings remains poor and consistent picture of a common susceptibility loci in autism is still remain largely unknown. With the dramatic advances in genome wide screening for copy number variations (CNV), there is a growing consensus among geneticists that rare structural variants including de novo CNVs significantly contribute to the autism etiology. Hence there is paradigm shift away from the previously held “common disease – common variant” hypothesis to a “common disease – rare variant” model for the genetic architecture of autism. Here we tried to identify de novo rare CNVs in Japanese autism subjects.

**Methods:** We have a collection of 250 ASD family samples recruited on collaboration with a non-governmental organization, Asperger Society Japan (<http://www.as-japan.jp/>) and various university hospitals in central Japan. Diagnosis was made either by DSM-IV diagnostic classification of pervasive developmental disorders or by using a Japanese version of Autism Diagnostic Interview-Revised (ADIR-R). The Affymetrix Genome-Wide Human SNP Nsp/Sty 6.0 array was used to screen the samples. A total of 108 trio samples were analyzed so far. Only the samples which passed the required quality control (contrast QC >0.4) were used in subsequent analyzes. PennCNV software (University of Pennsylvania) was used to call the CNVs.

**Results:** Samples with an LRR\_SD [ $\log_2$  Copy Number – 1] > 0.35 were omitted. Only the CNVs with atleast 20 probes within its length were considered to avoid any false positive calls. An average of 37.21 autosomal CNVs are detected per patient samples, after all the QC corrections. There is no significant difference in the total number/average size of CNVs per sample between patients and their parents. However, the percentage of deletions in the total length of CNVs is 42% in case of patients, while it is only 31.2% in parents. Almost 90% of the CNVs in children are found to be inherited; almost equally from both the parents. Among the CNVs which are spanning genes, 74.5% are deletions in the case of de novo events; it's only 52.4% in inherited cases. To identify the most important de novo events, the CNVs are further screened based on the mean LRR values of all the probes within a CNV. Finally, 140 highly confident de novo CNVs were identified; among them 124 are spanning exons, and hence supposed to have some functional significance. Among these 124 CNVs, 18 are found be rare de novo events.

**Discussion:** The lack of common predisposition genes and the accumulating number of distinct, individually rare genetic causes have led to the suggestion that rare variants constitute the majority of ASD risk. De novo events have consistently shown the greatest genetic effect and were more frequent in ASD probands with only one affected child (the simplex families) as reported in previous studies. The burden of rare de novo CNVs (percentage of individuals carrying  $\geq 1$

rare de novo event) in our simplex probands (10) is also similar to the studies reported previously (5 to 11). Following the logic that CNV deletions should decrease the dosage of affected genes, our results that almost 75 of CNVs affecting genes are deletions, is particularly interesting. Previous studies have identified many genes enriched for CNVs which are involved in neuronal functional pathways including synaptogenesis, axon guidance and other related molecular processes. The likely morphological consequences of genes hit by rare de novo variants in the present study are now being investigated.

**Case-control Mapping of 16P13.11 Copy Number Variation in Neurodevelopmental Disorders Implicates a Core Pathogenic Region Including the Genes NDE1 and ABCC6**

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**Background:** Copy number variants (CNVs) at chromosome 16p13.11 have been associated with a range of specific neurodevelopmental disorders including autism, intellectual disability and schizophrenia. The locus contains several candidate genes, but their association with neurodevelopmental phenotypes is still unclear.

**Methods:** Aiming to capture the full range of phenotypic diversity associated with these pleiotropic variants, we used a CNV-led approach and tested the presence of CNVs at 16p13.11 in a UK sample of 10,397 children and young adults with a range of neurodevelopmental conditions, clinically referred for array comparative genomic hybridisation (aCGH; Agilent oligonucleotide array 44 K platform); copy number variants in this population are available in the Brain and Body Genetic Resource Exchange database (BB-GRE; <http://bbgre.org>). Cases were compared with a control sample of 14,343 individuals. Seeking to identify the dosage-sensitive genes in the 16p13.11 region, we also performed a CNV gene content analysis and we investigated the presence of ohnologs at 16p13.11 using the method described by Makino and McLysaght (2010). Finally, we searched the DECIPHER database for previously identified 16p13.11 copy number variants..

**Results:** In the clinical referral series, we identified 46 cases with CNVs of variable size at the 16p13.11 locus, including 28 duplications and 19 deletions. Analysis of the inheritance pattern of the variations within families identified five de novo events and twenty-two inherited variations. Patients were referred for various phenotypes, including developmental delay, autism spectrum disorders, speech delay, learning difficulties, behavioural problems, epilepsy, microcephaly and motor delay. CNVs at 16p13.11 were also present in 33 controls. We found a significant excess of large CNVs (>250kb) (OR=2.38;  $p=4 \times 10^{-4}$ ) in cases compared with controls, with the effect coming mostly from deletions (OR=4.14;  $p=0.003$ ), present in 0.15% of cases versus 0.04% of controls. Gene-based analysis revealed a significant enrichment of case CNVs containing a core set of nine genes, located in the 0.83 Mb genomic region between 15.49 and 16.32 Mb, including NDE1 (OR=2.48;  $p=2.7 \times 10^{-4}$ ), one of the strongest candidate genes in the 16p13.11 region because of its crucial role in the process of mammalian encephalisation and human cerebral cortex growth. Ohnologs search identified a total of five ohnologs at the 16p13.11 locus, including the four genes NDE1, MYH11, ABCC1 and ABCC6, contained within the core pathogenic region significantly overrepresented in our case CNVs, and which therefore represent the most likely source of deleterious phenotypes associated with the 16p13.11 copy number variants. The DECIPHER database search identified 85 patients with CNVs at 16p13.11 with a range of phenotypes similar to the one observed in our cases.

**Discussion:** Our data confirm that duplications and deletions at 16p13.11 represent incompletely penetrant pathogenic mutations, predisposing to a range of neurodevelopmental phenotypes, and provide new evidence for the identification of the dosage-sensitive genes responsible for the pathogenic consequences of these CNVs.

## Neurodevelopmental Phenotype in Pitt-Hopkins Syndrome

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**Background:** Pitt-Hopkins syndrome (PTHS) is characterized by intellectual disability, distinctive facial characteristics, breathing abnormalities, epilepsy and repetitive behaviors. It is caused by deletions/mutations in Transcription Factor 4 (TCF4) on chromosome 18 (18q21). Published descriptions of some 100 patients with TCF4 changes have mainly investigated genetic and somatic aspects. To date no study of psychiatric/neuropsychological assessments in PTHS has been performed. Here we present results of such a study in 10 individuals.

**Methods:** Recruitment of 4 girls and 6 boys with molecularly confirmed TCF4 mutations was through the Dutch PTHS Family Association. Distance between residence and research center, and availability within the study timeframe, determined participation. Age range was between almost 3 and 24 years, median age was 10 years. Informed parental consent and medical ethical permission were obtained. All participants underwent individual psychiatric examination. Neuropsychological assessments consisted of Bayley Scales of Infant Development (BSID-II), Vineland Adaptive Behaviour Scales – Survey Form (VABS), and Developmental Behavior Checklist-Primary Carer (DBC-P) for the children and the Developmental Behavior Checklist for Adults (DBC-A) for those above 18 years. The Autism Diagnostic Interview-revised (ADI-R) was used, in two cases parents could not be interviewed due to practical reasons.

**Results:** Clinical psychiatric assessments (n=10) Most participants had an amiable demeanor, but difficulties engaging socially. All participants made repetitive hand and/or finger movements, 9 repetitively fiddled with toys and showed a fascination with a (part of) a specific object. 6 participants played repetitively with the same toy or enjoyed the same activity (music, video) repeatedly. 9 participants were non-verbal or had only single words. Breathing abnormalities were present in 6. Self injury was seen in 5, aggression in 4 participants. Parents of 5 participants noted difficulties when changes in daily routine occurred. BSID-II (n=10). All participants had severe intellectual disability and only age-equivalent scores were determined. The chronological age of the participants lies between 32 and 289 months and the developmental age for the mental scale was between 3.5 and 15 months, and between 4 and 19 months for the motor scale. VABS (n=10) Age-equivalent scores were determined. None of the participants, except the eldest, performed beyond a developmental age of 20 months. Adaptive functioning on the domains of daily living skills and communication appeared better than functioning on the socialization domain. With increasing age very little progress in adaptive functioning seemed to be accomplished. DBC (n=10) The DBC assessment showed that only 2 participants scored above the clinical cut-off level for problem behaviors for age group (with elevated scores on the Self-Absorbed, and the Communication Disturbance and Disruptive Behaviour scales). All participants had high scores on self-absorption. Five of the 7 subjects below 18 years scored just above threshold on the DBC Autism Screening Algorithm. This algorithm is not available for adults. ADI-R (n=8) Highest scores for all participants were found on the domain of social interactions and play. All subjects scored at or above cut-off scores on social and communication domains. Two participants did not score above cut-off for the behavioral domain. These ADI-R results, while not conclusive for autism, added to and corroborated other findings.

**Discussion:** In this first study of neurodevelopment and behavior in PTHS all subjects shared a phenotype of (very) profound intellectual disability, severe impairments in social interactions, communication and language, and highly frequent, intense stereotyped behaviors. Psychiatric assessments additionally showed repetitive play, fascinations, and insistence on sameness. This behavioral phenotype is clearly similar to ASD. We conclude that the quality and intensity of social, communication and behavioral difficulties in our sample are beyond what would be expected even for the very low cognitive level found and cannot be readily explained by it. Thus, we conclude that ASD may be part of the phenotype of PTHS. Changes in TCF4 have been implicated in outcomes of intellectual disability, epilepsy, autism, and schizophrenia, but their precise impact on neuronal networks is unclear. Understanding the neurodevelopmental phenotype in PTHS is useful for understanding other disorders with some of the same behavioral, cognitive, and possibly genetic features.

### Glutamatergic Candidate Genes in Autism Spectrum Disorders

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**Background:** Autism spectrum disorders (ASD) are heterogeneous disorders characterised by qualitative impairment in social interaction and communication, and restricted repetitive and stereotyped patterns of behaviour. ASD are predominantly genetically determined with a heritability of around 70 - 90% and a prevalence of around 0.5 -1% (Hallmayer et al. 2011; Freitag et al. 2010). Fragile-X-Syndrome (FXS) is one of the most studied monogenetic disorders with core symptoms of ASD caused by a trinucleotide expansion in the promoter region of the FMR1 gene. The reported dysregulated mRNA translation due to disturbances in the FMR1 signalling pathway leads to altered synaptic function and loss of protein synthesis-dependent plasticity, which is a potential link to autism (Lüscher and Huber 2010). The gene product of FMR1 (FMRP) is a downstream target of the metabotropic glutamate receptors 1 and 5 (GRM1, GRM5) (Bassell and Warren 2008). The aim of this study was to assess common variants in candidate genes upstream and downstream of this pathway as risk factors for ASD. Therefore, we selected a set of candidate genes implicated in ASD and in FMRP signalling, and genotyped several SNPs in these genes.

**Methods:** In total, we selected 14 annotated SNPs within six glutamatergic genes (GRM1/5, CYFIP1, CaMKIV, CREB1, eIF4E) with a minor allele frequency (MAF) >10% which are located in (potentially) functional domains or tag-SNP regions. SNPs were analyzed by Real-time PCR (genotyping assays) or RFLP. 200 parent-child trios were used as detection sample for validation of minor allele frequencies and preliminary association tests. Ten of these 14 SNPs were followed up in an age, sex, and sub-diagnosis matched replication sample of 256 parent-child trios. Association of single variants and haplotypes was calculated using UNPHASED, and a scoring based approach, comparing cases and pseudo-controls, was chosen to assess possible epistatic effects.

**Results:** In the detection sample we observed a nominal association with ASD for SNP rs7170637 in CYFIP. In the combined sample we could confirm this result for rs7170637 with a p-value of 0.021 and additionally we observed a nominal association for a second variant rs25925 in the CaMKIV gene (p-value 0.025). Database mining showed that both SNPs are suggested as functional, as predicted splice site enhancers or silencers, respectively. Epistatic effects will additionally be assessed.

**Discussion:** Our results point towards the relevance of variants of the glutamatergic system in the etiology of autism spectrum disorders. In our combined sample we detected a nominal association with ASD for two SNPs out of 14 (rs7170637 in CYFIP1 and rs25925 in CaMKIV). CYFIP1 has been recently implicated in ASD (Nishimura et al. 2007; van der Zwaag et al. 2009) and a physical interaction with FMRP and eukaryotic translation initiation factor 4E (eIF4E) could be demonstrated (Napoli et al. 2008). SNP rs7170637 putatively influences the stabilization of CYFIP1 interaction with these translation associated proteins, and thus may alter synaptic plasticity. On the other hand, CaMKIV contributes to the regulation of FMRP

by phosphorylation of the major transcription factor cyclic AMP-responsive element-binding protein 1 (CREB1), and thus acts at the transcriptional level, which again plays a key role in synaptic plasticity (Wang et al. 2009; Toyoda et al. 2010). Therefore, SNP rs25925 may have an impact on transcriptional regulation of ASD genes. Though we observed a trend in the detection sample for additional SNPs (rs3693, CYFIP1; rs6923492, GRM1), this finding was not confirmed in the combined sample. This might either be due to a too small sample size for a small effect, the heterogeneity of the disorders, or it may have been a false positive finding in the detection sample. To validate the functional impact of our results, further investigations of these SNPs in a cellular model are in progress.

### Genetic Studies of Consanguineous Pakistani Pedigrees with Pervasive Developmental Disorders

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**Background:** Pakistan has the highest rate of consanguineous marriage, as well as a high fertility rate, in the world due to historical, religious, cultural and social reasons. This population characteristic makes Pakistan an excellent location for collecting large families for genetic studies, particularly for autosomal recessive traits and for severe psychiatric disorders plagued by extreme heterogeneity and reduced reproductive fitness in outbred Western populations.

**Methods:** In the past few years, with the cooperation from local geneticists and clinicians, we have identified and characterized 5 consanguineous pedigrees with 3-5 affected siblings/first-cousins per pedigree with variable PDD, which fits with a recessive inheritance pattern. With the collaboration of local clinicians, we have thoroughly investigated all the symptomatic individuals, including standard clinical examinations, comprehensive psychological evaluations, routine laboratory tests, MRI and EEG investigations; and have collected blood samples from all the affected individuals, their unaffected parents and siblings. We have carried out classical linkage analysis combined with homozygosity mapping in these 5 PDD pedigrees using high density DNA microarrays, as well as whole exome sequencing in selected affected individuals.

**Results:** We have identified shared homozygous chromosomal region(s) among all the affected individuals in each of these 5 pedigrees independently. Joint homozygosity mapping and whole exome sequencing data analyses are underway.

**Discussion:** Our preliminary results strongly indicate that each of this PDD pedigree is caused by a different recessive gene mutation. Further genetic validation of the potential disease causing mutation will be needed.

### Association between Polymorphisms in Sex Steroid Related Genes and Autism Symptoms in a Swedish Population

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**Background:** Sex differences in psychiatric disorders are common, which is particularly striking in autism that is five times more prevalent in boys. It has been hypothesized that high levels of testosterone during early development may be a risk factor for autism. This theory has been supported by several studies showing fetal testosterone levels, as well as indirect measures of prenatal androgenization, to be associated with autism and autism-related personality traits. Further, the importance of sex steroid related genes in autism is supported by studies reporting associations between polymorphisms in genes involved in sex steroid synthesis/metabolism and autism and/or autistic traits. The aim of the present study was to investigate possible associations between 26 polymorphisms in 6 genes related to sex steroids and autism symptoms in a general population.

**Methods:** Subjects used in the study are a subset from The Child and Adolescent Twin Study in Sweden (CATSS, N=1771). The parents of the subjects were asked to fill out the telephone interview Autism-Tics, ADHD, and Other Co morbidities inventory (A-TAC). Factor analyses in CATSS, using A-TAC, have revealed that the three dimensions of autism symptoms were impairments in social interaction, language impairments and restricted and repetitive behavior. DNA was extracted from saliva samples using OraGene® DNA self-collection kit. The polymorphisms were genotyped with KASPar® PCR SNP genotyping system (KBiosciences, Herts, UK).

**Results:** The genotyping success rate was >95% and all SNPs were in Hardy-Weinberg equilibrium. About 14 associations between any of the investigated polymorphisms and autism dimensions were found at p<0.05. For two SNPs (in ESR1 and SRD5A2) the associations survived Bonferroni correction for multiple testing.

**Discussion:** In conclusion, polymorphisms in sex steroid related genes known to affect gene expression (the polymorphism in ESR1) and enzymatic activity (the polymorphism in SRD5A2) seem to increase the risk of autism symptoms in boys and girls respectively.

### Variants of the Oxytocin Receptor Gene Associate with Human Social Behaviors

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**Background:** The neuropeptide oxytocin has crucial effects on bonding behaviors and other social behaviors in rodents. Intriguing recent evidence indicates that this may be true also in humans. I will present recent data from our laboratory indicating that polymorphisms of the oxytocin receptor gene (OXTR) associate with social bonding behaviors, aggression as well as with the risk of autism.

**Methods:** Inspired by the well-established importance of oxytocin for social behaviors, we investigated twelve genetic variants in OXTR in several large samples assessed for different aspects of social behaviors.

**Results:** Based on the well-known importance of oxytocin for pair-bonding behavior in voles, we investigated twelve genetic variants in OXTR in relation to pair-bonding behavior in two independent samples. One variant, the SNP rs7632287, was in both cohorts, TOSS (N=2309) and TCHAD (N=1240), associated with different measures of pair-bonding behavior. Moreover, using longitudinal data available in the TCHAD cohort we found that pair-bonding behavior in adulthood was partly predicted by social problems assessed, in the same individuals, at the age of eight. Intriguingly, this measure of social problems was associated with the same OXTR variant, rs7632287, in girls. In a third sample, CATSS (N=1771) comprising children assessed with respect to symptoms related to Autism Spectrum Disorder (ASD), the rs7632287 SNP again was, specifically in girls, associated with the abilities to socially interact and communicate with others. In a separate experiment the same twelve OXTR SNPs were genotyped in 116 Finnish men, which have undergone an experimental paradigm of aggression (Response Choice Aggression Paradigm) during alcohol intoxication or placebo. In this sample an interactive effect between the SNP rs4564970 and alcohol on experimentally induced aggression was revealed. Interestingly, in a population-based sample of Finnish men and women (N=3577) the effect of alcohol consumption on aggressive behavior was again moderated by the rs4564970 polymorphism.

**Discussion:** In conclusion, these results provide further support for the notion that oxytocin is crucial for social behaviors in humans.

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### Analysis of Genotyping Reliability in Multiplex Technical Replicates of Affymetrix Human SNP Array 6.0 Microarrays

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**Background:** Genome-wide association studies (GWAS) are a widely used approach to investigate genetic components of complex traits. High-density Single Nucleotide Polymorphism (SNP) microarrays nowadays can provide genotype information for millions of markers scattered all over the genome at once. Two complementary quality control strategies are employed to assure correctness of genotype calls: First, the default cutoff values for a given genotyping algorithm to achieve high confidence calls, as specified by the manufacturer's recommendations. Second, the standard post-genotyping quality controls (QC), such as minor allele frequency (MAF), rate of missingness per SNP and deviations from the Hardy-Weinberg-Equilibrium (HWE). Reliability of genotype calls obviously is a key feature that provides the basis for solid research in high throughput genetics. Therefore we assessed the reliability of microarray measured SNP genotype calls in multiplex technical replicates.

**Methods:** We systematically evaluated the genotyping performance of the 891'227 autosomal SNPs represented on the Affymetrix Human SNP Array 6.0 in a series of 7 multiplex technical replicates (MTR), which comprise 3- to 9-fold hybridizations of the same DNA.

**Results:** A total of 844'958 SNPs (94.8%) showed consistent genotype calls over all technical replicates, when missing genotypes were not taken into account. Thus, a total of 46'319 (5.2%) SNPs yielded contradictory genotype calls in at least one MTR group. For 3918 SNPs, differing genotype calls were generated for two to five MTR groups. For intra-group comparisons, the number of inconsistent genotypes increased with size of the MTR group. More than 90% of the differing genotype calls split across two genotype groups. Interestingly, from the 139 SNPs that yielded three different genotype calls for a given SNP within one MTR group, only 102 were removed by the standard post genotyping QC criteria (removal of markers with HWE  $p < 0.01$ , MAF  $< 10\%$ , Per-SNP-missingness  $> 5\%$ ). These QC criteria also only removed 22'641 (48.8%) out of the total of 46'319 SNPs with inconsistent genotype calls.

**Discussion:** The analysis of technical replicates reveals a high rate of consistency for genotype calls given the application of common QC criteria, but still leaves a certain fraction of divergence. The number of inconsistently genotyped SNPs that would enter statistical analysis is greatly reduced, emphasizing the importance of a post-analysis QC. Further investigation is required for inconsistent markers that were not eliminated by any of the QCs in order to determine potential systematic differences, e.g. greater variance in the confidence scores. Such additional QC criteria could help to extend the standard QC in order to obtain a high quality subset of reliably genotyped SNPs, where pre-analysis QC could help to save computational costs for data-mining approaches that come with a high computational burden.

## Allele Specific Expression Analysis of Human Transcriptome Suggests Distribution of Chromatin States between Homologous Chromosomes

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**Background:** The studies of molecular mechanisms of common disease demonstrated that genetic and epigenetics factors such as SNPs and chromatin marks would affect cells and organisms predominantly not on the level of protein sequence but rather on the level of gene expression, splicing and mRNA stability. If genetic composition is fixed during the life-span, chromatin landscape is highly dynamic not only during organism development but also spatially, i.e. in different cell types and tissues. Genome-wide association studies (GWAS) identified many SNPs highly associated with complex multifactorial disorders. However, considering this information in connection with chromatin state is of great importance since chromatin marks could easily change the state of chromatin making transcription factor binding site or microRNA target inaccessible for transcription factors or microRNAs, respectively, making known functional SNPs irrelevant for further investigation. With availability of ChIP-Seq data chromatin profiling provides information about association of different chromatin mark combinations. Applying unsupervised learning algorithms these combinations could be learned across the entire genome and these genome states can be turned into interpretable annotations such as euchromatic and heterochromatic regions, different level of promoter or enhancer activities. However, these computed chromatin states cannot distinguish level of homologous chromosome expression. There are known SNPs marking transcripts with differential expression between chromosomes aka expression quantitative trait loci (eQTLs). However, analysis of eQTLs is complicated with genetic and epigenetic variability among individuals. To avoid this type of noise allele-specific expression (ASE) analysis has been used by researchers where RNA samples from heterozygous individual have been probed. High throughput Next Generation Sequencing (NGS) such as RNA-Seq has made it significantly easier to analyze human individual transcriptome. NGS allows distinguishing of homologous chromosome transcripts expression through the reads containing alternative alleles. Identifying the level of expression of the transcripts in heterozygous loci and associating them with the chromatin states would allow validating this chromatin states and, perhaps, suggesting these states distribution between homologous chromosomes.

**Methods:** For the pilot project we downloaded RNA-seq alignment from ENCODE/Caltech project for GM12878, H1-hESC, HUVEC cell lines. Reads obtained from strand specific protocol (1x75 bp reads) were aligned to the hg19 human reference genome using TopHat. Bed-files of chromatin state segmentation by Hidden Markov Model (HMM) for 9 human cell types have been downloaded from ENCODE/Broad. Samtools mpileup command generated pileup output for BAM files. Pileup format where each line represents a genomic position, reference and genomic base and read qualities served as an input for VarScan program. This program has used been used for pileup file filtering and SNP calling. The heterozygous SNPs with the counts of reads overlapping each allele were extracted and replicates were combined. This file has served as an input for DESeq, a part of R Bioconductor package, which we used for ASE SNPs calling. We used Bedtools to find intersection between heterozygous SNPs and chromatin state segments. Nationale Genomforschungsnetz.

**Results:** Initially to test validity of the ASE approach to chromatin states on homologous chromosomes we used three ENCODE project cell types GM12878, H1ESC and HUVEC. We downloaded

alignments of two replicates in each cell type with reads aligned to hg19. Samtools mpileup provided output in pileup format with a quantity of overlapping reads and read quality scores for each nucleotide base. This output was piped to VarScan with options to remove low quality bases in the reads. VarScan called SNPs and provided number of counts for each allele. The total number of SNPs called by VarScan was highly unequal between replicates which could be attributed to a different depth of sequencing. We turned VarScan output into count data tables to use in DESeq R program usually applied to gene differential expression. Each column with counts was corresponding either to a reference or to an alternative allele. DESeq estimates the dispersion of each ASE allele and identify differentially expressed alleles through negative binomial test. We identified several thousands of heterozygous SNPs and less than 100 ASE SNPs. The large number of ASE SNPs was located in HLA complex. Subsequently, we intersected ASE SNPs with chromatin state regions of 3 cell type and found that the same SNPs differentially expressed in one cell type but not in the other can fall into different chromatin states, e.g. poised promoter or active promoter region. The subsequent analysis and tuning of the method is underway.

**Discussion:** This pilot project demonstrates the possibility of distinguishing of the chromatin states of homologous chromosomes. One region can be a poised promoter on one chromosome and active promoter region at the same loci on homologous chromosome. This can imply that a SNP identified as a functional could be a truly functional in one case or neutral in another case where this chromatin region could be in heterochromatic state. The accumulation of RNA-seq and ChIP-seq data in different tissues including normal and pathogenic ones will lead to finer resolution of chromatin states and increase in number of identified ASE loci.

### The Essentials for Schizophrenia Phenomics

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**Background:** EU-GEI (European network of national schizophrenia networks studying Gene-Environment Interactions) is a multinational schizophrenia research project ([www.eu-gei.eu/](http://www.eu-gei.eu/)). Its aim is to identify interactive genetic, clinical and environmental determinants involved in the development, severity and outcome of schizophrenia. EU-GEI employs family-based and multidisciplinary study paradigms for efficient assessment of gene-environment interactions. Translation of results to clinical practice will be facilitated by additional experimental research and risk assessment bioinformatics research. Extensive and deep phenotyping to characterize several thousand affected subjects across Europe and Australia will be realized by clinical interviews using a series of standardized instruments, brain fMRI imaging and the application of psymate, a purpose-made device facilitating the monitoring of daily life experience and behaviour. This may be the best opportunity to date to characterize the schizophrenia phenome with implications for the wider psychiatric research community.

**Methods:** We referenced from our past experience of using existing phenotype databases for research purpose, also surveyed from the literature on endophenotypes and phenomics in general to draft a schematic framework for a schizophrenia phenomics. Attention was paid particularly to the practicality of constructing the data repositories, understanding the research process from hypothesis formation to data collation and its ultimate use for inference generation. EU-GEI (European Network of National Schizophrenia Networks Studying Gene-Environment Interactions).

**Results:** The proposed framework is a 3-tier system. The mid-tier is conceptual and mainly designed for mapping selective endophenotypes to three search spaces: genotype[G], phenotype[P] and environment[E]. All three search spaces are multi-dimensional. Although most of the data will probably be genetic, it is relatively easy to handle as it is standardized and rigidly specified. In contrast, the phenotypic data and the environmental data will be mapped to a plethora of phenotype types and environmental risk factor types.

**Discussion:** From the past experience, data cleaning and the transformation of the raw data into refined phenotypes is always very resource consuming. It is resource effective to carry out data transformation centrally and hold the derived multivariate data in a separate refined data repository. By keeping the raw data as they are in a phenotyping database helps to maintain information content. The top-tier is a dynamic engine for formulating hypothesis, defining and refining endophenotypes. Hypotheses can concern a combination of familial and heritable endophenotypes. Each endophenotype is mapped to the three search spaces to obtain the sub-sample for hypothesis testing. The proposed framework takes into consideration the need to support reverse-phenotyping, phenome-wide association and GxE studies which become more commonly practiced to complement the recent genetic research analyses such as GWAS. Although the construction of the schizophrenia phenome has not yet materialised, the framework facilitates exploring the benefits of having a proper phenomic database for a large-scale project such as EU-GEI.

### Family Load Estimation in Schizophrenia, Bipolar and Anxiety Disorders -- An Approach to Target the Selection of Families in Genetic Studies

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**Background:** Selection of appropriate patients for genetic sampling is often constrained by limited resources. Therefore a sound statistical as well as epidemiological approach to target the selection of high risk probands is crucial. This study aims to provide an easy implemented approach to target the selections of individuals in which the disease in question might be caused by genetic factors; this can be achieved by calculating the family load. The estimation of the family load will be exemplified through the use of a nation-wide register-based study providing information on psychiatric illnesses across three generations. This estimation of the family load makes it possible to target the selections of families for DNA analyses in large datasets. The study will focus on estimating family loads in Schizophrenia, Bipolar Disorder and Anxiety Disorder.

**Methods:** A matched dataset including all patients diagnosed with a child and adolescent psychiatric diagnosis between 1969 and 2004 and registered in the Danish Central Psychiatric Register (caseprobands) along with a maximum of three matched control per case (controlprobands) was analyzed. The controlprobands were matched on age (year and month of birth), sex and region (at the index time of the caseproband). Psychiatric diagnoses were also obtained on the relatives, i.e. parents, siblings, and offspring as a part of the Danish Three Generation Study (3GS). The family load is estimated using the statistical approaches mixed logistic regression and Cox regression with shared frailty in order for it to be controlled for known risk factors, i.e. sex, year and month of birth and degree of urbanization.

**Results:** Data illustrating the family load in families affected with Schizophrenia, Bipolar Disorder and Anxiety Disorder compared to the family load of control families will be presented; these data demonstrate clear differences in the family load among the different diagnostic subgroups.

**Discussion:** A limitation to the method is its lacking ability to take the distance among family members into consideration, therefore it would not be an appropriate method to estimate family loads in large families. Since the method takes well known risk factors into account it provides a useful estimate of the family load in small families.

**Linking GWAS and Genetic Heterogeneity of Brain Cells in Neuropsychiatric Diseases: A Meta-analysis**

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**Background:** Despite of a plethora of data on candidate genes determining the susceptibility to neuropsychiatric diseases revealed by genome-wide association studies (GWAS), no consensus is reached on their intrinsic contribution to the pathogenesis. Alternatively, single-cell analyses of the brain have shown that somatic genome variations (somatic mosaicism) do affect neuronal cell populations and are likely to mediate pathogenic processes associated with brain dysfunctions. One can hypothesize that, at least in a proportion of cases, inter- and intragenic variations associated with major brain disorders (schizophrenia and Alzheimer's disease) lead to genetic dysregulation resulting in somatic genetic mosaicism. To clarify the possible relationship between candidate genes or pathways (identified by GWAS) and somatic genome variations, we have performed an analysis of data stored in a Catalog of Published GWAS by NHGRI with special emphasis on schizophrenia and Alzheimer's disease (AD) and own data on brain-specific somatic mosaicism.

**Methods:** Data on somatic mosaicism in the schizophrenia and AD brain were retrieved from our previous studies (Yurov et al., 2008; Iourov et al., 2009) and additionally obtained by multiprobe FISH and interphase chromosome-specific multicolor banding. We have retrieved candidate genes from Catalog of Published GWAS by NHGRI (excluding intergenic sequence variations, because their effects are questionable). Gene functions and the involvement in the "pathways of interest" were addressed using the resources provided by NCBI and UCSC. "Pathways of interest" included mitotic regulation, chromosome missegregation, abnormal DNA damage response (DNA repair), DNA replication, programmed cell death, differentiation, number regulation, and proliferation. Tissue-specific expression distribution of GWAS genes was retrieved using BioGPS.

**Results:** Using these criteria, we identified 54 schizophrenia GWAS genes, among which 4 genes lack functional assignments and 11 genes are implicated in "pathways of interest". Thus, 22 of schizophrenia GWAS genes are likely to play a role in generating genomic instability and the persistence of genetically abnormal cells. The expression of these genes demonstrates a brain-area-specific increase in some instances. AD was associated with 26 genes meeting our criteria, one gene of which lacks functional assignments and 9 genes (36) are implicated in "pathways of interest". The expression of the latter genes is usually increased in the brain. Apart from these ones, a number of other genes are implicated in transcriptional regulation or chromatin remodeling, potentially possessing a phenotypic effect either through epigenome variations specific for these diseases. These data correlated with observations on somatic mosaicism in the diseased brain manifesting as area-specific chromosomal mosaicism (5-15 of chromosome 21-aneuploidy in the AD brain and 3-5 of chromosome 1-aneuploidy in the schizophrenia brain) or genomic instability (progressive aneuploidization).

**Discussion:** Our meta-analysis demonstrates that variations in GWAS genes can cause susceptibility of neural cells to genomic instability or susceptibility to unexpected programmed cell death unable to clear abnormal neuronal cells and allow to propose an original hypothesis. Inherited and/or de novo mutations (together or apart) affecting the GWAS genes cause such susceptibility to abnormalities

in genome stability maintenance, cell cycle and death regulation. Then, an environmental effect on cellular genome leading to genomic instability, persisting because of failures in cellular machineries due to the mutations, initiates genomic instability. In this instance, genomic instability possesses prime effect and initiates accumulation of deleterious somatic mutations, which are able to cause brain dysfunctions. Finally, we speculate that approaches considering both GWAS and somatic genomic variations are likely to have bright perspectives for disease-oriented genome research.

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### YAMAS Provides a New Imputation-free Meta-analysis Approach for Differing Genome-wide SNP Panels

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**Background:** Meta-analysis (MA) is widely used to pool genome-wide association studies (GWASes) in order to a) increase the power to detect strong or weak genotype effects or b) as a result verification method. As a consequence of differing SNP panels among genotyping chips, imputation is the method of choice within GWAS consortia to avoid losing too many SNPs in a MA. YAMAS (Yet Another Meta Analysis Software), however, enables cross-GWAS conclusions prior to finished and polished imputation runs, which eventually are time-consuming.

**Methods:** Here, we present a fast method to avoid forfeiting SNPs present in only a subset of studies, without relying on imputation. This is accomplished by using reference linkage disequilibrium (LD) data from 1,000 Genomes/HapMap projects to find proxy-SNPs together with in-phase alleles for SNPs missing in at least one study. MA is conducted by combining association effect estimates of a SNP and those of its proxy-SNPs. Our algorithm is implemented in the MA software. Association results from GWAS analysis applications can be used as input files for MA, tremendously speeding up MA compared to the conventional imputation approach. We show that our proxy algorithm is well-powered and yields valuable ad hoc results, possibly providing an incentive for follow-up studies. We propose our method as a quick screening step prior to imputation-based MA, as well as an additional main approach for studies without available reference data matching the ethnicities of study participants.

**Results:** YAMAS has been used for MA of various GWASes, e.g. Type II diabetes, Parkinson's or Alzheimer's disease. Currently, one focus is the MA of seven case-control studies of bipolar disorder, which were genotyped on different platforms. In this context, the field of application for YAMAS ranges from fast prior MA to the evaluation of imputed data, the results hopefully will provide new insight into the etiology of bipolar disorder.

**Discussion:** YAMAS is an efficient and fast meta-analysis program which offers various methods, including conventional MA as well as inserting proxy-SNPs for missing markers to avoid unnecessary power loss. MA with YAMAS can be readily conducted as YAMAS provides a generic parser for heterogeneous tabulated file formats within the GWAS field and avoids cumbersome setups. In this way, it supplements the meta-analysis process. The Software is available for free on the YAMAS web page (<http://yamas.meb.uni-bonn.de>).

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### Mechanism of Schizophrenia: Bioinformatic Approach

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**Background:** Genome-wide association studies (GWAS) and copy-number variation (CNV) studies of schizophrenia have uncovered hundreds of potential common and rare susceptibility loci for schizophrenia risk. Bioinformatics approaches can integrate results of these studies into better understanding of disease mechanisms of schizophrenia. To generate biological hypothesis on the mechanism of schizophrenia, we used bioinformatic approaches to explore all possible interactions between known protein products of published candidate genes of schizophrenia.

**Methods:** We conducted a systematic collection of genes which were at least nominally associated with schizophrenia in GWAS and CNV studies from public databases, followed by application of bioinformatic tools, to determine all known protein products of these candidate genes and all possible interactions between these proteins. Computational algorithms, which recognize areas of dense interconnections within the global network of protein-protein interactions, were used to characterize clusters of molecular complexes. Finally, information from ontological databases were used to annotate the resulting clusters or pathways of schizophrenia.

**Results:** Four hundred of the best ranked genes were selected. Based on stringent criteria, the Search Tool for the Retrieval of INteracting Genes/Proteins (STRING) identified 326 genes with probable relationships between each other, resulting in 4632 probable interactions between their protein products. Overall synaptic transmission ( $P=1.91 \times 10^{-21}$ ), transmission of nerve impulse ( $P=3.58 \times 10^{-21}$ ), and cell-cell signaling ( $2 \times 10^{-19}$ ) were the most representative biological functions. The best ranked disease pathway, consists of complexes of immune function molecules interacting with complexes of molecules involved in neurotransmission through an intermediate group of 'linker proteins' that interact directly with molecules in the latter two clusters.

**Discussion:** Our findings provide a new line of evidence for relevance of immune complexes in the mechanisms of schizophrenia and highlighted pathways and novel genes, which can be targeted in molecular studies of schizophrenia.

### GWAS on a Desktop: Using Next-Gen Sequencing to Support Assembly, Analysis and GWAS Comparisons on a Desktop Computer

Biostatistics/Bioinformatics

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**Background:** DNASTAR offers an integrated suite of software for assembling and analyzing sequence data from all major next-generation sequencing platforms supporting key workflows on a desktop computer including Genome Wide Association Study (GWAS) analysis.

**Methods:** The GWAS workflow includes assembling and analyzing multiple samples using one reference template; probabilistic identification of SNPs, small indels and genotype calls with known variants correlated to their dbSNP and COSMIC IDs and GERP reference data; review of SNPs from multiple samples within a single project; identification of structural variations; and, for large multi-sample projects with hundreds or thousands of individual data sets, GWAS analysis tools including tools for SNP quantitation, filtering, set comparison, clustering and indication of the gene disruption impact from called SNPs.

**Results:** Interactive views within the software facilitate fast, comprehensive analysis, helping scientists move quickly from raw next-gen sequencing data to genetic and genomic impact, including gene ontology.

**Discussion:** By using innovative algorithms within the software, scientists can have all of the assembly and analysis capabilities available to them on their desktop computer, supporting large data sets generated by traditional next-gen sequencing instruments and large numbers of small data sets beginning to be produced by bench-top next-gen sequencers.

### Association Study of the Serotonin Transporter Polymorphism RS12150214 with Heroin Addiction in Bulgarian Roma

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**Background:** Drug addiction is a serious psychiatric disorder with negative consequences for both individual and society, which determines the need for identification of genetic factors associated with predisposition, unfavorable treatment response or relapse. The serotonin neurotransmission system, which controls the mood, emotions and cognition, is deregulated in opioid addiction. Heroin and cocaine inhibit serotonin reuptake and increase its concentration in brain. The 5-HTT, a presynaptic transporter, plays major role in the turnover of this neurotransmitter and there is marked scientific interest in its association with predisposition to addiction. Several polymorphic variants in the gene coding for this transporter, SLC6A4, have been found to modify the individual's predisposition to drug dependence. Many of these polymorphisms affect the regulation of the gene. Bulgaria is located on the road of heroin from Asia towards Europe, which results in wide distribution of this drug among Bulgarian abusers. For this reason our attention was focused on heroin dependence and its association with different variants in SLC6A4. More specifically, we chose to study rs12150214 SNP in intron 1, because it is tagged with multiple polymorphisms in the 5' end of the gene, known to be involved in regulation of its expression.

**Methods:** The DNA samples for the association study were isolated from blood drawn from heroin dependant individuals (based on DSMIV criteria). Population control samples were obtained from the repository of the Molecular medicine Center and the National Genetics Laboratory in Bulgaria. The study has been approved by the Ethics Committee of Medical University – Sofia and Washington University of St. Louis. Genotyping was performed in a large set comprising of samples from 2044 population controls and 2545 heroin addicts by means of TaqMan assay (Applied Biosystems). Statistical analysis was carried out using Plink toolset.

**Results:** A significant association was observed for rs12150214 in Roma subsample. The rare C allele was found in 19.6% of the drug users and 12.6% of the population control sample (206 controls; 441 cases;  $p=0.0019$ ), and appears to confer protection for the carriers (OR=1.69). No significant association was observed for the Bulgarian subsample. There was no deviation from Hardy Weinberg Equilibrium in any of the ethnic groups.

**Discussion:** In summary, we observed a statistically significant association of the rs12150214 single nucleotide polymorphism in SLC6A4 with heroin addiction in Bulgarian Roma samples. This genetic variant is located in intron 1 and is tagged with several other SNPs in the proximal end of the gene for the serotonin transporter, where the regulatory elements are situated. The rs12150214 was previously found to be associated with depression. A further detailed phenotypic analysis of the entire sample set would allow us to determine if the association observed in the Roma group correlates with the presence of depressive symptoms and whether it also exists in a subset of Bulgarian heroin addicts with depression.

**Alterations in Hippocampal Gene Expression and Epigenetic Methylation in a Mouse Model of Fetal Alcohol Spectrum Disorder: Towards Understanding Cognitive Deficits**

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**Background:** Alcohol abuse during pregnancy can lead to a range of neurological abnormalities termed Fetal Alcohol Spectrum Disorder (FASD). The mechanisms by which alcohol (ethanol) induces such disorders is heterogeneous and poorly understood. We have shown that ethanol treatment in pregnant C57BL/6J mice also generates FASD-like learning and memory impairment. Further, these mice show persistent (70 days of age) changes in whole-brain gene expression. However, the mechanisms maintaining these long-term alterations remain unknown. In this research we are attempting to explain such changes by associated changes in epigenetic programming, particularly DNA methylation and histone modification. This study uniquely focuses on hippocampus which is known to play a critical role in memory.

**Methods:** Mouse pups were injected with saline or 5g/kg of ethanol on each of postnatal days 4 and 7, the period equivalent to human trimester three. The pups were allowed to develop under normal housing conditions to post-natal day (PND) 70. Whole-hippocampus was isolated on PND 70 and used for RNA, DNA and chromatin isolation. Gene expression microarray analysis followed by Real-Time PCR confirmation was used to discover altered transcripts. DNA methylation was assessed using methylated DNA immunoprecipitation and microarray (MeDIP-chip). Chromatin immunoprecipitation followed by promoter microarray (ChIP-chip) followed by ChIP-qPCR was used to assess changes in histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 lysine 27 trimethylation (H3K27me3).

**Results:** We have established that thirty genes were differentially expressed in response to ethanol treatment. Some of the genes identified are involved in fatty acid metabolism (Sgpl11, Cpt2) neuron function (Mrgprh, Serpinb1b, Creld2) and signal transduction (Rab37, Rgs1, Krt8, Tcf7l2). Furthermore, Sgpl1 and Tcf7l2 are known to be involved in learning and memory. qPCR confirmation data are being generated for these genes currently. DNA and histone methylation results are forthcoming, and will be presented at the conference.

**Discussion:** The results suggest that alcohol exposure during neurodevelopment affects gene expression differently in different brain regions that includes hippocampus. The specific genes identified suggest a complex “footprint” of fetal ethanol effect. These changes have potential to explain the aetiology of FASD associated learning and memory impairment. These genes may eventually serve as diagnostic or therapeutic targets. This study is the first simultaneous examination of gene expression, DNA methylation, and histone methylation in response to fetal ethanol. The high-resolution, whole-genome analysis of histone methylation presented here adds a novel dimension to the growing body of evidence that alcohol acts on the brain in a complex fashion on many levels, leading to the broad range of outcomes.

**MGLUR5 Receptors Located on Dopamine D1-Expressing Neurons Mediate Natural and Drug Conditioned Seeking Behaviours through the Endocannabinoid System**

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**Background:** It has been suggested that synaptic transmission in the nucleus accumbens (NAc) is involved in reinstatement of reward-seeking behaviour induced by exposure to drugs or natural reward. In this context, glutamatergic and endocannabinoid (ECS) systems have been focus of intensive research, as both have been largely shown to modulate behavioural and synaptic events involved in reward-learning processes. The fact that both systems have been pharmacologically targeted in the NAc and successfully shown to inhibit reward seeking behavior in animal models, strongly suggests common molecular mechanisms compromising their actions. But, which is the detailed molecular mechanism within the NAc by which natural and drug rewards affect conditioned responses?

**Methods:** Using a transgenic mouse line with a selective knockdown of the mGluR5 receptors in dopamine D1 receptor expressing neurons (D1miRmGluR5), we first examined the conditioning processes and relapse-like behavior for ethanol and saccharin using an operant discriminatory paradigm and pleasure-attenuated startle response (PAS).

**Results:** Lever pressing for ethanol and saccharin associated cues during the conditioning didn't differ in D1miRmGluR5 compared to control mice. However, after an extinction period the ability of the contextual cues to reinstate ethanol as well as saccharin seeking behavior was found to be strongly attenuated in mutant mice. Likewise, D1miRmGluR5 showed no attenuation of the startle response, when the sweet milk conditioned cue was presented. This phenotype was not derived from impaired motor, anxiety or cognitive processes. These data indicate that glutamate signaling through mGluR5 located on dopamine D1 receptor expressing neurons is necessary for natural and drug conditioned reward seeking responses. This finding was further supported pharmacologically, demonstrating a lack of effectiveness of the mGluR5 antagonist, MTEP, on suppressing cue-induced reinstatement of saccharin seeking in the mutants. Furthermore, the blockade of CB1 receptors with AM251 was neither effective in these mutants, while it attenuated cue-induced saccharin reinstatement in control mice.

**Discussion:** Collectively, these results indicate that in D1-expressing neurons, mGluR5 receptor modulates the salience or incentive value of reward-conditioned stimulus and identify the common pathway within the NAc by which CB1 and mGluR5 receptors control behavioural adaptations underlying reward seeking processes.

### Association of NPY Receptor 2 Polymorphism with Alcohol Dependence

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**Background:** Neuropeptide Y (NPY) is an ubiquitous neurotransmitter and expressed in CNS and peripheral tissue. NPY is one of the most evolutionarily conserved peptides with 92% sequence identity between species with an evolutionary distance of more than 400 million years. NPY and its receptors play an important role in the stress response system and are involved in the control of appetite and body weight homeostasis. These genes have been implicated in multiple diseases related to the stress response system (e.g. vascular diseases, obesity). In a similar manner these genes have repeatedly been reported to influence ethanol sensitivity and alcohol consumption. In a recent study on male fruit flies (*Drosophila melanogaster*) the NPY homologue NPF has been suggested to be part of the brain's reward system and experimentally induced down regulation of NPF receptors has been shown to influence ethanol preference of the subjects under study. Interestingly candidate gene studies have found polymorphisms located in NPY and its receptor genes associated with alcohol dependence (AD). We systematically explored single nucleotide polymorphisms (SNPs) located in these genes regarding their association with AD.

**Methods:** We systematically examined SNPs of the human NPY/ NPY-receptor system in an already available GWAS data set comprising of 1333 inhouse patients with DSMIV-AD and 2168 population based controls.

**Results:** 24 markers of NPY and its receptor genes were represented in the data set. SNP rs6857715 located in the promoter region of the NPY2R receptor gene was significantly associated with AD ( $p=4.7e-3$ ).

**Discussion:** Findings are consistent with the literature. Interestingly we found the same SNP as Wetherill et al. (2009) had reported to be associated with comorbid alcohol and cocaine dependence.

### Novel Quantitative Trait Locus for an Alcoholism-related Phenotype

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**Background:** Linkage studies of alcohol dependence (AD) have implicated several chromosome regions, with some leading to the successful identification of susceptibility genes, most notably ADH4 and GABRA2 on chromosome 4. Quantitative endophenotypes that are closer to gene action than clinical endpoints offer a means of obtaining more refined linkage signals that can further our understanding of the genetic component underlying alcoholism. In this study we perform linkage analysis on a self-reported measure of maximum number of drinks in a 24-hour period (abbreviated as MaxDrinks), a phenotype that is highly correlated to AD.

**Methods:** Family-based samples (n=695) from the San Antonio Family Study were analyzed, with IBD allele sharing estimated from a pruned set of GWAS SNPs not in linkage disequilibrium and standard variance component linkage methods implemented in the program SOLAR. GWAS genotyping was conducted with Illumina microarrays. Data on alcohol abuse and dependence was collected via the MINI-Plus psychiatric screening questionnaire.

**Results:** The MaxDrinks measure ranges from 0 to 216 and was normalized for analysis. Our results show that MaxDrinks has an estimated heritability of 0.32 ( $P=4.61 \times 10^{-14}$ ), with a genetic correlation of 0.98 ( $P=1.40 \times 10^{-10}$ ) with lifetime history of alcohol abuse/dependence, indicating extensive overlap in the genes influencing the two phenotypes. On chromosome 6, a highly significant multipoint LOD score of 4.17 ( $P=5.85 \times 10^{-6}$ ) was detected at a region flanked by SNPs rs6928074 and rs9295639 (GRCh37: 24,680,290 - 24,985,188 bp). When broken down by pedigree, the QTL signal was found to be shared among many families, indicating that it is not the product of a single rare variant of major effect. Among the genotyped SNPs from this region (n=139), rs6918210 exhibits the strongest association to MaxDrinks ( $P=7.50 \times 10^{-5}$ ; Bonferroni-corrected  $P < 0.01$ ; covariates age and sex).

**Discussion:** Under this linkage peak is a number of compelling candidate genes, including an aldehyde dehydrogenase gene, ALDH5A1, involved in the metabolism of neurotransmitter 4-aminobutyric acid (GABA). In an effort to identify the susceptibility gene(s) contributing to this signal, we are currently testing complete sequence data from this linkage region for association to MaxDrinks and alcoholism.

### Alterations in Genomically Imprinted MIRNA and SNORNA Clusters In A Mouse Model Of Fetal Alcohol Spectrum Disorders (FASD)

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**Background:** Alcohol abuse by a mother during pregnancy results in a common and heterogeneous disorder known as Fetal Alcohol Spectrum Disorders (FASD). While much of the research on FASD has focused on behavioural and neuro-structural changes, prenatal alcohol exposure also results in long-term alterations in gene expression; however, the mechanisms underlying the persistence of these changes are not known.

**Methods:** In this research, we used four ethanol treatment protocols to model developmental ethanol exposure in mice: injections at 3 specific neurodevelopmental time points that model a “binge” exposure, and a voluntary maternal consumption model, which represents moderate chronic exposure throughout development. We then assessed small RNA brain gene expression in resulting adult offspring (PD 70) using miRNA expression arrays, mouse gene expression arrays, and quantitative PCR. Finally, we assessed brain DNA methylation using methylated DNA immunoprecipitation followed by hybridization to DNA arrays (MeDIP-Chip).

**Results:** The analysis revealed that a large number of microRNAs and snoRNAs are altered, both up and down, depending on treatment paradigm. Some of these expression profiles are unique to a treatment protocol while others overlap. Strikingly, approximately 20% of the altered noncoding RNAs (ncRNAs) localized to three imprinted clusters. The first two, *Snrpn-Ube3a* (Murine 7qC/Human 15q11–q13) and *Dlk1-Dio3* (Murine 12qF1/Human 14q32.2), are associated with processes involved in neuronal plasticity and several neurodevelopmental disorders. The third cluster contains *Sfmbt2* (Murine 2qA1) and an overlapping antisense transcript that is unique to mice and rats. Furthermore, the MeDIP-Chip analysis revealed that fetal alcohol exposure has a genome-wide effect on DNA methylation with imprinted regions of the genome appearing to be particularly sensitive.

**Discussion:** Ultimately, our results suggest that imprinted ncRNAs, many of which play a critical role in neurodevelopment and brain function, may have a role in the long-term maintenance of altered gene expression and cognitive endophenotypes associated with FASD.

### Elevated Exhaled Carbon Monoxide in Interaction with Serotonin Transporter Gene is Associated with Depressive Symptoms in Smokers

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**Background:** Bidirectional relationship between smoking and depression is well known; there is a higher prevalence of smoking among patients with depression and smokers suffer from depression in a greater number than in general population. Shared genetic and environmental factors are involved in both psychiatric disorders but exact pathological ways are not known in details. There is a growing evidence that carbon monoxide (CO) can play an important role in neuropsychiatric conditions as an atypical neurotransmitter (Snyder and Ferris, 2000) and clinical data suggest that CO poisoning may induce depression (Borras et al. 2009). Furthermore, results from animal studies showed a significant effect of CO on the serotonergic system which is a key component in the pathomechanism of depression (Muraoka et al. 1998). Although smoking is regarded as a covariate in depression studies, carbon monoxide level has been not investigated in gene-environment model of depression so far.

**Methods:** We analyzed smoking habits (background questionnaire), exhaled carbon monoxide (CO) level, nicotine dependence (Fagerström Test for Nicotine Dependence, FTND) and depressive symptoms (Zung Self-Rating Depression Scale, ZSDS) of 255 smokers from 15 Hungarian Quitting Centers. Four tag SNPs (rs1042173, rs3794808, rs140700, rs2020942) of the serotonin transporter gene were genotyped with Sequenom Mass Array. For interaction tests CO level was transformed into a categorical variable based on the median point resulting in low (1) and high (2) CO group. ANOVA tests and generalized linear model were performed for association studies. Statistical analyses were performed using R 2.8 statistical software. All tests were adjusted for age and gender.

**Results:** Nicotine dependence and depression score did not show direct associations with genetic polymorphisms separately in single marker tests. However, we detected significant interactions between high CO level and two SERT SNPs on depressive phenotype in overdominant models. Subjects with GT rs1042173 scored significantly higher on ZSDS scale in the high CO group compared to low CO group (ZSDSGT1=36.8±1.07 vs. ZSDSGT2=40.0±1.16) and to GG or TT genotypes (ZSDSTT1=38.3±1.34; ZSDSGG1=38.2±1.19; ZSDSTT2=36.6±1.84; ZSDSGG2=35.9±0.958; pinteraction=0.04). Similarly, carriers of AG rs3794808 showed elevated ZSDS score in high CO subgroup compared to low CO (ZSDSAG1= 37.0±1.02 vs. ZSDSAG2=40.0±1.15 ) groups and other genotypes also in overdominant models (ZSDSAA1=38.8±1.22; ZSDSGG1=38.8±1.48; ZSDSAA2=35.9±1.0; ZSDSGG2=35.2±1.31; pinteraction=0.01).

**Discussion:** Our results suggest that effect of SERT on depressive phenotype is modified by exhaled carbon monoxide. Since smoking is over-represented among patients with depression compared to general population, CO level can be a crucial component in gene-environmental models of depression. Further analyses are required for clarifying the molecular background of this genetic interaction.

**Heterogeneous Behavioral Manifestations in a Mouse Model of Fetal Alcohol Spectrum Disorders (FASD): Assessing the Effects of Gestational Time and Gene Expression**

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<sup>1</sup>Western University

**Background:** Considerable attention has been given to the effects of prenatal alcohol abuse, resulting in well-known cognitive and behavioral abnormalities termed fetal alcohol spectrum disorders (FASD). FASD is among the most common (~1%), lifelong and highly complex disorders in some populations. Although 100% preventable, measures to properly diagnose, treat and reverse this disorder have been ineffective, primarily due to poor understanding. Towards an explanation of its complexity, we have used an animal model and numerous exposure paradigms to assess its impact on the heterogeneity of behavioral manifestations. The results are assessed in the context of underlying changes in gene expression.

**Methods:** We have modeled acute alcohol exposure in the developing brain using ethanol injections in C57BL/6J mice on gestational days (G) 8 and G11 (first trimester), G14 and G16 (second trimester) and postnatal days (P) 4 and P7 (third trimester equivalent). The resulting offspring are followed from birth until early adulthood using a battery of behavioral tests. These tests include assessment of developmental milestones, activity levels, anxiety-related behaviors (thigmotaxis), as well as spatial learning and memory using the Barnes maze. Further, at maturity (P70), mice are sacrificed and whole brains are used for genome-wide expression analysis and assessment of epigenetic features using mice microarrays. The nature and significance of genes affected by ethanol treatment is considered using bioinformatic tools.

**Results:** Results of this study show that behavioral heterogeneity of FASD can be explained, at least partially, by timing of alcohol exposure. Each ethanol treatment caused motor skill deficits, reflected by delayed surface righting and forelimb grasp milestones compared with controls ( $p < 0.05$ ). Ethanol treatment in trimester one and two increased activity levels ( $p < 0.05$ ) in juveniles (trimester 1, ethanol =  $2835.6 \pm 136.8$ , control =  $2332.1 \pm 142.9$  beam breaks; trimester 2, ethanol =  $3429.1 \pm 93.8$ , control =  $2291.5 \pm 98.6$  beam breaks). No changes in daytime activity were observed in trimester 3 mice; however, thigmotaxis was increased ( $p < 0.01$ ) for trimester 3 ethanol-treated mice ( $51.4 \pm 6.1$  sec in Center zone) as compared to control mice ( $78.1 \pm 6.9$  sec in Center zone). Interestingly, we observed the opposite effect ( $p < 0.001$ ) of thigmotaxis between trimester 2 ethanol ( $477.3 \pm 26.9$  sec in Center zone) and control mice ( $175.5 \pm 26.9$  sec in Center zone). Barnes maze results indicate that, independent of timing of exposure, ethanol-treated mice have difficulty performing spatial learning tasks compared to controls ( $p < 0.05$ ). Furthermore, trimester 2 and 3 ethanol-treated mice have difficulty remembering the location of the target hole (trimester 2 =  $4.5 \pm 0.3$ ; trimester 3 =  $6.3 \pm 0.5$  target hole explorations) compared with their respective control groups (trimester 2 =  $3.2 \pm 0.3$ ; trimester 3 =  $3.7 \pm 0.5$  target hole explorations),  $p < 0.01$ . These results argue for time of ethanol exposure dependent behavioral effects in FASD. Further analysis of the transcriptomes between ethanol-treated and control mice result in relevant pathways such as interleukin and cell cycle signaling networks (Eomes, Manf, Camk1g, Aft1, Xbp1, Egr3, Cdkn1a, Manf, Tnfrsf19, Cnr1, and Htr5a). Using quantitative PCR, we have validated the dysregulation of many of the genes including Tnfrsf19 ( $p = 0.02$ ) in trimester 1, Cdkn1a ( $p = 0.04$ ) in trimester 2 and Cnr1 ( $p = 0.004$ ) in trimester 3. Our results on gene expression and pathway analysis

argue that such effects are brought about by epigenetic changes that downregulate a set of imprinted non-coding RNAs. Further, the genes affected explain most, but not all, of the phenotypic consequences.

**Discussion:** These results represent a comprehensive comparison of behaviors representing a range of behavioral alterations in the mouse model of FASD. Further, FASD-related abnormalities are correlated with changes in global brain gene expression. Interestingly, many of the differentially expressed genes belong to common networks between trimesters, some of which have been previously implicated in other models of FASD. We conclude that although FASD-related phenotypes are dependent upon timing of in utero alcohol exposure, common mechanisms, such as epigenetic modifications, play a critical role in regulating developmental pathways in specific brain regions thereby contributing to this spectrum disorder. Our study adds a dimension to the current literature on FASD and provides further evidence that no time is safe from alcohol consumption during pregnancy. Furthermore, potential biological systems underlying alcohol disorders are complex and involve epigenetic mechanisms.

### Pathway-based Analysis For Alcohol Dependence

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**Background:** Alcohol dependence (AD) is a complex trait, and ~40 - 60 of its phenotypic variance is accounted for by genetic factors. Testing for the joint effect of multiple variants (polygenic analysis, Frank et al. *Addict Biol.* 2012) has shown that the genetic component of the risk for AD involves numerous SNPs of small effect. This approach captures associations that are missed when focusing on the level of single-markers. Further elucidation of this polygenic component may be facilitated by investigation of functionally related genes, selected on the basis of existing biological knowledge.

**Methods:** We conducted a pathway-based analysis for AD in a large genome wide association study from the German population. This involved application of the global test analytical method (Manoli et al. *Bioinformatics* 2006) to gene sets from the Molecular Signatures Database MSigDB (BioCarta, KEGG, Reactome, Gene Ontology, MIR, TFT, positional gene sets).

**Results:** Several promising pathways and gene sets were identified. Significant pathways included, e.g., 'ethanol oxidation' and 'non-homologous end joining'. The most promising gene was X-ray repair complementing defective repair in Chinese hamster cells 5 (XRCC5), which is located in chromosomal region chr2q35.

**Discussion:** XRCC5 receives strong independent support from linkage studies in humans and animal studies. Now we try to investigate specific traits in an invertebrate animal model (*Drosophila*) of alcohol dependence.

### A Case-control Genetic Study of ZNF699 Gene Markers in the University College London Alcohol Dependence Sample

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**Background:** The Zinc-finger protein 699 (ZNF699) gene has previously been associated with Alcohol dependence (AD) (BP. Riley, 2006). This protein, a transcription factor of unknown physiological function was initially identified as the most likely orthologue of hang, a gene crucial for ethanol tolerance in *Drosophila*. Riley et al. genotyped several ZNF699 SNP's in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) cohort, finding significant single-marker and haplotypic associations based upon the markers rs7254880, rs12460279, rs7252865 and rs10854142. In order to replicate this work, we have genotyped these SNP's in the University College London Alcohol dependence sample. Our analyses have failed to replicate the associations previously observed. We hypothesised that previous results may have occurred due to an association with another comorbid disease phenotypes in the IASPSAD population such as Alcoholic liver disease (ALD) or Opioid dependence (OD). Thus we have performed a subgroup analyses of these phenotypes in our sample as well as genotyping the markers in a separate Opioid dependence sample.

**Methods:** Subjects: Patients were recruited from a variety of Centers and all fulfilled the DSM-IV criteria for alcohol dependence. Control subjects were screened for psychiatric disorders including Alcohol dependence by interview (SADS-L). All subjects were Caucasian and of British ancestry, with a maximum of one grandparent from Northern Europe permitted. All subjects gave informed consent for participation in the study and approval was obtained from local and central NHS research ethics committees. Genotyping and analysis: Genotyping was performed in house using the K-Biosciences Allele Specific Primer (KASP) genotyping platform. Approximately 15% of samples were re-genotyped on a separate plate to validate genotype calls. Haplotype and genotype case-control analysis was performed using Haploview version 4.2.

**Results:** For all four SNP's we found no significant difference in allele frequencies between the Alcohol dependent (n=962) and control populations (n=766), the SNP rs7254880 approached significance (Allelic P = 0.08). A haplotype analysis found a block (rs12460279, rs7252865, rs10854142) composed of three main haplotypes concurring with previous data. Analysis found the least frequent of these haplotypes (ACG) to be significantly associated, surviving 10000 permutations (Permutation P-value = 0.03). Upon a subgroup analysis of the ALD phenotype we found that none of the markers were associated. However, for the marker rs7254880, we found a significant association between two different ALD phenotypes. Further, we observed a marked difference in the allele frequencies between the Opioid dependence (n=95) and control samples.

**Discussion:** The fact that our analyses fail to replicate the previous strong associations with Alcohol dependence is interesting. Riley's study is rigorous, with solid genotyping and functional experiments validating data. However since this paper six years ago, no study has been published replicating these strong findings, which is indicative that others may have also encountered our negative result. Our data is still needs further experimentation and analysis. Therefore we are going to genotype, rs724880 in a separate unscreened control

population with long term phenotypic data. Further we will genotype all the markers in a larger Opioid dependence sample and also perform a sub-group analysis of co-morbid Alcohol-Opioid dependence. Hopefully these studies will provide a clearer picture as to whether the ZNF699 locus is associated with Alcohol dependence per se, or another co-morbid disease.

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### Profiling DNA Methylation in Period 1, Negative Life Events and Alcohol Intake in Adolescents

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**Background:** Early onset of alcohol consumption is closely associated with negative life events occurred during adolescence. One major factor that modulates stress reactivity and alcohol consumption is circadian rhythmicity. The interaction between circadian gene PERIOD 1 (PER1) and stress reactivity in alcohol consumption has been previously demonstrated, where adolescents carrying the risk allele of rs30271712 is associated with increase psychosocial stress and alcohol intake (Dong et al., 2011). This study explores the role DNA methylation in PER1 and its relationship with negative life events and alcohol intake in adolescents.

**Methods:** Subjects: 14-year old adolescents (N = 707) were selected from the IMAGEN project (<http://www.imagen-europe.com>). The individuals were recruited during 2007-2010 across 8 cities in the Europe. DNA methylation analysis: Sodium bisulfite treatment was carried out on 250 ng whole blood DNA samples. For each individual 1ul bisulfite-treated DNA was amplified using the Period 1 primers generated by the Sequenom EpiDesigner. Human genomic DNA with 100%, 50% and 0% methylation were used as the internal controls. DNA methylation at the selected PER1 promoterregion was analysed on the Sequenom MALDI-TOF mass spectrometry platform. The selected region (about 1kb) is known to contain the first exon and transcription factor binding sites including E-box and GRE. Behavioural measurement: Self-report data from the Life-Events Questionnaire (Newcomb, Huber & Bentler, 1981) was selected to examine the frequency and ratings of negative life events experienced by the individuals. Data from the AUDIT questionnaire (WHO, 1992) was selected to measure the frequency of alcohol intake. IMAGEN.

**Results:** Differential DNA methylation has been observed across 42 CpG units in PER1. The first exon and the GRE site displayed DNA methylation ratio of  $0.026 \pm 0.016$  and  $0.003 \pm 0.009$  respectively. The 6 CpG units surrounding the E-Box displayed DNA methylation ratio ranging  $0.491 \pm 0.058$ ,  $0.891 \pm 0.029$ ,  $0.736 \pm 0.059$ ,  $0.584 \pm 0.046$ ,  $0.494 \pm 0.056$  and  $0.150 \pm 0.028$ . Regression analyses were performed to assess the relationship between DNA methylation at the 6 CpG units surrounding the E-Box and the frequencies of negative life events and alcohol intake. The frequency of alcohol intake has no significant association with the DNA methylation at these 6 CpG units (CpG1:  $\beta = -.832$ , 95CI [-3.07, 1.41]; CpG2:  $\beta = .087$ , 95CI [-4.15, 4.32]; CpG3:  $\beta = .725$ , 95CI [-1.74, 3.19]; CpG4:  $\beta = .315$ , 95CI [-2.34, 2.97]; CpG5:  $\beta = -1.11$ , 95CI [-3.36, 1.14]; CpG6:  $\beta = -.445$ , 95CI [-4.83, 3.94]). The frequency of negative life events has no significant association with the DNA methylation at the CpG units (CpG1:  $\beta = .138$ , 95CI [-1.40, 1.68]; CpG2:  $\beta = .178$ , 95CI [-3.21, 3.56]; CpG3:  $\beta = .526$ , 95CI [-1.49, 2.54]; CpG4:  $\beta = .189$ , 95CI [-1.74, 2.12]; CpG5:  $\beta = -.019$ , 95CI [-1.53, 1.57]; CpG6:  $\beta = -.376$ , 95CI [-3.50, 2.74]). Gender did not show any significant effect on DNA methylation, alcohol intake or frequency of negative life events.

**Discussion:** The first exon and the GRE site are located at the PER1 CpG island and they are largely unmethylated. Although inter-individual differences in DNA methylation around the E-Box has been observed, such variations in DNA methylation has no apparent associations with the frequencies of negative life events and alcohol intake. Further investigation is required to dissect the significance of DNA methylation at the transcription regulatory elements on PER1.

# **POSTER SESSION II**

## **ABSTRACTS**

**High Density Imputation of the ASD-associated MACROD2 Gene Region Identifies eQTL for Plausible ASD-related Genes**

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**Background:** Establishing a functional consequence of association is required to determine a causal link between marker and disease. In a recent GWA the Autism Genome Project (AGP) identified a strong association within the gene MACROD2 and autism (Anney et al., 2010). Very little is known about the function of MACROD2 in humans, although there are suggestions in the wider literature associating variation within MACROD2 with other neuropsychiatric disorders such as schizophrenia. Using molecular approaches we have previously characterised the spatial expression of MACROD2 and shown that variation in the 5' flanking region of the gene influences gene expression. We sought to identify whether additional genotype information could better describe the ASD association signal. Using detailed clinical data we also explore whether the association signal was strengthened for groups of ASD individuals including those that differ according to symptom severity, IQ, language status and gender. Additionally, we sought to examine whether the ASD associated region was also associated with gene expression in the human brain. These data may better explain causal relationships between association in ASD and biological pathways.

**Methods:** Using reference haplotypes from the 1000 genomes project data we performed high-density imputation of a 1Mb region surrounding the associated MACROD2 signal. Imputation was performed using BEAGLE in approximately 2900 probands and 2900 pseudo-controls from the AGP ASD sample (Anney et al., 2010) genotyped on the Illumina 1M Beadarray and 193 samples from a human cortical gene expression eQTL dataset (Myers et al., 2007) genotyped using the Affymetrix GeneChip Human Mapping 500K Array Set. Corresponding expression array data was derived from the HumanRefseq-8 Expression BeadChip and was made available from (<http://labs.med.miami.ed/myers/>). All regression analyses were performed for the imputed region only using PLINK.

**Results:** This association study confirmed the association identified in the 2010 AGP study, and following imputation additional supporting markers were identified. However, there was no association signal observed above the previously described association at rs4141463. Subsequently, the Myers data was used to identify trans-eQTL related to MACROD2. One of the significant trans-eQTL associations supporting the ASD-related MACROD2 association signal locus is between rs439451 and CNTN1 (Contactin 1), a gene previously implicated in ASD. This association maps most closely to the non-coding RNA MACROD2-AS1, nested on the reverse strand between exon 5 and 6 of MACROD2.

**Discussion:** The imputation of the MACROD2 locus in this study demonstrates that 'filling in' missingness in your phased dataset can enrich the original association signal and provide additional supporting associated markers around the original association. Theoretically, imputation can also identify new independent associations within the same locus, although we did not identify any new associations at the MACROD2 locus in this study. Post-hoc analysis of traits may offer additional insight into the underlying drivers of the association and thus inform future validation studies. This study also demonstrates the power of using the 1000 Genomes dataset in imputation, as a previous analysis using genotype information from the HapMap project did

not enrich the signal. We are unable to refine the association signal to a specific gene due to the presence of 3 genes in close proximity to the association signal: MACROD2, FLRT3 and the non-coding RNA MACROD2-AS1. Interestingly, the observation of a trans-eQTL between MACROD2-AS1 and CNTN1 may indicate that molecular follow-up studies should consider exploring the role of these intragenic genes.

### Genotyping Accuracy in a Series of Technical Replicates of Affymetrix Human SNP Array 6.0 Microarrays

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**Background:** The recent development of high-density Single Nucleotide Polymorphism (SNP) microarrays allowing the simultaneous measurement of millions of genetic marker has dramatically changed the field of population genetics. Genome-wide association studies (GWAS) that rest upon microarray generated marker data allow an unbiased view on the genome to identify molecular underpinnings of various complex traits and diseases, have become a very popular and successful research approach. Since the success of GWAS and the replicability of research findings is also highly dependent on the reliability of microarray data, we set out to investigate the reliability of microarray measured SNP genotype calls.

**Methods:** We systematically assessed the genotyping performance of the ~930000 SNPs represented on the Affymetrix Human SNP Array 6.0 in a series of N=157 technical replicates.

**Results:** A total of 577'177 SNPs showed consistent genotype calls over all technical replicates, when missing genotype calls were not considered in genotype call comparisons, i.e. counted as different genotype calls. For 54'327 SNPs, different genotype calls were generated for more than 2 of the technical replicates. Only a small fraction of SNPs (N = 6002) yielded incongruent genotype calls in more than 5% percent of the technical replicates, of which N=4809 would have been removed from analysis if rather lax standard quality control (QC) criteria (removal of markers with HWE\_pval < 0.001, MAF < 5%, Per-SNP-missingness > 10%) were employed. More stringent QC criteria (removal of markers with HWE\_pval < 0.1, MAF < 10%, Per-SNP-missingness > 5%) removed 5461 of 6002 genotype calls that were incongruent for >5% percent of technical replicates.

**Discussion:** The analysis of technical replicates reveals a high rate of consistency for genotype calls given that common QC criteria are applied. Further investigation of genomic regions that harbor markers with varying genotype calls in technical replicates is warranted to determine potential special sequence features (e.g. Copy Number Variations) that underlie the genotyping inconsistencies.

## Poster 107

### Genetic Risk Factors for Depression in Alzheimer's Disease Patients

Dementia

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**Background:** Alzheimer's Disease (AD) is often associated with depressive symptoms developing at any time before and after AD onset. The aetiology of depression in AD has not sufficiently been characterized, but biological aspects due to neurodegeneration and/or genetic risk factors may play a plausible role and may distinguish it from common depressive disorders.

**Methods:** To investigate the possible relationship between genetic risk factors and depression in AD, we assessed genetic polymorphisms reported to be associated with depression (MAOA VNTR, ACE 288bp Insertion/ Deletion, 5HTTLPR, COMT Val158Met, BDNF Val66Met, TPH1 A218C, HTR2A T102C, P2RX7 Q460R, FKBP5 rs1360780 and CRHR1 rs242941) in a cross-sectional study on 246 AD patients with or without clinically significant major depressive disorder (MDD) according to DSM-IV.

**Results:** Significant associations between AD and MDD have been found for three polymorphisms in females only (TPH1 A218C, MAOA VNTR and BDNF Val66Met) and one polymorphism in the total population (FKBP5 rs1360780). There was an increased risk of having MDD in homozygous female carriers of the TPH1 A-allele (odds ratio: 5.0) and homozygous carriers of the MAOA VNTR low activity allele 3R (odds ratio: 3.4).

**Discussion:** We detected allelic or genotypic associations of MAOA, TPH1, FKBP5 and BDNF in clinically significant MDD in AD. Odds-ratios were generally higher in female AD-patients, which might be due to the composition of the study population. Further studies on the neurotransmitter systems affected by the genetic polymorphisms found to be associated with MDD in AD may help to elucidate the underlying pathomechanisms of MDD.

### Impaired Cognitive Function in a Non-aging Non-demented Population is Associated with an Interaction between Major Depressive Disorder and the TOMM40 Risk Allele

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**Background:** Genetic influences on the susceptibility of developing dementia, specifically Alzheimer's Disease (AD), have been well investigated. Both the apolipoprotein E(ApoE) gene and the translocase of outer mitochondrial membrane 40 (TOMM40) gene have been strongly implicated in the development of AD. However, it remains unknown what effects AD susceptibility genes may have in earlier age, years before the first signs of dementia become apparent. It is possible that if depression is prodromal to AD then these genetic mechanisms may be more prevalent in influencing a depressed state at this early phase.

**Methods:** We conducted a battery of neuropsychological measures of working memory (n-back), executive function (the Stockings of Cambridge), and affective memory bias (Emotional Word Memory) with currently depressed participants compared to controls and remitted individuals in a cohort of 264, investigating the effect of the strongest TOMM40 SNP (rs2075650) based on previous AD GWAS studies.

**Results:** In our study a diagnosis x genotype interaction was found for our measure of executive function ( $F(6,402)=3.270$ ,  $p=0.004$ ), with post-hoc comparisons revealing differences between those participants who were both risk allele carriers and MDD sufferers (all  $p<0.01$ ). In addition, a significant diagnosis x genotype interaction was found in the emotional word memory task ( $F(6,448)=2.510$ ,  $p=0.021$ ), suggesting that those individuals with current MDD symptoms, who also carried the protective allele, had a significantly increased number of positive intrusions on delayed recall ( $p=0.016$ ). No diagnosis x genotype effect was apparent in the working memory task.

**Discussion:** In our non-aging non-demented population genetic effects of rs2075650 on our neuropsychological behavioural tests were only discernable in those individuals who were currently depressed and carried the risk allele. The strongest deficits can be seen in executive function suggesting that the prefrontal cortical top-down control mechanism is the most vulnerable to mitochondrial dysfunction caused by variations in the TOMM40 gene. It can also be hypothesised that this mechanism increases the probability of developing depression in TOMM40 risk allele carriers. Further studies are needed to investigate this hypothesis.

### Genetic Services and Autism Spectrum Disorder: Parental Knowledge, Awareness and Attitudes

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**Background:** Genome copy number changes identifiable by chromosome microarray (CMA) are believed to contribute to ~1 in 5 of cases of ASD (Bremer 2011). Practice guidelines now stipulate that genetic testing, including CMA, be offered as a first-tier test for ASD (Miller 2010). Translating genetic findings to clinical tests is juxtaposed with the lack of a standard for delivery of results and raises both ethical and social issues (Coulter 2011). Little is known about the psychosocial, experiential, and educational needs of parents whose children may receive genetic testing (Arribas-Ayllon 2009). To address these concerns we surveyed parents of children with ASD about their knowledge of, experiences with, and expectations for genetic testing in ASD.

**Methods:** A 54-item parent survey was developed by clinical psychologists and genetic counselors familiar with ASD and genetic testing for ASD and was approved by the University of Miami Institutional Review Board. The survey asked about parent experiences with genetic services for their child with ASD. Participants were recruited to participate in an on-line, anonymous survey sent out by the Center for Autism and Related Disabilities (CARD). The email invitation described the purpose of the study and provided a link to the online consent document and survey. Participants were restricted to parents who were a part of one of three CARD registries in Florida as of January 2012.

**Results:** 345 participants (~10%) completed at least part of the online survey; 26 were excluded due to insufficient data. Of the 319 remaining participants (80% White; 52% Hispanic), the average age of the child of the respondent is 10.9 years. Eleven respondents had children with ASD and a genetic syndrome (e.g., Fragile X, Rett, Down, and Prader-Willi). Among parents whose child does not have a genetic syndrome 26% indicated that genetic testing should not be done at all. When specifically asked about genetic testing for ASD, 10% of respondents indicated that genetic testing should not be done at all. The majority of respondents agree that genetic testing is useful in healthcare (89%) and are interested in finding out if genetic factors are the cause of their child's ASD (84%). Among parents of children without a genetic syndrome 49% reported that they believed the main cause of ASD was genetic. When asked to estimate recurrence risk, 37% of respondents placed their risk in the "moderate chance" category. Among all respondents, 16% reported that their child had received microarray testing and 13% reported that their child had received karyotype testing. However, while 42% indicated that they understood the results of karyotype testing, only 28% of respondents whose child received microarray testing noted that they understood the results. Interestingly, 76% of parents reported that their treating physicians did not refer them to or discuss genetic services. Among those parents who were referred for genetic tests or services, the most frequent referring specialist was a pediatric neurologist (68%). When asked to indicate benefits of genetic testing, 68% endorsed "to answer the question of why my child has ASD." When asked about potential barriers, 63% endorsed "cost and/or insurance coverage issues".

**Discussion:** This survey is a first step in understanding the expectations for genetic services among parents of individuals with ASD. Our preliminary data suggest that parents of individuals with ASD have a limited familiarity with genetic services and, among those who have received them, a limited understanding of the results. Nonetheless, nearly half of the parents surveyed believe that genetics may cause ASD. Finally, the reported involvement of genetic specialists in our sample was low. This is concerning given the potential increased demand for genetic services associated with its status as a first-tier evaluation. More work is needed to optimize delivery of genetic services for families of individuals with ASD who desire such services.

## Endophenotypes

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#### Family Based Genome Wide Association Study (GWAS) of Externalizing Disorders

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**Background:** Twin studies indicate that there is a common genetic predisposition to several classes of externalizing disorders, including alcohol dependence, other illicit drug dependence, childhood conduct disorder and adult antisocial behavior. Here we created externalizing factor scores based on a composite of alcohol abuse and dependence symptoms, antisocial personality disorder symptoms (or conduct disorder symptoms if participant was under age 18), and total illicit drug (cocaine, marijuana, sedatives, stimulants, opiates, and other drugs) abuse and dependence symptoms. We report results from a family based genome-wide association study (GWAS) of this externalizing factor score to identify novel variants that may predispose individuals to broad externalizing-type problems.

**Methods:** A genome-wide association study (GWAS) was performed in 2322 individuals from 118 families with a high density of AD individuals; subjects were drawn from the Collaborative Studies on the Genetics of Alcoholism (COGA). The primary phenotype was the externalizing factor score, although we performed GWAS for separate phenotypes used in factor analysis for comparison. Association analysis was performed using kmix procedure from kinship package under R. kmix uses kinship matrix based on family structure. Covariates in the analysis model included age at last interview, sex, and birth cohort.

**Results:** No single nucleotide polymorphism (SNP) reached genome-wide significance (10<sup>-8</sup>). The most significant results were on: a) chromosome 18 in the ARHGAP28 gene (ARHGAP28; 2 SNPs, p=2.73\*10<sup>-7</sup> and p=6.88\*10<sup>-6</sup>); and b) chromosome 12 in the TMTC2 gene (rs12579483).

**Discussion:** We find evidence for several genes and genomic regions that appear to confer risk to a variety of forms of externalizing psychopathology. Although none of the results reached genome-wide significance, ARHGAP28 is involved in the regulation of small GTPase mediated signal transduction, and may have potential value in understanding underlying mechanisms involved in both alcohol dependence and related externalizing disorders.

### Studying Brain-based Intermediate Phenotypes in Schizophrenia: From Candidate Genes to Genome-wide Approaches

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**Background:** Despite a number of twin studies indicating high heritability in complex neuropsychiatric disorders such as schizophrenia, the mechanisms of susceptibility for these disorders remain to be clarified. Possible reasons for this problem include the polygenic inheritance, the genetic and the phenotypic heterogeneity of the disorders and the low reliability and long-term stability of psychiatric diagnoses. To address the latter, it has been suggested to use intermediate phenotypes instead of diagnosis. Intermediate phenotypes are heritable, disease-associated and stable traits which were suggested to show a strong association with risk genes due to their proximity to the underlying biology. Hippocampal volume reduction, decreased cortical thickness and DLPFC dysfunction during working memory processing have been shown to be heritable markers closely related to schizophrenia. In this presentation I will summarize the results of our recent studies investigating the central nervous system effects of several widely-acknowledged risk genes for schizophrenia. However, candidate gene approaches rely on prior and possibly ill-defined assumptions about the underlying biological pathways of disorders or intermediate phenotypes. Therefore, in our latest study we choose a genome-wide association study (GWAS) approach to identify single-nucleotide polymorphisms (SNP) that are highly associated with hippocampal volume without making prior assumptions about possible candidate genes.

**Methods:** The Mind Clinical Imaging Consortium (MCIC) Study of Schizophrenia obtained multimodal imaging and genetic data on a total of 321 individuals from four participating sites. Structural and functional magnetic resonance imaging (MRI) data was acquired with either a 1.5T Siemens Sonata or a 3T Siemens Trio. Cortical reconstruction and volumetric segmentation based on high resolution structural MRI scans was performed with the FreeSurfer surface reconstruction software. Functional data were analyzed using the FBIRN Image Processing Stream (FIPS), a pipeline using the FMRIB Software Library of FSL. Genotyping was performed using the Illumina HumanOmni-Quad BeadChip. Quality control steps were carried out in PLINK following standard procedures. After quality control, genotype data was available for 115 schizophrenia patients and 126 matched controls. To account for population stratification, we either excluded participants to form a more homogeneous group or applied principal component analysis on a pruned SNP data set using EIGENSOFT 3.0, and included the 10 principal components into the regression analysis. Depending on the specific study we also modelled diagnosis, age, gender and acquisition site. Furthermore, we used independent data such as neuropsychological functioning or gene expression profiles of human hippocampal tissue to cross-validate the effects of the studied genetic risk variants.

**Results:** In our candidate gene studies we found associations between the COMT Val158Met polymorphism and hippocampal volume, between DISC1 Leu607Phe and cortical thickness in the infraparietal lobe and between neurogranin risk markers and increased neural activity during working memory processing in prefrontal brain areas. Using a hypothesis-free approach none of the genetic markers reached the proposed genome-wide significance threshold. However, six highly correlated SNPs on chromosome 19p13.11 had p-values up to  $8.3 \times 10^{-07}$ . Allelic differences of these SNPs were highly associated with memory functioning in patients and controls as well as differential mRNA expression in human hippocampus tissue.

**Discussion:** Taken together, the results of our studies highlight the effects of schizophrenia risk variants on established intermediate phenotypes. Furthermore, our findings based on the GWAS approach allow new but preliminary insights into the allelic architecture of a brain structure closely linked to schizophrenia. Identification and verification of causal variants and their functional effects may unveil yet unknown players in the neurodevelopment and the pathogenesis of burdensome neuropsychiatric disorders such as schizophrenia.

### A Joint Endophenotype and Polygenic Approach Reveals Association between Neurocognitive Gene Sets and Psychiatric Disorders

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**Background:** Psychiatric disorders, such schizophrenia (SCZ) and bipolar disorder (BD), are among main causes of disability worldwide. Since such patients often present serious cognitive deficits, it has been suggested that cognitive ability measures could serve as endophenotype. In addition, it has been shown that the manifestation of complex genetic disorders, such as SCZ and BD, may be explained by the small effects of multiple genetic variants, including not only the GWAS top hits but also the weaker association signals that do not reach genome-wide significance (Valdar, et al. 2006; Lee et al., 2012).

**Methods:** In order to validate cognitive ability as genetic endophenotypes for psychiatric disorders, we generated gene sets associated to different neurocognitive domains (general cognition, learning, memory, speed of processing and attention) tested in a GWAS of a healthy Norwegian sample (NCNG) phenotyped for cognitive abilities. The different gene sets were built by assigning SNPs to genes and scoring them using an adjusted minimum p-value approach with LDsnpr package (Christoforou et al., 2012). The candidate gene sets were then tested by gene set enrichment analysis (GSEA) against 3 independent SCZ and 3 independent BD GWASs. This method, initially developed for gene expression studies, assesses the enrichment of signal of a priori gene set in a ranked list of genes (Mootha et al., 2003; Subramanian et al., 2005). The SCZ and BD GWAS SNPs were also binned and scored using LDsnpr.

**Results:** We observed significant enrichment for several neurocognitive gene sets. Most interestingly, one set replicated in all the 3 SCZ datasets and another gene set in all 3 BD samples.

**Discussion:** Our work suggests that the combination of endophenotypes and polygenic analysis might be important in overcoming the difficulties of studying complex psychiatric traits and may be useful when exploring GWAS outcomes.

### Neuropsychological Profile of Adults with Down Syndrome and Moderate Intellectual Disability: Verbal and Visual-spatial Processing

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**Background:** The term intellectual disability is used to refer to a wide range of clinical conditions with quite diverse etiologies, involving a wide range of neural deficits and mental abilities (Carvajal, Fernández-Alcaraz, Rueda & Sarrion, 2012). Numerous studies conducted with specific etiological groups show that people with intellectual disability with common etiology often display similar neuropsychological profiles (Facon & Nuchadee, 2010). Consequently, more recently researchers have investigated these profiles, taking into account the etiology of the intellectual disability (Karmiloff Smith et al., 2004). Down's syndrome (DS) is the most common genetic cause of intellectual disability accompanied by additional, specific cognitive strengths and weaknesses (Chapman & Hesketh, 2000). In other words, DS is a genetic disorder that exhibit a specific neuropsychological profile with some abilities more preserved and others more impaired. Specifically, their neuropsychological profile appears to be characterized by a remarkable deficit in verbal skills that usually exceeds impairments in visual-spatial skills (Vicari et al., 2006). However, recent studies have found results that are not consistent with this profile. Therefore, we ask ourselves what exactly occurs at verbal and visual-spatial processing in adults with DS in comparison with adults who have intellectual disability due to other aetiologies.

**Methods:** The sample was composed of 60 participants with moderate intellectual disability. The first group was composed of 30 users who presented diagnosis of DS and the second group was composed of 30 users with moderate intellectual disability due to other aetiologies. Each of the participants carried out the Wechsler Intelligence Scale for Children-WISC-IV-(Wechsler, 2003), the Boston Naming Test (Goodglass & Kaplan, 1983), the Peabody Pictures Vocabulary Test-PPVT- (Dunn, 1981), verbal fluency of the Illinois Test of Psycholinguistic Abilities-IPTA- (Kirk, McCarthy & Kirk, 1968) and the Frostig Developmental Test of Visual Perception- Frostig IV- (Frostig, 1964).

**Results:** On one hand, the two factors ANOVA (Group x Indexes of WISC-IV) revealed that DS group did not showed significant differences between their performance in Verbal Comprehension Index (VCI) and Perceptual Reasoning Index (RPI)  $F(1, 29) = 611.23, p = 0.23$  whereas the control group exhibited a significant higher performance in Verbal Comprehension Index than Perceptual Reasoning Index  $F(1, 29) = 272.77, p < 0.001$ ; subsequent analyses demonstrated that the DS group obtained lower scores than the control group in Verbal Comprehension Index  $t(50,14) = -2.37, p < 0.05$ . On the other hand, the two factors ANOVA (Group x Verbal tasks) revealed that DS group did not showed significant differences between their performance in receptive vocabulary (PPVT-III) and verbal fluency (IPTA subtest)  $F(1, 29) = 17.83, p = 0.34$  whereas the control group exhibited a significant higher performance in receptive vocabulary than verbal fluency  $F(1, 29) = 17.83, p < 0.001$ ; subsequent analyses demonstrated that the DS group obtained lower scores in receptive vocabulary than the control group,  $t(58) = -2.1, p < 0.05$ . Additionally, the t-tests showed that the DS group displayed significant worse performance in verbal short-term memory (digit span)  $t(58) = 2.35; p < 0.05$  and similar performance in verbal fluency, naming (BNT) and IQ (WISC-IV)  $t(58) = -0.39, -1.285, -0.03; ps = 0.70, 0.204, 0.98$ . Finally, the two factors ANOVA (Group x Frostig

subtests) revealed that the two groups showed lower performance in shape perception (Frostig III) than spatial processing (Frostig V)  $F(1, 58) = 336.12, p < 0.001$ ; later analyses indicated that the DS group obtained lower scores than the control group in shape perception  $t(58) = -3.52, p < 0.005$ .

**Discussion:** The results of this study indicate, against expected, that the DS showed similar performance in verbal and visual-spatial skills and similar performance in verbal production and comprehension. Furthermore, the two groups exhibited lower performance in shape perception than spatial processing. However, the DS group demonstrated significantly poorer performance in receptive vocabulary, verbal short-term memory and shape perception but similar performance in naming, verbal fluency and IQ in compared with adults with intellectual disability due to other aetiologies. These results highlight that those adults with DS showed strengths and weaknesses in different verbal and visual-spatial sub-processes. We think that these results would be useful to clarify the characteristic neuropsychological profile of adults with DS.

#### Poster 114

##### Investigating the Association between Rare Copy Number Variation in Neurodevelopmental Disorders: Autism Spectrum Disorders and Schizophrenia

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**Background:** There is emerging evidence that copy number variants (CNVs) provide a new vista on understanding susceptibility to neuropsychiatric disorders. Recent work also suggests that there may be some common genetic and environmental factors underlying neurodevelopmental disorders, in particular Autism Spectrum Disorders (ASD) and schizophrenia. Specifically, both independent Genome Wide Association Studies (GWAS) and studies of CNVs have identified genes that have been reportedly associated with both disorders, and both disorders present with overlapping neurodevelopmental symptoms with deficits in cognition and social interaction systems.

**Methods:** Data were derived from the Autism Genome Project and an Irish sample of schizophrenia cases that have been included in genetic studies in the Neuropsychiatric Genetics Group, Trinity College Dublin. The association between rare CNVs (<1% frequency in cases and controls) impacting genes previously implicated in ASD or Intellectual Disability (ID) (Pinto, et al. 2010), or genes that are differentially brain expressed (Raychaudhuri, et al. 2010) and clinical phenotypes of interest were examined. Clinical phenotypes with probable neurodevelopmental origin including IQ, gait disturbances, epilepsy, and adaptive function were included in the ASD analyses. In addition to the variables included in the ASD analyses, positive and negative symptom severity, social cognition and other neuropsychological measures were included in the schizophrenia analyses. Finally, parental age associations were examined for both disorders. All statistical analyses were carried out in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Additional exploratory analyses using recursive partitioning techniques and latent profile analyses were completed. Autism Genome Project.

**Results:** Contrary to expectations, no statistically significant association between CNVs that impact ASD- or ID-implicated genes or differentially brain-expressed genes and selected phenotypes were identified for either ASD or schizophrenia. Exploratory analyses suggest sub-phenotypes that would provide good targets for association analyses in future studies.

**Discussion:** Despite large initial sample size this study was likely to have been underpowered to detect an association between the rare CNVs impacting candidate gene lists and phenotypes of interest due to very small numbers in some of the phenotypic measures. Risk factors and associated features are not systematically assessed for genetic studies, and these sub-phenotypes of interest should be rigorously assessed in future studies.

## Poster 115

### Application of MRI Anatomical Changes as Bipolar Disorder Biomarkers

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**Background:** High resolution Magnetic Resonance Imaging (MRI) combined with automated brain surface and volume segmentation can provide accurate information of individuals anatomical measurements. These measurements can be applied to study the anatomical changes that occur during neuropsychiatric disorders. Neuroanatomical regions implicated in Bipolar Disorder (Brambilla et al., 2003) and schizophrenia (Rimol et al., 2010) have been identified in structural imaging studies.

**Methods:** During this study, MRI brain anatomy was acquired for 46 subjects. Scans were acquired at El Paso University Medical Center North East Clinic with a 1.5 Tesla Siemens scanner and at the The University of Texas Health Science Center Research Imaging Institute with a 3 Tesla Siemens scanner. T1 weighted MPRAGE sequences were used with TR= 2200 ms, TE 2.83 ms, and FOV=256 mm. Voxel Based Morphometry was implemented to study brain structure size and shape differences between Bipolar Disorder (BPD) and healthy subjects. FreeSurfer software was used to reconstruct brain's cortical surface from structural MRI data. Gray matter volume, cortical thickness and cortical curvature were measured. Discriminant function analysis was used to determine which variables could segregate BPD patients from healthy controls.

**Results:** Significant changes in right hemisphere rostral middle frontal volume, right hemisphere entorhinal volume, right hemisphere medial orbitofrontal volume, left hemisphere middle temporal volume and left hemisphere temporal pole provided accurate segregation of BPD patients from healthy subjects.

**Discussion:** Automated brain reconstruction and segmentation analysis can provide detailed information of brain abnormalities associated with neuropsychiatric disorders. These abnormalities in brain structure may be implicated with cognitive deficits observed in BPD and can be validated as biomarkers and aid in the future identification of BPD predisposition genes.

## Poster 116

### The Stability of Prepulse Inhibition of the Startle in Schizophrenic Patients: A 6 Year Follow-up Study of a Cohort of First-episode Drug-naïve Patients and Matched Healthy Controls

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**Background:** Deficits in information processing appear core features in the pathogenesis of schizophrenia. Prepulse inhibition of the startle reflex (PPI) and habituation are operational measure of early information processing. Impaired PPI in schizophrenia has been replicated in many studies and are generally regarded as an endophenotype for schizophrenia. The results on habituation in schizophrenia are inconsistent. There is growing evidence that second generation antipsychotic can improve PPI in chronic schizophrenic patients. However, in an earlier study, we reported that neither a three months treatment with zuclopenthixol (first generation antipsychotic) nor with risperidone (second generation antipsychotic) improved the PPI deficits of antipsychotic naïve, first-episode patients with schizophrenia. Furthermore, the follow-up periods of prior studies had a maximum of 6 month; therefore the stability of PPI and habituation over long time has been deduced from cross-sectional studies. The current study is a 6 year follow-up investigation of that original study, i.e. a 6 year follow-up investigation of schizophrenic patient that were drug-naïve at baseline.

**Methods:** At baseline, 20 drug-naïve first-episode schizophrenic patients and 21 healthy controls matched for gender and age, participated in the project. Three PPI measures (SOA 30 msec, 60 msec. and 120 msec.) and habituation were examined at baseline, after 3 month of randomized antipsychotic treatment and after 6 years. 16 patients and 17 healthy controls were re-examined at the 6 year follow-up.

**Results:** Patients had PPI deficits compared to healthy controls at baseline. At the 6 years follow-up, no significant group differences were found and the PPI60 (SOA 60 msec.) had improved significantly in the patients. Furthermore, PPI60 in healthy controls decreased over the same period. Patients habituated significant less than healthy controls and habituation was stable in both patients and healthy controls.

**Discussion:** The present results support the notion that PPI deficits are fundamental trait markers of schizophrenia that are already present at an early stage in the development of the disease. However, the deficits seem to diminish over time. Since PPI in matched healthy controls decreased over the same period as PPI increased in patients it is likely that the increase was caused by disease related factors such as disease process, clinical state, or medication. Habituation was deficient in patients compared to healthy controls and did not change over time.

### A Genome-wide Association Study of a Brain-based Phenotype Related to Schizophrenia

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**Background:** Patients with schizophrenia and their siblings show subtle changes in brain structures, such as a reduction of hippocampal volume. Abnormalities of hippocampus structure and function have been associated with deficits in memory and executive function, suggesting that these changes could reflect a central pathophysiological process associated with the illness. In addition, sibling and family studies provide evidence for the high heritability of hippocampal volume, and it is thus considered an intermediate phenotype for schizophrenia. By combining the powerful approach of a genome-wide association study (GWAS) with imaging and neuropsychological measurements, the aim of this study was to identify single-nucleotide polymorphisms (SNP) that are highly associated with hippocampal volume without making prior assumptions about possible candidate genes.

**Methods:** We obtained high-quality structural MRI, genetic and neuropsychological data of 126 healthy controls and 115 patients with a DSM-IV diagnosis of schizophrenia from the Mind Clinical Imaging Consortium study of schizophrenia. An automated atlas-based segmentation algorithm (FreeSurfer) was used to generate volumetric measures of the hippocampus. Genotyping was performed at the Mind Research Network Neurogenetics Core Lab using the Illumina HumanOmni-Quad BeadChip. Quality control steps were carried out in PLINK. A total of 743,591 SNPs were tested for association by fitting an additive linear regression model with minor allele count, sex, age, scanner field strength and intracranial volume as predictors of average hippocampal volume. We also modelled diagnosis to account for possible additional environmental factors specific to psychiatric patients such as treatment effects or stress. To correct for population stratification, we applied principal component analysis on a pruned SNP data set using EIGENSTRAT of the EIGENSOFT 3.0 software package, and included the 10 principal components into the regression analysis. Furthermore, gene expression profiles of human hippocampal tissue were investigated for the gene regions of significantly associated SNPs.

**Results:** None of the genetic markers reached the genome-wide significance threshold of  $5 \times 10^{-8}$ . However, six highly correlated SNPs on chromosome 19p13.11 had p-values up to  $8.3 \times 10^{-7}$ . Allelic differences were highly associated with differential mRNA expression in human hippocampus tissue.

**Discussion:** Our findings support previous reports demonstrating that GWAS with a quantitative brain-based intermediate phenotype as a dependent variable is a viable method to identify associated gene variants. Although encouraging, our results require replications in independent cohorts. Identification of causal variants and their functional effects in the regulation of neurodevelopment and synaptic (re)organization could improve our conceptual framework of processes related to hippocampal volume reduction and facilitate a better understanding of schizophrenia.

### Genetic Effects on Basic Human Information Processing are Moderated by Age - Results from Two Genome-wide Association Studies

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**Background:** Human information processing is a complex trait consisting, in particular, of psychomotor speed, cognitive flexibility, and working memory. The Trail Making Test (TMT) is a well established method for the assessment of these components. Results from twin studies suggest moderate to strong heritability of test performance in the TMT with  $h^2$  estimated between 0.35 and 0.6. However, in addition to heritability, age of the subject plays an important role in this trait. A number of genetic associations with the TMT have been reported, including variants in APOE, COMT, and the BDNF genes. None of these studies, however, evaluated the effects of age as a potentially important mediator for genetic associations with human information processing.

**Methods:** Genome-wide association (GWA) studies have been performed in two representative Caucasian population samples of the Munich metropolitan area ( $N_1 = 544$ ;  $N_2 = 348$ ). Participants of both samples did not have any mental axis I disorder and were heterogeneous in age (mean age 45 years, age range between 20 and 75 years). The best hits obtained in both genome-wide studies were investigated in a third sample of community-dwelling elderly Caucasians ( $N_3 = 1037$ , mean age 79 years, age range between 70 and 90 years) participating in the Sydney Memory and Ageing Study.

**Results:** While the GWA analysis did not reveal genetic main effects on the TMT that could be replicated in the respective other GWA sample, we found an age-moderated effect between variants in the DSG1 gene region at 18q12.1 and TMT-A performance in both GWA samples, with a detrimental effect on information processing speed in younger cohorts and a beneficial effect in older cohorts (meta-analysis =  $1.3 \times 10^{-7}$ ). This effect was not observed in the sample of the Sydney Memory and Ageing Study.

**Discussion:** The results of two German GWA studies in population representative samples suggest an age-moderated effect of variants in the DSG1 gene on information processing speed, while no effect was found in an elderly, age-homogenous cohort. While the two GWA samples are representative for a non-psychiatric population with an age range between 20 and 75, the participants of the Memory and Ageing Study were between 70 and 90 without major health problems resulting in a selective sample with higher education and higher mobility compared with census data. These factors might have contributed to a limited age-moderated variability of cognitive function in this sample, and thus, to the failure to replicate the GWA findings. Therefore, we conclude despite the failure of replication in the elderly sample that genetic variants in the DSG1 gene region affect basic human information processing in an age-dependent manner.

### Dopamine-D2 (DRD2) and Type 3 Metabotropic Glutamate Receptor (GRM3) Genotypes are Differentially Associated with Eye Tracking Performance in Untreated Patients with Psychotic Disorders

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**Background:** Alterations in both dopaminergic and glutamatergic synaptic neurotransmission have been implicated in the pathogenesis of psychotic disorders. How these alterations are related to different intermediate phenotypes, and their modulation under antipsychotic treatment, are unclear. Two eye tracking abnormalities in psychotic disorders supported by different functional brain systems were examined in the present study: 1) tracking initiation that is driven primarily by sensory/visual motion information processed to motor systems (open-loop), and 2) tracking maintenance that is driven by higher-order cortical processes including analysis of visual feedback from retinal error signals (closed-loop).

**Methods:** 138 untreated patients with a psychotic disorder (schizophrenia N=96, psychotic bipolar N=26, psychotic major depression N=16) from two university hospitals in Pittsburgh and Chicago, USA, were included in this study. At both sites, patients were assessed on identical eye tracking tasks. 93 patients were followed after 6 weeks of antipsychotic treatment. To account for potential site effects, patient's eye movement data were compared to data from site-specific healthy controls (N=100). Patients were genotyped for four markers in the DRD2 gene and four markers in the GRM3 gene. Candidate polymorphisms were selected based on functional consequence or prior association with disease risk, pathology, or antipsychotic treatment response. 100 ancestral informative markers were genotyped to assess and covary for genetic ancestry.

**Results:** Genetic markers did not deviate from Hardy-Weinberg Equilibrium and pre-treatment performance was not related to genetic ancestry. Marker rs1799732 (-141C Ins/Del) in DRD2 was associated with open-loop eye velocity during tracking initiation independently of antipsychotic treatment. Deletion-carriers had slower open-loop eye velocity than patients with a CC insertion ( $p = 0.001$ ). Second, deletion carriers were more delayed in initiating eye tracking than CC insertion carriers ( $p = 0.001$ ). Antipsychotic treatment resulted in speeded eye tracking initiation in deletion carriers but delayed tracking initiation in CC insertion carriers (time x genotype  $p = 0.006$ ). Two markers in GRM3 were associated with tracking maintenance, not with tracking initiation. Patients with a rs274622\_CC genotype had poorer maintenance performance (slower closed-loop eye velocity) than T-carriers ( $p=0.001$ , see Figure). This deficit was not affected by antipsychotic treatment. Additionally, patients with an rs6465084\_GG genotype tended to have better tracking maintenance than A-carriers ( $p=0.058$ ). Antipsychotic treatment resulted in poorer tracking maintenance in GG homozygotes but not A-carriers (time x genotype  $p=0.015$ ).

**Discussion:** Our findings have important implications for a better understanding of how different brain systems are modulated by variants in DRD2 and GRM3 genes in psychotic disorders. First, alterations in DRD2 that is highly expressed in caudate nucleus were associated with tracking initiation ability. This is in line with the fact that movement initiation is highly dependent on intact caudate function. The -141C deletion allele has been related to reduced DRD2 promotor activity and lower D2 receptor expression. Our findings suggest that this may result in poorer movement initiation (lower open-loop eye velocity and prolonged latency to start a tracking movement). In contrast, variants in GRM3 were associated with eye tracking maintenance. GRM3 codes for the mGluR3 protein and is essential for optimal signaling of glutamate in the brain, notably in prefrontal cortex. Eye tracking maintenance requires adjustments within the complex cortical networks that include prefrontal areas. Within this neocortical network, continuous integration of the retinal tracking error and updating of predictions about target motion are processed. Thus, DRD2 may disrupt eye tracking in psychotic disorders by disrupting basic motor functions, while alterations in GRM3 may regulate higher cortical perceptual and cognitive processes that maintain accurate eye tracking over time, after the motor initiation phase. Additionally, antipsychotic treatment seems to modulate both DRD2 and GRM3 effects on tracking performance to some extent, again by influencing motor and higher order cortical processing respectively.

### Pleiotropic Effects of COMT VAL158MET Polymorphism on Working Memory and Emotional Episodic Memory

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**Background:** MET polymorphism influences dopamine level mainly in prefrontal brain regions. Therefore COMT is an important target gene for behavioral studies, imaging genetics and gene-disease associations. There are hints from imaging genetics, that the Val158MET polymorphism has pleiotropic effects on executive functioning (including N-back task) and emotional processing, using a meta-analytic approach (Mier et al,2010).

**Methods:** Our actual behavioral data from an ongoing fMRI-study supports this view investigating 753 young and healthy subjects (40% male, mean age 22.5, Val/Val n = 189, Val/Met n = 371, Met/Met n = 193, Hardy-Weinberg-Equilibrium p = 0.72) with a typical working memory task (N-back 2-back minus 0-back) and an emotional episodic memory task (emotional pictures 10 minutes delayed free recall). Phenotype task performances were separately z-transformed after taking the residuals from a linear model with sex, age and the interaction term between sex and age as covariates. The interaction term between phenotype category (N-back versus pictures delayed recall) and genotype (Met-dominant genetic model, 75% Met-allele carrier) was calculated using a mixed model with subject as random effect. Genotype, phenotype category and the interaction term between genotype and phenotype category were fixed effects. Dependent variable was task performances.

**Results:** In line with the findings of the meta-analysis our results show a significant interaction effect ( $t(751) = 3.84, p = 0.00014$ ) between phenotype category and genotype. Post hoc tests reveal that Met-allele carriers outperform Val-homozygous subjects in the N-back task ( $t(751) = 3.39, p = 0.00074$ ). Furthermore there is tendency for Val-homozygous subjects to outperform Met-allele carriers in an emotional memory task ( $t(751) = -1.91, p = 0.057$ ).

**Discussion:** Our actual data support the view that the Val158MET polymorphism has an impact on executive functioning and memory performance but that these effects can be highly task dependent. Reference: Mier, D., Kirsch, P. & Meyer-Lindenberg, A (2010). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Molecular Psychiatry* 15, 918-927.

### The Interaction between a Composite Genetic Risk Score and Birth Weight is Associated with Social Anhedonia

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**Background:** Social anhedonia is a clinically relevant feature in schizophrenia, and it has been proposed to be a vulnerability marker for schizophrenia-spectrum disorders. Yet it is an enduring trait that can be measured in the general population. By studying the development of social anhedonia we are likely to gain insight also into the developmental pathways of schizophrenia. The developmental background of social anhedonia is not well studied, but it is reasonable to assume that genes and environment are both involved and they are likely to interact. Our aim was to examine whether the interaction between recently identified risk alleles and known environmental risk factors for schizophrenia is associated with social anhedonia.

**Methods:** The study sample was derived from the 1966 Northern Finland Birth Cohort (total N = 12 058), which is a sample representing general population and prospectively followed from perinatal period to adulthood. Environmental risk factors considering the early years, e.g. birth weight, were collected from child welfare clinic registries and with questionnaires filled in by the mothers during years 1965-1967. Social anhedonia was self-rated in 1997 as a part of the cohort's 31-year follow-up, using the Chapman Social Anhedonia Scale. We computed a composite genetic risk score from 8 best SNPs found in a recent large European GWAS meta-analysis for schizophrenia (The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium et al., 2011). Two of the SNPs were genotyped (rs10503253 and rs7004633) and 6 of them imputed (rs1625579, rs17662626, rs2021722, rs7914558, rs11191580 and rs548181).

**Results:** We examined the interaction between the composite genetic risk score and early environment (birth weight) on social anhedonia in adulthood. Preliminary data show that having more risk alleles together with high birth weight is associated with higher social anhedonia in adulthood ( $p < 0.05$ ).

**Discussion:** Our results suggest having several genetic risk alleles together with unfavorable environment may predispose to social anhedonia. Together with earlier studies this suggests that gene-environment interaction may play a role in the development of clinically relevant phenotypes. Whether these results have relevance to schizophrenia should be further explored.

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### Association of DRD4\_VNTR and Performance in Speeded Tasks

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**Background:** Individual differences of cognitive functioning show substantial heritability, candidate gene studies to date concentrated on dopaminergic polymorphisms. The 7-repeat allele of the variable number of tandem repeat polymorphism in the dopamine receptor 4 gene (DRD4 VNTR) has been studied extensively with relation to psychological traits: Association with Novelty Seeking, attention problems and Attention Deficit Hyperactivity Disorder has been reported, however, replication of these results were contradictory. Candidate gene studies of non-clinical adult reaction time performance to date focused on a single task grasping a particular cognitive process (e.g. Fossella et al., 2002).

**Methods:** In the present study we analyzed association of reaction time data and four dopaminergic polymorphisms: DRD4-VNTR, COMT, DRD2/ANKK1, and DAT1 GDNF in a healthy young adult sample of 245 Caucasians. The obtained genotype frequencies were in Hardy-Weinberg equilibrium. Data from six reaction time tasks were assembled to investigate information processing endophenotypes detectable in different cognitive tasks.

**Results:** Significant association of the DRD4-VNTR and speed of performance was found ( $p=0.0001$ ), the group with the 7-repeat allele showed slower responses. This effect was present in both sexes and was not due to fatigue. Other studied dopaminergic polymorphisms did not show association with performance speed measures.

**Discussion:** These results (Szekely et al., 2011) are the first report of an association between the DRD4 VNTR polymorphism and reaction time performance in a non-clinical adult sample. Endophenotypes developed here could be promising tools to detect genetic effects on performance in speeded tasks.

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### Effect of the NCAN Genetic Risk Variant on Cortical Folding in Schizophrenia

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**Background:** Imaging genetics allows investigating the neuronal mechanism of specific genetic risk variants in schizophrenia (Schultz et al., 2011). Recent advances in identifying the genetic foundations of major psychosis revealed the NCAN gene to be a main contributor to psychosis etiology (Cichon et al., 2011; Mühleisen et al., in press). In order to identify a potential cortical pathomechanism of the NCAN risk variant rs1064395 in schizophrenia and healthy controls, the present study is the first to explore the impact of the NCAN risk variant on cortical morphology.

**Methods:** 128 individuals (63 patients and 65 matched healthy controls) were genotyped for the rs 1064395 SNP and underwent high resolution T1 weighted MRI. Cortical thickness and local cortical folding was computed using an automated surface based approach (FreeSurfer). Both anatomical parameters were compared between risk and non-risk carriers in patients and healthy controls on a node-by-node basis covering the entire cortex.

**Results:** Risk carrier status in patients was significantly associated with altered cortical folding of the V1 visual cortex. Additionally, a strong trend for altered prefrontal cortical folding was found in patients with risk carrier status. No significant effects were found for cortical thickness. In healthy controls there were no significant associations.

**Discussion:** To our best knowledge, this is the first study elucidating a neuroanatomical pathomechanism underlying the NCAN genetic risk variant in schizophrenia. We demonstrated that NCAN is associated with altered cortical folding of prefrontal and occipital areas implying a potential role of NCAN in bottom-up visual processing and top-down cognitive functioning. Both major cognitive processes are known to be disturbed in schizophrenia. Moreover, our study reveals first evidence for a specific genetic underpinning of altered cortical folding in schizophrenia. References Cichon S., Mühleisen T. W., Degenhardt F. A., Mattheisen M., Miro X., Strohmaier J., et al. Genome-wide association study identifies genetic variation in neurocan as a susceptibility factor for bipolar disorder. *Am J. Hum Genet* 2011; 88: 372-81. Mühleisen T. W., Mattheisen M., Strohmaier J., Degenhardt F., Priebe L., Schultz C. C., et al. Association between schizophrenia and common variation in neurocan (NCAN), a genetic risk factor for bipolar disorder. *Schizophr Res* in press. Schultz C. C., Nenadic I., Koch K., Wagner G., Roebel M., Schachtzabel C., et al. Reduced cortical thickness is associated with the glutamatergic regulatory gene risk variant DAOA Arg30Lys in schizophrenia. *Neuropsychopharmacology* 2011; 36: 1747-53.

### Association Analysis of Bipolar Risk Variants with Mrna Blood Levels, Neurocognitive Factors and Structural MRI

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**Background:** Bipolar disorder (BD) is a spectrum of psychiatric conditions characterized by severe mood fluctuations. The aim of this project was to identify key endophenotypes associated with the top candidate risk variants identified in large bipolar genome-wide association studies.

**Methods:** sMRI, neurocognitive, mRNA and clinical data were collected from participants from two Norwegian research projects: The Bipolar Research And Innovation Network (BRAIN) and TOP (Thematic Research Area Psychosis) including both patients and controls (~1000-1500 individuals). Genotyping was performed with Affymetrix Gene Chip Genome-Wide SNP 4.0.

**Results:** Association results for the previously identified risk variants in ANK3, CACNA1C, SYNE1 and ODZ4 with neurocognition, structural MRI and clinical data (including suicide attempts) as well as association between ANK3 and CACNA1C risk variants and blood mRNA levels will be presented.

**Discussion:** The understanding of the possible connection between key bipolar endophenotypes and risk variants will widen our knowledge of the biological significance of the risk variants.

### Cumulative Genetic Risk Predicts Dorsolateral Prefrontal Cortex Activity in Schizophrenia Patients

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**Background:** Considering the polygenic etiology of schizophrenia, the aim of this work was to derive a measure of cumulative genetic risk, that is based on the additive effects of several known genetic susceptibility loci for schizophrenia, and study its effect on neural activity during working memory (WM) using fMRI. Relating genetic polymorphisms to underlying physiological aspects of schizophrenia (so called intermediate phenotypes) may be more reliable and can help to circumvent the problem of often ill-defined clinical phenotypes for psychiatric disorders.

**Methods:** We obtained functional MRI, behavioral and genetic data of 99 healthy controls and 79 patients with a DSM-IV diagnosis of schizophrenia from the Mind Clinical Imaging Consortium study of schizophrenia. Neural activity during the Sternberg Item Recognition Paradigm was acquired with either a 1.5T Siemens Sonata or a 3T Siemens Trio and analyzed using FSL. A genetic risk score (GRS), which combined the additive effects of 41 single nucleotide polymorphisms (SNPs) from 34 risk genes for schizophrenia, was calculated for each participant. These risk SNPs were chosen according to the continuously updated meta-analysis of genetic studies on schizophrenia available at [www.schizophreniaresearchforum.org](http://www.schizophreniaresearchforum.org). We performed whole brain fMRI data analyses investigating the relationship between GRS and WM-induced brain activity in FSL. All models were cluster-corrected and controlled for the effects of acquisition site and diagnosis. To control for population stratification, we used linkage agglomerative clustering in PLINK and included the first six dimensions as covariates.

**Results:** We found a positive relationship between GRS and left dorsolateral prefrontal cortex (DLPFC) inefficiency during WM processing, which was the only surviving cluster in a whole-brain analysis for a GRS main effect (Fig. 1). GRS was not associated with intelligence, WM performance, age or medication effects and did not differ between diagnostic groups, gender or acquisition sites. There were no significant diagnosis-by-GRS interaction effects. In the main model GRS accounted for 3.6 of the total variance (adjusted R<sup>2</sup>) at the most activated DLPFC locale (x, y, z: -26, 50, 8), corresponding to a significant R<sup>2</sup>-change of 0.04 (p=0.008).

**Discussion:** These findings indicate that cumulative genetic risk, combining the effects of many small impact genes, is associated with a known brain-based intermediate phenotype for schizophrenia. The GRS approach supports an additive genetic risk model for a complex phenotype and could provide an advantage over studying single genes in studies investigating the genetic basis of polygenic conditions such as neuropsychiatric disorders. Furthermore, a neural characterization of genetic risk will help to define system neuroscience models of schizophrenia.

### MC4R Gene, Weight Regulation, And Eating Behavior In Binge Eating Disorder

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**Background:** Binge eating disorder (BED) is characterized by regular episodes of binge eating in the absence of compensatory mechanisms. Considering its role in appetite regulation, the hypothalamic melanocortin system is a prime candidate to investigate in BED. Although extensively researched in type II diabetes, obesity (Loos et al, 2008), and antipsychotic-induced weight gain (Malhotra et al, in press), thus far the melanocortin 4 receptor (MC4R) gene has not been studied in BED. The goals of this study were threefold: (1) to investigate the role of MC4R in BED; (2) to explore the link between body mass index (BMI) and MC4R risk alleles in BED patients; and (3) to conduct an in-depth analysis on eating behaviour and MC4R polymorphisms.

**Methods:** We genotyped 471 probands (114 participants with BED, 150 obese controls, 207 normal weight controls) on the following five MC4R markers selected from the literature on obesity and antipsychotic-induced weight gain: rs571312, rs17782313, rs489693, rs11872992, and rs8087522. Height, current weight, highest and lowest lifetime weights, as well as several measures on eating behaviour were collected during the in-person assessment.

**Results:** Analyses on eating behaviour and the MC4R gene are currently underway. We observed a trend (rs571312,  $p = 0.076$ ) toward overweight probands carrying the high-risk MC4R allele compared to normal weight controls. Maximum lifetime BMI was significantly associated with rs17782313, previously linked to obesity and weight gain, in the entire sample ( $p = 0.009$ , Nyholt corrected).

**Discussion:** To our knowledge, this is the first study to look at the MC4R gene in BED. Based on our preliminary findings, although it may not be linked to BED diagnosis, MC4R is strongly associated with weight regulation in the general population. If MC4R is found to be associated with dysfunctional eating behaviours, there may important clinical implications, including potential for the use of MC4R agonists in the treatment of obesity and disordered eating.

### Literature Mining for the Discovery of Hidden Connections Between Endophenotypes and Candidate or Putative Genes in Antisocial Personality Disorder

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**Background:** Currently, Pathways and Processes of modern biomedical research involves the analysis of large volumes of experimental data to identify new patterns or correlations, ultimately leading to novel hypotheses. Data mining methodologies, such as literature mining have been extensively used to infer new targets related to biochemical signals, certain neurotransmitter keys, metabolic data, imaging and neurocognitive markers. This may indicate the presence of quantifiable deviation on a genetically influenced dimension that underlies ASPD.

**Methods:** Using STRING (functional protein association networks), VisANT 3.86, and Medsum; here we were addressing the scientific literature to identify a novel pathways that indicating one or more biological or neurocognitive cues to showing a network of interactions to understand a ASPD disorder underlying mechanisms to create new strategies to search better in personality disorders. Biological and clinical expressions related to traits in ASPD were used to search the compiled Medline text files. In addition, a full gene name involved in the control of both dopamine and serotonin brain systems were checked. We also analyze the relationship between neurocognitive task which could help to explain the expression of biochemical responses and neuroimaging activation.

**Results:** The scoring of hidden relationships between biomedical concepts was obtained a through the number of interactions between covariables of interest depending on the number of total papers regarding to neurocognitive findings and molecular pathways involved.

**Discussion:** We have made a simple method to infer an interesting neurobiological pathways involved in ASPD.

### Prenatal Programming of Gene Expression; Effect of Mothers Attachment Style on Offspring's Methylation of DNA

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**Background:** Attachment theory is a psychological theory that describes formation of bond between child and his/her primary caregiver. It shapes close relations to other people throughout whole life and has been shown to be fairly stable. It is associated with mental health, many different somatic conditions and health behavior. It is also central in many psychotherapeutic approaches. However, only a little is known about its biological basis. There are some evidence from rodents that maternal care affects offspring's methylation of DNA and further on offspring's behavior. In humans, early rearing environment like abuse or maternal depression has its effect on methylation of DNA, but so far it has not been linked to attachment.

**Methods:** In this study we use samples of a pilot study of a large prospective FINNBRAIN- birth cohort that will be collected during 2011-2015. We focus on epigenetic regulation of candidate genes as mediators of prenatal and postnatal rearing environment on future attachment style. We will measure methylation of promoter region of candidate genes and search for new candidate genes by using bisulfite sequencing and whole-genome array technology (Infinium 450 Bead Chip from Illumina). We explore whether mothers' attachment style or possible prenatal depression affects methylation of DNA from blood leukocytes of offspring and, consequently, offspring's attachment style. Collection of the main sample is in progress and analyze of the pilot sample is underway.

**Results:** Preliminary results show that the methylation pattern of the mothers is fairly congruent with high correlation between the methylation sites of the same gene ( $P < 0.0001$ ,  $\beta > 0.80$ ) and also between different genes of same biological pathways ( $P < 0.05$ ,  $\beta \sim 0.2-0.3$ ). In newborn, this correlation is weak (intra-genic correlations  $\beta \sim 0.2-0.6$ ). According to the preliminary data we observe some correlation of the methylation pattern and attachment pattern of the mothers ( $P < 0,01$ ).

**Discussion:** Our preliminary data evidences for regulation of attachment pattern via methylation of biologically relevant candidate genes. In adults, this is a fairly stable phenomenon while in newborn this epigenetic programming is only about to start. The pattern in father remains yet to be elucidated. These results will be eventually replicated in another large prospective birth cohort from the same population.

### Environmental Stress Affects Genome-Wide DNA Methylation in a Finnish Nurse Cohort

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**Background:** DNA methylation has been suggested to play a pivotal role in the interplay between gene and environment and has been studied in relation to stressful life events and early life stress. Methodological advances in studying DNA methylomes have opened up new interesting opportunities to investigate methylation changes in the origin and progression of stress. In this study, we compared DNA methylation differences between shift-working nurses from high work stress environment ( $n=10$ ) to nurses from low work stress environment ( $n=10$ ) using the Human Methylation 450 BeadChip from Illumina.

**Methods:** Our study sample is part of an ongoing Finnish Public Sector Study and comprises of 20 shift-working nurses from high- ( $n=10$ ) and low ( $n=10$ ) work stress environments. Subjects were initially drawn from a large cohort of 5615 female health care professionals who responded to the Karasek's Job Content Questionnaire. Inclusion criteria was: age between 30-58 years old, mother tongue Finnish, BMI under 35, at least 3 years work experience in the same ward and absenteeism from work of no longer than 6 months during this time. Exclusion criteria were: use of medication affecting cognitive function, use of hormonal medication, heavy smoking (reported daily smoking for at least 10 consecutive years) and high use of alcohol (average of 4 or more doses of alcohol over 4 times a week). We used the 450K DNA methylation microarray from Illumina to assess methylation differences on group level (between high- and low stress environments) and individual level. To compare DNA methylation on individual level, each individual from the high work stress group was paired with an individual of the same age (or up to 2 years age difference) in the low work stress group. As controls, we used commercially available fully methylated (100) and unmethylated (0) human genomic DNA.

**Results:** Our preliminary results suggest that environmental stress affects global DNA methylation. A cluster analysis separates fully methylated and unmethylated controls into distinct and separate groups. A cluster analysis of all 20 individuals shows a distinct difference between high- and low stress environments. Individuals from low stress environment are clustered together whereas individuals from high stress environment are clustered into two groups of high and low methylation respectively.

**Discussion:** There appears to be a certain interplay between environmental stress and DNA methylation on a global level. The specific molecular mechanisms remain to be elucidated and the study is ongoing.

### Genome-Wide Studies of Methylation in the Mouse Frontal Cortex Reveals Novel Imprinted Differentially Methylated Regions and Non-CG Methylation

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**Background:** Current data support approximately 90 validated imprinted genes in mice, but recent estimates indicate that this number may be well over 1,000. Many of the genes are imprinted in a tissue/cell type specific pattern and little is known of these genes. Previous evidence indicates that imprinted genes influence the development of specific brain regions and we sought to identify imprinted genes contributing to the development of the frontal cortex.

**Methods:** We created crosses and reverse crosses of mouse strains to determine the parent-of-origin of expressed genes. We used next generation sequencing of RNA (RNA-seq), chromatin immunoprecipitation to histone modifications (ChIP-seq), and bisulfite sequencing (MethylC-Seq) of DNA from the frontal cortex of the adult F1 mice to identify transcripts and epigenetic markers with parent-of-origin effects.

**Results:** Using a FDR of .01, and requiring that the same parent-of-origin bias was evident in 6 biological replicates, we identified known imprinted genes as well as novel patterns of expression for known imprinted genes. We also identified 109 novel transcripts with parent-of-origin effects. Using Methyl-seq we correctly identified known differentially methylated regions (DMRs) as well as 23 novel DMRs most within known imprinted regions. Interestingly, we found significant amounts of non-CG methylation in frontal cortex (35% of methylation), some showing evidence of imprinting. None of the novel imprinted transcripts that we identified using RNA-seq were marked by a DMR or by chromatin patterns that were indicative of imprinting. Further, pyrosequencing of a subset of the novel candidates did not replicate their imprinted expression. Thus the status of these genes as imprinted was not supported.

**Discussion:** The results thus do not support the existence of many more imprinted genes than previously identified in frontal cortex and suggest a potential bias in RNA-seq data. The finding of significant amounts of non-CG methylation was surprising given that this epigenetic mark, previously documented only in ES cells, preimplantation embryos and oocytes, and was thought to be a marker of pluripotency. The role of this epigenetic mark in brain is currently under investigation.

### A Model of Epigenetics Effects on Neuroimmune Mechanisms of Mental Illness

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**Background:** Nearly 30% of all adult psychiatric disorders could be attributed to early life adversities [1]. Early life stress (ELS) events such as childhood maltreatment have been implicated in developing cognitive and emotional deficits at a later stage in life [2]. The stress effect of ELS is primarily mediated by an altered hypothalamus-pituitary-adrenal (HPA) axis function [3]. ELS affect the neuroendocrine-immune function at a sensitive period in development and gene x environment interactions usually involves epigenetic modifications. Recently epigenetic changes in inflammation related genes have been associated with childhood trauma/neglect in humans [6, 7]. However, there are no reports on MS mediated immune function alterations and epigenetic modifications addressing adult brain and behaviour. ELS also enhance the risk of developing inflammatory disorders in adulthood, as reported by increased levels of the inflammation in adults maltreated as children [4]. Inflammation has been associated with depression, cognitive dysfunction and other mental illnesses and their associated neuropathologies [5]. Maternal separation (MS) stress, an animal model of ELS has been implicated in behavioural and neurobiological alterations in adulthood [8] and in epigenetic modifications such as DNA methylation and histone modifications in the genes involved in the HPA pathways [9, 10]. In this study, we investigate the longitudinal effects of ELS on the development of psychiatric symptoms by applying an animal model of ELS. We hypothesise that ELS induces neuroimmunological dysfunction of both humoral and cellular immune cells via epigenetic modifications of the immune cell genome. It is assumed, that these epigenetic immune changes incurred at early developmental stages increase the susceptibility of the development of psychiatric disorders during adulthood.

**Methods:** We will use an ELS model to induce an immune response leading to neurobiological changes. To assess the behavioural effects of the ELS induced immune response, we will apply a behavioural test battery on cognition-like, depression-like, anxiety-like and sociability behaviours in mice. Immune markers and function such as T cells will be assessed quantitatively and qualitatively.

**Results:** Our work describes a unique model of ELS related to immune function as a critical mechanism for the susceptibility of adult mental illness. We show that specifically the alterations of immune function including cytokine dysregulation and T cell dysfunction are induced by ELS. Moreover, we will present that these immune effects mediate the long-term effects of ELS on adult behaviours. Specifically, our results will show that humoral and cellular immune changes lead to impaired neuroplasticity within brain regions such as hippocampus, prefrontal cortex and amygdala. These neurobiological changes are closely related to behavioural alterations such as anxiety, cognition, depression and sociability like behaviours. Finally, our results demonstrate epigenetic modifications within the immune cell genome as the primary mechanism by which ELS exerts effects on the development of mental disorders.

**Discussion:** The results demonstrate that epigenetic modifications of immune cells due to early life stress will lead to adult hippocampal changes associated with impaired cognition and depression-like behavior. Our results will stimulate translational human studies investigating the immune genome for both identifying genes

and epigenetic modifications involved in early life stress and the development of psychiatric disorders. References 1.Kessler, R.C., et al., *Br J Psychiatry*, 2010. 197(5): p. 378-85. 2.Heim, C. and C. B. Nemeroff, *Biol Psychiatry*, 2001. 49(12): p. 1023-39. 3. Van Voorhees, E. and A. Scarpa, *Trauma Violence Abuse*, 2004. 5(4): p. 333-52. 4.Danese, A., et al., *Proc Natl Acad Sci USA*, 2007. 104(4): p. 1319-24. 5.Yirmiya, R. and I. Goshen, *Brain Behav Immun*, 2011. 25(2): p. 181-213. 6.Uddin, M., et al., *Proc Natl Acad Sci USA*, 2010. 107(20): p. 9470-5. 7.Naumova, O.Y., et al., *Dev Psychopathol*, 2012. 24(1): p. 143-55. 8.Oitzl, M. S., et al., *Eur J Neurosci*, 2000. 12(10): p. 3771-80. 9.Murgatroyd, C., et al., *Nat Neurosci*, 2009. 12(12): p. 1559-66. 10.Weaver, I. C., et al., *Nat Neurosci*, 2004. 7(8): p. 847-54.

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### The Nature of the Epigenetic Contribution to Psychosis

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**Background:** The case for an epigenetic contribution to the etiology of psychosis has been argued on the grounds that no sequence variation has been firmly established either through linkage1 or genome-wide association. The most promising empirical candidate has been the “paternal age effect” 2, although this might also be explained on the basis of increased rates of conventional mutations in spermatozoa. Now two new sources of evidence combine to suggest that the epigenetic contribution may 1) be larger than the paternal age effect suggests, and 2) may have a physiological basis in an established genetic mechanism, and 3) may be relevant to the earliest stages of neurodevelopment.

**Methods:** 1) Re-appraisal of the maternal grandfather effect reported by Frans et al (2011). 2) Investigation of relative handskill in relation to parental age at the birth of the child.

**Results:** 1) Taking account of the earlier upper limit of female compared to male age of parenthood the paternal grandmother effect cannot be dismissed and may be as large as the paternal grandfather effect. 2) If as Frans et al argue the mgf effect requires X linkage then Y linkage is also necessary to explain the paternal age effect. 3) Frans et al’s observations suggest that the epigenetic influence passes through both male and female meiosis, and may be transferred from the X to the Y. 4) Directional handedness deviates from expectation independently with age of both mother and father in ways that suggest X and Y chromosomal influence.

**Discussion:** An XY gene is postulated for cerebral dominance4 and now appears necessary to account for the parental and grandparental effects. Such genes are eminently susceptible to the mechanism of meiotic suppression of unpaired chromosomes (MSUC) 5,6: it has been suggested that they play a particular role in mammalian speciation. It appears that an epigenetic imprint that relates to the species most recently acquired and sexually dimorphic characteristic is passed between generations. According to this interpretation its function might be to establish as species-specific the identity of the cells of the embryo that are dividing at a time when other gene expression is suppressed. 1. Crow, T. J. How and why genetic linkage has not solved the problem of psychosis: review and hypothesis. *Am J Psychiatry*164, 13-21 (2007). 2. Malaspina, D. et al. Advancing paternal age and the risk of schizophrenia. *Arch Gen Psychiatry*58, 361-367 (2001). 3. Frans, E. M., McGrath, J. J., Sandin, S., Lichtenstein, P., Reichenberg, A., Langstrom, N., Hultman, C. M. Advanced paternal and grandpaternal age and schizophrenia: a three-generation perspective. *Schizophrenia Research*, doi:10.1016/j.schres.2011.09.027. (2011). 4. Crow, T. J. The case for an X-Y homologous determinant of cerebral asymmetry. *Cytogenetics and Cell Genetics*67, 393-394 (1994). 5. Turner, J. M. A. Meiotic sex chromosome inactivation. *Development*134, 1823-1831 (2007). 6. Lee, J. T. Sex chromosome inactivations The importance of pairing. *Curr Biol*15, R249-R252 (2005).

**Monoamine Oxidase A Gene-Environment and Epigenetic Associations with Depression in Females, and Association of Early Parental Death with Hypermethylation of the Glucocorticoid Receptor**

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**Background:** Monoamine oxidase A (MAOA) harbors a polymorphic upstream variable-number tandem repeat (u-VNTR). The MAOA-L allele of the u-VNTR leads to decreased gene expression levels in vitro. MAOA-L increases the risk of conduct disorder in males with childhood adversities. Early-life adversities have been associated with hypermethylation of the glucocorticoid receptor (NR3C1) in agreement with lower NGFI-A binding and lower NR3C1 expression.

**Methods:** Individuals with depression (n = 392) and controls (n = 1276) from a longitudinal population-based study in Sweden were used for the genetic association analysis. Smaller subgroups were used for DNA methylation analyses of MAOA and NR3C1 in saliva and blood samples.

**Results:** Adult MAOA-L females with childhood adversities were at a higher risk of developing depression (P = 0.006). Overall MAOA methylation levels were decreased in depressed females compared to controls (P = 0.04). One specific childhood adversity (early parental death) was associated with hypermethylation of NR3C1 close to an NGFI-A binding site (P = 0.005). Regression analysis indicated that this association may be mediated by the MAOA-L allele (adjusted R<sup>2</sup> = 0.24, ANOVA: F = 23.48, P < 0.001). Epigenetic comparisons between saliva and blood samples of the same individuals revealed gender and tissue differences for MAOA but not for NR3C1.

**Discussion:** 1) Depression in females may result from a gene x childhood-adversity interaction and/or a dysregulated epigenetic programming of MAOA. A possible explanation for this is that MAOA-L carriers probably have increased levels of neurotransmitters during a critical period of brain development in early-life, which may then amplify the effects of childhood adversities. On the other hand, higher-than-normal MAOA levels later in life (achieved by epigenetic mechanisms) may lead to a decrease in important neurotransmitters that are essential for maintaining mental health. 2) The DNA methylation status of NR3C1 has previously been recognized as a neurobiological hallmark of early-life trauma and this study showed both that individuals who lost a parent early in life had increased DNA methylation levels of NR3C1 and also that MAOA-genotypic variants may mediate NR3C1's methylation. 3) Both gender and tissue-type should be taken into consideration when studying MAOA methylation levels whereas NR3C1 methylation level was similar in DNA from blood and saliva DNA, and from both genders.

**Epigenetic Changes in Alcohol-Dependent Individuals: Influence of Withdrawal Characteristics?**

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**Background:** Evidence mainly from laboratory studies indicates that ethanol ingestion may cause changes in methylation of CpG promoter regions of candidate genes. These changes may result in subsequent alteration of protein expression. However, evidence for these epigenetic modifications in alcohol-dependent patients and their relationship to clinical phenotypes is scarce. The aim of this initial study is to investigate CpG island methylation changes of GABRA2 and their relationship to clinical phenotypes like withdrawal, tolerance or delirium.

**Methods:** 47 alcohol-dependent individuals (DSM IV) and 20 controls were characterized according to their methylation rate in 29 GABRA2 promoter CpG sites using quantitative PCR (Polymerase Chain Reaction). Clinical phenotypes like withdrawal, tolerance and delirium tremens were obtained by a semi-structured interview (SSAGA).

**Results:** Alcohol-dependent subjects had a significantly lower CpG methylation rate compared to controls (p < 0.001). Among patients those with tolerance, alcohol withdrawal and delirium, a significantly lower rate of CpG methylation was detected too.

**Discussion:** This initial study in alcohol-dependent individuals confirms epigenetic changes as a consequence of alcohol intake which may be related to physiological characteristics of alcohol dependence. Subsequent studies are required to investigate if alcohol intake-related GABRA2 promoter CpG methylation causes changes in mRNA or protein expression.

**Epigenetic Regulation of Stress Reactivity Genes in Adolescents:  
The Trails Study**

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**Background:** Negative stressful events early in life can alter the neuroendocrine stress response, serving as a risk factor for both psychiatric and non-psychiatric disorders. This effect is thought to be partly mediated through epigenetic modifications in the promoter regions of stress-reactivity genes. Animal research has made great advances in understanding the processes underlying epigenetic regulation via methylation marks regulating hippocampal glucocorticoid receptor (GR) expression. Differences in maternal care affected calibration of the hypothalamic-pituitary-adrenal (HPA) axis in offspring through different methylation rates of the GR gene. A post-mortem study of human suicide victims with and without a history of abuse also revealed more hippocampal methylation marks in the promoter region of the GR gene in victims of chronic abuse, suggesting a similar epigenetic mechanism in humans. We will investigate if exposure to stressful events during childhood and early adolescence leads to adverse health outcomes (anxiety, depression) via epigenetic processes that influence the neuroendocrine response, across 10 years of development from early adolescence into adulthood. Using a candidate gene approach, we will investigate methylation of the GR gene as well as the serotonin reuptake transporter (5-HTT) gene and the catechol-O-methyltransferase (COMT) gene in relation to stress, anxiety and depression. This study is embedded within TRAILS (TRacking Adolescents' Individual Life Survey), a longitudinal cohort study that follows children from the age of ten into early adulthood and assesses their psychological, social, physical development every two years.

**Methods:** DNA methylation analysis: DNA was extracted from blood samples of 950 TRAILS participants (14-18y). Quantitative DNA methylation was analyzed using the Sequenom EpiTyper Assay. Following bisulfite conversion, DNA was amplified (PCR), transcribed in vitro and RNA was cleaved base-specifically. Cleavage products were analyzed using MALDI-TOF mass spectrometry. Primer sequences for methylation analyses were selected based on previous studies (McGowan et al., 2009; Wong et al., 2010; Philibert et al., 2008; Zhao et al., 2010; Xu et al., 2010). Four additional GR amplicons were designed using the online EpiDesigner software from Sequenom. Adversity measures: Adversities include sexual and physical abuse, parental rejection and stressful events at different ages (perinatal until mid-adolescence). Outcome measures: Anxiety and depression were assessed with validated questionnaires. Neuroendocrine response/ HPA activity: Cortisol response is measured during and following a Social Stress Test (n=475, age 14-18y). Cortisol awakening response is available at age 10-12y (N=950).

**Results:** Preliminary results are based on a pilot study (n=116) of subjects with high scores on our measures of anxiety, depression, abuse and parental rejection. GR: GR methylation rates showed positive correlations with perinatal stress, gender specific correlations for stressful events between ages 0-5 and ages 6-11, and negative correlations with stress in adolescence (P5-HTT: Higher 5-HTT methylation rates predicted anxiety and depression scores (pCOMT: High methylation rates of COMT were associated with rejection, physical abuse and sexual abuse).

**Discussion:** These preliminary findings suggest that methylation of the GR, 5-HTT and COMT genes is influenced by stressful events early in life. This is in accordance with previous studies using humane tissues (blood or brain derived DNA). Methylation of the 5-HTT gene also predicted anxiety and depression scores in adolescence. The relationship between methylation rates and stressful events, depression or anxiety scores, however, was variable across different CpG sites within the three candidate genes. This suggests that differences in type or timing of stressful events may affect methylation of individual CpG dinucleotides differently. It will be interesting to see if any patterns can be found relating methylation of individual CpGs dinucleotides to specific stressful life events.

### DNA Methylation of the Glucocorticoid Receptor Gene Promoter is Linked to PTSD Risk in Genocide Survivors

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**Background:** Stress induces a complex set of mechanisms that affect the entire organism. The primary function of those changes is to prepare the organism for the direct consequences of stressful events and to ensure a quick return to homeostasis. Additionally, stress is triggering long-term adaptive responses, which result in enhanced memory of stressful events. The biological mechanisms underlying the response, accommodation and restitution of the "normal state" are highly conserved among vertebrates. Failing to recover from the initial acute response and to keep the adaptive biological alterations under control leads to impaired homeostasis. Severe traumatic experiences can lead to development of posttraumatic stress disorder (PTSD). It is characterized by the presence of three distinct, but co-occurring, symptom clusters: Intrusions, Avoidance and Hyperarousal. Studies with PTSD patients point to impaired physiological homeostasis. One of the critical features is loss of the auto-regulation of the stress-induced alterations in HPA signalling and increased inhibition of the HPA axis. These changes are maintained over a long period of time, although the underlying mechanisms remain unclear.

**Methods:** Via direct bisulfite pyrosequencing and methylation sensitive High Resolution Melting PCR, as well as quantitative PCR, we investigated promoter methylation and expression of NR3C1 (nuclear receptor subfamily 3, group C, member 1) gene and global methylation of LINE-1 (Long Interspersed Nuclear Element 1) in survivors of Rwanda genocide (n=189) and healthy Swiss individuals (n=161). To capture the variability of the genes encoding glucocorticoid receptor (NR3C1) and DNA methyltransferase (DNMT1, DNMT3A, DNMT3B) 71 intragenic SNPs that are present on the Affymetrix Human SNP-array 6.0 were selected and analyzed for association with PTSD symptom clusters and risk (n=473).

**Results:** In the present study we investigated epigenetic alterations of the glucocorticoid receptor (GR) gene promoter in saliva samples from survivors of the Rwandan genocide and healthy Swiss individuals. We found a strong, negative correlation of PTSD symptoms like intrusions ( $P \leq 0.002$ ), avoidance ( $P \leq 0.006$ ), and PTSD diagnosis ( $P \leq 0.007$ ) with DNA methylation of the GR gene promoter in genocide survivors. Furthermore, the epigenetic changes were specific to the NGFI transcriptional factor-binding site of the GR promoter and also correlate with GR gene expression ( $P \leq 0.001$ ). Furthermore, we did not observe any significant difference in the overall genomic methylation levels between Rwandese and healthy Swiss, as assessed by determining global LINE-1 element promoter methylation (17% of human genome). Interestingly, we detected a significant negative correlation of LINE-1 methylation and PTSD symptoms in the Rwandan population (Avoidance,  $P \leq 0.003$ ; PTSD diagnosis,  $P \leq 0.003$ ). Additional analysis of the LINE-1 promoter locus unravelled the existence of a steroid binding site consensus sequence, previously pointed as one of the regulators of LINE-1 expression.

**Discussion:** Here we report for the first time that epigenetic alterations at GR gene promoter are strongly associated with symptom clusters and PTSD risk. DNA hypomethylation at the CpG site important for transcriptional control of the NR3C1 gene and up-regulation of GR expression could explain the increased HPA axis inhibition and long

term impairment of homeostasis in PTSD cases. Together with the findings linking GR signalling with active epigenetic remodelling of the GRE sites, our data suggests that epigenetic alterations of genomic loci important for the regulation of steroid-related gene expression contribute to the pathophysiology of PTSD and may offer new targets for PTSD treatment.

### Epigenetic Alterations in Neuroblastoma Cells after Antipsychotic Treatment

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**Background:** There is growing evidence that epigenetic mechanisms play not only an important role in the development of schizophrenia, but also in the action of antipsychotic drugs, such as clozapine or haloperidol. Several studies have demonstrated aberrant DNA methylation in single genes of schizophrenic patients after antipsychotic drug treatment, but the impact on global methylation and the underlying mechanisms is still poorly understood. Thus, the main aim of the present study was the in vitro investigation of global methylation in a neuroblastoma cell model after incubation with different antipsychotics.

**Methods:** SH-SY5Y neuroblastoma cells were inculcated with haldol, clozapine, olanzapine, quetiapine, risperidone and ziprasidone for 6, 24 and 96 hours using the normal and the 10-fold therapeutic plasma concentrations. Global DNA 5-methylcytosine content was measured with the Methylight method by the measurement of genomic DNA methylation of the repetitive LINE-1 element.

**Results:** We detected drug-, time- and concentration dependent alterations of global methylation in neuroblastoma cells. After 6 hours of incubation there were no significant alterations of the DNA methylation for both concentrations; after 24 hours an overall decrease of global methylation was obvious for all antipsychotics after incubation with the normal therapeutic plasma concentration, but not statistically significant. A significant alteration, in terms of an increased methylation was detectable after 96 hours incubation with the 10-fold therapeutic plasma concentration of clozapin, haldol, olanzapine and risperidone (ANOVA:  $p < 0.03$ ).

**Discussion:** The preliminary results of the present study suggest that alterations in global DNA methylation seem to be involved in the action of antipsychotic drugs. Our findings of drug-, time- and concentration dependent alterations of global DNA methylation might be a hint on different epigenetic mechanism probably related to short and long term treatment effects and are partially consistent with recent methylation studies in single genes. Further investigations are needed to confirm the present observations and to investigate their impact on antipsychotics' action in more detail.

### Global DNA Methylation Analysis in Major Depression

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**Background:** Aberrant transcriptional regulation may be one of the key components in the pathophysiology of mood disorders. DNA methylation acts as a epigenetic gene silencing mechanism, not only in the context of development, but also due to environmental stimuli during the whole life span. Several lines of evidence have suggested aberrant DNA methylation in single genes of depressed and schizophrenic patients, but the underlying mechanisms are still poorly understood. Moreover, levels and impact of genome wide global DNA methylation in the pathophysiology of depression have not been investigated in detail up to now. Therefore the main aim of the present project was to analyse the global methylation pattern in peripheral leukocyte DNA of depressed patients and healthy controls.

**Methods:** 130 patients suffering from major depression and 100 healthy controls were included in the study. Blood DNA 5-methylcytosine content was measured by two methods, a commercially available fluorometric ELISA kit (Biocat, Heidelberg) and with the Methylight method by the measurement of genomic DNA methylation of the two repetitive elements LINE-1 and Alu-M2.

**Results:** We found significant elevated global DNA methylation levels applying the Elisa test in depressed patients compared to controls (one way ANOVA:  $F = 5.19$ ,  $p = 0.02$ ). This observation was not confounded by age or gender in both samples. This finding was supported by the Methylight method (Alu M2: one way ANOVA:  $F = 3.83$ ,  $p = 0.05$ ; Line-1: one way ANOVA:  $F = 4.16$ ,  $p = 0.04$ ). Correlation analysis between global methylation levels in depressed patients and clinical variables, as age of onset, duration of hospitalisation, number of episodes, HAMD-17 scores or cortisol concentrations from the Dex-CRH test did not reveal any significant results.

**Discussion:** These preliminary results of the present study suggest that alterations in global DNA methylation seem to be involved in the pathophysiology of major depression. Our findings of elevated global DNA methylation in depression might be a hint on decreased overall gene expression as major pathogenic factor in the development of affective disorders. We are aware that this conclusion is highly speculative and more patient and control samples have to be analyzed to confirm the present results and to investigate their impact on clinical characteristics in more detail.

### Epigenetic Investigation of the Angiotensin Converting Enzyme (ACE) Gene in Depression

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**Background:** Recent genetic studies with ACE gene polymorphisms in major depression (MD) propose an involvement in the pathophysiology of the disorder, in the therapeutic outcome, in HPA-axis dysregulation and ACE serum concentrations. Especially the ACE Ins/Del polymorphism, which accounts for ~ 40 of the ACE serum concentration variance, was one of the main topics, although the detailed functional consequences of the Ins/Del polymorphism are poorly understood. Interestingly, within an interdisciplinary study investigating cardiovascular markers, we determined the serum ACE concentrations and inflammatory biomarkers in 100 healthy controls and 100 depressed patients. Besides the observation that many inflammatory markers were increased in depressed patients, we observed a significant correlation between the ACE serum concentrations and several inflammatory markers as ICAM-1, VCAM-1, E-selectin, P-selectin and MCP-1 in these patients, independent from the functional ACE Ins/Del polymorphism. We hypothesize that epigenetic mechanisms of the ACE gene might be a reason for these observations. Therefore the main aim of the present study was the analysis of the DNA methylation pattern in the regulatory region of the ACE gene in peripheral leukocytes of depressed patients and compared to healthy controls.

**Methods:** A sample of 81 patients suffering from major depression and 81 healthy controls was included into the study. DNA methylation was determined by bisulfite sequencing of ~1000bp of the ACE promoter region.

**Results:** We detected intensive DNA methylation within a recently described, functional important region of the ACE gene promoter including hypermethylation in depressed patients ( $p = 0.008$ ) and a significant inverse correlation between the ACE serum concentration and ACE promoter methylation frequency on the total sample ( $p = 0.02$ ). Furthermore, a significant inverse correlation between the concentrations of the inflammatory CVD risk markers ICAM-1, E-selectin and P-selectin and the degree of ACE promoter methylation in MD patients could be demonstrated ( $p = 0.01 - 0.04$ ).

**Discussion:** The results of the present study suggest that aberrations in ACE promoter DNA methylation may be an underlying cause of MD and probably a common pathogenic factor for the bi-directional relationship between MD and cardiovascular disorders.

### GWAS Analysis of Polymorphic CPG Sites: Genetic Association in Suicide Attempt and Schizophrenia

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**Background:** Studies of various genes have shown that SNPs are associated with suicidal behavior rather than with primary psychiatric diagnosis. Given the above rationale, the primary aims of the current study are: 1) To identify SNPs that are associated with suicide attempt in schizophrenia; 2) Create a SNP map in our sample, by selecting SNPs that affect the potential methylation across the genome.

**Methods:** We have collected detailed clinical information and DNA samples from 70 schizophrenia patients, allowing us to perform GWAS analyses in suicide attempters and non-attempters. Using the structured research interview we determined the presence of suicide attempts lifetime that can be tested using DNA variants in the serotonin system to detect the genetic markers associated with suicide risk in schizophrenia. This cross-sectional DNA sample included subjects with a diagnosis of SCZ, all carefully assessed for lifetime suicide attempt by the means of Beck Scale for Suicide Ideation (BSS). In the initial step, we will apply a conventional genetic association strategy in order to find any SNP associated with suicide attempt in schizophrenia. The association study was performed using the duration of illness as main covariate incorporated in an additive model. A novel mapping analysis will be conducted using a specific bioinformatic tool we have developed, which analyzes only the polymorphic CpG sites genome wide. This analysis will look at the presence or absence of methylation sites affected by the SNP allele. Using this analysis, each subject can have zero, one or two methylation sites for each SNP locus, which in turn can be translated in a methylation level of 0, 50 or 100%. This bioinformatic tool can detect the SNPs that are affecting the polymorphic CpG sites across the genome. In this analysis, the SNPs will be studied under a different perspective considering their direct contribution to the availability of methylation sites within the gene of interest. Furthermore the total number of potential methylation sites at gene level, system and chromosome level will be calculated.

**Results:** Among the candidate genes we considered the RGS4 was significantly associated with suicide attempt. There were approximately 33% of CpG SNPs in our sample that was investigated using a two millions SNP chip.

**Discussion:** The overall results show no association between CpG SNPs and suicide attempt however the information of the SNP CpG methylation analysis can be used as covariate in future methylation analysis. The coverage of the traditional GWAS chips maybe inadequate for experiments aimed to link sequence and methylation variation in the DNA.

### Epistatic Interactions Between Histone Deacetylase (HDAC) Genes Influence the Risk of Schizophrenia: A Family-based Association Study

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**Background:** Growing evidence supports the importance of epigenetics in the etiology of schizophrenia. Among other mechanisms, the acetylation of the core histones has a critical influence on chromatin architecture and transcriptional activity. Histone deacetylases (HDAC) are key enzymes of histone acetylation, and abnormalities in histone modifications and in the level of HDAC proteins have been reported in schizophrenia.

**Methods:** The objective of the present study was to systematically test the classical HDAC genes for association with schizophrenia. A family-based genetic association study (multi-Center enrollment in France, Spain and Quebec) using tag-single nucleotide polymorphisms was conducted. Participants were 951 Caucasian subjects in 313 nuclear families (325 cases and their 626 parents forming 325 case-parents trios).

**Results:** Ten markers were significantly associated with  $P < 0.01$ . Four significant markers were located in the HDAC10 locus: rs7290710 (OR: 1.46, CI95: 1.15 – 1.84,  $P = 0.0015$  highest significance level; rs1076649, rs1076649 and rs742184); and three in the HDAC9 gene (rs1726596, rs12531908 and rs7801662). Multimarker analysis showed the association of small haplotype blocks in HDAC3, HDAC4, HDAC10, and HDAC11. By using the pedigree-based generalized multifactor dimensionality reduction, a four-locus interaction model was detected involving rs14251 (HDAC3), rs12531908 and rs7801662 (HDAC9), and rs7290710 (HDAC10).

**Discussion:** This first exploratory systematic study of the HDAC genes already provides consistent support for the involvement of the HDAC3, HDAC9 and HDAC10 genes in the etiology of schizophrenia.

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### Gabaergic Dysfunction in a Genetically Modified Mouse Model of Schizophrenia and Bipolar Disorder

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**Background:** Schizophrenia (SZ) and bipolar disorder (BD) are devastating psychiatric disorders likely influenced by genetic and non-genetic factors and which can be seen as complex disorders of neurotransmission. Glutamatergic and GABAergic neurotransmission have been implicated in both diseases not only by positive genetic association findings among glutamate and GABA genes but also by post-mortem studies in humans as well as by detailed studies in animal models. We have generated a genetically modified mouse model for SZ and BD (R mice) by inactivating one allele of the *Brd1* gene that previously has been associated with both SZ and BD in humans. The aim of the present study was to investigate whether the behavioral phenotypes observed in R mice (equivalents of depression, psychosis and impaired cognition) could be explained by an underlying GABAergic dysfunction.

**Methods:** Male R and wild type (WT) littermates (each  $n = 10$  mice) were anesthetized with isoflurane and decapitated P18-P25. Brains were rapidly excised and transferred to artificial cerebrospinal fluid, sliced coronally and stored for 1 h before recording. Patch-clamp recordings were carried out from pyramidal neurons of somatosensory cortex.

**Results:** Action potential dependent inhibitory GABAA receptor-mediated neurotransmission was evaluated by measurements of spontaneous inhibitory postsynaptic currents (sIPSCs) in voltage clamped cells ( $-70$  mV) in the presence of kynurenic acid to block ionotropic glutamate receptors. Preliminary data showed a decrease of sIPSCs mean frequency from 12.5 in WT to 8.7 Hz in pyramidal neurons of R mice. Extracellular paired-pulse stimulation was applied to inhibitory axons to evoke IPSCs and evaluate function of GABAergic nerve terminals. The IPSC paired-pulse ratio was increased in R mice reflecting that the presynaptic terminals displayed a reduced probability of GABA release. Tonic currents were measured by the shift in holding currents upon application of SR95531 (a competitive GABAA receptor antagonist). In presence of the GABAA receptor superagonist THIP, we found increased tonic currents in R mice ( $50 \pm 5$  pA) versus controls ( $38 \pm 5$  pA). Finally, postsynaptic GABAB receptor-mediated inhibition was evaluated in the presence of 100  $\mu$ M baclofen (a GABAB receptor agonist). We found that baclofen-induced currents were significantly decreased in R mice.

**Discussion:** In conclusion, our results indicate both presynaptic and postsynaptic alterations in GABAergic neurotransmission in the neocortex of R mice indicating a functional association to the phenotypes observed. The alternations in GABAergic function could be explained by: 1) a decrease in the number of GABAergic neurons, 2) a decrease in neurotransmitter availability, 3) alterations in presynaptic GABA release, and/or 4) a dysregulation of GABA receptor mediated signaling. Future studies will aim at clarifying these aspects.

### High Throughput Proteomics Analysis of BRD1 Identifies PBRM1 and YWHAE as BRD1 Interaction Partners

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**Background:** The schizophrenia (SZ) and bipolar disorder (BD) associated bromodomain containing 1 (BRD1) gene encodes a transcription factor, BRD1, that is important for H3K14 acetylation and plays a key role in embryonic development and survival. The BRD1-S and BRD1-L isoforms are widely expressed in brain.

**Methods:** To further investigate the function of BRD1 and its potential role in SZ and BD, we have: 1) cloned and stably expressed epitope tagged BRD1-S and BRD1-L isoforms in HEK293T cells, and 2) performed repeated co-immunoprecipitations of V5-tagged BRD1-S and BRD1-L followed by high throughput liquid chromatography coupled to tandem mass spectrometry (Co-IP HT-LC-MS/MS).

**Results:** Co-IP HT-LC-MS/MS confirmed BRD1 interaction with ING4, ING5, and EAF6. Only BRD1-S-V5 was found to interact with the acetyltransferase MYST2 which is important for H3K14 acetylation. Interestingly, both BRD1-S-V5 and BRD1-L-V5 showed consistent interaction with the 14-3-3 tyrosine monooxygenase epsilon (YWHAE) compared to controls. Additionally, we found indications that YWHAB and YWHAZ interact with BRD1-S-V5 and BRD1-L-V5, and that YWHAH interacts with BRD1-L-V5. We further identified the poly bromo-1 (PBRM1/PB1) to interact with both BRD1-S-V5 and BRD1-L-V5. The YWHAE, YWHAH and YWHAZ genes have previously been associated with SZ and BD and the PBRM1 gene was recently identified as a SZ and BD risk gene in a GWAS meta-analysis. To further address the interaction of BRD1 with PBRM1, we immunoprecipitated cell extracts from BRD1-S-V5 and BRD1-L-V5 expressing cells as well as HEK293T cells as a control with antibodies against endogenous PBRM1. Western blotting analysis of the immunoprecipitates using anti-V5 antibody showed that both BRD1-S-V5 and BRD1-L-V5 were co-immunoprecipitated with PBRM1, suggesting that PBRM1 interacts with BRD1-S and BRD1-L.

**Discussion:** Our data indicates that BRD1 form complexes with proteins encoded by the SZ and BD risk genes YWHAE, YWHAH, YWHAZ and PBRM1. The functional consequences of these interactions will be the subject for further studies.

### Switching Set Is Modulated by NOS1EX1F-VNTR

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**Background:** Impulsivity is a not only a character trait, but also an accompanying symptom to many psychiatric disorders such as Attention-Deficit-Hyperactivity-Disorder (ADHD), bipolar disorder (BD) and cluster B personality disorders. For ADHD and the manic phase in BD, it is a core symptom. Therefore impulsivity can be an important feature of certain psychiatric disorders and could even serve as an endophenotype when trying to discover the underlying mechanisms of these disorders. In recent years, a new polymorphism has been discovered which short allele is linked to heightened impulsivity: the NOS1ex1f-VNTR. It is expressed mainly in the striatum, a structure involved in the inhibition of already planned behavior, but also important for set switching, an executive function involved in the learning of new rules and abandoning old ones. The short allele of the polymorphism results in less NOS1 expression, which in turn leads to less NO synthase, and less NO being available to modulate neurotransmitter systems such as the dopamine system in the striatum. The NOS1ex1f-VNTR has been associated with ADHD and cluster B personality disorders.

**Methods:** 60 healthy subjects were stratified into a homozygous long alleled group (20), a homozygous short alleled group (20) and a heterozygous group and given a set switching task known to be disturbed in Parkinson's patients, a disorder affecting the striatum. While performing that task, prefrontal brain activity was measured using near-infrared spectroscopy.

**Results:** Behavioral results show a heightened error rate for the short alleled group, as compared with the heterozygous and homozygous long alleled group. This effect is modulated by a varying difficulty for the switching tasks, the most difficult switch produces the most errors in the short alleled group. Heterozygous and homozygous long alleled subjects did not differ. No differences between groups could be found in reaction times. Brain activation showed a robust dorsolateral prefrontal activation pattern for all subjects, switch trials activated the dorsolateral prefrontal cortex (dlPFC) more than the no-switch trials. As for group differences, again, homozygous long alleled and heterozygous subjects did not differ. Short alleled subjects activated the dlPFC significantly more than did the other 2 groups.

**Discussion:** The activation pattern of the short alleled group can be interpreted as a compensation mechanism in the fronto-striatal-frontal loop hypothesized to be active in switching set. In order to perform as fast and as well as the other two groups, the short alleled subjects have to make up for their loss of function in the striatum with a higher activation in the prefrontal cortex. However, with rising difficulty, short alleled subjects can not make up for their loss of function of the NO Synthase, and this leads to an increased error rate in switching set, which in turn could be used as a new measurement in the search for impulsive behavior as an endophenotype.

### Molecular Mechanisms of D-Cycloserine In A Fear Extinction Posttraumatic Stress Disorder (PTSD) Animal Model

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**Background:** Posttraumatic stress disorder (PTSD) is a severe, chronic and debilitating psychiatric disorder that can occur after exposure to a potentially traumatic event, significantly impairing normal functioning and quality of life. The disorder occurs in about 7 of the general population. D-cycloserine (DCS), a partial N-methyl D-aspartate receptor (NMDA) receptor agonist, has been found to be effective in facilitating extinction learning in animal studies and human trials of anxiety disorders. However, the precise mechanism by which DCS reduces the fear triggered by the traumatic context remains to be elucidated.

**Methods:** 48 Male Sprague-Dawley rats were used in the PTSD animal model (Siegmund and Wotjak, 2007) which incorporates both memory processes in the development and maintenance of PTSD. DCS or saline (depending on treatment group) was administered intrahippocampally allowing the direct assessment of the effects of the drug in this brain region. The rats were subjected to behavioural tests (light/dark avoidance test, modified holeboard novelty-induced suppression feeding test and the forced swimming test) to identify well adapted (rats that do not exhibit fearful behavior) and maladapted (rats that exhibit fearful behavior) animals. Six well adapted and 6 maladapted animals were selected from each group for transcriptome sequencing. RNA extracted from the left dorsal hippocampus (LDH) were paired-end sequenced on the Illumina HiSeq 2000 platform and bio-informatic analysis will be conducted to identify genes that are differentially expressed between the groups. These results will be confirmed via real-time PCR. The different treatment groups that are being compared on gene expression level are: 1. Control (well-adapted) + Saline vs. Fear-conditioned (maladapted) + Saline – this should give an idea of expression changes brought about during extinction training alone. 2. Fear-conditioned (maladapted) + Saline vs. Fear-conditioned (maladapted) +DCS – this should give an idea of the expression changes brought about by DCS during fear conditioning.

**Results:** Whole transcriptome sequencing yielded an average of 63.8 million reads per sample. These consisted of very high quality paired end 100bp reads. Genes that are differentially expressed between the different treatment groups are currently being investigated. Real time PCR verification results will also be generated for a selected subset of differentially expressed genes. These results will be reported at the conference.

**Discussion:** These results will provide information on the molecular underpinnings of PTSD and the mechanism and pathways whereby DCS facilitates fear extinction. Relevant results (currently being generated) regarding gene expression profiles and pathways involved in fear extinction and the aetiology of PTSD as well as potential treatment strategies will be discussed at the conference.

### Conditional Inactivation of the Schizophrenia and Bipolar Disorder Associated BRD1 Gene in the Central Nervous System of Mice

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**Background:** Schizophrenia (SZ) and Bipolar Disorder (BD) are devastating lifelong psychiatric disorders with a significant genetic component in their complex etiology (heritability up to 80%). Accumulating evidence from genetic studies form strong evidence that the Bromodomain-containing 1 gene (BRD1) is involved in the pathogenesis of SZ and BD. We have previously found that homozygous constitutive Brd1 knockout mice are not viable. To overcome this we aim at generating a mouse strain carrying a conditional inactivated allele of the gene in the central nervous system (CNS) neurons in order to establish the importance of Brd1 in the adult brain and in behavior.

**Methods:** C57BL/6 NTac mice carrying a targeted allele of Brd1 with loxP sites flanking exon 3-5 were crossed with Nes-Cre mice, carrying the Cre-recombinase gene under control of the CNS-specific Nestin (Nes) promoter to obtain offspring carrying a deleted allele uniquely in the CNS. Brd1 transcripts derived from the knockout allele are expected to be degraded by nonsense mediated mRNA decay (NMD) due to a premature stop codon in exon 6 and otherwise, the encoded protein is expected to be non-functional due to the deletion of functionally important domains. To confirm the conditional genotype, we have analyzed the Cre efficiency and the mRNA expression levels of Brd1 by means of quantitative RT-PCR (qPCR) in whole brain, liver, kidney and heart genomic DNA (gDNA) and RNA from wildtype (FW), heterozygous Brd1 knockout (LC) and homozygous Brd1 knockout (FC) mice. Each genotype group included 3 male and 3 female mice.

**Results:** The Cre-efficiency was measured by determining the amount of full-length Brd1 gDNA in a tissue sample. We found a significant reduction in full-length Brd1 gDNA relative to FW mice in brain to 62% in LC mice and to 20% in FC mice ( $p=0.0005$ ). In kidney, the amount of full-length Brd1 gDNA is significantly reduced ( $p=0.0071$ ) to 92% in LC mice and to 89% in FC mice. No significant reduction was observed in the other tissues. The derivation in brain from the expected 50% and 0% reduction in respectively LC and FC mice can be explained by either an inefficient Cre-activity, resulting in neuronal cells still carrying both alleles or by the presence of cells in the tissue homogenate where the Nes-promoter is not activated. Secondly, we measured the relative expression levels of two amplicons within the Brd1 mRNA by means of qPCR: one amplicon determines the total Brd1 expression (transcripts from both wildtype and Cre-deleted alleles), a second amplicon amplifies solely the wildtype transcripts. We found a significant reduction ( $p=0.0005$ ) of total Brd1 transcripts in brain to 60% in LC mice and to 22% in FC mice relative to the FW mice. No significant change in expression of Brd1 was seen in the other tissues. When determining the amount of wildtype transcripts, we found a significant reduction ( $p=0.0006$ ) to 54% in LC mice and to 7% in FC mice relative to the FW mice. In kidney, we see a significant change ( $p=0.0387$ ) in Brd1 expression to 107% in LC mice and to 85% in FC mice relative to FW mice. No significant changes were observed in other tissues. The difference in expression between total Brd1 transcripts and wildtype transcripts indicates an incomplete NMD. No phenotyping results are available yet, though there is an indication of

reduced growth in both LC and FC mice. Breeding of the mice (FW x LC) to obtain FC offspring also revealed the offspring genotypes deviates from the mendelian expectation indicating partial embryonic loss of FC mice.

**Discussion:** We have created a conditional Brd1 knockout mouse model with loss of function of the Brd1 gene in the CNS but not in other tissues. In kidney there is a slightly reduced amount of full-length Brd1 gDNA and expression of Brd1 mRNA, but not to the same level as in the CNS. The Nes-promoter was reported to be active to a lesser extent in kidney by the manufacturer of the Nes-Cre mouse. Our mouse strain carrying a conditional deleted allele of the Brd1 gene will be an important tool to determine the importance of the Brd1 gene in the adult brain and in the pathogenesis of mental illnesses.

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### A Novel BRD1 Knock-out Mouse Model for Schizophrenia Exhibits Cognitive Deficits, PPI Disruption and Increased Drug-Induced Locomotor Hyperactivity

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**Background:** We have repeatedly shown association of the gene, BRD1, to both schizophrenia and bipolar disorder. The gene encodes the Brd1 protein for which recent data suggests a role in transcriptional regulation, neuro-development, and brain disorder susceptibility. We have created a mouse strain heterozygous for a constitutively inactivated allele of the gene on a congenic C57BL/6NTac genetic background (R mice). In the present study, we wished to perform a phenotypic characterization of this R mouse, and establish the involvement of Brd1 in behavioral traits which are comparable to the endo-phenotypes displayed by patients with schizophrenia.

**Methods:** The physical and behavioral functions of male R mice were examined in a modified Irwin test. Subsequently, the R mice were phenotyped in a series of behavioral models related to schizophrenia symptomatology. The behavior was explored at baseline and after psychostimulant challenge (cocaine and PCP). Behaviors reflecting positive symptomatology of schizophrenia were explored by prepulse inhibition (PPI), a translational measure of pre-attentive information processing, and by psychostimulant-induced locomotor hyperactivity. To assess aspects of negative symptoms social interaction was explored. Cognitive function was examined in a comprehensive battery of maze-based tests supported by tests of fear conditioning and social memory. We aimed at making a broad characterization of learning and memory ranging from simple short term memory/working memory to long term/episodic memory (spontaneous alternation, continuous alternation, delayed alternation, Morris water maze, social preference and memory and fear conditioning).

**Results:** No signs of physiological abnormalities or reduction in fitness were noted for R mice when compared to WT litter mates. However, our data shows a significantly larger PPI disruption in R compared to WT mice when challenged with PCP. We furthermore show that R mice display a significantly higher initial startle response, which may indicate elevated stress and/or anxiety levels in these mice. R mice showed an increased locomotor response to PCP and cocaine. PCP (5 mg/kg) and cocaine (30 mg/kg), respectively, resulted in increased activity in R mice compared to WT littermates. When assessing direct social interaction, R mice did not differ from WT mice in the time they spent investigating an unfamiliar mouse. However, a significantly higher number of aggressive encounters were observed. In the three chamber social preference test, R mice displayed a decreased social preference, but initially had an intact social memory, suggesting a selective deficit in social interest. Upon repetition of the experiment one week later, R mice, in contrast to WT mice, no longer showed preference of novel social stimuli over familiar, indicating that their long-term memory of the encounter with the familiar mouse was impaired. Supportive of memory/learning deficits in the R mice, we found deficits in tests for spatial working memory. The impairment was less pronounced in tests with short retention intervals. However, in contrast to WT mice, the R mice were impaired also on these tests when challenged with PCP. In the fear conditioning test, R and WT mice displayed large basal differences in freezing, both in acquisition and in contextual spatial memory. No difference was observed in the Morris water maze task.

**Discussion:** In conclusion, we have shown that Brd1 plays a role in several behaviors comparable to schizophrenia endophenotypes. Although these deficits are most evident in cognitive tasks requiring retention of learned information as seen in the fear conditioning and delayed alternation task and test for remote social memory, Brd1 also seems to be involved in mechanisms relating to both psychosis and social deficits. The next immediate step will be to elucidate the molecular and cellular pathways that underlie the observed phenotypes through mRNA expression analysis, magnetic brain imaging and assays for neuronal connectivity.

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### A Novel Gene Knock-out Mouse Model Exhibits Reversible Depressive Phenotype and Cognitive Deficits

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**Background:** A number of studies have reported the association between the BRD1 gene and schizophrenia as well as bipolar disorder (BD) in human populations. We have developed a heterozygous BRD1 knock-out mouse model (R mice). The BRD1 gene encodes a potential regulatory factor and is important for the development of central nervous system. However, its contribution towards depressive disorders remains unknown.

**Methods:** We maintained all mice on a 12 hours light cycle with continuous access to food and water. We employed forced swim test (FST) and tail suspension test (TST) to assess the depressive equivalent behaviors of R mice. We assessed their general locomotion in an open field (OF). We employed bright open field (BOF), light and dark box (LDB) and elevated plus maze (EPM) to assess their anxiety equivalent behaviors. We used a fear conditioning system (FCS) to assess the context as well as cue dependent learning and 8 arm radial maze (8ARM) to assess the spatial as well as working memory. Each experiment involved 15 male and 15 female R mice with corresponding age matched wild type (WT) littermates as controls. Then, we assessed the depressive equivalent behaviors of another 15 female R mice and age matched WT controls with imipramine (two doses: 1mg/kg and 10mg/kg SC) and Fluoxetine (5 mg/kg SC). We scored the experiments by Ethovision XT 8.0 and employed appropriate multivariate statistics with STATA12.1. We sacrificed the mice, one hour after the experiments, and saved their brain samples.

**Results:** R mice and WT controls did not differ on general locomotion and on their anxiety equivalent behaviors during BOF, LDB and EPM. R mice exhibited more depressive equivalent behaviors than WT controls during TST ( $p=0.003$ ) and FST ( $p=0.001$ ). Such depressive phenotypes were more prominent among female R mice. Depressive phenotypes of R mice were reversed by both Imipramine and Fluoxetine. Imipramine at the dose of 10mg/kg produced larger effect sizes than Fluoxetine. During FCS, R mice learnt slower than WT controls ( $p=0.002$ ) and had context dependent learning deficits ( $p=0.0003$ ). Moreover, male R mice exhibited cue dependent learning deficits ( $p=0.02$ ). During 8ARM, male R mice showed working ( $p=0.007$ ) and visuospatial ( $p=0.04$ ) memory deficits.

**Discussion:** Depressive phenotype of BRD1 gene knock-out mice and the potential of anti-depressant medications to reverse such phenotype indicate the importance of BRD1 gene in the complex etiopathogenesis of major depressive disorders (MDD). Varying levels of cognitive deficits are present in schizophrenia, BD as well as MDD. Neurodevelopmental abnormalities, secondary to BRD1 dysfunction, may contribute towards such cognitive deficits. We plan for next generation RNA sequencing methods to analyze differential expression of various genes, regulated by BRD1 gene in specific brain regions of BRD1 gene knock-out mice. We desire to study the clinical predictors, identifying the subgroup of patients with MDD, whose illnesses are likely to be influenced by BRD1 gene.

### SF2/ASF-1 Regulates Pre-mRNA Splicing In Tryptophan Hydroxylase-2

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**Background:** Dysregulation of brain serotonin homeostasis has been implicated in various psychiatric disorders. Tryptophan hydroxylase-2 (TPH2) is the rate-limiting enzyme in brain serotonin synthesis, and is an ideal candidate gene to study psychiatric disorders. Although many single nucleotide polymorphisms (SNPs) in TPH2 have been identified, few have been functionally characterized. A rare missense mutation c.907 C>T (rs120074176) in TPH2, which encodes R303W mutation, has been reported as a loss-of-function mutation in protein expression and enzyme activity in attention-deficit/hyperactivity disorder (ADHD). Here, we studied the functional consequence of rs120074176 SNP at the genomic DNA level.

**Methods:** We designed minigene constructs with partial genomic DNA sequences flanking rs120074176 SNP to assess the efficiency of pre-mRNA splicing using GFP and Renella luciferase as a reporter gene.

**Results:** Compared to the wide-type construct, luciferase activity was decreased by 60% in the presence of rs120074176 SNP when mutant construct was expressed in PC12 cells. Similarly, real-time RT-PCR revealed 70% reduction in normal mRNA transcript in the presence of rs120074176 SNP. Since no aberrant mRNA was identified, these data suggested that rs120074176 SNP exhibited loss-of-function phenotype in TPH2 pre-mRNA splicing. Moreover, bioinformatic approach indicated that rs120074176 SNP could abolish the recognition of SF2/ASF-1, a splicing factor. To further verify this prediction, SF2/ASF-1 RNAi knockdown was carried out and the results indicated that RNAi knockdown severely reduced the pre-mRNA splicing efficiency in WT minigene construct to similar level as compared to that in minigene construct carrying rs120074176 SNP. Therefore, rs120074176 SNP abolished SF2/ASF-1 recognition of exonic sequence, leading to severely reduced TPH2 pre-mRNA splicing efficiency.

**Discussion:** Our data provided potential novel loss-of-function mechanism of TPH2 in pre-mRNA transcriptional regulation, further extending our knowledge to understand the pathophysiology of serotonin-related psychiatric disorders.

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### The Prevalence of Depression and its Associated Factors Among Resident Doctors Working in a Training Hospital in Karachi, Pakistan

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**Background:** Residency training programs and the level of stress on the trainees has been a topic of concern for over a century. Workload, poor learning environment, financial constraints are among a few of the stressors. The trainees suffer physically, mentally as well as emotionally as a consequence of these stressors, making them more susceptible to depression and in some severe cases leading to suicide.

**Methods:** This cross-sectional study will be conducted in a teaching hospital in Karachi, Pakistan. The study population will be stratified proportionally according to hospital departments and a random sampling method will be used. Informed consent will be taken by participants and permission will be taken from respective departments to maintain autonomous decision. Participants will only choose their participation by their free will. Study will be started after receiving approval from Hospital's ERC. The Participants will be informed that their respective BDI Scores will be returned to them in sealed envelopes. Participants if found with high scores on the Depression scale, will be offered appropriate psychiatric help in the form of Pharmacological and Psychological Options. Data will be collected by questionnaire consisting of socio-demographic variables, Beck Depression Inventory (BDI), and Minnesota Job Satisfaction Questionnaire (MJSQ). Associations will be studied through univariate and multivariate analyses.

**Results:** The Relationship between Depression and Job satisfaction will help in determining the mental well being of the trainees. Findings will be disseminated via publication, poster, oral presentations to raise awareness, foster a resident-friendly working environment and to help improving the quality of Residency Training Structured Programs.

**Discussion:** The Relationship between Depression and Job satisfaction will help in determining the mental well being of the trainees. Findings will be disseminated via publication, poster, oral presentations to raise awareness, foster a resident-friendly working environment and to help improving the quality of Residency Training Structured Programs.

**Area-Specific Distribution of Neuronal Aneuploidy in the Alzheimer's Disease Brain**

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**Background:** Aneuploid cells can populate the normal and diseased human brain. However, aneuploidy origins and the way how it alters prenatal or postnatal neurogenesis are not specified. The human developing brain demonstrates extremely high rates of mosaic aneuploidy affecting 30-35% of cells. During following ontogenetic stages the brain exhibits a significant decrease of the rate achieving 10% in adulthood. This phenomenon supports the idea that neural cells are generated during prenatal life, and excess of aneuploid neurons is the result of early developmental disturbances due to unchecked proliferation, abnormal cell cycle regulation and altered regulation of the natural cell number by programmed cell death (apoptosis). Assuming that these processes can be altered, it was hypothesized that aneuploidization of the brain is a susceptibility factor for complex psychiatric diseases, including schizophrenia and autism.

**Methods:** Here, using molecular neurocytogenetic approaches we provide the evidences that alteration to adult neurogenesis could be also involved in the pathogenesis of Alzheimer's disease (AD). We have analyzed aneuploidy rate in different areas of the AD brain differentially affected by neurodegeneration. The frequency of aneuploid cells was determined in different regions of the brain affected by neurodegeneration (prefrontal cortex and hippocampus) and non-affected regions (cerebellum) using modern molecular-cytogenetic and immunohistochemical techniques (interphase MFISH and MCB FISH, immunoFISH with neuronal-specific antibodies). To carry out the study, we have obtained cell suspensions from 3 brain areas of 5 individuals with pre-mortem diagnosis of AD and 5 individuals without neurological/psychiatric diseases, conspicuous brain malformations and injuries (in total 30 cell suspensions). We have applied interphase chromosome-specific multicolour banding (ICS-MCB) that allows to analyze chromosomes in their integrity regardless cell cycle stage, i.e. provides for chromosome analysis in any tissue of the human organism. We selected four targeted chromosomes (chromosomes 1, 14, 21 and X) according previous molecular cytogenetic findings in the diseased human brain and previous cytogenetic studies of blood lymphocytes in AD patients (for more details see Iourov et al., *Int Rev Cytol* 2006; 249:143-191; Yurov et al., *Schizophr Res* 2008; 98:139-147). Analysis of AD and control brain samples was performed through scoring 300-500 interphase nuclei per sample per probe. In total, there were performed 120 analyses of brain samples that make total amount of cells analyzed to approach 50000.

**Results:** Increased aneuploidy (monosomy and trisomy) affecting chromosome 21 and chromosome X was observed in AD brain. However, the incidence of abnormal neural cells affected by aneuploidy was significantly higher in degenerating brain areas (hippocampus, prefrontal cortex — AD) as to unaffected areas (cerebellum). The frequency of aneuploidy varied from 2 to 20.7 in the AD cerebrum, whereas it varied from 0.3 to 2.7 in the unaffected cerebrum. In the cerebellum, aneuploidy were observed in 0,7-3,7 of cells in the AD brain and in 0.3-1.7 of cells in the normal brain. Aneuploidy rate was 3-29.3 in the AD hippocampus and was 0.7-2.3 in the unaffected hippocampus. We observed significant increase of aneuploidy level within the AD cerebrum and hippocampus in case of autosomes, whereas chromosome X showed less significant differences

in aneuploidy level as to control, but it was still appreciable. The difference between the AD and control cerebellum was less evident as to the cerebrum and hippocampus. However, the AD cerebellum had more aneuploid cells as to controls. The high level of aneuploidy was observed for chromosome 21 in the AD cerebrum and hippocampus. The later achieved from 10 to 30 of cells analyzed. Furthermore, we were able to depict that AD brain is selectively affected by aneuploidy, inasmuch as the highest rates were observed in the cerebrum and hippocampus, but the aneuploidy incidence of the AD cerebellum approached to that in controls.

**Discussion:** Therefore, our data indicates that brain areas, which are damaged by neurodegeneration in AD, are more seriously affected by aneuploidy and the most commonly involved chromosome in AD aneuploidy is chromosome 21. Nevertheless, aneuploidy involving remaining chromosomes in the cerebrum and hippocampus was higher as to controls, as well. Our data propose widespread aneuploidization of selected brain areas as a mechanism of AD. This has appreciable implications for numerous issues concerning increasing life quality of AD patients including new treatment strategies and drug design considering aneuploidy or aneuploidization as drug targets as it is the case for malignant diseases (cancer), which are also associated with aneuploidization and chromosome instability. These regional specific distribution and accumulation of aneuploidy in brain areas differentially affected by degeneration could be explained by accumulation of aneuploid cells during postnatal life. We speculate that acquired neural aneuploidy of the diseased brain could be generated during developing and adult neurogenesis. Supported by BMBF/DLR (RUS 09/006) and DLR/BMBF (BLR 11/002).

### A Genome-wide Association Study of Suicidal Behavior in Two Independent Bipolar Disorder Samples

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**Background:** Suicidal behaviour is a serious public health concern. Twin studies have shown that suicidal behaviour, including suicidal ideation, suicide attempt and suicide completion, has a prominent genetic component (reviewed in (Zai et al. 2012)). Candidate gene studies have suggested a number of promising leads, including genes coding for the serotonin transporter (SLC6A4), the brain-derived neurotrophic factor (BDNF) (Zai et al. 2011e) and its receptor (NTRK2). Recently, genome-wide association studies on large samples from multiple sites have identified a number of novel candidate genes, including glial cell line derived neurotrophic factor family receptor alpha 1 GFRA1 (Schosser et al. 2011), sorbin and SH3-domain containing-1 (SORBS1) (Perroud et al. 2012), and acid phosphatase ACP1 (Willour et al. 2011).

**Methods:** The purpose of the study was to look for novel candidate genes for suicidal behaviour in two independent samples of 212 small nuclear families with bipolar probands and 428 bipolar cases both recruited from Toronto. After quality control and removal of duplicate subjects and population outliers, data analysis was conducted using PLINK for the case sample and SOLAR for the family sample. We performed quantitative analyses on the suicide specifier scores (from Structured Clinical Interview for DSM-IV SCID: 0=non-suicidal; 1=thought of one's death; 2=suicidal ideation; 3=suicide plan; 4=suicide attempt) in the family sample, and on the suicidality scores (from the Schedule for Clinical Assessment in Neuropsychiatry SCAN: 0=non-suicidal; 1-3=degree of loathing of life; 4=suicide plan/ideation; 5=suicide attempt without serious harm; 6=suicide attempt with serious harm; 7=suicide attempt designed to end life) in the cases-only collection. We included age, sex, history of alcohol use/abuse, and presence of mixed/manic episodes as covariates in our analyses. As the two samples were genotyped using different platforms, we performed the clump-best analysis in PLINK to compare the significant p-values.

**Results:** In our preliminary analysis of suicidal behaviour, we found suggestive significance with markers in the ARNTL gene, which is previously implicated in schizophrenia and bipolar disorder, in our family and case samples ( $p < 0.001$  and  $p < 0.01$  respectively). For the top hit in our family sample, which is located in the follicle-stimulating hormone receptor (FSHR) gene, its proxy marker also showed suggestive significance in our case sample ( $p = 0.006$ ).

**Discussion:** Our preliminary findings suggest that some of the genes previously associated with psychiatric disorders may play a role in suicidal behaviour. We will attempt to replicate these findings in other suicide attempt data sets. We will also be performing targeted re-sequencing of these and other novel candidate genes from our GWAS in our ongoing search for new suicide-associated variants.

### Genetic Epidemiological Studies in Twins with Mental Disorders in Cuba

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**Background:** The Cuban Twin Registry is a population-based volunteer register comprising pairs of twins who accept to participate in medical and health related research aimed at investigating the roles of genetic and non-genetic risk factors, in the origin of common complex diseases in the Cuban population. It was established in 2006 and contains 55400 multiple births of all ages and regions of the country. The registry has facilitated more than 140 genetic epidemiological studies in complex diseases with very high participation rates all over the country. The focus to date has been primarily in asthma, schizophrenia, major depression, hypertension, alcohol addiction and bipolar disorder.

**Methods:** Twins and/or their closest relatives are asked to fill in a questionnaire on physical similarity consisting of 24 items. Additional questionnaires exploring birth defects, genetic disorders, complex diseases as well as social variables have been also conducted in most of the twins. To establish a zygosity classification algorithm we used items from zygosity questionnaire, blood group tests and HLA antigens identification for a subset of 188 same sex twins' pairs. Using this algorithm we correctly classify 86.2% of monozygotic pairs and 100% of dizygotic pairs, the global rate of correct classification is 92.5%. Of the total classified twin pairs, 31.3% are monozygotic. Most of the initial analyses were based on classical twin studies focusing on one trait at a time. More recently we have started to explore co-morbidity mainly in mental disorders.

**Results:** The heritability of affective illness was 0.62 for bipolar disorder and 0.54 for major depression. Bipolar illness, major depression and alcohol addiction were the most frequent disorders in 1<sup>st</sup> and 2<sup>nd</sup> degree relatives of twins with bipolar disorder meanwhile major depression, alcohol abuse and generalized anxiety disorder were the most frequent in relatives of twins with major depression.

**Discussion:** The Cuban twin registry is administered by the National Center of Medical Genetics and it has become a powerful resource of data for conducting biomedical research in our country.

### The Genes in Irritable Bowel Syndrome Research Network Europe (GENIEUR)

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**Background:** GENIEUR (The Genes in Irritable Bowel Syndrome Research Network Europe) is a pan-European interdisciplinary network to identify genetic factors (human genetics, epigenetics, metagenomics) contributing to IBS etiopathogenesis. Irritable bowel syndrome (IBS) is a highly prevalent functional gastrointestinal (GI) disorder with a major impact not only on the healthcare system but also on the patient's quality of life. More than 15% of the population in developed countries suffer IBS. IBS is characterized by a high comorbidity with psychiatric conditions such as anxiety and depression as well as with pain syndromes. The interdisciplinary connection of clinicians specialized in functional GI disorders, immunology and psychiatry and basic scientists focusing on (epi-) genetics, microbiomics and phenotypic analysis of case-control cohorts under the umbrella of GENIEUR is an important prerequisite for success in this field.

**Methods:** The COST Action is focusing on the following objectives: Standardization and harmonization of criteria for case-control definition and recruitment and IBS patient characterization, establishing a phenotyping tool as gold standard for large-scale studies, creation of a database collecting data which will be created during the course of the Action such as harmonized inventories, inventories for the assessment of data such as exposure to environmental factors (exposure to certain germs, infection, gastroenteritis, nutrition), phenotyping tool, SOPs guidelines, presentations etc., establishment of a biobank fostering a better data availability by collecting patient and control material (blood, tissue, stool) not only for genetic studies but also for analyses of functional / phenotypical consequences of coined genetic factors. Such a biobank could also be used to test novel pharmacological targets developed by pharmaceutical or "agro-alimentary" industries, Identification of (epi-)genetic risk factors for IBS, and interaction analysis of gene-environment as well as human genome and microbiome (metagenome).

**Results:** In order to reach these aims and to share workload, the following working groups (WGs) have been established: WG 1) Establishment of a gold standard for patient recruitment and characterization, WG 2) Definition of quantitative traits as intermediate phenotypes, WG 3) Genetics: Molecular genetics and epigenetics, and WG 4) Microbiomics. The above listed objectives will be achieved under the umbrella of the Cost Action GENIEUR by joining forces of experts working in different complementing disciplines with their main research interest on IBS: (neuro-) gastroenterologists, psychiatrists, physiologists, pathologists, immunologists, nutritionists as well as geneticists, microbiologists and molecular biologists have joined the Action with the main goal to unravel the etiopathogenetic mechanisms of this multifactorial condition.

**Discussion:** GENIEUR will provide an excellent platform for unifying and harmonizing research strategies, which is a prerequisite to nail down crucial factors involved in IBS. With this Action, Europe-wide collaboration will be facilitated with a significant impact on IBS research all over Europe by networking of renowned scientific experts from different disciplines from different countries. GENIEUR will accelerate the successful coordination of national Actions into an integrated project by meetings and training workshops on a regular basis.

### SLC19A3: Screening and Association with Wernicke-Korsakoff's Syndrome

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**Background:** Wernicke-Korsakoff's Syndrome (WKS) is a neurological disorder characterized by the acute phase Wernicke Encephalopathy and the chronic stage Korsakoff Syndrome. Wernicke's encephalopathy is a neuropsychiatric condition arising as a result of low levels of thiamine reaching the brain; Korsakoff syndrome develops when the brain becomes permanently damaged leading to ataxia, ophthalmoplegia and memory disorders. WKS, found in 0.1% of the population, is commonly associated with chronic alcohol abusers due to malnutrition and liver damage that promotes thiamine deficiency. SLC19A3 on chromosome 2q37 encodes the thiamine transporter 2 in humans. This transporter is responsible for the uptake of thiamine in cells fundamental for maintaining the body's homeostasis. The human SLC19A3 gene is widely expressed, with the most abundant expression observed in placenta, kidney and liver. Studies have shown that SLC19A3 mutations were responsible for the impaired thiamine uptake activity and the development of Wernicke's encephalopathy in two compound heterozygote brothers. Furthermore, this gene was found to be associated with biotin-responsive basal ganglia disease an autosomal recessive metabolic disorder characterized by encephalopathy and ophthalmoplegia.

**Methods:** SLC19A3 was screened in more than 100 WKS cases through High Resolution Melting (HRM) curve analysis. SNP genotyping for both the WKS case and controls including alcoholic subjects were conducted using KASPar genotyping.

**Results:** HRM analysis and sequencing detected 20 SNPs of which 7 are novel including 5 non-synonymous mutations. 3 SNPs were detected in the intronic regions whereas 5 were detected in the 5'UTR and 3'UTR.

**Discussion:** The mutations identified may affect thiamine uptake in the affected individuals but further research has to be carried out on the role that these variants have on the gene. All cases and controls will be screened for the identified mutations and functional assays will be performed to study the effects of these changes in vitro.

### Genome-wide Association Study of Criminal Behavior in Finnish Cohort of Criminal Offenders

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**Background:** Criminal behavior is a complex trait that derives from both genetic and environmental influences. Previous studies indicate that certain characteristics of personality, such as antisocial personality, impulsivity, and aggression apparently underlie criminal behavior. This study investigates the genetic variation behind criminal behavior.

**Methods:** DNA from 761 criminal offenders, comprising 691 males and 70 females, was collected from prisons in Finland. A genome-wide association study was carried out in the criminal cohort at the Wellcome Trust Sanger Institute with Illumina 670 platform comprising 594398 common single-nucleotide polymorphisms (SNPs). After quality control, the total of 486903 SNPs were analyzed in 479 of the male criminals. A sample comprising 3528 males from the general Finnish population, genotyped with Illumina 670 platform, was used as controls. Logistic regression test adjusted for age and multidimensional scaling clusters was applied in association (case/control) analysis by using PLINK 1.07. Based on the GWAS results, the most significant variants were chosen for re-genotyping in the cases (Finnish criminals, n = 745) and in a large Finnish population based control cohort (Health 2000, n = 7774) by using Sequenom MassArray technology.

**Results:** Over 70% (564/761) individuals were diagnosed with antisocial personality disorder according to the criteria from Structured Clinical Interview for DSM-IV Axis Disorders (SCID-II). Several variants were found to associate with criminal behavior and antisocial personality according to the genome-wide criteria. Confirmatory results from the re-genotyping study are presently pending.

**Discussion:** This study identifies putatively several signals for association, which indicate that common genetic variations may, in part, provide explanation to criminal behavior. However, further work is necessary to clarify the genetic variations associated with the characteristics of personality involved in criminal behavior and their interplay with environment.

### Discovery of Clinical and Metabolic Genetic Syndromes Manifesting as Neuropsychiatric Disorders

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**Background:** Many clinical and metabolic genetic syndromes are known to present with psychiatric disorders or features. Significantly, many of these conditions require surveillance for other, sometimes preventable or treatable, clinical manifestations, such as hormonal disturbances, heart disease, or neurodegenerative processes. Additionally, many metabolic conditions are treatable by adjustments in diet, medication and/or enzyme replacement therapy. The overall goal of this study is to determine the role of hidden metabolic and clinical genetic conditions in patients with psychiatric disorders/features and comorbidities. Thus, we will define the prevalence of genetic syndromes within this population, therefore potentially revolutionizing the management and treatment of selected individuals with psychiatric impairment.

**Methods:** By prospectively recruiting patients 16 years of age and older with a psychiatric disorder or features and at least one of 1) neurologic abnormality, 2) developmental delay, autism spectrum disorder or pervasive developmental disorder (PDD), 3) dysmorphic features, 4) congenital anomalies or 5) family history of developmental delay, autism spectrum disorder or PDD, a clinical database of phenotypic correlates is being established in order to delineate the highest yield data that lead to the most effective and efficient diagnosis of genetic and metabolic syndromes in patients with neuropsychiatric disorders. Additionally, novel adult phenotypes in genes associated with known genetic syndromes can be delineated. Samples from recruited patients are investigated for the presence of alterations and copy number variants in high-priority candidate genes (NPC1 and NPC2, FMR1, NRXN1) and for novel variants by high-resolution SNP array.

**Results:** We present examples of genetic syndromes and variants identified in the patient cohort to date. Patient recruitment and sample analysis is ongoing.

**Discussion:** Achieving the aims of this study will contribute to identifying genetic syndromes that can mimic or contribute to psychiatric illness, as well as determining the prevalence of these syndromes in psychiatric populations. It will facilitate education of primary care physicians, patients and families about the important implications for surveillance, management, treatment and family planning once a genetic diagnosis is made in psychiatric patients with dual/multiple diagnoses. Furthermore, it will lead to the establishment of standardized protocols for diagnosing, managing and treating patients with psychiatric disorders and comorbidities.

### The UST Variant Linked to the Job-Related Exhaustion Associates with General Exhaustion in the Large Finnish Population Cohort

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**Background:** Job-related exhaustion is the core dimension of burnout, a work-related stress syndrome that has several negative consequences for individual's health. In our previous study on genome-wide analysis of job-related exhaustion, we obtained the strongest signal for a variant located in the intron of UST, and the finding was replicated in the second data set (presented in the XVIIIth WCPG 2010, PP-219 Genetic Background of Burnout). In this study we explored association of the UST variant to general exhaustion, regardless of the context, in another Finnish population-based sample comprising 20 813 individuals.

**Methods:** The FINRISK study consists of independent random population samples that have been collected every 5 years in Finland. In this study we included samples collected in 1997, 2002 and 2007. The general exhaustion was evaluated with the question "Do you feel exhausted or overloaded?". Linear regression analysis of the UST variant and the exhaustion score was performed for 20 813 individuals from the FINRISK sample.

**Results:** The UST variant of UST associated significantly with general exhaustion ( $P < 0.05$ ). The association was slightly stronger among the workers as compared to non-workers.

**Discussion:** The allelic variant of UST (uronyl-2-sulfotransferase) may be a modest risk factor for both job-related and general exhaustion. The predictive potential of the variant is probably negligible, but our findings open interesting functional hypothesis about the contribution of dermatan/chondroitin sulfate modification in the etiology of exhaustion symptoms.

### A Systematic Review and Meta-Analysis of Genetic Associations with Violence and Aggression

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**Background:** A large number of promising candidate-gene studies for aggression and violence have been conducted. Successful identification of associations between genetic markers and aggression would contribute to understanding the neurobiology of antisocial behavior and potentially provide useful tools for risk prediction and therapeutic targets for high risk groups of patients and offenders. Therefore, we systematically reviewed genetic association studies of aggression and subjected all polymorphisms studied in more than three independent samples to meta-analyses.

**Methods:** Studies were identified through PubMed and HUGO Navigator databases search and additional data was sought through reviewing reference lists and correspondence with investigators. Genetic association studies were included if outcome data on aggression or violent behavior either as a binary outcome in a case control design or as a quantitative trait were provided. From 1331 potentially relevant investigations, 185 studies constituting 277 independent associations on 31 genes fulfilled the predetermined selection criteria. Data from variants investigated in three or more samples were combined in meta-analyses. Potential sources of heterogeneity were investigated using subgroup analyses.

**Results:** Information on over 60,000 individuals was included. In the primary analyses, which used relaxed inclusion criteria to incorporate maximum data available, we found no association between any polymorphism analyzed and aggression at the 5% level of significance. Subgroup analyses, including by severity of outcome, age group, characteristics of the sample, and ethnicity, did not demonstrate any consistent findings.

**Discussion:** Despite half the individual studies included in this review reporting significant findings, when meta-analyzed, candidate gene studies did not yield any consistent associations with aggression and related outcomes. Current evidence does not support the use of such genes to predict dangerousness or as markers for therapeutic interventions.

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**Epistatic Effects Of CACNA1C and PCLO Depression Risk Alleles on Subgenual Cingulate FMRI Response During Self-Referential Processing**

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**Background:** Depressive disorders are associated with an abnormal representation and regulation of mood and emotion and characterized by negative self-referential beliefs. Multiple studies have implicated the subgenual cingulate, Brodmann Area 25 (CG25), in negative mood and depressive symptoms. Common single nucleotide polymorphisms (SNPs) in genes encoding the presynaptic active zone protein Piccolo (PCLO; rs2522833) and the neuronal calcium channel CAV1.2 (CACNA1C; rs1006737) have previously been linked to increased risk for affective disorders (Sullivan et al., 2009; Green et al., 2009). Given the known disturbances of self-image in depressed patients, we now investigated the impact of these genetic risk variants on brain activation patterns during a self-reference task.

**Methods:** We acquired event-related functional magnetic resonance imaging (fMRI) from 68 young, healthy adults while they performed a well-established self-reference paradigm (Kelley et al., 2002). Subjects were presented with words describing positive and negative personality traits and were supposed to either judge whether the trait described them (self-reference), whether it applied to the German chancellor Angela Merkel (other-reference) or whether it had exactly two syllables (control condition). Data analysis was performed using SPM8 and a two-stage mixed effects model with PCLO and CACNA1C genotypes as between-subject factors.

**Results:** We found a significant interaction of PCLO and CACNA1C risk alleles in CG25 ( $p < .05$ , small volume FWE-corrected). Carriers of no risk allele and carriers of both risk alleles showed higher CG25 activation during self-reference as compared to carriers of only one risk allele.

**Discussion:** We tentatively suggest that, since all subjects had no lifetime history of depression, increased risk allele load might co-occur with the presence of resilience factors (genetic or environmental) that exert protective effects in healthy humans at genetic risk for depression. The finding is also in line with a comparable observation using a social memory encoding task in a large cohort (see companion abstract Schott et al.). Since these results were found during two different cognitive tasks, the interaction of PCLO and CACNA1C in CG25 could be region-specific but relatively task-independent.

**Quantitative Genetic Modeling of Cortical Thickness Change in Twin Pairs Discordant for Schizophrenia: A Longitudinal Study**

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**Background:** Progressive structural brain changes have been found in schizophrenia. Moreover, an earlier report of this sample suggested that the progressive changes in whole brain, frontal and temporal lobe volumes were partly attributable to genetic factors related to the illness. Whether change in cortical thickness is mediated by genetic or disease-related factors is unknown. Our objective is to investigate whether genetic and/or environmental factors are associated with cortical thickness change in anatomically defined regions of interest for schizophrenia.

**Methods:** We used a longitudinal 5-year follow-up design in monozygotic (MZ) and dizygotic (DZ) twin-pairs discordant for schizophrenia and healthy comparison twin-pairs using structural MRI brain scanning. Subjects were recruited from the twin-pair cohort at the University Medical Center Utrecht, the Netherlands. A total of 90 subjects completed the study: 9 complete MZ and 10 complete DZ twin-pairs discordant for schizophrenia and 14 complete MZ and 11 complete DZ healthy twin-pairs plus additional 2 single DZ healthy twins. Cortical thickness change over time was calculated. Structural equation modelling was applied to estimate contributions of additive genetic and environmental factors on cortical thickness change.

**Results:** Bonferroni correction for multiple comparisons resulted in a significant difference in superior left temporal cortex change between the discordant twins relative to that in healthy controls. Figure 1 shows the change for each group (ie., patients, co-twins and controls) for left superior temporal cortex. Structural equation modelling revealed a significant genetic correlation ( $r_g$ ), which shows that two traits show the same genetic effects between cortical thickness change and schizophrenia in left superior temporal cortex ( $-0.39$  95%CI:  $-0.73$  to  $-0.11$ ). The environmental correlation ( $r_e$ ) was significant in the opposite direction ( $0.65$  95%CI:  $0.19$  to  $0.90$ ) and thereby the  $r_g$  and  $r_e$  cancel each other out for the phenotypic correlation ( $r_{ph}$ ). Part of the phenotypic correlation which accounts for the genetic effects ( $r_{ph-a}$ ) between schizophrenia and changes in left superior temporal cortex was found significant ( $-0.25$ , 95%CI:  $-0.41$  to  $-0.07$ ). Part of the phenotypic correlation which accounts for environmental effects ( $r_{ph-e}$ ) was found significant ( $0.13$ , 95%CI:  $0.08$  to  $0.17$ ).

**Discussion:** Associations between schizophrenia liability and cortical thickness change seems to be particularly prominent in the left superior temporal cortex. In addition, this region of interest is thicker in co-twins relative to that in controls at baseline. In figure 1 we can clearly see that the co-twins are showing the most pronounced cortical thinning. One could only speculate, but perhaps the co-twins who are genetically related to the patients show a delayed thinning process which get started later in life? Genetic factors drive the decrease in thickness and environmental factor seems to dampen the decrease seen in patients which perhaps is due to medication.

### Systematic Search for Genetic Factors Influencing the Thickness of the Cerebral Cortex

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**Background:** The human cortex is structurally and functionally segregated. It varies more than any other part of the brain between subjects. The identification of gene variants contributing to this inter-subject variability will help to elucidate molecular mechanisms underlying brain functions. Through systematic correlation of each variant's genotype with the phenotypic variability, assessed through magnetic resonance imaging (MRI), those genetic variants can be identified that explain the phenotypic variability. In the present study, we focused on cortical thickness (CT) which is a heritable, quantitative trait, assumed to reflect the architecture of neuronal and glial cells in the cortex. Because CT undergoes changes in neuropsychiatric disorders, it has become a promising endophenotype for many imaging genetic studies in our field.

**Methods:** We analyzed T1-weighted structural brain images from 98 healthy volunteers using a 3T MRI scanner, and performed genome-wide genotyping (HumanOmniExpress and HumanOmni1S), resulting in 1.4 million single-nucleotide polymorphisms (SNPs) per subject after quality control. Thickness was estimated using tools provided by FreeSurfer software. To determine those data which explain most of the thickness variability, we applied a principal component analysis (PCA) on the pooled data from all cortical regions. We selected the first 15 principal components which together explained 80% of the total variance and performed genome-wide association studies (GWAS) on each component. Across PCA GWAS, SNP P-values were combined using a meta-analysis approach.

**Results:** Overall, 31 SNPs showed P-values of less than 1E-04. The most significant finding was a cluster of SNPs located between CDYL2 and C16orf61 on chromosome 16q23.2 (P=9.48E-06).

**Discussion:** The identification of genetic factors contributing to inter-individual variability in cortical thickness provides new important insights into the biological processes involved in corticogenesis. Our study has identified several common genetic variants that show suggestive evidence for an involvement in cortical thickness. We are currently analyzing the respective genes and available knowledge of their function. The P-values do not exceed the formal threshold for genome-wide significance which may be a result of the relatively limited sample size and/or the small or medium effect-size conferred by each of these genetic variants.

### Genome-wide Analysis to Identify Genetic Correlates of Brain Imaging Measures and Cognitive Test Performance in the Betula Study

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**Background:** Psychiatric disorders are complex and often accompanied by prominent cognitive deficits that severely compromise quality of life. Alterations in brain morphometry and brain connectivity have also been reported to be potential endophenotypes of high relevance to psychiatric disorders. In this context, an integration of brain imaging, neuro-cognitive and genetic data can be used to enhance our understanding of genetic factors that could influence brain functioning and be relevant to its dysfunction in psychiatric disorders.

**Methods:** Here we describe the ongoing Betula-Bergen genome-wide imaging genetics study aimed at identifying genetic correlates of brain imaging measures and cognitive abilities. Since 1988 the Betula study has recruited a unique and large sample of individuals tested and followed up every five years from the region of Umeå. At the latest wave of the study, 376 participants were tested for various functional and morphometric imaging measures as well as measures of cognitive function. The genotyping was performed using a combination of Illumina HumanOmni Express and HumanOmni 1S Bead chip kits. After appropriate genotype quality checks a total of 1.4 million SNPs remained in the final data set. The genetic association of these markers with two measures, white-matter diffusion-tensor imaging (DTI) and Episodic Memory are currently being analysed. Single marker association analysis was performed using linear regression with age and sex as covariates. SNPs were then assigned to genes; using linkage disequilibrium based binning approach, as implemented in the LDsnP tool (Christoforou et al., 2012, AJHG 90(4):727-33), and scored.

**Results:** No single SNP association reached genome-wide significance, best P values being  $7.29 \times 10^{-7}$  and  $3.32 \times 10^{-6}$  for the DTI and Episodic Memory measures, respectively. However, 169 SNPs showed a nominal significance with  $P \leq 10^{-5}$  for the DTI measure and 156 SNPs for Episodic Memory. Replication in additional samples is ongoing.

**Discussion:** As part of the Betula imaging genetics study we have so far identified several interesting candidate genes for DTI and episodic memory for further investigation. Next steps will include examination of functional MRI data. The identification of the genes affecting individual differences in cognition may help in elucidating the mechanisms underlying brain dysfunctions in disorders such as neurodegenerative or psychiatric disorders.

### Association Of SNPs in the FKBP5 Gene Region with Hippocampal and Amygdala Volume in a Healthy Control Sample

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**Background:** FKBP5 has repeatedly been reported to play a role in the etiology of PTSD and response to antidepressant treatment. Further hippocampal and amygdala volume have been linked to the risk of developing depression, PTSD and a variety of other psychiatric disorders. We hypothesized that the implication of FKBP5 in psychiatric diseases might be mediated through effects of genetic variation and differences in amygdala and hippocampal volumes. Therefore we examined whether single nucleotide polymorphisms (SNPs) in the FKBP5 gene region are directly associated with hippocampal and amygdala volume differences in a sample of healthy young subjects.

**Methods:** We used automated segmentation procedures of structural Magnetic Resonance images to determine measures of hippocampal volume in 719 healthy Swiss individuals. Hippocampal and amygdala volume was corrected for age, gender and intra-cranial volume. Genetic variation in FKBP5 and additionally 50kb up- and downstream of the gene was assessed through 48 SNPs represented on the Affymetrix Genome-Wide Human SNP Array 6.0. Genetic associations were tested under the assumption of a dominant and an additive model using the Plink software package.

**Results:** The analysis for mean hippocampal volume revealed four SNPs where associations withstood Bonferroni correction for multiple testing ( $p < 0.001$ ). The analysis for mean amygdala volume did not show any SNPs withstanding correction for multiple testing.

**Discussion:** These results make it tempting to speculate that the role of the FKBP5 gene region for developing psychiatric disease phenotypes like PTSD is mediated through its effects on hippocampal properties.

### An Association Between DISC1 Genotype and White Matter Integrity Using DTI

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**Background:** Disrupted in Schizophrenia 1 (DISC1) is a gene involved in brain development, and studies have shown that DISC1 is important for the dendritic structure and positioning of neurons in the cortex, particularly in the hippocampus (1). DISC1 has been associated with a number of neuropsychiatric disorders, suggesting affection of neurodevelopment as a risk factor in these disorders. Diffusion-tensor imaging (DTI) is an MRI technique that allows for the analysis of white matter integrity by measuring diffusion of water molecules in the brain. Reduced diffusion in white matter has been shown both for disorders such as ADHD and for DISC1 genotypes (2,3). Here, we investigate the relationship between DISC1 genotypes and white matter integrity in a Norwegian sample of adults with ADHD.

**Methods:** For this purpose, 28 patients with ADHD and 38 controls were scanned using a 3.0 T GE Signa System MRI machine, using a protocol that included a DTI sequence. DTI data were analyzed using tract-based spacial statistics (TBSS) of fractional anisotropy (FA) (4). All participants were genotyped for DISC1 variants as part of a larger DISC1 study (Jacobsen et al, under revision). We chose to test three coding variants in DISC1, as well as the top SNP (rs1598979) from our larger study to define groups which were then tested for differences in FA. The coding SNPs rs821616 (S704C), rs3738401 (Q264R) and rs6675281 (L607F) are well known in the literature (5).

**Results:** After controlling for gender and ADHD-status, we found an association between rs3738401 genotype and FA. Individuals homozygous for the G-allele had a bilateral significantly ( $p < 0.05$ ) lower FA in the white matter adjacent to the hippocampus than individuals carrying the A-allele. We found no association with the other SNPs, including rs821616.

**Discussion:** Sprooten et al. (3) recently reported a general reduced FA in the entire brain of carriers of the A-allele of rs821616, a coding SNP in DISC1. We could not confirm this association, but instead found an association between rs3738401 genotypes and white matter integrity in the hippocampus region in both hemispheres. This suggests that variations in DISC1 is important for the white matter in the hippocampus, where DISC1 is known to be particularly important for neurodevelopment. Although the present study has limited power to detect differences between the ADHD patients and the controls, the results suggest that future studies, particularly in neuropsychiatric disorders such as ADHD, might benefit from analyzing DISC1 together with DTI data. References 1, Bradshaw N. J., Porteous D. J. DISC1-binding proteins in neural development, signalling and schizophrenia. *Neuropharmacology*. 2010 Dec. 31. 2, Pavuluri M. N., Yang S., Kaminen K., Passarotti A. M., Srinivasan G., Harral E. M., et al. Diffusion Tensor Imaging Study of White Matter Fiber Tracts in Pediatric Bipolar Disorder and Attention-Deficit/Hyperactivity Disorder. *Biol Psychiatry*; 2009 Apr. 1;65(7):586–93. 3, Sprooten E., Sussmann J. E., Moorhead T. W., Whalley H. C., French-Constant C., Blumberg H. P., et al. Association of white matter integrity with

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### The Effect of Genome-wide Supported Variant in CACNA1C on Functional Correlates of Episodic Memory Encoding and Retrieval

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**Background:** Genetic studies implicated variation in CACNA1C as a susceptibility locus for bipolar disorder, schizophrenia and major depression.

**Methods:** We investigated the influence of the CACNA1C single nucleotide polymorphism (SNP) rs1006737 on functional correlates of episodic memory encoding and retrieval. Brain activation was measured with functional magnetic resonance imaging (fMRI) during an episodic memory encoding and retrieval task in 94 healthy individuals who were genotyped for rs1006737.

**Results:** In the fMRI experiment, while there were no differences in behavioral performance, neural activation –mainly in the superior frontal gyrus and inferior and middle temporal gyrus - was associated with CACNA1C genotype during encoding and retrieval.

**Discussion:** Our data suggest that the CACNA1C rs1006737 variant influences the neural systems related to memory processes: Brain activations during encoding and retrieval of new material might compensate for otherwise insufficient performance. These findings are in line with results of imaging studies in affective disorder and schizophrenia. It may explain some of the brain activation variation found in these disorders and healthy subjects.

### Common Variants in Psychiatric Risk Genes are Associated with Brain Structure at Birth

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**Background:** A better understanding of how genetic variants affect neurodevelopment, as indexed by magnetic resonance imaging (MRI), could help identify children at risk for psychiatric disorders early in life and allow early intervention. Studies in adolescents and adults have demonstrated that polymorphisms in putative risk genes are associated with differences in brain structure, but cannot address when in development changes arise. To fully understand how genetic variants alter neurodevelopment, it is necessary to perform studies at much earlier ages.

**Methods:** To determine if common genetic variants in disrupted-in-schizophrenia-1 (DISC1, rs821616 and rs6675281), catechol-O-methyltransferase (COMT, rs4680), neuregulin 1 (NRG1, rs35753505 and rs6994992), apolipoprotein E (APOE;  $\epsilon 3\epsilon 4$  vs.  $\epsilon 3\epsilon 3$ ), estrogen receptor alpha (ESR1, rs9340799 and rs2234693), brain-derived neurotrophic factor (BDNF, rs6265), and glutamate decarboxylase 1 (GAD1, rs2270335) are associated with individual differences in brain tissue volumes in neonates, we performed structural MRI scans on 272 neonates (152 Male, 120 Female; 144 singletons, 128 twins) using a Siemens head-only 3T scanner and employed automated region of interest volumetry and tensor-based morphometry (TBM). Selected genes have received significant research attention as putative psychiatric risk genes, have well-documented roles in brain development, and have been related to neuroimaging phenotypes in older samples. For region of interest volumetry, tests were conducted at a significance level of 0.005 (Bonferroni corrected for the number of variants examined). For TBM, cluster-based inference was used to determine corrected significances while accounting for the high level of spatial dependencies between adjacent voxels.

**Results:** ESR1 (rs9340799) predicted intracranial volume (ICV) with AA homozygotes having 3.7% larger ICV than G allele carriers ( $p = 0.0028$ ). TBM revealed that local variation in gray matter (GM) volume was significantly associated with polymorphisms in DISC1 (rs821616), COMT, NRG1, APOE, ESR1 (rs9340799), and BDNF. No associations were identified for DISC1 (rs6675281), ESR1 (rs2234693), or GAD1. Of particular note, neonates homozygous for the DISC1 (rs821616) serine allele exhibited numerous large clusters of reduced GM in the frontal lobes and in lateral temporal cortex including the middle temporal gyrus; regions which anatomical likelihood meta-analyses indicate are altered in schizophrenia and bipolar disorder. Neonates homozygous for the COMT valine allele exhibited reduced GM volumes in the temporal lobe, including right hippocampus, and increased volumes in frontal and parietal areas. Neonate carriers of the APOE  $\epsilon 4$  variant, a major susceptibility factor for Alzheimer's disease exhibited reduced gray matter volume in medial temporal cortex, including hippocampus and parahippocampus. The above findings are similar to those reported in adults and may represent stable markers of risk. Other findings, such as an extensive area of reduced volume in TT homozygotes (NRG1, rs35753505) encompassing right temporal, parietal, and occipital regions, appear to be unique to this critical developmental period.

**Discussion:** Variation in putative psychiatric risk genes affects neural systems implicated in the pathophysiology of psychiatric disorders prior to birth. Results highlight the importance of prenatal brain development in mediating psychiatric risk. Ultimately, by identifying genes and by extension, molecular pathways that contribute to abnormal developmental phenotypes, it may be possible to develop treatments targeted at correcting these abnormal developmental trajectories, ultimately preventing the onset of such disorders or reducing their severity.

### Effect Of Genome-wide Supported Risk Variants For Schizophrenia and Bipolar Disorder on the Cortical Thickness of Healthy Individuals

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**Background:** To date, more than 50 Genome-wide association studies (GWAS) of bipolar disorder (BD) and schizophrenia have been reported in samples of European and Asian ancestries (NHGRI GWAS Catalog, as of 04/20/12). The most promising risk variants include ZNF804A (O'Donovan et al., 2008), the MHC region (ISC, 2009; Shi et al., 2009; Stefansson, Ophoff, Steinberg et al., 2009), ANK3 and CACNA1C (Ferreira et al., 2008; Moskvina et al., 2009; Green et al., 2010; Nyegaard et al., 2010; Ripke et al., 2011), NCAN (Cichon, Mühleisen et al., 2011), MIR137 (Ripke et al., 2011), and ODZ4 (Sklar et al., 2011). Imaging genetics represents one promising strategy to obtain insight into the functional and structural effects of these genetic risk variants on the brain, thereby hopefully leading to a better understanding of disease mechanisms. Given that disturbed neurodevelopmental processes have long been speculated in the etiology of neuropsychiatric disorders, we tested the effect of common risk genotypes for BD and schizophrenia on structural properties of brains in healthy individuals. As an indicator of possible developmental effects, we used the endophenotype cortical thickness (CT) which is a heritable, quantitative trait of the cortex structure. CT is assumed to reflect the overall architecture of neurons and glia in grey matter.

**Methods:** We computed a measure estimating the CT by a surface-based method (FreeSurfer) in 98 healthy adults from the German population using T1-weighted images from a 3 Teslamagnetic resonance imaging scanner. For each risk variant, CT of both hemispheres was compared between risk allele and non-risk allele carriers on a node-by-node basis using clustering and monte-carlo simulation (correcting the P-value for multiple-testing).

**Results:** Among the most significant findings indicative of locally reduced CT in risk allele carriers were CSMD1 rs10503253 (P=6.0E-03) from the recent Psychiatric GWAS Consortium (PGC) study of schizophrenia (Ripke et al., 2011), the well-characterized psychosis variant ZNF804A rs1344706 (P=6.7E-03), and the intergenic single-nucleotide polymorphism (SNP) rs7296288 from the PGC study of BD (Sklar et al., 2011).

**Discussion:** Our study suggests that several common genetic variants that are strongly associated with BD and/or schizophrenia have an effect on the effect on MRI-based CT measures in healthy risk allele carriers. We are currently investigating whether the highlighted cortical regions are linked to both phenotypes using available knowledge of their function. We have also initiated replication studies for the top SNPs to follow-up and strengthen our results.

### A Complex Interaction of CACNA1C And PCLO Depression Risk Alleles in the Subgenual Cingulate Activity During Associative Memory Encoding

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**Background:** Recent genome-wide association studies (GWAS) have pointed to single nucleotide polymorphisms (SNPs) of the presynaptic active zone protein Piccolo (PCLO, rs2522833) and of the neuronal calcium channel Cav 1.2 (CACNA1C rs1006737) as risk factors for affective disorders, particularly major depression (Sullivan et al., 2009; Green et al., 2009). Previous studies have implicated the subgenual cingulate (CG25) in negative mood and depressive phenotypes, and CACNA1C rs1006737 has already been linked to altered CG25 function (Erk et al., 2010). Here we tested for a potential interaction of CACNA1C and PCLO depression risk alleles in CG25, using a social memory encoding task.

**Methods:** We performed functional magnetic resonance imaging (fMRI) in 300 healthy adults at threesites (Berlin, Bonn, Mannheim). During fMRI acquisition, participants performed a blocked-design face-profession associative memory encoding task (Erk et al., 2011). Data analysis was performed using SPM8 and a two-stage mixed effects model with PCLO and CACNA1C genotypes as between-subject factors and scanning site as covariate of no interest.

**Results:** PCLO and CACNA1C risk alleles showed an epistatic interaction in CG25 (p<.05, small volume FWE-corrected), with carriers of no risk allele and of both risk alleles showing higher CG25 activation during memory encoding when compared to carriers of only one risk allele.

**Discussion:** Together with a comparable pattern in a self-reference task (see companion abstract by Assmann et al.), our findings suggest that the interaction of Piccolo and CACNA1C depression risk alleles might be region-specific, but generalizable to different cognitive tasks. Our observation of a complex epistatic interaction of two depression risk alleles supports the notion that healthy carriers of multiple depression risk alleles might additionally possess genetic or environmental resilience factors that exert protective effects, which can be observed at the level of neuroimaging endophenotypes.

### The Neural Correlates of Reward Processing in Major Depressive Disorder: A Meta-Analysis of Functional Magnetic Resonance Imaging Studies

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**Background:** A growing body of functional magnetic resonance imaging (fMRI) studies had been conducted in major depressive disorder (MDD) to elucidate the reward-related brain functions which may relate to the core symptom of anhedonia. But the heterogeneity of the results in conjunction with the occasional opposing patterns makes it difficult to obtain a clear picture of the abnormal brain reward circuitry. The aim of this meta-analysis was to examine the common reward network in MDD brain and further distinguish the brain activation patterns between emotional and monetary rewards as well as reward anticipation and outcome.

**Methods:** A series of quantitative Activation Likelihood Estimation (ALE) meta-analyses were performed across 22 fMRI studies about reward processing with a total of 327 MDD patients and 339 healthy controls. First we performed a global meta-analysis of all studies to examine the common reward network in MDD. At the second stage, ALE analyses were also conducted based on the sub-lists that categorized different experiments into emotional and monetary rewards as well as monetary reward anticipation and outcome.

**Results:** We observed several frontal-subcortical brain areas that participated in the reward processing of MDD, included the medial, middle and superior frontal gyrus, anterior cingulate, superior temporal gyrus, thalamus, brainstem, putamen, caudate, claustrum, insula and cerebellum. The core reward network in MDD is characterized by decreased striatum activity and increased prefrontal cortex (PFC) response during reward processing. In addition, the cerebellum, thalamus, parahippocampal gyrus, superior temporal gyrus and fusiform gyrus preferentially responded to emotional rewards, whereas the insula, precuneus, cuneus, posterior cingulate and brainstem selectively responded to monetary reward. Results showed reduced caudate response during both monetary anticipation and outcome stages and increased activation in the middle frontal gyrus, precentral gyrus and dorsal anterior cingulate during reward anticipation in MDD.

**Discussion:** We provided evidence for common and distinct neural correlates of reward processing in MDD. Three frontal-subcortical circuits are involved in the common reward network in MDD, the brain activation patterns are characterized by decreased striatum activity and increased prefrontal cortex (PFC) response during reward processing suggest that a dissociation of function in frontostriatal regions. There exist distinctions in the brain networks that are sensitive to processing emotional and monetary rewards as well as those are involved in reward anticipation or outcome. These finds suggest that there exist emotional or motivational pathway dysfunctions in MDD. Future behavioral and neuroimaging studies of reward reactivity in MDD may be strengthened by giving careful consideration to the type of reward used as well as different stages of reward processing, and potential implications for psychosocial treatments bear further investigation.

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### Serotonin Receptor Promoter Polymorphism 5-HTTLPR Does not Interact with Oxytocin Receptor Gene Variants to Predict Childhood-Onset Aggression

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**Background:** Childhood-onset aggression is costly to society, both financially and socially. Aggressive behaviours during the childhood and adolescent years are associated with many negative consequences. Although the etiology of aggression is still elusive, it is recognized that aggressive behaviours are heritable. Many studies have observed that genetic variants of monoamine oxidase A (MAOA), the serotonin transporter and other serotonergic and dopaminergic genes are associated with aggression. Both serotonin receptor polymorphism (5-HTTLPR) and oxytocin receptor (OXTR) gene polymorphism have previously been implicated in aggression. The purposes of this study are 1) to replicate the findings implicating 5-HTTLPR polymorphism in extreme, persistent and pervasive aggressive behaviours in children, and 2) to determine whether any OXTR polymorphisms interact with 5-HTTLPR genotypes to predict aggressive behavior.

**Methods:** The cases include 182 Caucasian children clinically referred from the Center for Addiction and Mental Health (CAMH) and Youthdale Treatment Center (Toronto, Canada). Inclusion criteria were as follows: age 6-16, a minimum 2-year history of aggressive behaviours; at or above the 90th percentile on subscale of aggressive behaviors on both the Achenbach Child Behaviour Checklist (CBCL), Teacher Report Form (TRF). Children with chronic medical illnesses and psychiatric disorders, such as schizophrenia, mania, autism and Tourette's Syndrome, were excluded. These cases were matched with 182 Caucasian controls based on gender and age. Genomic DNA was extracted from blood, cheek swab or saliva from each child using commercially available kits. PCR based TaqMan Single Nucleotide Polymorphism (SNP) assay was used to genotype 9 makers in oxytocin receptor (OXTR) gene. 5-HTTLPR polymorphism was assayed with established procedures (MspI restriction digestion following PCR amplification of 5-HTT gene fragment). Genotypic analyses were performed using STATA, and interactions were tested with Multifactor Dimensionality Reduction (MDR) software.

**Results:** In the entire sample, 5-HTTLPR genotypic frequencies did not differ between cases and controls (Chi-sq=3.58, p=0.167). Sex-stratified analyses revealed that there were significant differences in genotypic frequencies between male cases and controls (Chi-sq=7.60, p=0.022) but not in female cases and controls (Chi-sq=0.41, p=0.806). In the male subsample, participants with the ll genotype were 1.67 times more likely to be in the aggressive cases group rather than the control group; there was no such trend for participants who carried one or two copies of the s allele. We were unable to detect any significant interactions between the 5-HTTLPR polymorphism and any OXTR SNPs using both the MDR program and logistic regression models in STATA.

**Discussion:** Our results suggest that the ll genotype for the 5-HTTLPR polymorphism is associated with aggression, however the polymorphism does not interact with previously examined OXTR SNPs. While previous studies have suggested a relationship between 5-HTTLPR ss genotype and aggressive behaviours in children and adolescents, there have been studies that were unable to find an association. And another study implicated the ll genotype in predicting callous-unemotional and narcissism subscales of psychopathy in

adolescents. The discrepancy between our finding and previous reports on the association between 5-HTTLPR polymorphism and children aggression, and the failure to detect significant interactions between the 5-HTTLPR polymorphism and OXTR SNPs in predicting aggressive behaviours, may be due to the relatively small sample size, and sample specific characteristics associated with differing allelic frequencies in the current control group compared to the previous one.

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### The Association of Genetic Variation in Genes Regulating the Oxytocin-Vasopressin in Neurohumoral System with Childhood-onset Aggression

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**Background:** Aggressive behaviours in children are a major public health problem as well as being the most common reason for referrals to mental health clinics. Early or childhood-onset aggression has been associated with antisocial personality disorder, substance dependence, and other antisocial behaviours later in adulthood. While the neurobiology of aggression is complex and the etiology of aggressive behaviours remains elusive, growing evidence suggests that aggression is heritable, and certain genetic variants have been implicated as contributing factors. The oxytocin-vasopressin (OXT-AVP) neurohumoral system has recently been implicated in social behaviours. It has been reported that the cerebrospinal fluid oxytocin level is inversely, but vasopressin level is directly, correlated with a lifetime history of aggression. The purpose of this study is to determine whether genetic variation in genes regulating oxytocin-vasopressin neurohumoral system is associated with childhood-onset aggression.

**Methods:** Our sample consists 182 Caucasian children (age 6-16) displaying extreme, persistent and pervasive aggressive behaviour (a minimum 2-year history of aggressive behaviours; at or above the 90th percentile on the subscale of aggressive behaviors of the Achenbach Child Behaviour Checklist (CBCL) and Teacher Report Form (TRF) Children with chronic medical illnesses and psychiatric disorders, such as schizophrenia, mania, autism and Tourette's Syndrome, were excluded. These cases were matched with 182 Caucasian controls based on gender and age. Genomic DNA was extracted from blood, cheek swab or saliva from each child using commercially available kits. PCR based TaqMan Single Nucleotide Polymorphism (SNP) assay was used to genotype 28 markers in OXT, AVP, oxytocin receptor (OXTR), vasopressin receptor (AVPR) and CD38 genes. Genotypic differences were analyzed with STATA, and allelic and haplotype analyses were performed using Unphased v3.1.

**Results:** All 28 markers were in Hardy-Weinberg equilibrium ( $p > 0.10$ ). In the entire sample, OXTR SNP rs237898 had a nominally significant main allelic effect (A allele overrepresented in cases;  $p = 0.018$ ) and a trend-like genotypic effect (AA genotype overrepresented in cases;  $p = 0.06$ ). A haplotype consisting of OXTR rs237898A and rs237902C was overrepresented in cases ( $p = 0.032$ ). In the female subsample, OXTR rs6770632 T allele ( $p = 0.028$ ), OXTR rs1042778 AA genotype ( $p = 0.048$ ), AVPR1A rs11174811G allele ( $p = 0.040$ ), are over expressed in cases and in females. A haplotype consisting OXTR rs6770632 T and OXTR rs1042778 A alleles is also significant ( $p = 0.014$ ). In the male subsample, OXTR rs237898 A allele ( $p = 0.006$ ) and AA genotype, rs237902 C allele ( $p = 0.007$ ) and CC genotype ( $p = 0.021$ ) are overexpressed in cases. Haplotypes consisting OXTR rs237898 A and rs237902 C ( $p = 0.025$ ), OXTR rs237898 A and rs52576A ( $p = 0.014$ ), are also significant in cases. AVP rs3761249 A ( $p = 0.008$ ) allele and genotype AA ( $p = 0.037$ ) are under-expressed in cases. Haplotypes consisting AVP rs2282018 G and rs3761249 C ( $p = 0.012$ ), 1410713 T and rs2740204 T ( $p = 0.035$ ), are over-expressed in cases.

**Discussion:** Our results showed that genetic variants of OXTR and AVP are nominally associated with extreme, persistent and pervasive aggression in children and adolescents, particularly when the sample is stratified by gender. The finding that the association is gender specific

likely reflects sexual dimorphism. It has been found that OXTR expression is relatively higher in females than in males, possibly because the expression of OXTR is regulated by steroid hormones, which may contribute to the lessened effects of certain genetics variants in females as compared to males or vice versa. The small sample size, and the number of females (65) is less than the number of males (117), are the limitations of this study. Future studies with a larger sample are needed to confirm these findings.

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### The Role of Rarely Studied Serotonin Receptors 1D, 1E, 1F, 2B and 3-7 In Depression Related Traits

Krisztina Mekli<sup>1</sup>, Gabriella Juhasz<sup>1</sup>, Hazel Platt<sup>1</sup>, Fabio Miyajima<sup>2</sup>, William Ollier<sup>1</sup>, Michael Horan<sup>1</sup>, Neil Pendleton<sup>1</sup>, Anthony Payton<sup>1</sup>  
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**Background:** Depression is a serious health problem worldwide. This disorder often co-occurs with various anxiety disorders with the personality trait neuroticism being a common background for them. Extensive research has focused on the roles of genes within the serotonergic pathway, with special attention given to the HTR1A-1B autoreceptors, HTR2A-2C heteroreceptors and the serotonin transporter. The aim of this study was to investigate the influence of rarely studied serotonergic receptors (HTR1D, E, F, HTR2B, HTR3-7) in depression and related phenotypes. We hypothesised that several of them, especially the 5-HT1D, 5-HT3, 5-HT4, 5-HT6 and 5-HT7 receptors may have a role in depression, or in related phenotypes, such as anxiety and neuroticism.

**Methods:** We used haplotype tagging SNPs in a Caucasian cohort of 1563 individuals (454 males and 1109 females; mean age 67.4 years at the time of sample collection) that had continuous phenotypic measures of depression (Beck Depression Inventory, Yesavage Geriatric Depression Questionnaire, Cornell Medical Index of Depression) anxiety (Cornell Medical Index of Anxiety) and neuroticism (Eysenck Personality Questionnaire). Genotypic data was obtained from the Illumina 610 Quad microarray platform, spanning the HTR1D, HTR1E, HTR1F, HTR2B, HTR3A-B, HTR3C-E, HTR4, HTR5A, HTR6 and HTR7 genes.

**Results:** The strongest associations were observed between the rs4912138\_A allele (in the HTR6 gene) and Yesavage depression score and three SNPs in the HTR4 gene (rs17108435\_C, rs4280857\_A and rs1011427\_T) that were associated with CMI anxiety scores, Yesavage depression scores and CMI depression scores ( $p < 0.005$ ). Two SNPs in the HTR3 gene also showed relatively strong association ( $p < 0.05$ ) to CMI anxiety and Beck depression scores (rs939335\_G and rs10891611\_C, respectively). After correction for multiple testing (LD based method) even with moderately strict parameters, only three results remained significantly associated with depression, two SNPs in the HTR6 gene and one in the HTR4 gene but none of them survived the Bonferroni-correction.

**Discussion:** We found several nominal associations. These SNPs were located in the HTR4 and HTR6 genes. We found indications in the literature of animal studies and human association studies to corroborate these findings. However, none of these associations survived Bonferroni correction for multiple testing. Despite the limitations of the candidate gene approach it is important to disclose nominally significant results as accumulating evidence that in polygenic multifactorial disorders the individual genetic effect may be very small and thus require replication.

**Association Between CYP2D6 Gene Dosage and Tardive Dyskinesia in English Caucasians**

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**Background:** An association between cytochrome P450 2D6 metabolizer status and susceptibility to typical antipsychotic-induced tardive dyskinesia (TD) has previously been reported. However, overall the data are inconclusive. Our aim was to examine whether there was an association between TD and number of functional CYP2D6 genes (CYP2D6 gene dosage).

**Methods:** A Caucasian sample of 72 patients was recruited in 1996-1997 from South London and the Maudsley National Health Service Trust. Subjects had a DSM-III-R diagnosis of schizophrenia treated with typical antipsychotics at doses equivalent to at least 100 mg chlorpromazine daily for at least 12 months prior to assessment. Subjects were examined for TD using the Abnormal Involuntary Movements Scale (Schooler & Kane, 1982). All patients were genotyped for CYP2D6 alleles\*3-5, \*41, and for amplifications of the gene. The AmpliChip CYP450 Test® was performed in eight subjects for whom CYP2D6 genotype could not initially be definitively called.

**Results:** Thirty-five out of 72 patients (48.6%) met the criteria for drug-induced parkinsonism (DIP), and 13 of 72 (18.1%) met the criteria for TD. Thirty-nine were male [54.2%, mean age 35.6 (±12.4) years, range 19-80 years], and thirty-three female [45.8%, mean age 49.5 (±18.9) years, range 23-87 years]. The mean age of the TD-positive subjects (39.6±11.6 years) did not differ significantly from that of the TD-negative group (42.5±18.4 years, independent t-test,  $t=0.540$ ,  $df=0$ ,  $p=0.59$ ). For the TD-positive group, the mean duration of typical antipsychotic treatment (189.7 months, range 60-348, SD 94.5) was significantly greater than that for the TD-negative group (132.5 months, range 14-396, SD 87.3; independent t-test,  $t=2.106$ ,  $df=70$ ,  $p=0.004$ ). The presence of TD was also significantly associated with the presence of DIP (one sample Kolmogorov-Smirnov test; Kolmogorov-Smirnov Z for TD= 4.233,  $p<0.000$ , and Kolmogorov-Smirnov Z for DIP=2.943,  $p<0.000$ ), but gender did not differ significantly between the TD-positive and TD-negative groups ( $\chi^2 = 3.47$ ,  $df = 1$ ,  $p=0.39$ , Fisher's exact test).

On logistic regression analysis (dependent variable: presence of the Research Diagnostic Criteria (RDC) probable TD, or RDC persistent TD) versus CYP2D6 gene dosage, controlling for potential confounding variables (age, gender, duration of antipsychotic treatment, and DIP), there was a positive association between CYP2D6 gene dosage (Wald  $\chi^2=4.222$ ,  $df=1$ ,  $p=0.04$ , odds ratio=0.206, 95%CI = 0.046-0.93) and between CYP2D6 genotypic category (Wald  $\chi^2 4.383$ ,  $df=1$ ,  $p=0.04$ , odds ratio=0.497, 95%CI=0.26-0.96) and TD.

**Discussion:** We found a significant association between CYP2D6 genotypic category and TD and between CYP2D6 gene dosage and TD, with the direction of effect being an increase in the number of functional CYP2D6 genes being associated with an increased risk of

TD. CYP2D6 is found in the nigrostriatal pathway. It is therefore possible that increased oxidative metabolism resulting from higher CYP2D6 gene dosage may be responsible for higher plasma or local neuronal levels of neurotoxic antipsychotic drug metabolites, and hence, increased risk for the development of TD. In line with this hypothesis, levels of superoxide dismutase (SOD), which is thought to reduce cellular oxidative stress and neuronal damage via the inactivation of oxygen free radicals, have been found to be increased in patients on antipsychotic treatment, and SOD is induced in response to increased oxidative tone, which would be likely to occur in those with higher CYP2D6 gene dosage (Allen 1991, Zhang et al., 2003).

### Establishing Biological Sampling Methodology for Genetic and Epigenetic Studies in Young People

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**Background:** Establishing appropriate biological sampling methodology for pharmacogenetic analysis in young people is part of the research funded in Workpackage 3 of the STOP study ([www.stop-study.com](http://www.stop-study.com)), Suicidality: Treatment Occurring in Pediatrics. The types of samples that are feasible and acceptable to patients, carers, and clinicians to collect, and fit for their intended purpose, may be different in children and adolescents compared to adults. Our aim was to extract DNA from various sample types and conduct quality control analysis thereof, including genotyping using various technologies, in order to generate a DNA sampling Standard Operating Procedure (SOP) for genetic and pharmacogenetic studies in this age group.

**Methods:** At King's College London (KCL), 30 adult volunteers provided four different sample types (5ml venous blood, buccal swabs x 10, 2 ml saliva using the Oragene kit, and 2.5 ml saliva using an in-house collection method) for DNA extraction. At the Central Institute of Mental Health (CIMH) in Mannheim, 30 adult volunteers provided three different sample types (buccal swabs x 10, 2 ml saliva using the Oragene kit, and 10 ml saliva collected using an antiseptic mouthwash). Quality control (QC) analysis was conducted by quantification using various methods (UV spectrophotometry, NanoDrop 2000, fluorimetry), and by agarose gel electrophoresis. Genotyping at KCL included long-PCR, DMETPlus® microarrays, and methylation analysis using Sequenom, and at CIMH variable number tandem repeat (VNTR), single nucleotide polymorphism (SNP) genotyping, and methylation analysis using pyrosequencing was conducted. The University of Barcelona (UB) collected samples using the Oragene kit from 3 different age groups: children (3-7 years), adolescents (13-15 years), and adults, <http://www.stop-study.com/>.

**Results:** QC analysis. In samples collected by KCL, the mean concentration (by UV spectrophotometer) of DNA extracted from blood samples was comparable to that extracted from Oragene kits (227±44ng/μl vs. 224±184ng/μl), and greater than that extracted from the latter two methods (72.5±45ng/μl and 74.8±60ng/μl respectively). Agarose gel electrophoresis revealed a more variable molecular weight profile for buccal swab DNA in comparison to DNA extracted using the Oragene kit. CIMH similarly found that the Oragene kit performed the best out of their three sample types (total yield on NanoDrop quantification: 220.9±119μg vs. 1.4±1μg vs. 53.1±46μg). UB found that the extracted DNA concentration was adults>adolescents>children, and that the yield for children <12 years could be increased by employing a modified extraction protocol. Genotyping. KCL genotyped 8 samples from the 4 sample types using the Affymetrix DMETPlus® Array, and found comparable call rates to 3 Affymetrix controls (99.68±0.19, 99.51±0.33, 99.56±0.21, 99.13±1.45 vs. 99.67±0.03). CIMH genotyping of the DAT1 intron 8 VNTR and SNP rs1006737 (using a TaqMan assay) was successful in all sample types. Methylation analysis at KCL and CIMH revealed an approximately equivalent number of assayable CpG sites, with tissue-specific methylation likely contributing to some minor observed differences in percentage methylation.

**Discussion:** Although DNA extracted from a variety of sample types may be successfully used for genotyping on a variety of platforms, DNA derived from the Oragene kit performed best overall on quality control analysis. SOPs for collection of DNA using the Oragene kit in various age groups have been generated and will be presented.

### Early Antidepressant Efficacy Modulation By Glutamatergic Gene Variants in The Star\*D

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**Background:** The glutamatergic system has been suggested as a modulator of rapid antidepressant response. Therefore, the Sequenced Treatment Alternatives to Relieve Depression (STAR\*D) genetic dataset was reanalyzed focusing on glutamatergic gene variants, timing and stability of antidepressant response.

**Methods:** 44 genes involved in the interconnections between the glutamatergic and monoaminergic systems were selected according to literature and investigated in 1541 major depressive patients from the STAR\*D genome wide dataset. Outcomes of interest were early response (2nd week) and later response (from the 4th to the 14th week) compared to non response and stability of response through the first 14 weeks of treatment in the STAR\*D level 1. In order to limit possible genetic admixtures within the database, only white (1109 non Hispanic and 192 Hispanic) and African-American (n=240) subjects were included. A complete agglomerative clustering, based on pairwise identity-by-state (IBS) matrix, was applied in order to further control for genetic admixture. Each of the 10 multidimensional scaling (MDS) values for each individual was tested for association with the phenotype. A chi-square test was employed to test the association of glutamatergic variants with phenotypes of interest under genotypic, allelic, dominant and recessive models. A logistic regression model was employed to corroborate SNPs associated to outcome at liberal  $p < 0.001$ . Clinical-demographic variables (age, gender and baseline severity) and MDS-based measure of ancestry were used as covariates. A Bonferroni correction for multiple testing was applied. PLINK served for the analysis.

**Results:** 1995 SNPs were available after quality control. Both chi-square test and logistic regression suggested that the rs1083801 within the GRM7 (glutamate receptor, metabotropic 7) gene was associated to early response under a recessive model (GG genotype observed in 14.34% of early responders vs 5.25% of late responders, OR=0.33, 95% CI=0.21-0.53,  $p=5.53e-06$ . GG genotype observed in 5.34% of non responders, OR=0.33, 95% CI=0.20-0.56,  $p=3.93e-05$ ). The result was confirmed in the white non Hispanic group (GG genotype observed in 17.46% of early responders vs 5.81% of the rest of the sample, OR=0.29, 95% CI=0.19-0.46,  $p=1.968e-07$ ). No marker predicted the stability of response after Bonferroni correction.

**Discussion:** Preclinical studies has suggested GRM7 as a promising candidate gene for involvement in antidepressant response, but this is the first study which supports that GRM7 may play a role in antidepressant efficacy in humans. The evidence of involvement of glutamatergic gene variants in early antidepressant efficacy may be relevant in further understanding the pathophysiology of the drug induced antidepressant effect.

### Association Study Of IMPA1, IMPA2 and INPP1 Genes and Lithium Response in Bipolar Disorder: A Pharmacogenetic Study

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**Background:** Lithium (Li) is still considered the first-line treatment in bipolar disorder (BD). In this regard, clinical data show that ~80% of chronically-treated subjects are at least partial responders and that ~30% of these patients are excellent responders with a complete remission of symptoms (Garnham et al., 2007). It has been suggested that lithium-responsive BD may be a distinct subtype of the disorder and that lithium response has a genetic component. It has been shown that i) response to Li appears to be a stable trait (Berghofer et al., 2008), ii) responsive patients tend to present a family history of BD (Smeraldi et al., 1984) and iii) concordant BD twins have better rates of Li response than discordant pairs (Mendlewicz et al., 1978). However, the molecular basis of Li's mechanism of action is still unclear. Although several putative pharmacological mechanisms of Li have been hypothesized, the pathway involving inositol metabolism is one of the most accepted. In this sense, findings support the hypothesis that lithium depletes free inositol, ultimately inhibiting this signalling pathway after chronic administration (Berridge et al., 1989). The aim of this study was to investigate the potential association of genetic variability at IMPA1, IMPA2 and INPP1 genes with response to lithium in BP.

**Methods:** Our sample consisted of 110 unrelated Caucasian bipolar outpatients from the Bipolar Disorder Unit of the Hospital Clinic of Barcelona and from primary care settings from Oviedo. Inclusion criteria were (a) bipolar I or II DSM-IV-TR diagnosis and (b) age > 18 years. Exclusion criteria were the presence of (a) mental retardation and (b) severe organic disease. Genomic DNA was extracted from blood samples from each participant, according to standard protocols. Several SNPs at the IMPA1 (rs915, rs1058401 and rs2268432), IMPA2 (rs669838, rs1020294, rs1250171 and rs630110) and INPP1 (rs3791809, rs4853694 and rs909270) genes were genotyped using Taqman 5'-exonuclease assay. All patients were grouped and compared according to their level of response to lithium treatment. The distribution of all genotypes was in Hardy-Weinberg equilibrium. Patients were classified as excellent responders, partial responders and non-responders. For statistical purposes, excellent and partial responders were grouped. Patients showing no tolerability to lithium were not considered. Categorical variables were compared using Chi-square or Fisher exact tests, as appropriate. Analyses were performed using PASW v18.0 and EpiInfo. Linkage disequilibrium (LD) between the two markers was tested with Haploview v4.1. Haplotype analyses were conducted using R-program v2.2.1 and Haploview v4.1. All procedures were approved by the research ethics committees in each institution.

**Results:** Our results showed that non-responders presented higher frequencies of the GG genotype of the rs630110-IMPA2 gene than responders (excellent and partial) (OR= 2.9; 95%CI [1.05-8.19];  $\chi^2=4.9$ ;  $p=0.023$ ). We also found that non-responders were more likely to present GG genotype of the rs909270-INPP1 gene (OR= 3.19; 95%CI

[1.08-9.52];  $\chi^2=5.66$ ;  $p=0.04$ ) when compared to responders to lithium. No other significant association was found when analyzing other SNPs. Haplotype association analysis showed that CG combination (rs1250171-rs630110) at the IMPA2 gene was more frequent in non-responders to Li than in responders (51.2%vs32.4%;  $\chi^2=5.79$ ;  $p=0.01$ ). On the contrary, CA combination of the same two SNPs was more frequent in responders than in non-responders (36.4%vs18.5%;  $\chi^2=5.8$ ;  $p=0.01$ ). No other significant haplotype combination was found.

**Discussion:** Our findings suggest that lithium-responsive bipolar disorder's phenotype seems to be associated with genetic variability at IMPA2 and INPP1 genes. Specifically, haplotype analysis seems to support the involvement of the IMPA2 gene in lithium response. In this sense, a previous positive association with Li response was shown for two polymorphisms in the IMPA2 gene (Dimitrova et al., 2005). Therefore, these results not only would strengthen those studies that have suggested the potentially significant role of genetic variability at the inositol pathway in lithium prophylactic efficacy but also reinforce its involvement in the pathophysiology of bipolar disorder. Acknowledgements: Centro de Investigación en Red de Salud Mental (CIBERSAM); Instituto Carlos III (PI080247)-PN 2008-2011-Subdirección General de Evaluación y Fomento de la Investigación; Fondo Europeo de Desarrollo Regional. Unión Europea. Una manera de hacer Europa; Spanish Ministry of Economy and Competitiveness; Comissionat per a Universitats i Recerca del DIUE (2009SGR827) and to the Bipolar Disorders Group (2009 SGR 1022).

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### The Pharmacogenomics of Bipolar Disorder - Acute And Longitudinal Treatment Aspects: A Systematic Review

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**Background:** Pharmacotherapy constitutes a mainstay in the treatment of both acute episodes and in maintenance therapy of bipolar disorder (BD). For more than two decades, there have been attempts to clarify the genetic basis of mechanisms of drug actions, aiming at the possibility to offer a more personalized medicine. Here we compile the current state of pharmacogenomics of BD.

**Methods:** First, we decided to focus on the pharmacogenetic investigation of first line treatments for BD on the basis of international guidelines. We focused on drugs recommended as monotherapies. PubMed was searched for articles published until September 2011 using the search terms "bipolar disorder" or "manic-depressive illness" cross-referenced with drugs in question. We also reviewed the lists of the identified publications manually. From these, we selected case-control-association studies, with the case-control-status being the drug response or the occurrence of drug induced adverse effects (ADR).

**Results:** As regards response, we selected 28 studies, with the following breakdown: lithium (25), lamotrigine (1), divalproex (1), olanzapine (1). As regards ADR, our search algorithm yielded one study on divalproex. Most of the pharmacogenetic research in BD is about lithium, while there are only few case-control-association-studies concerning the other first line treatments. The candidate genes studied for lithium included 5-HT2A, 5-HT2C, 5-HTTLPR, AP-2 $\beta$ , BCR, BDNF, COMT, DAT1, DGKH, DRD1, DRD2, DRD3, DRD4, FYN, G $\beta$ 3, GABRA1, GR, GRIA2, GRIN2B, GRK3, GSK3- $\beta$ , HTR2A, IMPA1, IMPA2, INPP1, MAO-A, MARCKS, NR1D1, NTRK2, ODZ4, SDC2, SERTPR, SV2B, and XBP1.

**Discussion:** There is a striking lack of replicated findings. For only two genes, the 5-HTTLPR and the BDNF gene, positive findings could be replicated in a second study. Also, most studies included very small samples, with the majority totaling less than 200 subjects. Future pharmacogenomic research should be based on larger samples, unified, exact criteria for clinical response, non-response and ADR, integrate knowledge from biochemical pathways, and also include pharmacokinetic and-dynamic aspects.

### Multiple Single Nucleotide Polymorphisms of Schizophrenia-Related DISC1 Gene in Lithium-Treated Patients with Bipolar Affective Disorder

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**Background:** Linkage between schizophrenia and chromosome 1q42.2 markers has been reported in different populations worldwide. This region contains DISC1 gene which had been found to be disrupted by a balanced translocation and to co-segregate with schizophrenia in a large Scottish pedigree. Association studies investigating DISC1 single nucleotide polymorphisms (SNPs) in schizophrenia and bipolar affective disorder in a number of samples were promising but not yet compelling. DISC1 disturbances are considered to be a component in neurodevelopmental processes postulated in schizophrenia but also in bipolar affective disorder [1]. Neurodevelopmental processes are among those implicated in the mechanism of action of mood stabilizers [2]. In the present study, we aimed to investigate the possible contribution of 1q42.2 markers to the prophylactic lithium treatment response in patients with bipolar affective disorder.

**Methods:** The study sample counted 87 patients (55 females, 32 males) with bipolar affective disorder, treated with lithium carbonate. All individuals were derived from the Polish population of Wielkopolska region. The diagnosis was based on DSM-IV criteria - with the use of structured interview (SCID I), and consensus by two independent clinicians. Alda's scale, ranging from 0 (no response) to 10 (excellent response) was used for the assessment of the clinical outcome (trait's average in the studied sample = 6,5). We investigated 34 SNPs at the 1q42.2 locus (TSNAX/DISC1), chosen on the basis of previous association findings in schizophrenia and bipolar affective disorder. A MALDI-TOF mass spectrometry-based SNP genotyping (Sequenom's iPLEX technology) technique was used. For statistical analysis FAMHAP18 software was used.

**Results:** All SNPs tested were in concordance with Hardy-Weinberg equilibrium. SNP rs11122330 resulted in positive association ( $p=0,03$ ) of the A allele with the better Alda's scale outcome than G allele (on average 1,5 point comparing to non A allele carriers). The association was also present at the genotype level – for AA genotype ( $p=0,02$ ) and AG genotype ( $p=0,02$ ), with an average response better of 1,7 point when compared to GG genotype carriers (trait's average=5). Another SNP rs821581 showed an association of a rare genotype GG with visibly poorer (of average 3,3 points in Alda's scale comparing to AA and AG genotypes) response to lithium ( $p=0,03$ ).

**Discussion:** We observed the association between therapeutic lithium action and polymorphism of DISC1 - gene related with neurodevelopmental processes. We were able to detect a modest effect of a single SNP (rs11122330) on the lithium treatment efficacy. However observed association must be treated with caution due to the fact that the allele A and genotypes (AA and AG) related with better response are only slightly above the overall average response. Therefore practical value may be to point at the individuals carrying the allele G as predicting poorer response. The main limitation of the study is a small sample size. For the effect of the second associated SNP (rs821581) it must be clearly emphasized that extremely small

number of individuals with GG genotype ( $n=3$ ) that was related with worse response to the lithium treatment does not allow for reliable conclusions. References: [1] Harwood, A. J., 2003 Neurodevelopment and mood stabilizers. *Curr Mol Med.* Aug;3(5), 472 - 82. [2] Perlis, R. H., Purcell, S., Fagerness, J., Kirby, A., Petryshen, T. L., Fan, J., Sklar, P., 2008 Family-based association study of lithium-related and other candidate genes in bipolar disorder. *Arch Gen Psychiatry.* Jan;65(1), 53 - 61. [3] Grof P., Duffy A., Cavazzoni P., Grof E., Garnham J., MacDougall M., O'Donovan C., Alda M., 2002 Is response to prophylactic lithium a familial trait? *J Clin Psychiatry.* Oct;63(10), 942-7.

### Haloperidol in Acute Psychosis: Impact Of 544 Genetic Variations among 98 Genes on Treatment Efficacy and Side Effects

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**Background:** Pharmacogenetics holds the potential to describe the pharmacodynamics and pharmacokinetics of the current prescribed medications. Genes whose variations drive the short term antipsychotic response and side effects are still to be clearly defined, despite decades of research. We previously investigated a sample of psychotic patients treated with haloperidol. As a main result, a variation harbored by the SLC6A5 (the glycine transporter a regulator of the glutamatergic system) modified the time course of haloperidol induced motor side effects, but no significant association was detected among the genetic variations and the efficacy of antipsychotic treatments. Aims: in the present study, we extend the list of candidate genes that could impact the efficacy of haloperidol treatment based on recent findings in literature. We included genes investigated in candidate associations and genes that belong to molecular pathways whose activity is consistent with the results from the genome-wide investigations. Overall, the influence of 98 candidate genes on both efficacy and tolerability to haloperidol treatment was evaluated.

**Methods:** An investigation sample and a sample for independent replication were analyzed. In the investigation sample, 96 acutely psychotic patients treated with haloperidol were genetically characterized for 544 SNPs located in candidate genes and weekly assessed through Positive and Negative Syndrome Scale (PANSS), Extrapyramidal Symptom Rating Scale (ESRS) and Udvalg for Kliniske Undersogelser side effects rating scale (UKU) scales during the first month of treatment. Multivariate analyses were employed to test possible influences of polymorphisms on clinical and safety variables. Analysis of haplotypes was also performed. The replication sample was constituted of 129 schizophrenic patients from the Clinical Antipsychotic Trials in Intervention Effectiveness (CATIE) study. Outcomes in the replication sample were response vs. non response and the presence vs. absence of motor side effects at one month of treatment.

**Results:** Concerning haloperidol tolerability, rs2242480 located in the CYP3A4 was associated with a different distribution of the UKU neurologic scores through time (permutated  $p = 0.047$ ), in absence of a major impact on haloperidol plasma levels. This finding was not replicated in the CATIE sample. Concerning haloperidol short-term efficacy, rs2242592 within ANKK1 gene and rs1124493 within DRD2 gene were associated with clinical improvement (respectively  $p=0.008$  and  $p=0.001$ ) in the investigation sample. Results were confirmed in the allelic analysis. Three haplotype blocks, one among ANKK1 and two among DRD2 gene were associated with better clinical improvement. Our results were not replicated in the CATIE sample, although rs11604671, which is in strong linkage disequilibrium with rs2242592, was associated with response in the replication sample. Further, rs2298723 (GRIK4) and rs183294 (GABRG2) showed trends of association with clinical response in both samples.

**Discussion:** CYP3A4 is involved in the pharmacokinetics of haloperidol and its activity is induced by this drug. According to our results, the rs2242480 located in CYP3A4 may impact on the resilience of neurons to haloperidol toxicity. Further, our findings support a

possible role of ANKK1, DRD2, GRIK4 and GABRG2 variability on haloperidol efficacy. However, cautiousness is mandatory in interpreting the results because of the low level of associations and the clinical discrepancies between the original and replication sample.

### Quantitative Trait Loci Localized for Spatial Working Memory: General and Family Specific Loci

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**Background:** Working memory is a locus of dysfunction in schizophrenia and is associated with genetic liability for the illness. In two prior samples, we demonstrated that a spatial delayed response task (SDRT) is sensitive to genetic liability for schizophrenia. Individuals with schizoaffective disorder and psychotic bipolar disorder are impaired on this SDRT while non-psychotic bipolar patients were not impaired. Thus, identifying the genetic factors that influence this SDRT should provide candidate genes for psychosis. Here, we examined the genetic underpinnings of this SDRT using standard and pedigree specific linkage analysis.

**Methods:** 883 Mexican-American individuals from extended pedigrees received the SDRT. 432 dinucleotide repeat microsatellite loci, 10 cM intervals apart, were assessed and linkage was performed in SOLAR.

**Results:** SDRT performance was 82% and heritability was estimated to be 0.246,  $p=9.0 \times 10^{-7}$ . Using standard linkage analyses, a QTL was localized at chr 12:143cM (LOD=3.64). Post-hoc analyses reveal that at least four pedigrees contribute to this QTL signal suggesting the presence of either common functional variants or multiple rare variants in the region of the linkage peak. Using pedigree/founder-specific linkage analysis, we localized an additional three QTLs, with respective LODs of 3.85 (chr 4:22cM), 3.09 (chr 13:12cM) and 3.08 (chr 8:46cM).

**Discussion:** We identified both common and family specific genome-wide significant loci influencing performance on a spatial working memory test that is an endophenotype for schizophrenia. Our results strongly suggest that the traditional approach to quantitative trait linkage analysis may miss QTL signals due to rare variants within specific pedigrees.

### Small Candidate Gene Studies of the Acute Response to Amphetamine Fail to Replicate

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**Background:** Humans vary in their responses to d-amphetamine, and this variation is heritable. Sensitivity to the subjective effects of a drug may predict its abuse liability; therefore, genetic variation underlying subjective responses may also influence drug abuse risk. Thus, the acute response to the drug can be viewed as an intermediate phenotype for dependence and abuse. Intermediate phenotypes are suggested to be more directly linked to genetic variants, which would in turn show greater effect sizes, potentially allowing for the use of smaller samples.

**Methods:** We have previously reported genetic associations with ten genes and responses to d-amphetamine in 99-162 healthy, non-drug abusing volunteers. Participants received d-amphetamine (10 or 20 mg) or placebo in randomized order and completed self-report questionnaires (POMS, DEQ, ARCI) regular intervals.

**Results:** We observed significant associations with the following genes: ADORA2A, BDNF, COMT, CSNK1E, DRD2, FAAH, OPRM1, SLC6A2, SLC6A3, and SLC6A4. We have now collected an additional 236 participants tested using identical methodology, providing us with a strong sample for a replication study, without the presence of sample and methodological heterogeneity. Despite this strong sample, none of the 12 previously observed associations replicated, strongly suggesting that the initial findings were false positives.

**Discussion:** Possible explanations for the prevalence of false positives in our prior studies may be the use of small sample sizes, failure to completely account for multiple testing within and between these studies, and the presence of publication bias. Thus, our results have broad implications for human candidate gene studies that use small samples, and suggest the need for a paradigm shift away from small, likely underpowered candidate gene studies.

### BDNF and NTRK2 Polymorphisms and Antidepressant Treatment Outcome

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**Background:** Data from clinical studies and results from animal models suggest a major involvement of the neurotrophin system in the pathology of depression and antidepressant treatment response. Genetic variations within the genes coding for the brain-derived neurotrophic factor (BDNF) and its key receptor Trkb (NTRK2) may therefore influence the response to antidepressant treatment.

**Methods:** We performed a single and multi-marker association study with antidepressant treatment outcome in 398 depressed Caucasian inpatients participating in the Munich Antidepressant Response Signature (MARS). Two Caucasian replication samples (N=249 and N=247) were investigated, resulting in a total number of 894 patients. 18 tagging SNPs in the BDNF gene region and 64 tagging SNPs in the NTRK2 gene region were genotyped in the discovery sample; 16 nominally associated SNPs were tested in two replication samples.

**Results:** In the discovery analysis, 7 BDNF SNPs and 9 NTRK2 SNPs were nominally associated with treatment response. Three NTRK2 SNPs (rs10868223, rs1659412 and rs11140778) also showed associations in at least one replication sample and in the combined sample with the same direction of effects (P<sub>corr</sub>=.018, P<sub>corr</sub>=.015 and P<sub>corr</sub>=.004, respectively). We also observed an across-gene BDNF-NTRK2 SNP interaction withstanding correction for multiple testing (rs4923468 and rs1387926) and a significant interaction effect of rs10868223 and rs11140778 with BDNF serum levels as a predictor for treatment outcome.

**Discussion:** Although not all associations in the discovery analysis could be unambiguously replicated, the findings of the present study provide further substantiation for a possible involvement of genetic variations in the BDNF and NTRK2 genes in antidepressant treatment outcome.

### A Potential Role for The Melanocortin-3 Receptor Gene in Antipsychotic Induced Weight Gain in Schizophrenia Patients

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**Background:** Many effective antipsychotic medications treat core symptoms of schizophrenia (SCZ), including the onset of substantial weight gain. Early diagnosis of metabolic disturbances through genetic testing may become very important to prevent the morbidity and mortality resulting from medication use. Variants in the melanocortin-4 receptor (MC4R) genomic region have been strongly associated with antipsychotic induced weight gain. Given the expression of the melanocortin-3 receptor (MC3R) in the hypothalamus, and recent associations with general obesity, we investigated the potential role of the melanocortin-3 receptor (MC3R) SNPs with antipsychotic induced weight gain.

**Methods:** Nine MC3R SNPs (rs6127698, rs6024731, rs1543570, rs6024730, rs6014649, rs1926064, rs6014646, rs11697509, rs3827103) were selected by tagSNP for full gene coverage. Variants were genotyped using Illumina GoldenGate Genotyping Assays. The MC3R SNPs were genotyped in 217 chronic schizophrenia patients who underwent treatment and were evaluated for AIWG for up to 14 weeks. We compared weight change (%) across genotypic groups using analysis of covariance.

**Results:** Significant genotypic associations were found between the MC3R polymorphisms and weight gain (p<0.05). In the overall sample, the following SNPs were significantly associated with weight gain: rs6024731 p = 0.033; rs1543570 p = 0.045, rs6024730 p = 0.021; rs6014649 p = 0.000; rs11697509 p = 0.001; rs3827103 p = 0.003. We evaluated MC3R variants in a refined sub-sample consisting of patients who were of European ancestry and treated with either clozapine or olanzapine and found that the MC3R variants that remained significantly associated with weight gain included: rs1543570 p = 0.004; rs6014649 p = 0.025; rs11697509 p = 0.004.

**Discussion:** In this study, we observed that MC3R gene variants were nominally associated with antipsychotic induced weight gain. These findings, in combination with previous reports of melanocortinergic system gene variants, suggest that these hypothalamic genes may have a role in the development of antipsychotic induced weight gain. If the association between melanocortinergic gene variants and weight gain continue to be replicated, this may aid in antipsychotic drug development and result in personalized treatment.

### Pharmacogenetics of Twelve Candidate Genes and Antidepressant Response in Obsessive-Compulsive Disorder

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**Background:** Obsessive-compulsive disorder (OCD) is a chronic and debilitating disorder with a strong genetic component. The serotonergic, dopaminergic, glutaminergic, and neuro-inflammatory systems have been implicated in the pathoetiology of OCD. Genetic associations between OCD and several candidate genes including solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1 (SLC1A1), monoamine oxidase (MAOA), glutamate receptor, ionotropic, N-methyl D-aspartate 2B (GRIN2B), serotonin 2A receptor (5HTR2A), solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4), and catecholamine-O-methyl-transferase (COMT) genes have been reported albeit with inconsistent results. Pharmacogenetics represents an important alternate to investigate inter-individual genetic variation and drug response, which may further aid to clarify the role of these candidate genes in OCD. In this preliminary study, we investigated 12 different genes including those mentioned above in addition to the disks large (drosophila) homolog-associated protein 2 (DLGAP2), myelin oligodendrocyte glycoprotein (MOG), serotonin 1B receptor (5HT1B), chromosome 9 open reading frame 68 (C9orf68), adenosine deaminase, RNA-specific, B2 (ADARB2), and oligodendrocyte lineage transcription factor 2 (OLIG2) genes.

**Methods:** Thirty, two, six, sixteen, eleven, six, two, ten, sixteen, ten, ten, and ten single nucleotide polymorphisms (SNPs) in the DLGAP2, MOG, 5HT1B, SLC1A1, C9orf68, MAOA, ADARB2, GRIN2B, 5HTR2A, SLC6A4, OLIG2, and COMT genes respectively were genotyped in 117 individuals with OCD and retrospective response data collected on multiple serotonin reuptake inhibitor (SRI) trials. Individuals were grouped into those who improved following an adequate trial of one or more SRI(s) as compared with those who reported “minimal”, “no change”, or “worsening” in response to SRI(s) tried. Genotypes and response data were examined by exploratory analyses on a drug-by-drug and combined basis.

**Results:** Significant response associations were detected in DLGAP2 and paroxetine/clomipramine (P=0.008-0.042), 5HT1B and clomipramine/any SRI(s) (P=0.0003-0.045), SLC1A1 and sertraline/fluvoxamine/citalopram/any SSRI(s)/any SRI(s) (P=0.0008-0.035), C9orf68 and clomipramine/fluvoxamine/any SSRI(s) (P=0.007-0.031), MAOA and citalopram (P=0.028), GRIN2B and fluoxetine/paroxetine/fluvoxamine/citalopram/clomipramine (P=0.004-0.024), 5HTR2A and clomipramine (P=0.024), SLC6A4 and paroxetine (P=0.0006-0.010), OLIG2 and paroxetine (P=0.008), and COMT and paroxetine/sertraline/citalopram/any SSRI(s)/any SRI(s) (P=0.007-0.044). Analyses for association with response to other drug trials were negative.

**Discussion:** These results suggest that genetic variants may play an important role in SRI response to OCD. However, replication in larger and independent samples is required.

### Genome-wide Association Study of Weight Development During Antidepressant Treatment

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**Background:** Weight gain during psychopharmacological treatment frequently constitutes a crucial factor regarding compliance, continuation of treatment and development of secondary metabolic disorders. Besides drug-specific effects, high inter-individual differences in weight gain during antidepressant treatment exist.

**Methods:** In order to elucidate genetic risk factors underlying weight development during psychopharmacological treatment we performed a genome-wide association analysis in the MARS (Munich Antidepressant Response Study)-sample consisting of 720 inpatients with major depression. Genotyping was performed using Human610-Quad BeadChip (Illumina Inc, San Diego, California).

**Results:** We were not able to detect genome-wide significant signals for weight change analyzed for five weeks of treatment and overall duration of hospitalization after quality control and correction for multiple testing. The best association results (p-values~10<sup>-6</sup> to 10<sup>-7</sup>) were located on chromosomes 7, 16, and 17.

**Discussion:** Identifying genetic risk factors for weight development could provide insights into biological mechanisms underlying weight gain during psychopharmacological treatment. Furthermore, these results could have an important clinical impact on individualized disease management and metabolic surveillance strategies. The results of this study need to be replicated in independent samples and followed up by functional studies.

### Association between Antipsychotics-Related Restless Legs Syndrome and Clock and NPAS2 Genes in Schizophrenia

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**Background:** This study aimed to investigate whether polymorphisms of CLOCK and NPAS2 genes are associated with antipsychotic-related restless legs syndrome (RLS) in schizophrenia. Restless legs syndrome (RLS) has been reported to be more prevalent in schizophrenic patients who take antipsychotics. The cause of RLS is unknown but associated with dopaminergic deficiency. Additionally, circadian oscillation can be a possible mechanism of antipsychotic-related RLS because it presents only in evening or night time.

**Methods:** We assessed antipsychotic-related RLS symptoms in 190 Korean schizophrenic patients and divided the subjects into two groups using the IRLSSG (International Restless Legs Syndrome Study Group) diagnostic criteria: (i) subjects that met all of the criterion who were considered as the definite RLS patients (n=44) (ii) the other subjects who were not considered as RLS, even though some have a few RLS-like symptoms (n=146). Genotyping were performed for the CLOCK gene (rs2412646 and rs1801260) and the NPAS2 gene (rs2305160 and rs6725296) by PCR based methods. The method of multifactor dimensionality reduction was used to analyze gene-gene interactions.

**Results:** There were significant differences in the genotype and allele frequencies of rs2412646 in CLOCK (p=0.031 and 0.010, respectively). However, the genotypes and allelic frequencies of rs1801260 of CLOCK and NPAS2(rs2305160 and rs6725296) between the two groups were not significantly different. Furthermore, MDR analyses provided significant evidence for gene-gene interactions (CLOCK and NPAS2) in the development of RLS.

**Discussion:** Our Study results suggest that CLOCK gene is associated with the increased susceptibility to RLS in schizophrenic patients. These finding are different from the previous genetic studies finding of primary RLS, which suggests that RLS in schizophrenia may be different from primary RLS in etiology. We hypothesize that antipsychotic-related RLS in schizophrenia may be very mild form of akathisia presenting during night time under the control of circadian oscillation.

### Gene-Gene Interaction Between the SLC6A4 and HTR2A Gene Predicts Treatment Response to Venlafaxine XR in Generalized Anxiety Disorder

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**Background:** Anxiety Disorders are chronic psychiatric disorders with significant morbidity and mortality. Antidepressant drugs and psychotherapy are the preferred choice for treatment; however, treatment response is often variable. We will review current state of the field of pharmacogenetic and psychotherapygenetics in anxiety disorders. In addition, we will present new data in our generalized anxiety disorder (GAD) cohort focusing on gene-gene interaction between the serotonin transporter gene (SLC6A4) and serotonin receptor gene 2A (HTR2A) in treatment response to antidepressants.

**Methods:** We examined whether an interaction between the SLC6A4 5-HTTLPR/rs25531 haplotype and HTR2A SNP rs7997012 would be associated with antidepressant treatment outcome in GAD. Treatment response was assessed in 156 patients that participated in a 6-month open label clinical trial of venlafaxine XR for GAD. Primary analysis included HAM-A reduction at 6 months. Secondary outcome measure was the CGI-I score at 6 months. Genotype and allele frequencies were compared between groups using chi-square contingency analysis.

**Results:** Our data show that subjects with genotypes La/La G/G or La/La G/A (n=28) showed significantly lower HAM-A scores than those with genotypes La/S A/A or S/S A/A (n=12) at 6 months outcome (HAM-A difference=10.7; p < 0.0001). Single marker analysis only showed significant HAM-A differences of 4.3 (5-HTTLPR/rs25531: La/La versus La/S S/S) and 4.8 (rs7997012: G/G G/A versus A/A), indicating a significant gene-gene interaction between these markers with a possible additive effect.

**Discussion:** We report for the first time a significant gene-gene interaction between SLC6A4 and 5HTR2A in antidepressant treatment response in GAD. Future prospective studies with larger sample sizes are necessary to further characterize this effect in treatment response to antidepressants in GAD.

### Use of Genetic Polymorphisms as Markers of Resistance to Treatment in Schizophrenia

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**Background:** Pharmacogenetics studies have confirmed the role of several brain neurotransmitter systems in the efficiency profile and side effects of medications. Nevertheless, only few studies associating therapeutic response to antipsychotics and genetic variations in target receptors and in genes related to neuroregulation and neurodevelopment were performed. Genetic variants in dopamine receptors have been associated with response to antipsychotics. The most significant results correlate genetic variations of the dopamine receptor D2 (DRD2) and dopamine receptor D3 (DRD3) and response level. The second-generation antipsychotics have high affinity for serotonin receptors that, hypothetically, can at least influence its antipsychotic activity. Several polymorphisms in HTR2A and HTR2C genes have been associated with response to clozapine and risperidone. Due to the importance of the dopaminergic system in the activity of antipsychotic drugs, proteins that regulate the availability or function of dopamine may influence the treatment response. The catechol-O-methyltransferase (COMT) enzyme catalyzes the degradation of dopamine. Some associations have been described between the functional polymorphism Val108/158Met and drug response, with individuals carrying the Met allele showing better response to treatment. On the other hand, BDNF (brain-derived neurotrophic factor) is involved in the development, survival and maintenance of neuronal function and has a role in regulating the expression of dopamine-related systems. The aim of this study was to investigate the role of genetic polymorphisms in genes COMT, HTR2A, HTR2C, CHRNA7, BDNF, DRD3 and GRM8 as possible markers of treatment resistance in schizophrenia.

**Methods:** 88 schizophrenia patients were enrolled from the ambulatory of the Group of Psychoses of the LIM-27, Institute of Psychiatry, University of São Paulo, between 2009 and 2011. All patients met the DSM-IV criteria for schizophrenia or schizoaffective disorder and were divided in two groups: 58 refractory patients according to IPAP algorithm (International Psychopharmacology Algorithm Project) and 30 non-refractory patients. Genomic DNA was extracted from peripheral blood. Each of the following polymorphisms: rs4680 (COMT); T102C, rs6314, rs1928042 (HTR2A); rs6318, rs3813929 (HTR2C); rs7164043 (CHRNA7); rs6265 (BDNF); rs6280 (DRD3); rs7778604 (GRM8) was determined using TaqMan® SNP Genotyping Assays. Genotyping was performed by real-time PCR allelic discrimination.

**Results:** Thirty non-refractory and 58 refractory patients were genotyped for the polymorphism rs4680 (Val158Met) of the COMT gene. Among the non-refractory patients we found 7% of the AA genotype, followed by 50% of the AG and 43% of the GG genotype. Among refractory patients, we found 20% of AA, 59% of AG and 21% of GG genotypes. After the Chi-Square test, we found that there was a difference in genotype distribution between the refractory and non-refractory groups ( $p=0.043$ ) (Table 1). For the other investigated polymorphisms in genes HTR2A, HTR2C, CHRNA7, BDNF, DRD3 and GRM8 we found no significant difference between refractory and non-refractory patients.

**Discussion:** In our study we found a higher prevalence of individuals homozygous for the Met allele in the refractory group and a higher prevalence of individuals homozygous for the Val allele in the non-refractory group ( $p=0.043$ ). Previous studies found an association between the presence of the Met allele and better response to antipsychotics. However, these studies have focused on the response to negative and, specifically, cognitive symptoms. Our study, however, showed an association between the presence of the Met allele and a poorer antipsychotic response to positive symptoms, leading to a condition of refractoriness, in which the persistence of positive symptoms is the central feature. Taking into account the assumption that the excess of functioning of the dopaminergic system in the mesolimbic area could be secondary to the reduced dopamine function in the prefrontal cortex, we can argue that the reduced function of the COMT enzyme leads to an oversupply of dopamine in the synaptic cleft, or alternatively, an increased density or sensitivity of the postsynaptic dopaminergic receptors. This would explain the decreased response to antipsychotics, particularly those dopamine antagonists, and the persistence of positive symptoms in refractory patients.

### The Role of the Pteridine Tetrahydrobiopterin Pathway in Mood Disorders and Their Treatment

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**Background:** The pteridine tetrahydrobiopterin (BH4) is an essential co-factor for production of many neurotransmitters including serotonin. The BH4 pathway has been linked to several diseases, including depression & bipolar disorder, and their treatment. Several of these studies have looked at the BH4 pathway metabolites, neopterin and biopterin, as biomarkers of depression and treatment response.

**Methods:** Several methodologies were employed in this work including, proteomics, transcriptomics, QPCR, promoter assays and gene association studies. Contact author for further details.

**Results:** This research has shown a relationship of this pathway to mood disorders, antidepressant treatment response and antidepressant function. I observed that the antidepressant paroxetine, a selective serotonin reuptake inhibitor, regulates sepiapterin reductase (SPR) gene expression and substantially affects the level of the SPR protein in neural cells. SPR catalyzes the final step in the biosynthesis of BH4. Furthermore, I have shown that variants of two genes in the BH4 pathway, GTP cyclohydrolase feedback regulator (GCHFR) and SPR, are associated with clinical SSRI response.

**Discussion:** This work suggests a need to further examine the BH4 pathway in the context of the pathobiology of mood disorders and response to antidepressant therapies. Improved understanding of the BH4 pathway's role in the aetiology of mood disorders and its interaction with antidepressants will potentially provide simple tools which will allow us to not only predict disease but also response to current antidepressant therapies. Moreover, it will also enable us to develop novel therapies and improved treatment strategies for the treatment of mood disorders.

### The Role Of CNR1 Gene in Clinical Response and Remission after Citalopram Treatment (SSRI) in Major Depression: A 12-Week Follow-Up Study

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**Background:** Major depressive disorder (MD) has been described as a clinically heterogeneous disease that results from the interplay of multiple genes interacting with environmental factors such as early stressful life events. A recent meta-analysis reported the anxiogenic and depressive effects when CB1-R is blocked by antagonist (Christensen et al., 2007). Furthermore, it has recently shown that genetic variability at the cannabinoid receptor 1 gene (CNR1) confers an increased risk of resistance to antidepressant treatment, particularly in female patients with MD and high comorbid anxiety (Domschke et al., 2008). Given the evidence for the involvement of the endocannabinoid system in the pathogenesis of MD as well as in the mediation of antidepressant drug effects, the aim of this study was to analyse the role of genetic variability in the CNR1 gene in clinical response (4th week) and remission (12th week) after citalopram (SSRI) treatment.

**Methods:** The sample consisted of 155 depressive outpatients (78.7% females) of Spanish origin. All patients were treated with citalopram (CIT) and followed along 12 weeks. Clinical response was evaluated at 4th week and remission at 12th week by means the 21-item Hamilton Depression Rating Score (HDRS) (Hamilton, 1960). Genomic DNA was extracted from blood samples according to standard protocols. SNPs at the CNR1 gene (rs1535255 (G/T), rs806377 (C/T), rs806371 (G/T), rs1049353 (A/G), rs80636 (C/T)) were genotyped using Sequenom MassArray technology. Analyses were performed using PASW v18.0 and EpiInfo. Haploview 3.2 was used to generate a linkage disequilibrium map and to test for Hardy-Weinberg equilibrium. The 'R' software (V. 2.2.1) was used to calculate haplotype frequencies by the "haplo.stat" package.

**Results:** Genotype distribution of all SNPs was found to be in Hardy-Weinberg equilibrium. We reported significant differences between Remitters (Rm) and no-Remitters (N-Rm) at the 12th week for genotype and allele distributions for both rs806371 [genotype: X<sub>22</sub>= 6.18, P=0.043; allele: X<sub>12</sub>= 5.74, P=0.016; OR=2.42 95% CI (1.10-5.44)] and rs806368 [genotype: X<sub>22</sub>= 7.069, P=0.029; allele: X<sub>12</sub>= 5.27, P=0.021; OR=2.05 95% CI (1.06-4.00)] polymorphisms. The TT-homozygous of the rs806371 presented almost 3 times more risk of no-remission than the G-carriers [X<sub>12</sub>= 6.18, P=0.012; OR= 2.8 95%CI (1.14-7.01)], while TT-homozygous of rs806368 presented almost 1.5 times more risk of no-remission than the C-carrier [X<sub>12</sub>= 6.94, P=0.008; OR= 1.45 95%CI (1.20-5.89)]. Haplotype analysis showed that T-C combination (rs1535255-rs806377) was more frequent in N-Rm (40.2%) than Rm (26.6%) (P=0.04). No other significant haplotype combination was found. The longitudinal study for the rs806368 polymorphism showed a significant effects of time-sex interaction (F(2.76, 284.98)=6.85, P<0.001), time-genotype interaction (F(2.76, 284.98)=4.987, P=0.003) and a significant effect of time-sex-genotype interaction (F(2.76, 284.98)=3.233, P=0.026) showing that the men C-carriers presented a better response to antidepressant treatment along the follow-up than TT-homozygotes men and the whole women group.

**Discussion:** Our results show that CNR1 gene has an indirect effect on clinical response to CIT (SSRIs) treatment basically in remission at the 12th week. The rs806368 polymorphism seems to be associated with the differential response along the 12-week follow-up. A recent study reported that genetic variability of the rs1049353, which is in linkage disequilibrium with rs806368 in our sample, confers an increased risk of resistance to antidepressant treatment, particularly in female patients with MD (Domschke et al., 2008). Further studies focusing on other genes involved in the endocannabinoid system or other systems related with endocannabinoid system could help to elucidate the complex mechanism of clinical response to antidepressants treatment.

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### The Role of Glutamatergic Neurotransmission in Lithium Response in Bipolar Patients: Association with GRIK2 and GABRB2 Genes

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**Background:** Bipolar disorder (BD) is a serious mental illness with well-established, but poorly characterized genetic risk. Since the introduction of lithium in psychiatry, it is still considered the first-line treatment in bipolar patients (BP) given its proven efficacy in both acute and maintenance phases. However, treatment response is variable ranging from an excellent response to a complete lack of response. It has been suggested that lithium-responsive BD may be a genetically distinct phenotype of the disorder and that lithium response has a genetic component. However the molecular basis of its mechanism of action is still unclear. It is known that chronic lithium administration up-regulates glutamate reuptake and thus decreases glutamate availability in synapse. Glutamate is an excitatory neurotransmitter and its reduction could exert an antimanic effect (Shaldubina et al., 2001). Taking into account the action of lithium in the glutamatergic neurotransmission, the aim of this study was to investigate the potential association of genetic variability at GABRB2, GRIK 2, GRIK 5 and GRIA 2 genes with response to lithium in BP.

**Methods:** The sample consisted in 110 unrelated Caucasian bipolar outpatients from the Bipolar Disorder Unit of the Hospital Clinic of Barcelona and from primary care settings from Oviedo. Inclusion criteria were (a) bipolar I or II DSM-IV-TR diagnosis and (b) age > 18 years. Exclusion criteria were the presence of (a) mental retardation and (b) severe organic disease. Genomic DNA was extracted from blood samples from each participant, according to standard protocols. Several polymorphisms at the GABRB2 (rs592403, rs2910284, rs2962406, rs4426954), GRIK2 (rs2852525, rs2787554, rs2518261, rs2852620), GRIK 5 (rs8099939, rs10407506, rs4803523) and GRIA2 (rs9784453) genes were genotyped. All patients were grouped and compared according to their level of response to lithium treatment. Patients were classified as excellent responders, partial responders and non-responders. For statistical purposes, excellent and partial responders were considered Responders. Patients showing no tolerability to lithium were not considered. Categorical variables were compared using Chi-square or Fisher exact tests, as appropriate. Analyses were performed using SPSSv17 and EpiInfo. Linkage disequilibrium (LD) between the two markers was tested with Haploview v.4.1. Haplotype analyses were conducted using the 'R' software (v.2.2.1) by the "haplo.stat" package. All procedures were approved by the research ethics committees in each institution.

**Results:** Genotype distribution of all SNPs was found to be in Hardy-Weinberg equilibrium. We reported significant differences between Responders and non-Responders for genotype and allele distributions for rs2852620-GRIK2 polymorphism (genotype: X<sup>2</sup>=6.879, P=0.032; allele: X<sup>12</sup>=5.03; P=0.024; OR=2.08 95% CI (1.04-4.16)). The TT homozygous of this polymorphism presented 3.6 times more risk of no-response than the A-carriers [X<sup>12</sup>= 6.85, P=0.008; OR= 3.61; 95%CI (1.21-10.98)]. No other significant association was found when analyzing other SNPs. Haplotype analyses yielded a significant association with response to lithium in the GABRB2 gene showing

that the A-A combination (rs2910284-rs2962406) was present only in the non-responders group (10%) ( $P=0.008$ ). No other significant haplotype combination was found between any of the other analyzed polymorphisms and lithium response.

**Discussion:** Our findings suggest that lithium-responsive BD's phenotype seems to be associated with genetic variability at GRIK2 and GABRB2 genes. Previous studies have shown the involvement of these receptors in BD. The GRIK2 gene has been identified as a potential BD susceptibility gene (Buervenich et al., 2003). Also, this gene is located in a genetic linkage region (6q21) associated with BD (Dick et al., 2003; Schumacher et al., 2005). In the other hand, the GABRB2 gene was found to have positive association with BD (Perlis et al., 2008). More studies are needed in order to clarify the role of glutamatergic system in lithium response. Acknowledgements: Centro de Investigación en Red de Salud Mental (CIBERSAM); Instituto Carlos III (PI080247)-PN 2008-2011- Subdirección General de Evaluación y Fomento de la Investigación; Fondo Europeo de Desarrollo Regional. Unión Europea. Una manera de hacer Europa; Spanish Ministry of Economy and Competitiveness; Comissionat per a Universitats i Recerca del DIUE (2009SGR827) and to the Bipolar Disorders Group (2009SGR1022).

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#### No Association Between Genetic and Epigenetic Variation in the IGF Pathway and Antipsychotic-Induced Metabolic Disturbances

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**Background:** Antipsychotics, and especially second generation antipsychotics, are well known for their potency in inducing metabolic syndrome. Despite variation due to drug properties, large inter-individual differences in weight gain remain. Genetic and epigenetic differences between patients may explain a part of this variation. The Insulin Growth Factor (IGF) pathway provides a theoretical model for both an increased risk of schizophrenia and metabolic disturbances: Adults prenatally exposed to the Dutch Hunger Winter have an increased risk for schizophrenia and metabolic disturbances. A possible explanation are methylation differences in regions such as the IGF2 DMR, which have been associated with famine.

**Methods:** DNA from peripheral blood lymphocytes came from a naturalistic and dynamic patient cohort of patients suffering from schizophrenia spectrum disorder. 26 SNPs in the IGF pathway (IGF1, IGF1R, IGF2, IGF2R genes) were examined using the iPLEXTM platform by Sequenom inc. Methylation in 7 CpG islands of the IGF2 DMR was tested using the EpiTYPERTM platform by Sequenom inc. The effect of both genetic and epigenetic variation on BMI, weight, waist and hip circumference, correcting for age and antipsychotic was tested.

**Results:** The sample consisted of 438 patients (mean age 35.8, SD 11.1, 67.4% male) suffering from schizophrenia ( $n=348$ ) or schizoaffective disorder ( $n=90$ ). Six SNPs had insufficient variation for further analysis. None of the 20 SNPs in the current pathway had significant influence on any of the investigated parameters after correction for multiple testing. The strongest association was found between IGF1 rs10860861 and weight (uncorrected  $p$  value 0.012). IGF2 DMR methylation on CpG islands 1 and 2 showed a trimodal pattern, whereas islands 3, 4, 5-6, 8-9 and 10 were normally distributed. Mean IGF2 DMR methylation was associated with rs3741211 and rs1003484 in IGF2 (Kruskal-Wallis  $\chi^2=31.9$ ,  $df=2$ ,  $p<1.19e-7$  for rs3741211 and Wallis  $\chi^2=7.1$ ,  $df=2$ ,  $p<0.029$ ). No association was found between total DMR methylation or methylation on individual CpG islands and metabolic parameters.

**Discussion:** The current study suggests that there is no major effect of both genetic variation or variation in methylation in the IGF pathway on metabolic disturbances during antipsychotic treatment.

### Medication Induced Obesity in Schizophrenia and the Prominent Causative Role of Appetite/Satiety Regulating Genes in the Hypothalamus

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**Background:** The substantial inter-individual variability observed in response and side effects with antipsychotic drugs is likely to largely depend on genetic factors. One of the most debilitating side effects, emerging with many newer antipsychotic drugs, is substantial weight gain and obesity associated with cardiovascular complications and metabolic syndrome. Since previous findings by our group and collaborators strongly implicated the leptin-melanocortin energy homeostasis system to be associated with antipsychotic-induced weight gain, we investigated several important genes in the hypothalamus known to be involved in appetite and satiety regulation such as the neuropeptide Y receptor Y2 (NPY2R).

**Methods:** A total of 237 patients who underwent treatment for chronic schizophrenia or schizoaffective disorder were evaluated for antipsychotic response and induced weight gain for up to six months. The sample consisted mainly of individuals of European descent exposed to clozapine for their first time. We performed genotype analyses in fifteen SNPs in the NPY2R gene. These selected tag SNPs with a minor allele frequency of at least 5 allowed for a dense coverage including the regions 10kb upstream and 2kb downstream of the NPY2R gene. ANOVA and ANCOVA analyses were conducted as well as haplotype analyses.

**Results:** Our analyses with the NPY2R gene showed that there was a trend for the rs12507396 polymorphism to be associated with antipsychotic-induced weight gain in the overall total sample ( $p = 0.08$ ). Since the overall sample included subjects on low-risk medication for weight gain and due to the ethnic heterogeneity, we refined the sample for analyses in the clozapine and olanzapine treated patients of European ancestry. Carriers of the rs12507396 T-allele gained on average significantly more weight than non-carriers ( $p=0.025$ ). This result became even more significant when we corrected for duration of treatment ( $p = 0.01$ ). Haplotype analyses and findings in the other remaining SNPs yielded some interesting trends which will be summarized in the presentation.

**Discussion:** Our results tentatively suggest a novel associations between a promoter variant in the NPY2R gene in patients treated with antipsychotic medication for schizophrenia. The location of the gene variant in the promoter region strongly suggests a functional role of this SNP. Since replications are required to evaluate a novel finding, we are currently performing replication studies in a large sample of schizophrenia patients who were prospectively assessed over the course of one year in addition to replication analyses in the CATIE sample. Furthermore, we are conducting gene x gene interaction analyses with other investigated hypothalamus genes in order to help creating clinical test algorithms which will provide more efficient treatment strategies through personalized medicine.

### The RS2522833 (A/C) Polymorphism in the Piccolo Gene might be Associated with Early Improvement in Patients with Depressive Disorders -- Results from Two Independent Patient Samples

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**Background:** Early improvement has been shown to be a relevant predictor of suicidality and further improvement. The piccolo gene (PCLO) has recently been associated with major depressive disorder (MDD). In this study we were interested to detect possible associations of clinical predictors and a polymorphism in the PCLO gene with early improvement.

**Methods:** Several clinical predictors and the rs2522833 (A/C) polymorphism in the PCLO gene were tested in a naturalistic patient sample from the German Research Network on Depression (n=258) for their associations with early improvement. We used univariate tests to detect relevant factors, which were further tested in logistic regression and CART analyses. Results were further investigated in a second independent patient sample (n=215).

**Results:** We found the rs2522833 (A/C) polymorphism in the PCLO gene to be significantly associated with early improvement as categorical outcome in univariate tests (p-values (genotypic model) in the discovery/replication sample: 0.02/0.0123), logistic regression models ( $p = 0.03/0.0052$ ) and CART analyses ( $p = 0.03/0.033$ ) in both patient samples. Clinical predictors of early improvement were HAMD at admission, number of previous hospitalizations, job situation, antidepressive pre-treatment and type of treatment. We found opposite alleles associated to early improvement in the two patient samples.

**Discussion:** The presynaptic protein piccolo is involved in neurotransmitter vesicle exocytosis. We found the rs2522833 (A/C) polymorphism to be associated with early improvement in two independent patient samples. These findings might indicate involvement of the PCLO gene in the rapid onset of treatment response in MDD.

### Pharmacogenetics of Drug Transport at the Blood Brain Barrier: Which Transporters for Which Drugs?

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**Background:** A significant fraction of psychiatric patients poorly respond to multiple drug treatments. Genetic variations in drug metabolizing enzymes are known to cause substantial variability in the plasma concentration of psychotropic drugs. However, variations in drug plasma concentration have only partially correlated with variations in response and adverse drug effects in psychiatric therapy. Therefore, we hypothesize that genetic variations in membrane drug transporters at the Blood Brain Barrier might additionally influence drug concentration at the site of action, and thus predetermine the outcome of therapy. Within this scope, we first analyzed the membrane permeability of several drugs in order to identify drugs that may depend on active transport to penetrate the blood brain barrier. Secondly, we analyzed the gene expression of drug transporters in primary cells from the brain and total brain tissue homogenate and studied some of the identified transporters for their relevance in transporting psychiatric drugs.

**Methods:** The membrane permeability of different psychotropic drugs was analyzed by parallel artificial membrane permeability assay (PAMPA) using pre-coated 96-well plates (BD Biosciences, Heidelberg, Germany). Gene expression assays were performed, using custom TaqMan® Array Cards (Applied Biosystems, Darmstadt, Germany), in total brain RNA and RNA isolated from primary human brain microvascular endothelial cells (HBMECs), astrocytes and epithelial cells from the choroid plexus (EpiC). Each RNA sample was screened for the expression of 90 different membrane transporters. Drug uptake was studied by incubating HEK293 cells, stably expressing the polymorphic OCT1 transporter, with a radioactively labeled drug. The intracellular drug concentrations of the radioactively labeled drug were measured by scintillation counting.

**Results:** The membrane permeability of the psychotropic drugs tested ranged from  $Pe=0.26$  cm/s (Amisulpride) to  $Pe=25.69$  cm/s (Doxepin). The gene expression arrays revealed that, in the brain, the expression of efflux membrane transporters is higher than the expression of influx membrane transporters. ABCB1 (P-glycoprotein) and ABCG2 (Breast Cancer Resistance Protein, BCRP) were strongest expressed efflux membrane transporters in HBMECs. SLC22A5 (OCTN2) was the strongest expressed influx transporter in HBMECs. High correlation in transporter expression was observed between astrocytes and EpiCs ( $r=0.63$ ). The correlation between HBMECs and astrocytes ( $r=0.44$ ) or EpiCs ( $r=0.54$ ) was lower, indicating a specific transporter expression pattern at the blood brain barrier. The highly polymorphic SLC22A1 gene (OCT1), that has been previously proposed to be expressed at the blood brain barrier, was able to transport sulphiride, a low permeability drug. However, we did not observe significant OCT1 expression in HBMECs in our experiments.

**Discussion:** Systematic analysis of the pharmacogenomics of the blood brain barrier, including the relevant influx and efflux transporters and their genetic regulation, may contribute to a better understanding of interindividual variation in psychotropic drug response. This study shows that although most psychotropic drugs are highly membrane permeable, a small number of low membrane permeable drugs exist. These low membrane permeable drugs may strongly depend on influx membrane transporters like OCTN2 to enter the brain. The potential role of the efflux transporters P-glycoprotein and BCRP on the blood brain barrier permeability of highly membrane permeable psychotropic drugs should be investigated.

### Pharmacogenetic Aspects of QTc Interval Prolongation under Antipsychotic Treatment

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**Background:** Many first and second generation antipsychotics are considered to increase the risk of QTc interval prolongation which is associated with an increased risk of torsade de pointes (TdP) and sudden death in patients. The Long-QT syndrome is associated with a variety of genetic polymorphisms mainly related to the potassium-voltage-gated channel (IKr) but also to the sodium-voltage-gated-channel. Adrenergic pathways also seem to play a role in prolongation of the QT interval.

**Methods:** In the present study we investigated the effect of different antipsychotics on changes in QTc interval and searched for possible associations between genetic polymorphisms of the potassium and sodium channel as well as adrenergic receptor genes and QTc prolongation. 346 schizophrenic patients treated with antipsychotics were genotyped for three potassium channel, one sodium channel and seven adrenergic receptor gene polymorphisms. ECG's were conducted biweekly over a time interval of at least eight weeks.

**Results:** Ziprasidone and amisulpride showed the most significant prolongation in QTc interval ( $p<0.01$ ) followed by quetiapine ( $p<0.05$ ). Risperidone, olanzapine and haloperidole showed no significant prolongation while patients treated with aripiprazole exhibited a slight decrease of the QTc interval. One polymorphism of the potassium channel (KCNQ1, rs757092), one of the sodium channel (SCN5A, His558Arg) as well as one polymorphism of the adrenergic beta 1 receptor (ADRB1, Gly49Ser) showed a significant association with the QTc time at baseline. The SCN5A (His558Arg) and ADRB1 (Gly49Ser) polymorphisms also showed a significant association with the prolongation of the QTc interval over eight weeks of antipsychotic treatment.

**Discussion:** Although still preliminary our data are indicating that genetic polymorphisms which are associated with congenital Long-QT syndrome might also predispose patients to a more pronounced QTc interval prolongation during treatment with antipsychotics.

### Association Study between Variants in Histamine Receptor H1 And H3 With Antipsychotic-induced Weight Gain

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**Background:** Weight gain and development of metabolic syndrome are the most common deleterious side effects following treatment with second generation antipsychotic drugs. However, the mechanisms underlying these negative effects of second generation antipsychotic drugs are not fully understood. In this study we investigate whether variants in the genes coding for the Histamine receptors H1 (HRH1) and H3 (HRH3) are associated with antipsychotic-induced weight gain (AIWG). Clozapine and olanzapine, the antipsychotics associated with the highest risk of weight gain, have high affinity for HRH1. The orexigenic abilities of the antipsychotics correlate with their affinity of HRH1 and deletion of HRH1 in mice abolishes their orexigenic activity. HRH3 is an autoreceptor involved in the control of food intake.

**Methods:** We investigated 40 tag and/or putative functional SNPs (HRH1=34 and HRH3=6) in 219 schizophrenia or schizoaffective disorder patients treated mainly with clozapine and olanzapine for up to 14 weeks. Overall, these SNPs cover almost 100% of the common variation in the HRH1 and HRH3 receptors.

**Results:** We observed significant association of an intronic SNP, rs7639145, in HRH1 with AIWG ( $p=0.021$ ). Carriers of the GG genotype gained more weight when treated with clozapine or olanzapine (GG vs. GA+AA, 5.2kg  $\pm$ 4.8 vs. 2.9kg  $\pm$ 3.9,  $p=0.026$ ). In HRH3 trends of association were observed for rs1615746 ( $p=0.057$ ) and rs6587299 ( $p=0.06$ ). However, none of the other SNPs were significantly associated with AIWG. A limitation is that the above associations do not remain significant after correcting for multiple testing.

**Discussion:** We have carried out a comprehensive analysis of genetic variation in HRH1 and HRH3 genes with AIWG that yielded some interesting findings. However, our observations suggest that SNPs in the HRH1 and HRH3 may not play a major role to play in AIWG and downstream pathways need to be investigated further.

### Family-based Association Study of Attention-Deficit Hyperactivity Disorder and Genes Increasing the Risk for Smoking Behaviors

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**Background:** Attention-deficit/hyperactivity disorder (ADHD) and smoking behavior are highly comorbid, and this comorbidity could be due to shared genetic factors. Several genes increasing smoking behavior risk were reliably identified by Genome-Wide Association Studies. Here, we have investigated five top single nucleotide polymorphisms (SNPs) located in different genes and loci (CHRNA3, BDNF, DBH, and LOC100188947) that were highly associated with different dimensions of smoking behavior, in relation to ADHD.

**Methods:** Family-based association tests were used to study transmission of risk alleles within these five genetic markers in families of 454 children with ADHD aged between 6 and 12 years old. Clinical diagnosis of ADHD, and a number of behavioral and neurocognitive phenotypes relevant to the disorder were investigated.

**Results:** One SNP (rs1329650) from a noncoding RNA (LOC100188947) was significantly associated with overall ADHD diagnosis with the C\* risk allele being over-transmitted from parents to ADHD children ( $p=0.02$ ). It was also over transmitted to children with higher scores on Conners' Parents ( $p=0.01$ ) and Conners' Teacher ( $p=0.002$ ) index scores, Child Behavior Checklist withdrawn ( $p=0.001$ ) and aggressive ( $p=0.007$ ) behaviors. Children with poorer performance on executive and attention tasks were more likely to inherit the risk allele.

**Discussion:** The C\* allele of rs1329650 may be increasing the risk for ADHD and smoking behavior through a common mechanism, possibly externalizing behaviors and specific cognitive deficits that manifest as ADHD in childhood and are the gateway to smoking behavior later in life. This exploratory study illustrates the use of comorbid disorders to investigate ADHD genetics. Replication in future studies is warranted.

### Characterization of CACNA1C and ANK3 Risk Alleles for Bipolar Disorder in Hispanics

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**Background:** Over the past several years, genome-wide association studies (GWAS) have been successful in identifying genetic variants that contribute to complex human disorders. However, with a few exceptions, GWAS have been centered on populations of European descent, and the degree to which the findings are applicable to other populations has not been extensively investigated. Through recent GWAS studies, several groups have reported significant association of variants in the alpha 1C subunit of the L-type voltage-gated calcium channel (CACNA1C) and ankyrin-3 (ANK3) genes with bipolar disorder (BP) in Caucasian cohorts. To determine whether CACNA1C and/or ANK3 are associated with BP in the Latino population, we performed a family-based association study between CACNA1C/ANK3 polymorphisms and BP.

**Methods:** This study consisted of 250 Latino families (913 individuals) from the United States, Mexico, Guatemala, and Costa Rica, previously recruited as part of the Genetics of BP in Latino Populations NIMH study. A total of 482 participants were diagnosed with BP (263 BP Type I with Psychosis, 202 BP Type I without Psychosis, and 17 Schizoaffective BP Type) using DSM-IV criteria, by a best-estimation consensus procedure using the Diagnostic Interview for Genetic Studies (DIGS), Family Interview for Genetic Studies (FIGS) and available psychiatric records. Unaffected family members were used as controls. The Illumina GoldenGate Genotyping Assay was used to genotype SNPs in the CACNA1C and ANK3 genes previously shown to be associated with BP (rs7297582 and rs1006737; rs9804190 and rs10994336, respectively) and an additional 92 SNPs that spanned regions encompassing the CACNA1C and ANK3 genes. Markers were selected using a Tag SNP method and supplemented with additional SNPs in potential strong linkage disequilibrium (LD) with the SNPs previously associated with BP. Individual SNP and haplotype association analyses were performed using the family-based association test (FBAT, version 2.0.3) and Haploview software (version 4.2).

**Results:** Although we were not able to replicate findings of association between individual CACNA1C (rs7297582,  $p=0.760$ ; rs1006737,  $p=0.469$ ) or ANK3 SNPs (rs9804190,  $p=0.899$ ; rs10994336,  $p=0.054$ ) and BP, we were able to replicate the GWAS signal reported for these genes with haplotype analyses which encompassed these SNPs. An 8-locus and a 6-locus haplotype block were identified to be in strong LD with the two GWAS significant SNPs in CACNA1C and one SNP in ANK3, respectively. One common and three rare haplotypes in CACNA1C displayed significant association under an additive model. The  $p$  value of the whole haplotype permutation test was also statistically significant ( $\chi^2_{sum}$ ,  $p=0.002$ ). A significant association was also found between a common haplotype in ANK3 and BP (permuted  $P=0.026$ ; global marker permuted  $P=0.021$ ), which included rs10994336, which was associated with BP in previous GWAS studies.

**Discussion:** These preliminary results suggest that the SNPs associated with BP previously reported in GWAS studies are not necessarily the disease predisposing variants, but are more likely SNPs in strong LD with functional polymorphisms which were not genotyped. Allele frequencies of genotyped markers in strong LD with the previously associated SNPs are highly variable across ancestral populations, suggesting the likelihood of a differential profile of CACNA1C/ANK3 variants with ethnicity. Given the negative findings of our single locus tests compared to previous associations found in the Caucasian population, the possibility remains that the true, functional variants may be in strong LD with SNPs previously reported in GWAS studies which are incorporated in the haploblocks identified in this Latino population. These results provide additional evidence that CACNA1C and ANK3 are associated with BP and provide the first evidence that variations in these genes might play a role in the pathogenesis of this disorder in the Latino population.

### NROG: A Novel Gene Interrupted by T(3;11)(P26.1;P15.1) in Father and Son with Manic and Aggressive Behavior

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**Background:** A balanced chromosomal translocation between chromosome 3 and 11 was identified in a father and his son. The father has history of alcohol and drug abuse, aggressiveness, nonspecific learning disability, difficulties holding a job, spending out of his financial means. The son was diagnosed with bipolar disorder, and aggressive behavior, nonspecific learning disability, and normal IQ, no dysmorphic features. Our previous cytogenetic and bioinformatic data analyses showed that a novel gene named as NROG (non-coding RNA overlapping GRM7) located at 3p26.1 and a known gene SUR1 (sulfonylurea receptor 1) located at 11p15.1 were interrupted by this chromosomal translocation. We did further molecular biological study attempting to understand the structure and the expression pattern of NROG.

**Methods:** Total RNA from frontal cortex of human brain was used to purify poly(A)RNA, for RT PCR and 3'RACE. In order to study the expression pattern of NROG gene we used TaqMan real-time PCR to detect qPCR array plate (OriGene) with cDNAs from 24 different areas of human central nervous system. With radioactive labeled probe we detected different northern blots (Clontech) with poly(A)RNAs from different human tissues. In order to obtain full length cDNA of NROG, cDNA library screening was performed by a commercial service from OriGene with its Rapid-Screen Arrayed cDNA Library Panels. The latest version (2009 assembly) of UCSC human genome browser was used for bioinformatic analysis.

**Results:** Our research provided primary biological data to generate a hypothesis explaining the structure and the potential function of NROG gene. RT PCR results showed that several independent cDNA fragments (AI222038, BG205265, AK124857, BC020876, and AK124088) located in the area where the breakpoint occurs are parts of NROG gene. 3'RACE was able to enrich NROG cDNA from frontal cortex poly(A)RNA, but the cloning of RACE products was not successful. The commercial cDNA library screen did not detect the cDNA clone. The full length cDNA sequence of NROG gene is still not available. Results of northern blot hybridization and real-time PCR showed a specific expression pattern of NROG that expressed at relatively high level in some areas of human central nervous system such as pituitary gland, spinal cord, cerebellum, substantia nigra, thalamus, hippocampus, corpus callosum and medulla, and at low level elsewhere, including the peripheral tissues. Northern blot hybridization showed that the size of major NROG transcripts is up to 4 kb. Analysis of available cDNA for coding area showed that there was no meaningful ORF identified. The results also showed that NROG gene is overlapped with GRM7 at genomic DNA level, with opposite transcription directions.

**Discussion:** Based on current research we suggested that NROG gene, a long non-coding gene with a CNS expression pattern, may be involved in CNS function by playing a regulatory role as an antisense non-coding RNA (ncRNA). The importance of regulatory ncRNA is becoming increasingly evident. The regulatory ncRNA may play some important roles in the normal functioning of the CNS and in some neurological disorders. In order to dissect the pathways that NROG gene involves in the future research might include 1) to screen the cDNA library generated from poly(A) RNA with higher level of NROG transcript to obtain the full length NROG cDNA; 2) to identify the NROG gene sequence from other animal species; 3) to generate and study knock-out animal models.

### Rare Copy Number Variants In Obsessive Compulsive Disorder

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**Background:** Studies of copy number variation (CNV) have successfully characterized loci and molecular pathways involved in a range of neurodevelopmental disorder. Within this context, genome-wide screens for de novo mutation have become an essential approach for gene discovery in psychiatric disease. Given that obsessive-compulsive disorder (OCD) may be regarded as a neurodevelopmental disorder, we conducted a family-based studies of 80 individuals with OCD, including 144 parent-child trios to determine, the frequency of spontaneous de novo mutation, identify novel risk regions and relevant molecular pathways involved with OCD.

**Methods:** The CNV analysis was undertaken using 610K to 1M probe Illumina arrays. Transmitted and de novo CNVs present in < 1% of the population were evaluated.

**Results:** The rate of the novo CNVs was 0,05. We identified one de novo CNV in 17 probands. The pathway analysis using two algorithms program showed enrichment pathways of cell adhesion, tight junction signaling and cell proliferation and differentiation.

**Discussion:** In addition, we are conducting a whole-exome sequencing in 20 OCD families with OCD consisting of pedigrees with two unaffected parents, an affected proband to capture a larger fraction of rare genetic variation. We are selecting some algorithms to predict CNVs in sequence data. We believe this experiment will achieve more robust data and increase the proportion of genetic risk that can be explained.

### Glial Cell Line-Derived Neurotrophic Factor (GDNF) and Mood Characteristics: An Association Study Among Healthy Adults

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**Background:** Neurotrophic factors regulate the neuron's survival, growth and morphological differentiation, thus they have a key role in synaptic plasticity and efficiency. Several studies identified connections between glial cell-line derived neurotrophic factor (GDNF) and mood disorders (e.g. bipolar disorder, major depression) but little is known about the effect of GDNF variations among healthy individuals.

**Methods:** In the present study we analyzed association of self-report data on mood characteristics with eight GDNF SNPs in a healthy young adult sample of Caucasians. Anxiety and depression scores of 708 Hungarians within the age range of 18-35 years were measured by the Hospital Anxiety and Depression Scale (HADS). Candidate GDNF SNPs (rs11111, rs1549250, rs1981844, rs2910702, rs2973041, rs2973050, rs3096140, rs3812047) were assayed by real time PCR, the obtained genotype frequencies were in Hardy-Weinberg equilibrium.

**Results:** Association with the two scales of the HADS questionnaire was evaluated by univariate analyses, gender was used as covariant, since anxiety in females was significantly higher ( $p < 0.001$ ). Tested SNPs did not show significant effects on depression scores, while five out of the 8 studied SNPs showed nominal association with anxiety scores (rs3812047:  $p = 0.0029$ ; rs3096140:  $p = 0.0054$ ; rs2910702:  $p = 0.0130$ ; rs1981844:  $p = 0.0272$ ; rs1549250:  $p = 0.0399$ ). After correcting for multiple testing, only the effect of rs3812047 polymorphism remained significant (considering the two scales and the 8 SNPs studied, corrected level of significance was:  $.05/16 = 0.003125$ ). We also tested the possible gene – gender interaction in a two-way analysis of variance with gender and the rs3812047 GDNF polymorphism as grouping variables. The genetic main effect was significant ( $p = 0.001$ ), however, there was no significant gender main effect, and the interaction between GDNF and gender was not significant either.

**Discussion:** The present results are the first report of an association between GDNF rs3812047 and mood characteristics in a healthy young adult sample. Our findings support earlier findings that GDNF is important genetic factor in mood related disorders, and highlight the importance of investigating heredity of mood characteristics within the non-clinical spectrum.

### Association Analysis of Serotonin Transporter Promoter Polymorphisms with Heroin Abuse in Bulgarian and Roma Subjects

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**Background:** Heroin addiction is a chronic complex disease with a substantial genetic contribution. This study is a part of ongoing project on genetic epidemiology of opioide dependence in Bulgaria. We have chosen to investigate for association with heroin addiction a gene involved in the reward modulation, the human serotonin transporter gene (SLC6A4). In previous studies, serotonin (5-HT) system disturbance was found involved in a variety of behavioral disorders, psychopathologies, and substance use disorders. A functional polymorphism in the promoter region of the human serotonin transporter gene (5HTTLPR) was identified and the presence of the short (S) allele associated with a lower level of expression of the gene and lower levels of 5-HT uptake. The substitution of the G for A in the SNP (rs25531, A/G) in the Long (L) form of 5HTTLPR might also have functional significance, as the more common LA allele was associated with higher basal activity, whereas the less common LG allele had transcriptional activity no greater than S.

**Methods:** In the present association study, 2598 heroin addicts and 1290 healthy control subjects have been included. All heroin dependent cases have been interviewed and a diagnosis according to DSMIV confirmed. The DNA samples were isolated from blood, drawn from the cases after signing an informed consent. The study has been approved by the Ethics Committee of Medical University – Sofia and Washington University of St. Louis. The heroin cases are from Bulgarian (1742 males, 332 females) and Roma (448 males, 60 females) ethnic origin. The population control samples are also from Bulgarian (913 males, 303 females) and Roma (53 male, 11 female) origin. They have been selected from the DNA Bank of Molecular Medicine Center and National Genetic Laboratory. The genotyping was performed with RFLP analysis and agarose gel electrophoresis. The statistical analysis was performed using Pearson's chi-square test.

**Results:** No significant differences between the allele and genotype frequency distributions among heroin addicts and controls have been observed. However significant inter-ethnicity differences have been found, both for allele and genotype distribution between heroin addicted from Bulgarian and Roma origin. When the groups were stratified by sex, the high activity LA allele was more frequently found among Bulgarian male heroin addicts compared to Roma men ( $p = 0.01$ ). The opposite tendency was found for the low activity SA allele ( $p = 0.003$ ). The distribution among the female heroin addicts from both ethnic groups displayed similar tendencies for allele LA ( $p = 0.04$ ), and SA ( $p = 0.009$ ). For the female sub-sample meaningful comparison between cases and controls could be done only for the Bulgarian subsample due to lack of enough Roma controls.

**Discussion:** In summary, significant inter-ethnic difference both for allele and genotype distributions has been observed in the group of heroin addicted. Such differences could not be observed between the control samples from both ethnic groups. This could be due to the comparatively small sample size of the Roma control group or it can

reflect a genuine difference between Bulgarian and Roma addicted. A further increase of the control group and more balanced representation of both genders and ethnicities will help us to confirm the preliminary findings. The results of the current study do not support a major role of the functional promoter polymorphisms 5HTTLPR and rs25531 for heroin addiction in Bulgarian and Roma population.

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### Synaptic Scaling in Anxiety Disorders

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**Background:** The aetiology of anxiety disorders remains poorly understood; however there exists clear evidence for a genetic component in the aetiology of anxiety. There has also been an increasing focus concerning the interaction of neurobiological/familial influences and environmental factors mediating disorder pathogenesis. Although a number of genetic studies have been conducted, no singular gene or genetic abnormality has been explicitly identified as being involved in the development and/ or progression of anxiety disorders. The striatum is the primary input nucleus receiving excitatory impulses from the cortex and thalamus and a major site of synaptic plasticity in the basal ganglia. Neural circuitry involving the striatum have been implicated in anxiety disorders like panic disorder (PD) and social phobia (SAD), and obsessive-compulsive (OCD) and OCD spectrum disorders like trichotillomania (TTM). Looking at the striatum and identifying mechanisms and genes responsible for changes at neuronal synapses involved in the manifestation of symptoms typical of these conditions could prove crucial to the understanding and/ or progression of these anxiety-related disorders. Traumatic events have also been linked with the development of many of these conditions.

**Methods:** A Sprague Dawley rat model subjected to maternal and/or restraint stress was used to mimic the presence of major life events and mild stress in adulthood, respectively. The rats were then subjected to behavioural tests, namely forced swimming, elevated-plus maze and open-field, to assess anxiety levels relative to unstressed controls. Rat striatal synaptic plasticity was investigated using pathway-based PCR array technology to identify genes that are up-regulated and down-regulated as a result of stress in the synaptic plasticity pathway. Molecular characterization of the identified susceptibility genes was performed within a human cohort comprising OCD, PD, SAD and TTM afflicted individuals, as well as healthy controls. This was done using TagSNP analyses based on HapMap data, genotyping using a TaqMan assay approach, as well as bioinformatics for core domain and inter-species sequence conservation identification where applicable.

**Results:** The behavioural tests indicated an overall level of anxiety and stress among rats exposed to maternal separation and/ or restraint stress, relative to the unexposed control group. The PCR array identified several genes involved in the synaptic plasticity pathway (Egr2, Egr4, Arc, Ntf4, Grm2, Bdnf and Mmp9) to be aberrantly expressed relative to the control group. There was a trend for an increased number of genes, as well as increased severity of aberrance in expression for rats exposed to restraint stress, maternal separation and combination restraint stress/ maternal separation, respectively. TagSNP analyses and TaqMan genotyping assays were performed for human homologue genes BDNF and MMP9. EGR2 and EGR4, coding for transcription factors, were identified to have three zinc-finger binding domains. NTF4 possessed a nerve growth factor (NGF) binding domain and GRM2 was bioinformatically identified to possess several transmembrane binding domains. ARC indicated no core domains, but an area of high sequence conservation including both UTR and exonic sequence areas was identified.

**Discussion:** In this study, several novel candidate genes and/ or expression profiles were identified as susceptibility genes and/ or pathways for association testing in anxiety patients. Differentially expressed genes in rats with a trauma history and anxiety behaviours, pointing to candidate susceptibility genes for anxiety disorders in humans, can significantly enhance the understanding for the molecular basis of anxiety disorders to date and ultimately allow for novel drug targets, therapies and treatment options.

### Meta-Analysis of Genome-wide Association Studies for Panic Disorder in the Japanese Population

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**Background:** Panic disorder (PD) is a moderately heritable anxiety disorder whose pathogenesis is not well understood. Due to the lack of power in previous association studies, genes that are truly associated with PD might not be detected.

**Methods:** We conducted a genome-wide association study (GWAS) in two independent datasets with the Affymetrix Mapping 500K Array or Genome-Wide Human SNP Array 6.0. We obtained imputed genotypes for each GWAS and performed a meta-analysis of two GWAS data sets comprising 718 cases and 1717 controls. For follow-up, 12 single nucleotide polymorphisms (SNPs) were tested in 329 cases and 861 controls. Gene Ontology (GO) enrichment and candidate gene analyses were conducted using the GWAS or meta-analysis results. We also applied the polygenic score analysis to our two GWAS samples to test the hypothesis of polygenic components contributing to PD.

**Results:** In spite that genome-wide significant SNPs were not detected in either sample set, polygenic scores calculated from weakly associated SNPs ( $PT < .3$  and  $PT < .4$ ) in the discovery sample were significantly associated with those in the target sample in both directions (sample I to sample II and vice versa) ( $p < 0.05$ ).

**Discussion:** Our findings suggest that large sets of common variants of small effects may collectively account for risk of PD.

### DRD4 Gene and Obsessive Compulsive Disorder: Do Symptom Dimensions Have Specific Genetic Correlates?

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**Background:** The dopamine D4 receptor (DRD4) is a promising candidate gene in obsessive compulsive disorder (OCD). A 48-bp variable number of tandem repeats (VNTR) sequence in exon 3 has been studied previously, and alleles containing 2-11 repeats (2R-11R) have been identified. Five published studies comparing patients and controls have found a positive association between DRD4 VNTR polymorphisms and OCD. Most previous reports have had smaller sample sizes ( $< 150$ ). The association of specific OCD symptom dimensions with DRD4 VNTR polymorphism has not been examined. We investigated the association of DRD4 VNTR polymorphism with OCD and its relationship with various clinical parameters (age of onset, gender, family history, co-morbidity, factor-analysed symptom dimensions, insight and treatment response).

**Methods:** We recruited 173 South Indian OCD patients (DSM-IV) from a specialty OCD clinic and 201 healthy controls after written informed consent. All patients were evaluated using the Yale-Brown obsessive compulsive scale (YBOCS), YBOCS item-11 for insight, Mini International Neuropsychiatric Interview (MINI) plus, tic disorder subsection of the MINI – KID and the Clinical Global Impression (CGI) scale. They were followed up every 2-3 months using the YBOCS and the CGI. Exclusion criteria included presence of co-morbid psychosis, bipolar disorder, mental retardation and neurological illnesses (except tic disorders). Controls were evaluated using the MINI plus. All subjects were genotyped for the DRD4 VNTR polymorphism. Ethnicity of all subjects was determined by confirming that all four of their grandparents originated from South India. The study was approved by the ethics committee of the NIMHANS. The allelic distribution and genotype frequency in cases and controls were compared using chi-square test after checking the Hardy-Weinberg Equilibrium. To generate factors (symptom dimensions), principal component analyses was performed with Varimax rotation on the 14 symptom categories of the Y-BOCS checklist (excluding miscellaneous symptoms) and an eigenvalue of greater than 1 was used to select the number of factors. Factor loadings of greater than 0.50 were considered robust. Factor analyses resulted in 5 factor solutions: factor 1 (hoarding), factor 2 (doubts and checking), factor 3 (symmetry and ordering), factor 4 (contamination) and factor 5 (forbidden thoughts). Associations between phenomenological subtypes and genotype were detected using chi-square analysis and binary logistic regression for categorical variables; Mann-Whitney U test and linear regression analysis was performed for continuous variables. Bonferroni correction for multiple comparisons was performed.

**Results:** The mean age of the OCD sample (119 men, 54 women) was  $29.2 \pm 9.5$  years. The mean age at onset OCD was  $20.7 \pm 8.0$  years and the median duration of illness was 6 (1-35) years. Y-BOCS total, obsession and compulsion subscale scores (Mean  $\pm$  SD) were respectively  $24.9 \pm 6.9$ ,  $13.6 \pm 3.3$  and  $11.3 \pm 4.9$ . A family history of OCD in first-degree relative was present in 23 patients (13%). Poor insight was present in 13 patients (7.5%). The most common co-morbidity was major depressive disorder (35%). Treatment response data was available for 152 patients (88%). The median duration of follow-up was 31 (3-345) months. Genotype frequencies of the sample did not deviate significantly from the Hardy-Weinberg equilibrium. Case-control association analysis revealed that the 7R allele frequency was significantly greater in OCD patients than controls. Among

the phenomenological subtypes examined, factor 3 (symmetry) was associated with presence of 2R allele. Linear regression with factor 3 score as the dependent variable and genotype, alleles and clinically important variables as independent variables confirmed the association of symmetry dimension with the 2R allele (Beta = 0.23, t=2.96, p=0.004, CI=0.19-0.95).

**Discussion:** Our data provides further evidence that DRD4 VNTR polymorphism is associated with OCD. In addition, the presence of the 2R allele was significantly associated with the symmetry dimension. Symmetry and ordering have also been found to be more familial than other symptom groups in previous studies. This symptom dimension has also been found to be associated with a functional polymorphism in the promoter region of the serotonin transporter gene (S allele and SS genotype of the 5-HTTLPR). Our finding of an association of this factor with presence of the DRD4 2R allele contributes to the growing body of knowledge suggesting that symmetry and ordering represent a genetically significant symptom subtype of OCD.

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### Mosaic Chromosome X And Y Aneuploidy in the Autism Prefrontal Cortex

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**Background:** Autism is recognized as etiologically heterogeneous neurodevelopmental disorder frequently associated with gene mutations and genomic imbalances, including chromosomal rearrangements and aneuploidy. It was hypothesized that inherited and acquired somatic genome mutations alter brain development in utero generating the susceptibility to autism or related psychiatric conditions later in life. However, the incidence of de novo mutations in the autistic brain has not been empirically addressed. Here, we monitored the rate of chromosome loss and gain (aneuploidy) acquired during prenatal brain development in the autistic brain.

**Methods:** Here, we monitored the rate of chromosome loss and gain (aneuploidy) acquired during prenatal brain development in the autistic brain. Postmortem brain tissues were obtained from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, USA. Prefrontal tissue from 10 autistic and 10 control male subjects aged from 7 to 47 years were analyzed by interphase molecular cytogenetic approaches.

**Results:** The autistic prefrontal cortex was found to show a 3-fold increase of mosaic aneuploidy affecting chromosome X (mean, 1.64%; 95% CI, 1.08-2.20%) as compared with control subjects (0.54%, 95% CI, 0.11-0.97; P=0.0015). A neuploidy involving chromosome Y was also increased in the autistic brain (mean, 1.03%; 95% CI, 0.45-1.61%) as compared with controls (mean, 0.33; 95% CI, 0.18-0.48%; P=0.016). Individuals suffering from autism had 176% more neural cells in the prefrontal cortex affected by chromosome X and Y aneuploidy (autism: mean, 2.54%, 95% CI, 1.54-3.54%; control: mean, 0.92%, 95% CI, 0.37-1.4%; P=0.0048).

**Discussion:** These findings support the hypothesis that chromosomal mutations acquired during early brain development can predispose to autism. We speculate that mosaic neural aneuploidy interplaying with other genetic and environmental factors affects neuronal networks in the adult autistic brain and may be a key to a complex problem of autism genomics. Supported by BMBF/DLR (RUS 09/006) and DLR/BMBF (BLR 11/002).

### Rare and Common Variants of MicroRNA Genes in Autism Spectrum Disorder

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**Background:** MicroRNAs (miRNAs) are a class of non-coding RNA molecules of 19 to 23 bp that work as post-transcriptional regulators. They are involved in a wide range of biological processes including cell cycle control, cell growth, differentiation, apoptosis and neuronal differentiation. Common and rare variants in miRNAs genes are likely to have a significant role in phenotypic variation, including disease susceptibility. In this study we aimed to test the possible contribution to Autism Spectrum Disorder (ASD) of common variants in a selection of miRNAs genes through a case-control association study design in four European populations. We also investigated the presence of novel rare variants in pre-miRNAs genes (70-80 nt) using exome sequencing data in a small sample of autistic individuals.

**Methods:** The discovery sample (DS) for the case-control association study consisted of 636 autistic patients (311 Spanish, 247 Dutch and 78 German) and 673 gender-matched controls (322 Spanish, 269 Dutch and 82 German). A Replication study (RS) was conducted in an independent European sample of 449 cases (232 Italian, 124 German and 93 Spanish) and 415 gender-matched controls (175 Italian, 131 German and 109 Spanish). We genotyped 350 tagSNPs in the DS, capturing the allelic variability of 163 miRNAs genes that include isolated miRNAs, miRNA clusters and potential regulatory regions. The selection of miRNA genes followed these criteria: 1) Presence in the Tarbase v5 (<http://diana.cslab.ece.ntua.gr/tarbase>) and miRecords (<http://mirecords.biolead.org>) databases and targeting of genes with brain functions; 2) involvement in neuron development or in psychiatric diseases reported in the literature. The case-control association study was performed under the additive model using the Cochran-Armitage test. Additionally, whole exome sequencing (WES) data were investigated to identify rare variants in miRNA genes in 41 autistic probands, 21 of them from multiplex families.

**Results:** The most significant associations in the pooled DS were obtained for the following miRNAs: miR-133b/miR-206 cluster (rs16882131,  $P=0.00037$ ; rs17578851,  $P=0.00872$ ); miR-17/miR-18a/miR-19a/miR-20a/miR-19b-1/miR92a-1 cluster (rs6492538,  $P=0.00199$ ); mir-106b/mir-93/mir-25 cluster (rs4729575,  $P=0.00645$ ) and miR-219-1 (rs107822,  $P=0.00881$ ). The RS was performed on 28 SNPs that included all the polymorphisms of those genes showing association in the pooled DS sample and also those of miR-129-2 and the miR-15a/miR-16-1 cluster, where association was detected only in one of the DS populations. None of these SNPs achieved a significant association after Bonferroni correction in the replica study. However, the pooled analysis of the DS and RS showed significant association after applying permutation tests for markers rs16882131 ( $P=0.00157$ , perm $P=0.042$ ) and rs6492538 ( $P=0.00074$ , perm $P=0.0185$ ), both associated with ASD in the DS. A Mantel-Haenszel meta-analysis for

rs16882131C showed an OR of 1.23 (95% CI= 1.08 to 1.41), whereas for rs6492538C an OR= 1.27 (95% CI= 1.1 to 1.48) was obtained. Finally, in the context of a separate project aimed at evaluating the contribution of rare variants in miRNA genes to ASD, we are currently performing the Sanger validation of WES data in 41 patients and their parents.

**Discussion:** Our data suggest that common allelic variants in two miRNA clusters on chromosome 6 (miR-133b and miR-206) and 13 (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR92a-1) may be involved in autism susceptibility. Both clusters include miRNAs that regulate interesting candidate genes for autism: miR-206 has been described to regulate the DMD gene, in which duplications have been found in autistic individuals; miR-133b regulates the MET proto-oncogene, with SNPs that have been associated with autism in different populations; and miR-19a regulates PTEN, involved in cancer but also implicated in autism recently. Further investigation and replication studies are warranted to corroborate these findings.

# **POSTER SESSION III**

## **ABSTRACTS**

### Genetic Association Links Receptor Tyrosine Phosphatase-Alpha to Schizophrenia

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**Background:** Schizophrenia (SZ; OMIM database entry #181500) is diagnosed by the joint appearance of positive (hallucinations, delusions), negative (disturbed affective and social functioning), and cognitive symptoms. Receptor protein tyrosine phosphatase  $\alpha$  (RPTP $\alpha$ , encoded by PTPRA) is known to be a signaling molecule that regulates a variety of cellular processes including neurodevelopment, oligodendrocyte differentiation, radial cortical migration, and synaptic plasticity. The objective of our study was to investigate whether common polymorphism within PTPRA, may increase genetic risk for schizophrenia within Japanese population.

**Methods:** Five hundred and sixty unrelated patients with schizophrenia ( $43.46 \pm 14.83$  yrs) and 548 healthy controls ( $43.97 \pm 14.43$ ) were recruited in Japan for 1st stage analysis, and 850 cases ( $45.89 \pm 14.00$  yrs) and 829 ( $46.01 \pm 14.58$ ) controls for 2nd stage analysis. Diagnosis was made according to DSM-IV criteria, following assessment by two psychiatrists. All patients received information and signed a consent form for participation in genetic studies. In the 1st stage analysis, DNA samples were hybridized on GeneChip® Human Mapping 5.0 Array (Affymetrix, Santa Clara, CA). In the 2nd replication analysis, genotyping was done by using TaqMan® SNP Genotyping Assays (Applied Biosystems). Association analysis was performed using PLINK v1.0.6 and missing genotype was imputed by IMPUTE v1.0.

**Results:** In the first stage, 560 cases and 548 controls were genotyped using the GeneChip Human Mapping 5.0 Array (Affymetrix, Santa Clara, California). Of 21 SNPs genotyped across the PTPRA locus, six yielded nominally significant association with SZ (rs6132976, rs6132977, rs6132978, rs1016753, rs1178032, and rs16988201) (best uncorrected  $p=0.002$ ). In the replication, only rs1016753 showed significant association ( $p=0.04$ ), with the same direction of association (Breslow-Day  $p=0.218$ ). Pooled analysis of first and second stages (1420 cases, 1377 controls) showed highly significant association of this SNP with SZ ( $p=0.0008$ ).

**Discussion:** We report highly significant association of a SNP in PTPRA with schizophrenia in a Japanese population. The sample size (~2600) is enough to detect mild to moderate effects of SNPs, and the evidence of PTPRA association is robust because the two-stage analysis reduces the potential for type I error. On the basis of LD analysis in the first stage, we selected rs1016753 as a representative SNP for rs6132976, rs6132977, and rs6132978. Therefore, the association of rs1016753 might reflect possible association of these or other linked SNPs. Because the LD structure of PTPRA is relatively loose, we cannot narrow down the associated region to identify the “true” SNPs.

### Polymorphisms in Genes Encoding Enzymes in Dopamine, Serotonin And Noradrenaline Pathways are Nominally Associated with Cerebrospinal Fluid HVA, 5-HIAA and MHPG Concentrations in Patients with Psychosis

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**Background:** Schizophrenia and other psychotic disorders have been associated with variation in a large number of genes and it is likely that some biological parameters form intermediate steps between gene variation and an altered phenotype. Dopamine, serotonin and noradrenaline are the major monoamines in the human central nervous system. Following their basic biochemical pathways they are degraded to their major metabolites homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), respectively. The present study will shed further light on the hypothesis that the cerebrospinal fluid (CSF) monoamine metabolite concentrations, reflecting monoamine turnover rates, can serve as intermediate phenotypes between genes and the psychotic phenotype. The specific aim was to study whether variation in genes encoding enzymes in monoaminergic pathways are associated with monoamine metabolite concentrations in patients with psychosis.

**Methods:** Psychotic patients have participated in a longitudinal study. At the first investigation cerebrospinal fluid was sampled from all participants and the concentrations of monoamine metabolites HVA, 5-HIAA and MHPG were measured by mass fragmentography with deuterium-labeled standards. Three to 39 years after the first investigation, 74 patients (60 with schizophrenia spectrum disorders and 14 patients with other psychotic disorders), selected from the initial cohort, have been re-investigated and the psychiatric morbidity has been re-assessed. At this second investigation, whole blood was drawn from all participants and genomic DNA was extracted. Genes encoding enzymes implicated in the basic pathways of the monoamines (figure 1) were selected: CATECHOL-O-METHYLTRANSFERASE (COMT), MONOAMINE OXIDASE A (MAOA), MAOB, DOPAMINE BETA-HYDROXYLASE (DBH), DOPA DECARBOXYLASE (DDC), TYROSINE HYDROXYLASE (TH), TRYPTOPHAN HYDROXYLASE 1 (TPH1), TPH2. Single nucleotide polymorphisms (SNPs), previously reported to be associated with schizophrenia or reported to be functionally relevant, as well as tag-SNPs, were selected and genotyped at the SNP Technology Platform at Uppsala University and Uppsala University Hospital, Sweden (<http://www.genotyping.se>). Covariates were selected by preliminary analysis excluding genetic markers.

**Results:** Nominal associations (uncorrected  $p$ -values 0.001- 0.049) were found between HVA and SNPs in DDC (6 SNPs), TPH1 (1 SNP), COMT (1 SNP) and TH (1 SNP). 5-HIAA was nominally associated with DDC (6 SNPs), DBH (2 SNPs), TPH1 (1 SNP), MAOA (1 SNP) and MAOB (1 SNP), whereas MHPG was found to be nominally associated with DDC (4 SNPs), TPH1 (6 SNPs), MAOB (4 SNPs) and DBH (3 SNPs). None of the associations remained statistically significant when accounting for the number of tests conducted.

**Discussion:** During the last decades a large number of variants in the genes investigated in the present study have been associated with schizophrenia. However, it is usually not known by which mechanisms the variants affect the human brain and result in psychotic symptoms. Our results do not provide any firm evidence that variants in genes encoding enzymes implicated in the basic pathways of dopamine,

serotonin and noradrenaline affect monoamine turnover rates in psychosis but still cannot exclude that disturbed monoamine turnover may be a possible mechanism behind the observed associations between the investigated genes and psychosis in humans.

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### Clustering Analysis of Low-Frequency Risk Variants in the Schizophrenia-associated 1Q21.1 Microdeletion Region Suggests CHD1L as Disease-Relevant Gene

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**Background:** Recently, a rare, recurrent microdeletion on chromosome 1q21.1 spanning several genes was identified as one of the strongest genetic risk factors for schizophrenia known to date. At this point, it is unclear (1) whether the region covered by the microdeletion harbours additional low frequency disease variants in the population, and (2) whether a single gene or a combination of genes underlies the increased disease risk. The aim of this study was to investigate the presence of such low frequency variants and pinpoint the schizophrenia risk gene/genes lying in the region.

**Methods:** We performed exon-targeted Sanger resequencing of all single-copy RefSeq genes (n=7) in 94 DSM-IV-diagnosed schizophrenia patients and 94 controls. 28.4 kb of genomic sequence per individual was generated. The identified variants were filtered for a minor allele frequency of (MAF) <5% and were subjected to a spatial-clustering based statistical test developed specifically for the analysis of rare variants in sequencing data. The analysis was performed for the whole region and for single genes at different MAF cut-offs of 1, 3 and 5% and -values were calculated on the basis of 10,000 permutations. Variants in the pin-pointed risk gene are currently being followed up by individual genotyping in a sample of 2,000 German schizophrenia patients and 2,200 population-based controls.

**Results:** A total of 55 different sequencing variants with a MAF<5% were detected across the re-sequenced region. The analysis of all variants revealed a statistically significant risk association signal in the region for variants with MAF<3% (p=0.028) and MAF<5% (p=0.048), and a non-significant trend for MAF<1% (p=0.084). In order to detect the origin of signal in the region the analysis was performed for single genes and the only gene which yielded a significant risk signal was the CHD1L gene (p=0.034, MAF<3% and MAF<5%). All variants in this gene are now being followed-up in a larger cohort by genotyping. Preliminary results from the genotyping are supportive.

**Discussion:** Our study provides evidence for a significant clustering of low-frequency risk variants in schizophrenia patients in the 1q21.1 microdeletion region. We believe that the statistical analysis utilized in this study is a powerful approach since it takes not only the relative frequencies of sequencing variants but also their physical locations into account. Testing for spatial dependence of low-frequency variants is based on the biologically plausible hypothesis that different variants with the same effect (protective or risk) cluster in the disease-relevant genomic region. A more detailed analysis of the microdeletion region provides evidence that CHD1L may be the disease-relevant gene in the region as variants in this gene explain the overall significant signal across the whole region. We hope that our results will provide important insights into the biological processes affected by the 1q21 microdeletion.

### Genetic Contributions to Heterogeneity in Schizophrenia

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**Background:** Schizophrenia is a genetically and clinically heterogeneous disorder with substantial variability in age at onset and severity. Although similar numbers of men and women are diagnosed with schizophrenia, men often have an earlier onset and more severe course of illness. A genetic basis for this variation is strongly suggested, but most prior studies exploring variation in onset and severity and the relationship to gender have been limited and underpowered. Additionally, about 1/4 of people with schizophrenia have other affected family members, and an increased loading of genetic risk factors may exist in familial cases. Identifying genetic sources of heterogeneity in schizophrenia and has potentially far-reaching implications from accurate subtyping leading to more reproducible research findings using fewer subjects to the ultimate goals of efficacious, targeted prevention and treatment plans. In this study, genetic influences on age at onset (AAO) and severity were examined as well as whether different genetic risk factors exist between males and females or increased genetic loading exists in cases with a family history of schizophrenia.

**Methods:** Cases with documented AAO (N=2,387), severity (N=2,290), gender (N=2,537), and family history information (N=2,188) were drawn from the International Schizophrenia Consortium (ISC) sample. All cases and 3,587 population-matched controls were genotyped using Affymetrix 5.0 and 6.0 arrays at the Broad Institute. Analyses were conducted using PLINK, and statistical models involved linear or logistic regression accounting for collection site, population substructure, and sex (when not specifically tested). Polygenic profile scoring was used to assess differences in genetic risk burden between the family history positive and negative groups. International Schizophrenia Consortium.

**Results:** Consistent with prior reports, we observed later average AAO in women (24.7 yrs) compared with men (23.7 yrs;  $p=0.005$ ) and a more severe course of illness for men ( $p=0.002$ ). Subjects with a family history of schizophrenia demonstrated a lower average AAO ( $p=0.048$ ), but no significant differences in illness severity were observed by family history status ( $p=0.51$ ). The top hit for genetic association analyses of AAO in all schizophrenia cases was near the olfactory receptor gene OR2K2, but this was not statistically significant ( $p=4.78 \times 10^{-7}$ ). Family history positive subjects showed the greatest association with KIF5C ( $p=1.96 \times 10^{-8}$ ), however, genetic risk burden does not differ overall between family history positive and negative groups. Separate case-control association analyses for males and females revealed no significant sex-specific associations. Analyses of illness severity (high vs. low) tentatively implicated variation in ST18, but this association did not attain genome-wide significance ( $p=8.24 \times 10^{-7}$ ).

**Discussion:** These results confirm recognized demographic relationships but do not support a simplified genetic architecture for schizophrenia subtypes based on these variables. Dichotomization on the basis of family history is not supported based on the lack of differences in overall genetic risk burden. Results for males and females separately indicate no sex-specific genetic risk factors of strong or moderate effect, and association analyses for AAO and illness severity did not yield genome-wide significant results in this sample.

### Concordance Differences in Twins with Schizophrenia According to the Presence or Absence of Other Psychotic Syndromes

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**Background:** In genetic studies of psychotic disorders, a single lifetime diagnosis is usually employed, with schizophrenia at the top of the diagnostic hierarchy. Most investigations do not consider whether individuals with schizophrenia have also had episodes of other psychotic syndromes during their lifetime. This could be problematic if patterns of risks in relatives differ according to whether or not other psychotic syndromes have occurred.

**Methods:** We ascertained 224 probandwise twin pairs (106 monozygotic, 118 same-sex dizygotic), where probands had psychotic or manic symptoms, from the Maudsley Twin Register in London (1948-1993). Twin probands with Research Diagnostic Criteria (RDC) schizophrenia were divided according to the presence or absence of other psychotic syndromes (schizoaffective-manía, schizoaffective-depression, manía or depressive psychosis). The pattern of monozygotic probandwise concordance for psychotic syndromes was compared according to whether probands did or did not have other psychotic syndromes in addition to schizophrenia.

**Results:** The distributions of psychotic syndromes in co-twins differed significantly ( $\chi^2=16.76$ ,  $df=4$ ,  $p=0.002$ ): co-twins of probands with schizophrenia only had a higher risk of schizophrenia (19/36 (52.8%) versus 1/13 (7.7%)) and lower risk of a schizoaffective disorder (0/36 versus 4/13 (30.8%)). However, risk in co-twins for the broad phenotype of any psychotic syndrome did not significantly differ between the two groups.

**Discussion:** Schizophrenia occurring in the absence of other psychotic syndromes is likely to be more aetiologically homogeneous than hierarchical lifetime schizophrenia, and this refinement of the schizophrenia phenotype may allow better prediction of the form of illness most likely to occur in affected relatives.

### Beyond Individual Analysis of Common SNPs In GWAS: Rare Variant Analysis and Hypothesis-Driven Gene Set Analysis in a Genome-Wide Missense SNPs Association Study in Schizophrenia

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**Background:** Previously, we have carried out a genome-wide association study of missense SNPs. Our dataset after quality control procedures consisted of 5100 common missense SNPs and 3612 rare SNPs (frequency < 5) genotyped in 476 schizophrenic patients and 447 control subjects from Galicia, NW Spain. Individual analysis of common SNPs and replication in 4069 cases and 15128 controls from 11 European populations led to the identification of one SNP at SLC39A8 that remained significant after Bonferroni's correction. Here, we present additional analysis to test for the accumulation of rare variants in any gene, or in candidate gene sets. We also applied a hypothesis-driven gene set analysis to search for an overrepresentation of the DISC1 interacting proteins within the top results of our ranked list of genes based on our previous genome-wide association study of missense SNPs.

**Methods:** Accumulation of rare variants at each gene was tested with the software CCRaVAT. Four different gene sets were considered for accumulation of rare variants: 1) DISC1 interactome, 2) Cell Adhesion Molecules, 3) Gene Ontology categories related to synaptic transmission or neurodevelopment, 4) Candidate genes present at SchizophreniaGene database. Accumulation of rare variants at each gene set was tested using VTest. This program uses a variable frequency threshold for consideration of rare variant, and allowed weight of variants based on functionality scores predicted by Polyphen 2. Finally, the Gene Set Enrichment Analysis adapted for SNPs, as implemented in the GenGen software, was used to test for overrepresentation of the DISC1 interacting proteins within the top results, using the subset of 5100 common SNPs. Briefly, the test calculated an enrichment score based on the weighted Kolmogorov-Smirnov-like running-sum statistic. The significance of the enrichment score was assessed by 5000 permutations of the case-control labels, then, preserving the linkage disequilibrium structure and SNP density at each gene.

**Results:** Our rare variants analysis at the gene level revealed an association at a rare variant of AHI1, rs6940875, although the result did not resist Bonferroni correction. There was previous strong evidence of association of AHI1 with schizophrenia. There was not significant accumulation of rare variants at any of the gene sets. Finally, the hypothesis-driven gene set analysis detected an overrepresentation of the DISC1 interacting proteins (permuted P value = 0.0158). We identified seven leading edge genes, MACF1, UTRN, DST, DISC1, KIF3A, SYNE1, and AKAP9, responsible for the overrepresentation.

**Discussion:** On the one hand, we did not find evidence of the polygenic mutation-selection balance model suggesting that most low-frequency missense SNPs present in dbSNP prior to the upload of data for next-generation sequencing experiments are of minor effect in schizophrenia risk. On the other hand, there was a significant overrepresentation of common SNPs at the top of our association results, strongly suggesting the involvement of this network in schizophrenia susceptibility. The leading edge genes are involved in neuronal cytoskeleton organization and intracellular transport through the microtubule cytoskeleton, suggesting that these processes may be impaired in schizophrenia.

### Identification of Rare Functional Variants in Putative Schizophrenia Risk Genes by Targeted Resequencing of a Galician (Northwest Spain) Sample

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**Background:** Population genetics studies showed that a substantial proportion of missense SNPs confers deleterious effects but these effects are weak enough to avoid removal by natural selection. Thus, these SNPs are segregating in populations at low frequencies, probably contributing to common diseases. Genome-wide association studies (GWAS) using large sample sizes have led to the identification of several common variants of susceptibility to schizophrenia with low effect as well as rare copy number variants (CNVs) of considerably larger effect. There is some evidence that a susceptibility gene may present variants of different frequency and risk effects. In this work, we carried out a sequencing study of coding exons from some of the genes with higher evidence of association with schizophrenia in order to search for putatively mildly deleterious single nucleotide variants (SNVs) contributing to disease.

**Methods:** Genes were selected based on these criteria: 1) genes associated with schizophrenia at genome-wide significant level; 2) genes in CNV regions strongly associated with schizophrenia; 3) DISC1 and genes coding for DISC1 partners well characterized at the molecular level or with some evidence of association in our previous large scale association study of missense SNPs; 4) metal ions transporters SLC39A8 and SLC39A3, associated with schizophrenia in our previous large scale association study of missense SNPs or with bipolar disorder, respectively. DNA samples of 153 schizophrenic patients were normalized and pooled with 3 DNA samples per pool prior to targeted enrichment. These samples were chosen from our collection of 513 schizophrenic samples (DSMIV criteria) from Galicia (NW Spain) based on positive family history and early age at onset. Additional pools of three samples were done from 153 Galician controls. A total of 124472 bp of coding sequences from the selected genes were captured using custom-designed Agilent's SureSelect Target Enrichment System in solution. Barcoded pair-end fragment libraries were constructed for sequencing using the ABI Solid4 platform (50bp + 35bp reads). Mapping of reads were done with BioScope (ABI) and identification of single nucleotide variants (SNVs) were done following the BioScope (high stringency parameters) and GATK pipelines. As quality control, we have used data for previously genotyped SNPs at frequencies 0/6 (1976 data) or 1/5 (840 data) in each pool.

**Results:** Coverage was highly homogeneous between the different samples and between cases and controls. The average depth of coverage at each position was 376x. Depth of coverage was larger than 100x for 95% of positions, after removal of low-quality and duplicate reads. A total of 448 SNVs were called by both BioScope and GATK, 172 only by BioScope and 237 only by GATK. Using previously genotyped data as controls, we estimated that the combination of SNVs detected by BioScope or by GATK considerably reduced the false negative rate to low values (0.06) in comparison with the individual results of each SNV calling procedure, while still maintained a low false positive rate (0.01). Then, we focused our analysis in the total 857 SNVs. There was an excess of putative deleterious SNVs at low

frequency as compared with synonymous SNVs, an effect detected both in SNV at frequencies < 1% as well as at frequencies 2-5% ( $P < 0.01$  in trend tests). We will show data of those putative deleterious SNVs present in more than one case and absent from our controls and from databases.

**Discussion:** We present an analytical pipeline that led to the identification of SNVs with high sensibility and specificity. There was an excess of putatively functional SNVs at low frequency in our sample, suggesting the existence of mildly deleterious variants. Some of these variants might play a role in schizophrenia risk, deserving further studies.

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### Psychosis is XY Linked and Epigenetic

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**Background:** Nearly all researchers have assumed that the genetic basis of psychosis is related to one or more autosomal loci, perhaps many. However few such loci have been reliably established - the problem of the “missing heritability”. Here it is suggested that an XY homologous locus explains five features otherwise unaccounted for, does not exclude an autosomal pattern of transmission, and is consistent with the concept that the disorder is species-specific and related to the capacity for language.

**Methods:** Evidence for modes of transmission of different forms of illness, for species-specific characteristics, and for asymmetry as the brain correlate of language, have been examined. Meta-analyses of brain structure in psychosis have been reviewed to determine the role of the torque. The Protocadherin11X gene has been sequenced in the great apes and Protocadherin11XY genes have been sequenced in 200 Homo sapiens individuals. The Xq21.3 to Yp11.2 duplication has been dated. The distribution of Protocadherin11XY has been mapped immuno-histochemically in the human adult and embryonic brain.

**Results:** Five findings stand out:

- 1) there are sex differences in age of onset of schizophrenia.
- 2) form of psychosis is systematically related to age of onset and sex.
- 3) interactions between sex, hemisphere and diagnosis have been reported for ventricular size, gyrification, and verbal and spatial ability.
- 4) the genetic basis of species differences is strongly related to the X chromosome according to Haldane’s rule that in hybrids if one sex is disadvantaged with respect to fertility or viability it is the heterogametic sex ie in mammals the males.
- 5) evidence from sex chromosome aneuploidies of hemispheric deficits eg in verbal and spatial tasks, and from structural MRI studies indicates that the cerebral basis of asymmetry (the “torque”) is dependent on a genetic factor on both the X and the Y chromosomes.

**Discussion:** These five lines of independent evidence indicate that the that the genetic basis of psychosis is sex linked rather than autosomal. Where is the gene? 6 million years ago a 3.5Mb block transposed from Xq21.3 to Yp11.2 and subsequently paracentrically inverted. Of the 3 genes within this region two have been eliminated by subsequent deletion or frameshift mutation, to leave Protocadherin11Y (PCDH11Y) gene as the only gene expressed from the transposed block. The Protocadherin11XY gene-pair that has been subject to 16 non-synonymous changes in the Y, and 5 changes in the X sequence, codes for two cell surface adhesion molecules both expressed in the brain. Because the homology between the X and Y forms of Protocadherin11XY is less than 100% variability of expression both genes will be expected as a result of ‘meiotic suppression of unpaired chromosomes’ in male meiosis. It is proposed that this epigenetic variation constitutes a species-specific message delivered to the very early embryo to establish its most characteristic developmental feature.

### A Genome-wide Pathway Mega-analysis Suggests that the DISC1 and Dopaminergic Pathways are More Genetically Disrupted in Schizophrenic Patients Compared to Controls

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**Background:** Schizophrenia remains one of the leading causes of global disease burden in adults despite more than 50 years of drug development. Pinpointing its genetics is critical to future novel and more efficacious treatments. DISC1 (Disrupted in Schizophrenia 1) and the dopaminergic system are interesting candidates. Nevertheless, the genome-wide studies insofar did not consistently replicate the results on these pathways and more studies are warranted.

**Methods:** We undertook a genome-wide-pathway analysis on a sample of 1078 schizophrenic patients and 581 controls available from the NIMH website. Three studies were included, all characterized by the use of the Affymetrix 500: the pgc-scz-genotyped-carwtc-cases (Cardiff), pgc-scz-genotyped-zhh (Zucker Hillside) and the pgc-scz-genotyped-cat2-v1\_01 (CATIE). The case – control labeling was the phenotype under investigation, covariates were the genetic admixture of the sample, the study labeling and gender. SNPs were included when they had a minor allele frequency  $\geq 0.01$ , did not deviate from Hardy Weinberg Equilibrium ( $p \geq 0.0001$ ) and had a genetic missingness per marker  $\leq 99\%$ . Imputation was conducted employing the hapmap3\_r2\_b36 dataset, an info value  $\geq 0.95$  was set for the quality control after imputation. 341880 SNPs were available after imputation and quality control in the merged dataset. Genes involved in the DISC1 pathway and in the dopaminergic pathway were chosen from international dataset for molecular pathway analysis (<http://conceptgen.ncibi.org/core/conceptGen/index.jsp>), the SNPs located in each of those genes were also retrieved from international dataset (<http://www.ncbi.nlm.nih.gov>). Contingency tables were created with columns indicating the genome-wide frequency of SNPs found within or out of the molecular pathway, and rows indicating the genome-wide frequency of SNPs associated with phenotype below or under a certain p level (a p level of 0.05 was chosen after 1000 simulations on the DISC1 pathway). A Fisher test was then conducted to test whether or not the pathway labeling was associated with the distribution of significant associations with the phenotype genome-wide. The bilirubin pathway was employed to falsify the method. R, PLINK, SNPtest and IMPUTE were employed for the analysis.

**Results:** 100 genes (1488 SNPs) were available for the DISC1 pathway (“DISC1 interactions” was the keyword for the database search), 16 genes (378 SNPs) were available for the dopamine pathway (“Dopamine Plasma Membrane Transport Proteins” was the keyword for the database search) and 12 genes (146 SNPs) were available for the bilirubin pathway (“Bilirubin” was the keyword for the database search). SNPs associated with the phenotype were more than expected in the DISC1 (10.6% within the pathway vs 5.5% genomewide,  $p = 2.691e-14$ , OR = 2.01) and dopaminergic (10.6% within the pathway vs 5.5% genomewide,  $p = 6.699e-05$ , OR = 2.05) pathways but not in the bilirubin pathway at a p level of association for single SNP  $\leq 0.05$ .

**Discussion:** We found evidence that the DISC1 and the dopaminergic pathway harbor significantly more variations in Schizophrenic patients compared to controls in the sample under analysis. The method we employed proved to be sufficiently specific to exclude – as expected - that the bilirubin pathway is more genetically disrupted in Schizophrenic patients than in controls. This result stress the relevance of common variations with single small impact towards the phenotype in increasing the risk for Schizophrenia, which is consistent with the polygenic nature of the disease.

### Pathway Analysis of Six PGC Datasets Using Magenta, One of the Five Pathway Analysis Methods Used by the PGC Network and Pathway Analysis (PGC-NPA) Group

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**Background:** The PGC Network and Pathway Analysis group (PGC-NPA) carried out pathway analysis using five different methods (MAGENTA, ALLIGATOR, INRICH, FORGE, Set Screen) on the six major PGC datasets. Given the large scope of the project, general methods and results from one pathway analysis method (MAGENTA) are presented here.

**Methods:** Genetic data (SNP p-values) were obtained from the PGC for five individual disorders and the cross-disorder analysis with the following numbers of cases and controls: schizophrenia (9,370 / 7,736), bipolar disorder (6,990 / 4,820), major depressive disorder (9,227 / 7,383), autism (4,949 / 5,314), ADHD (2,787 / 2,635), and cross-disorder (33,332 / 27,888). Six databases (GO, KEGG, OMIM, PANTHER, Reactome, and TargetScan) were used to generate pathways for analysis (total = 14,793 pathways with 2-200 genes).

**Results:** Pathway size, measured in number of genes, was a significant predictor of pathway p-value (adjusted r-squared = 11%,  $p < 2e-16$ ). This is true for MAGENTA because possible p-values are constrained by pathway size. While this consequence of the MAGENTA algorithm precludes the possibility of very small p-values for small pathways (e.g. 2 or 3 gene pathways), it does not create false positive results. The strongest evidence of enrichment was for the VEGF signaling pathway (Panther database) in schizophrenia ( $p = 9.9e-6$ ). None of the other disorders yielded p-values markedly smaller than those expected by chance.

**Discussion:** The results (p-values) from pathway analysis programs including MAGENTA depend partially on pathway size, and this is particularly true when very small pathways are used. Given this phenomenon, the effect of pathway size on p-values should be taken into account when multiple testing corrections are performed. Alternatively, smaller pathways can be excluded prior to analysis. As reported by others, results vary across pathway analysis methods even when the same data set is analyzed. Thus, the most confidence should be afforded to pathways identified across methods, and pathways implicated by only one method should be interpreted with caution.

### BCL9 and C9ORF5 Are Associated with Negative Symptoms in Schizophrenia: Meta-Analysis of Two Genome-wide Association Studies

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**Background:** Schizophrenia is a chronic and debilitating psychiatric condition affecting slightly more than 1 of the population worldwide and it is a multifactorial disorder with a high degree of heritability (80) based on family and twin studies. Increasing lines of evidence suggest intermediate phenotypes/endophenotypes are more associated with causes of the disease and are less genetically complex than the broader disease spectrum. Negative symptoms in schizophrenia are attractive intermediate phenotypes based on their clinical and treatment response features. Therefore, our objective was to identify genetic variants underlying the negative symptoms of schizophrenia by analyzing two genome-wide association (GWA) data sets.

**Methods:** The subjects consisted of a total of 1,774 European-American patients and 2,726 controls. Logistic regression analysis of negative symptoms as a binary trait (adjusted for age and sex) was performed using PLINK. For meta-analysis of two datasets, the fixed-effect model in PLINK was applied.

**Results:** Through meta-analysis we identified 52 single nucleotide polymorphisms (SNPs) associated with negative symptoms with  $p < 10^{-4}$ . Especially we detected five SNPs in the first two genes/loci strongly associated with negative symptoms of schizophrenia (Pmeta-analysis  $< 6.22 \times 10^{-6}$ , Table 1), which included three SNPs in the BCL9 gene: rs583583 showed the strongest association at a Pmeta-analysis of  $6.00 \times 10^{-7}$  and two SNPs in the C9orf5 (the top SNP is rs643410 with a  $p = 1.29 \times 10^{-6}$ , Table 1). Through meta-analysis, we identified several additional negative symptoms associated genes (ST3GAL1, RNF144, CTNNA3 and ZNF385D).

**Discussion:** This is the first report of the common variants influencing negative symptoms of schizophrenia. These results provide direct evidence of using of negative symptoms as an intermediate phenotype to dissect the complex genetics of schizophrenia. However, additional studies are warranted to examine the underlying mechanisms of these disease-associated SNPs in these genes.

### Analysis of the Hexonucleotide Repeat Expansion at C9ORF72 in an Irish Psychosis Case-control Sample

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**Background:** A hexonucleotide repeat expansion 'GGGGCC' in an intronic region of the C9ORF72 (chromosome 9 open reading frame 72) gene has been found to account for up to 60 of familial amyotrophic lateral sclerosis (ALS) and up to 10 of sporadic ALS. The repeat expansion is located between exon 1a and exon 1b of this gene and although function is unknown, it is thought to impact on gene expression. One in seven ALS patients develops frontotemporal dementia (FTD). Analysis of an Irish population-based cohort of ALS identified a higher rate of FTD in ALS patients carrying the repeat compared to those that do not carry the repeat (PubMed ID (PMID): 22305801). Study of an independent FTD sample showed a strong association between C9ORF72 mutations and psychotic symptoms: delusions, hallucinations, paranoid ideation and disordered thinking (PMID: 22300873). Therefore, we sought to screen a large Irish psychosis case-control sample for evidence of association between the repeat expansion and psychosis.

**Methods:** Our sample included 742 schizophrenia, 261 bipolar disorder, 162 schizoaffective disorder cases and 1,283 control samples. We used a reverse primed PCR method to amplify the hexonucleotide repeat expansion. Analysis of PCR products was carried out using a 3130xl Genetic Analyzer and GeneMapper 3.0 software (Applied Biosystems). The pathogenic range of the variant is  $>30$  repeats and the expansion can extend up to 700-1600copies in ALS/FTD sufferers. All samples were genotyped in 96-well plate format and each plate contained two positive control samples that had both previously been confirmed to contain 34+ repeats.

**Results:** Overall the distribution of repeat numbers was very similar for cases and controls. We identified four samples that carried a repeat number approaching the pathogenic range. There were two controls samples (23 and 24 repeats respectively) and two schizophrenia cases (both 26 repeats).

**Discussion:** Initial reports on this repeat expansion indicated that the normal range of repeats does not usually exceed 23 copies of the hexanucleotide. A small number of apparently normal individuals have an intermediate number of repeats between 24 and 29, the significance of which is unclear. The repeat length in two cases with schizophrenia lies within this intermediate range, raising the possibility of an association between C9ORF72 repeat expansions and psychosis. As expansions may be tissue specific, further studies using Southern blotting may be warranted to test the hypothesis that some forms of psychosis are linked to C9ORF72 repeat expansions.

### Genome-wide Association Study of Schizophrenia Modifier Loci in the Psychiatric Genomics Consortium (PGC)

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**Background:** Genetic factors might contribute not only to schizophrenia risk, but might also modify symptomatology, severity, course, and outcome.

**Methods:** Our sample comprised the PGC Schizophrenia study (N=9,394 cases). Several different rating scales and checklists were used to assess lifetime symptoms. These included the Operational Criteria Checklist for Psychotic Illness (OPCRIT), Positive and Negative Syndrome Scale (PANSS), Lifetime Dimensions of Psychosis Scale (LDPS), Comprehensive Assessment of Symptoms and History (CASH), Schedule for Clinical Assessment in Neuropsychiatry (SCAN), and Structured Clinical Interview for DSM-IV (SCID). To develop dimensional phenotypes, exploratory factor analyses were performed in each sample separately. Previously performed or published factor structures were used if available, in their respective subsamples (LDPS, CASH, and SCID). Harmonization across sites and instruments was attempted at both the item and factor levels. Factor scores were calculated for each subsample separately, given considerable phenotypic heterogeneity across sites. GWAS of the resulting quantitative traits, as well as Age of Onset, was performed using linear regression in PLINK in cases only. QC and adjustment for ancestry covariates was as previously described (Ripke et al., 2011). Psychiatric Genomics Consortium Schizophrenia Working Group

**Results:** A four-factor model was retained, comprising the Positive, Negative, Manic, and Depressive symptom dimensions. No SNP attained genome-wide significance. However, 25 independent SNPs were suggestively significant in one of the five phenotypes (P<10<sup>-5</sup>), nine of which were in genes or UTRs. None of these were suggestively associated with schizophrenia in case-control analyses. Furthermore, none of the top SNPs in our previously published GWAS mega-analysis of schizophrenia were suggestively associated with these quantitative phenotypes. Polygenic analyses are ongoing and will be presented.

**Discussion:** While this GWAS yielded no definitive evidence of modifier loci, a number of SNPs implicated genes that have not previously been suspected in schizophrenia. Follow-up in additional samples is warranted.

### Genome-wide Association Study on Attention

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**Background:** There is evidence for a strong genetic component in the etiology of schizophrenia, as demonstrated by family, twin and adoption studies. The relative contribution of genetic factors has been estimated to be ca. 80. The mode of inheritance is complex and non-Mendelian.

**Methods:** Schizophrenia as a heterogeneous disorder displays a variety of clinical features. For this reason we put a major focus on intermediate phenotypes (e.g. neuropsychological) as they might be more elementary compared to clinical syndromes and are thus potentially informative regarding the pathophysiology of schizophrenia. Carefully selected intermediate phenotypes which are state independent, associate with the illness, and co-segregate in families with the disease were studied.

**Results:** Schizophrenia patients have difficulties to discriminate relevant from irrelevant stimuli over a wide range of tasks. Attention deficits in schizophrenia can be assessed by using e.g. the Continuous Performance Test (CPT). The typical variable measured is the decrement in signal/noise discrimination within the vigilance period, termed sensitivity. The accumulated evidence to date supports the idea that CPT performance deficits are reliable and valid genetic susceptibility indicators of schizophrenia.

**Discussion:** We performed a genome-wide association study on attention including over 2500 healthy controls and 550 schizophrenia patients with several replication steps. The poster will present these new results on attention and will discuss newest studies on this topic.

### Activation of the Immune System with Respect to TMT And Stroop Test Performance in Schizophrenia

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**Background:** Genetic factors that modulate the immune response have been implicated as risk factors both for schizophrenia as well as for cognitive impairments, which are considered to be endophenotypes of schizophrenia, i.e. subclinical, heritable and independent of clinical state traits associated with genetic susceptibility. Regulation of immune response is mediated by two related receptors: CTLA-4 and CD28, which mediate differentially T-cell activity. CD28 is a major co-stimulator, whereas CTLA-4 performs negative regulatory functions. The level of activation of immune response depends on the balance between co-stimulatory and inhibitory signals. One of the key mechanisms leading to immune dysregulation is the expression of the regulatory molecules due to their genes polymorphisms. The study was carried out to investigate the association between polymorphisms of the CTLA-4 gene (49A/G, -319C/T, CT60 A/G) and CD28 gene (+17C/T) and frontal lobe functions in patients with schizophrenia.

**Methods:** 118 patients diagnosed with schizophrenia according to ICD-10 criteria and 352 controls were included in the study. The participants were evaluated for lifetime symptomatology using the Operational Criteria for Psychotic Illness Checklist (OPCRIT). Cognitive functions were assessed by performance on the most commonly administered measures of frontal lobe functioning: Trail Making Test (TMT) and Stroop Color Word Interference Test (SCWT). TMT-A involves connecting numbers in succession, while TMT-B requires subjects to connect numbers and letters alternately in successive order. TMT-A requires mainly visuo-perceptual abilities, TMT-B reflects primarily working memory and secondarily task-switching ability, while the difference between TMT-B and TMT-A score provides an indicator of executive control function. Stroop Test involves reading printed color names when the name of the color is printed in a color not denoted by the name, thus allowing to measure the ability to inhibit a prepotent response tendency.

**Results:** There was no significant difference in distribution of genotypes in the polymorphisms of CTLA-4 gene between patients and controls. However, there were significant differences ( $p=0,0007$ ) in distribution of genotypes of CD28 gene between group of patients and controls (CC: 2% vs. 1%, CT:41 vs 23%, TT: 58% vs. 76% respectively). Patients performed significantly below the norms for general population on both TMT A and TMT-B tests. There were no significant differences between patients with respect to CTLA-4 and CD28 gene polymorphisms in TMT-A and TMT-B scores. However, with respect to +17C/T CD28 gene polymorphism there was a trend level difference: C allele carriers (CC and/or CT genotype) performed worse than T allele carriers (TT genotype) ( $p=0,054$ ), suggesting weaker executive control function. Additionally, there were no significant differences among patients with respect to CTLA-4 and CD28 gene polymorphisms and performance on Stroop Test.

**Discussion:** CD28 has been widely studied in autoimmune disorders. In our study, distribution of alleles and genotypes of CD28 gene polymorphism in patients with schizophrenia are similar that found in patients with autoimmune disorders such as: early onset type 1 diabetes and Behçet's disease. On the other hand in the study of rheumatoid arthritis there is an inverse distribution of alleles and genotypes of CD28 gene polymorphism that found in our group of schizophrenia

patients. This finding is consistent with well known fact that despite many similarities there is a strong negative correlation between the prevalence of these two disorders and once a person gets one of the diseases then they are relatively immune to the other. Additionally, +17C/T CD28 gene polymorphism might be considered as a risk factor for cognitive impairment in schizophrenia. To the best of our knowledge it is the first report showing this findings.

### An Association Analysis of the Cardiomyopathy-Associated 5 (CMYA5) Gene With Schizophrenia In A Japanese Population

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**Background:** Schizophrenia is a complex psychiatric disease that is suggested to result from multiple genetic and environmental factors. Although a large number of studies have been conducted, genetic factors and mechanisms responsible for the disease are still to be clarified. Recently, Chen et al. (2010) described that three SNPs on the cardiomyopathy-associated 5 (CMYA5) gene, rs3828611, rs10043986, and rs4704591, were associated with schizophrenia based on their data-mining analyses using the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and molecular genetics of schizophrenia genome-wide association study supported by the genetic association information network (MGS-GAIN) data sets (total n = 33,834). Their study was followed by Li et al. (2010), which tried to replicate the association by using 5,608 Chinese subjects, except for rs10043986 due to its monomorphism. As a result, a significant association of rs3828611 was observed in their study, while not with rs4704591; however, until now, to our knowledge, there has been no other study which investigates the association, and further study may be needed to confirm the results. Here, we investigated the association between the CMYA5 gene and schizophrenia by using the Japanese Genetics Initiative for Replicating Association of Schizophrenia (JIRAS) sample, one of the largest DNA sample set of Japanese schizophrenics.

**Methods:** The subjects comprised of 2,785 unrelated cases with schizophrenia (1,487 males and 1,298 females; age 44.7 ± 14.9, mean ± SD) and 2,785 unrelated healthy controls (1,487 males and 1,298 females; age 44.9 ± 14.0, mean ± SD). Two experienced psychiatrists independently made diagnoses according to the ICD-10 and DSM-IV criteria. Controls were mainly recruited from the hospital and faculty staffs. All controls had a short interview with one of the authors to confirm that they had no history of major mental illness. The objective of the study was clearly explained, and written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of the Faculty of Medicine, the University of Tokyo. Genomic DNA was extracted from leukocytes by using the standard phenol-chloroform method. Two SNPs in the CMYA5 gene, rs4704591 and rs3828611, were analyzed by TaqMan PCR method using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster, CA, USA). Rs10043986 was monomorphic in the Japanese population, and therefore dropped out from further analysis. The chi-square test was used to compare the allele frequencies and genotypic distributions between the cases and controls.

**Results:** The allele frequencies and genotypic distributions of the two SNPs are summarized in Table 1. The genotypic distribution of rs3828611 followed the Hardy-Weinberg equilibrium (HWE) both in the cases and controls. In contrast, that of rs4704591 significantly deviated from the HWE in the cases (p value = 0.015), while not in the controls. No significant difference was observed between the cases and controls in the allele frequencies or genotypic distributions of the two SNPs.

**Discussion:** We did not observe the significant association between the two SNPs in the CMYA5 gene and schizophrenia in the present study. Our study was conducted by using one of the largest sample sets in Japanese population, and the statistical power was 0.81 for rs3828611, and 0.82 for rs4704591, respectively ( $\alpha=0.05$ ), which means the sample size in the present study was mostly adequate and the possibility of type II error was relatively small. The genotypic distribution of rs4704591 significantly deviated from the HWE in the cases. This could be caused by chance or technical error, although another possibility is that the SNP might be relatively novel and have an association with the etiopathology of schizophrenia. The present result was not consistent with those of the previous two studies (Chen et al., 2010; Li et al., 2010); however, rs4704591 did not show a significant association in the Chinese population (Li et al., 2010). In addition, the association of rs3828611 was not significant in the northern area, while significant in the southern area and total Chinese population (Li et al., 2010). Thus, the results were not completely the same even between the previous studies, and these may reflect the ethnic difference in the role of the SNPs for the development of schizophrenia. In conclusion, the present study did not provide the evidence for the association between schizophrenia and the two SNPs of the CMYA 5 gene in the Japanese population. Further investigation may be needed to clarify the role of the gene for the etiopathology of schizophrenia.

### The Clinical Research Group 241: Genotype-phenotype Relationships and Neurobiology of the Longitudinal Course of Psychosis

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**Background:** Over the last decade, much research has been conducted on the genetic and biological basis of psychosis in schizophrenia, schizoaffective and bipolar disorder. Genome-wide association studies (GWAS) have identified several novel susceptibility genes for these diseases. However, variants identified so far account for only a fraction of disease liability. In order to gain more insight into the complex genotype-phenotype relationships, GWAS-based on single nucleotide polymorphisms (SNPs) have to be embedded in a framework of complementary research. One important approach, which has so far received insufficient attention, concerns the analysis of longitudinal phenotypes. The Clinical Research Group (CRG) 241 was established in Göttingen, Lower Saxony, in order to fill this gap as a multidisciplinary research framework focusing on the longitudinal course of psychosis.

**Methods:** The CRG comprises both clinical and basic scientific components and is therefore divided into several work-packages (WPs) with different focuses. In the clinical part, the aims are to carry out GWAS (WP1/2 in collaboration with WP5), candidate gene studies (WP1/2 in collaboration with WP5), brain imaging and neurobiological studies (WP2), as well as pharmacogenetic approaches (WP3). Analyses are based on a large patient sample comprising 3000 prospectively-followed individuals suffering from schizophrenia, schizoaffective or bipolar disorder and prospectively-followed 3000 controls. Patients are recruited at the Department of Psychiatry and Psychotherapy (University Medical Centre, Georg-August-University Göttingen) and at associated recruitment centres at 13 collaborating hospitals in Lower Saxony, Northern Hesse, Bremen plus several more distant co-operating recruitment sites. Over a period of at least 1.5 years, patients and controls will be interviewed at regular visits every 6 months, and earlier if needed (e.g. in case of relapse). Each visit includes taking blood samples and assessing phenotypic features like differentiated psychopathology, response to pharmacological treatment (including adverse events), data on alcohol/substance consumption, global functioning, neuropsychological performance, and life events. These assessments are carried out in parallel with structural and functional imaging (WP2). The clinical part of the project is complemented by basic scientific projects involving animal models on epigenetic regulation (WP4) and the influence of environmental factors on potential susceptibility genes for the longitudinal course of psychosis (WP6).

**Results:** No results yet to publish.

**Discussion:** The findings of the Clinical Research Group will contribute substantially to a better understanding of the molecular and biological determinants of the longitudinal course of psychosis and their complex interactions with the environment. This in turn will hopefully contribute to the development of therapies improving the long-term outcomes of psychosis.

### Are Gap-junction Proteins Associated with Schizophrenic Psychoses?

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**Background:** Pannexins are a group of brain expressed gap-junction proteins with multiple proposed functions in CNS. They were associated with signal transduction, synchronization of neuronal groups, EEG activity and brain development. Pannexins are thought to be regulators of neural stem and progenitor cell proliferation and involved in inflammation and apoptosis of neurons. The chromosomal loci of PANX1-3 are proposed as candidate regions for schizophrenia in genome-wide linkage studies: PANX1 locates in the chromosomal region 11q21 spanning 53 kb with 5 exons. PANX2 locates in the telomeric region of chromosome 22q13 and comprises 9.5 kb with four exons. PANX3 locates at chromosome 11q24 spanning 9 kb with four exons. We tried to evaluate the role of pannexins in schizophrenic psychoses in a polydiagnostic approach. Beside ICD10, we tested additionally clinical sub-phenotypes according to Leonhard's classification that divides schizophrenic psychoses into three main groups, systematic schizophrenias, unsystematic schizophrenias and cycloid psychoses. Different genetic backgrounds for each of these diagnostic categories have been supposed based on findings in family and twin studies.

**Methods:** In a case-control sample of German descent (cases: n= 1172; controls: n= 480) we genotyped six single nucleotide polymorphisms (SNPs) around PANX1, five around PANX2 and three around PANX3 for genetic association in a polydiagnostic approach (ICD 10; Leonhard's classification). Cases fulfilled criteria for schizophrenia according to ICD10 and were additionally diagnosed according to Leonhard's classification. Probands were recruited at the Department of Psychiatry at the University of Würzburg. PCR for allelic discrimination was performed with TaqMan™ SNP genotyping assays and analysed with the appropriate software package from Applied Biosystems Software FAMHAP and HAPLOVIEW was used to test for association.

**Results:** We found no significant association with schizophrenia according to ICD10 for the tested SNPs at PANX1-3, but we observed significant association of SNP rs4838858 at PANX2 with phasic cycloid psychoses analyzing clinical subgroups according to Leonhard's classification ( $p > 0.0001$ ; table). No marker reached significance level ( $p=0.068$ ) testing for association with systematic and unsystematic schizophrenias with subtype periodic catatonia. The statistical Power of our study was 95.9 % at  $\alpha = 0.05$ .

**Discussion:** Our findings do not support a major contribution of Pannexins to disease risk for schizophrenia according to ICD 10. That partially replicates a Japanese case-control study, reporting no association for PANX2. Regarding clinical phenotypes, we found a nominal association for the subgroup cycloid psychoses but not for the unsystematic or systematic subforms. Several independent groups demonstrated face-validity of the concept of cycloid psychoses (Perris 1974, Brockington et al., 1982, Beckmann et al. 1990, Peralta and Cuesta 2003, Jabs et al. 2004, Garcia-Andrade et al. 2011). Within the group of cycloid psychoses, the best association was found for the phenotype confusion psychoses characterized by excitation and incoherence of thematic choice in the one pole or inhibition of the thought process and ideas of reference in the other. As the associated marker locates intronic between exon 20 and 21, the causative factor is still undefined, but points to a potential, but limited role of Pannexin 2 in clinical subgroups with phasic course and thought disorder.

### Association of SHANK3 with Schizophrenic Psychoses

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**Background:** Shank3 is a member of the Shank gene family involved in brain development, neuroplasticity and signalling pathways. Shank proteins are multidomain scaffold proteins of the postsynaptic density that connect neurotransmitter receptors, ion channels, and other membrane proteins to the actin cytoskeleton and G-protein-coupled signaling pathways. Shank3 plays a role in neurodevelopment during synapse formation and dendritic spine maturation. Shank3 at chromosome 22q13.3 consists of 22 exons spanning a region of 58.7 kb. In vitro studies proposed an influence of Shank3 on glutamate signaling and LTP. Genetic mutations of SHANK3 were reported in patients with cognitive impairment and ASD and are thought causative factors of the 22q13.3 deletion syndrome (Phelan-McDermid syndrome). Beside ASD, Shank3 could play a role in schizophrenia. There is evidence of linkage in SCZ families at 22q11-13 locus, which includes SHANK3, from several studies. Another study reported two de novo missense mutations in shank3 in patients with schizophrenia. We tried to evaluate the role of Shank3 in schizophrenic psychoses in a polydiagnostic approach. Beside ICD10, we tested additionally clinical subgroups according to Leonhard's classification to increase sample homogeneity and to intensify phenotype characterization. Karl Leonhard divides schizophrenic psychoses into three main groups, systematic schizophrenias, unsystematic schizophrenias and cycloid psychoses. In family and twin studies based on Leonhard's classification, a different genetic background for each diagnostic category was demonstrated.

**Methods:** In a case-control sample of German descent (cases: n= 1172; controls: n= 384) we examined six single nucleotide polymorphisms (SNPs) around shank3 for genetic association in a polydiagnostic approach (ICD 10; Leonhard's classification). Cases fulfilled criteria for schizophrenia according to ICD10 and were additionally diagnosed according to Leonhard's classification. Proband were recruited at the Department of Psychiatry at the University of Würzburg. PCR for allelic discrimination was performed with TaqMan™ SNP genotyping assays and analysed with the appropriate software package from Applied Biosystems. Software FAMHAP and HAPLOVIEW was used to test for association.

**Results:** SNP rs756638 was significant associated with schizophrenic psychoses according to ICD 10 (table). Analyzing subgroups according to Leonhard's classification, we found an association of rs6010063 with the subgroup of cycloid psychoses (p=0.005) and rs756638 with the subgroup of hebephrenias (p=0.0004). Statistical power calculation resulted in 92.7 % at alpha = 0.05.

**Discussion:** Shank3 has been proposed as a risk factor for ASD. Our case-control results support a role as a possible risk factor for schizophrenia as well. Shank 3 locates in a prescribed candidate region and is involved in synaptical function and neurodevelopment as proposed underlying mechanisms for schizophrenia. The associated marker rs756638 locates intronic at 3' UTR with no known effect on the protein level but possible regulatory effects. Our positive results in clinical subgroups according to Leonhard's classification were restricted to subgroups with low familial loading with psychoses. Likewise another study reported de novo mutations in Shank3 analyzing sporadic cases with unaffected parents. Thus, Shank 3 could be involved in genetic risk for schizophrenia, especially in sporadic cases.

### Schizophrenic Psychoses and Epigenetic Regulation: A Case Control Study

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**Background:** A genome-wide case control study reported associations of single nucleotide polymorphisms (SNPs) at SMARCA2 to schizophrenia in a Japanese sample. The SMARCA2 gene encodes BRM in the SWI/SNF chromatin-remodeling complex which is thought to regulate transcription of certain genes by altering the chromatin structure around those genes. SMARCA2 expression is induced to a high level during differentiation to neurons and astrocytes, suggesting an important role in neural cell differentiations. SMARCA2 locates in a candidate region for autism spectrum disorder (ASD) at 9p22.3 spanning 178 kb with 34 exons. De novo mutations in SMARCA2 have been reported as causative factors for Nicolaides-Baraitser syndrome (NBS) which is characterized by sparse hair, distinctive facial morphology, distal-limb anomalies and intellectual disability. We tried to evaluate the role of SMARCA2 in schizophrenic psychoses in a polydiagnostic approach. Beside ICD10, we tested additionally clinical subgroups according to Leonhard's classification for better phenotype characterization. Karl Leonhard divides schizophrenic psychoses into three main groups, systematic schizophrenias, unsystematic schizophrenias and cycloid psychoses. In family and twin studies based on Leonhard's classification, a different genetic background for each diagnostic category was demonstrated.

**Methods:** In a case-control sample of German descent (cases: n= 1180; controls: n= 480) we genotyped six single nucleotide polymorphisms (SNPs) around SMARCA2 for genetic association in a polydiagnostic approach (ICD 10; Leonhard's classification). Cases fulfilled criteria for schizophrenia according to ICD10 and were additionally diagnosed according to Leonhard's classification. Proband were recruited at the Department of Psychiatry at the University of Würzburg. PCR for allelic discrimination was performed with TaqMan™ SNP genotyping assays and analysed with the appropriate software package from Applied Biosystems. Software FAMHAP and HAPLOVIEW was used to test for association.

**Results:** None of the genotyped marker reached significance level for an association with schizophrenia according to ICD10 (table). Testing for the clinical subtypes revealed nominal significant association in the group of unsystematic schizophrenias (p=0.029) and cycloid psychoses (p=0.01) without Bonferroni correction. Statistical power was 95.9 % at alpha = 0.05.

**Discussion:** Our results cannot replicate the previous findings in a Japanese sample as we found no association with schizophrenic psychoses according to ICD10. This could be due to reduced MAF in our sample for the previously reported markers or due insufficient sample size although our sample encompassed more than 1600 cases and controls. Analyzing subgroups according to Leonhard's classification, we found no significant association after Bonferroni correction. Taken together, we cannot confirm a major contribution of SMARCA2 to susceptibility of schizophrenic psychoses.

**Rethinking Checkerboard Pattern Reversal Evoked-Potential as (Universal) Biomarker in Schizophrenia and Other Mental Disorders**

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**Background:** Functional abnormalities inside the visual system are commonly uncovered in schizophrenia with the help of different techniques and differentially associated with the pathophysiology. However, despite the tremendous advances, yet a definitive consensus in the scientific community about their accuracy rates as useful biomarkers for the clinical praxis does not exist. Actually, none study has demonstrated and least visualized so far, with a single task, neurobiological deficits inside the visual system in all individual patients. In this work we attempt to demonstrate and visualize neurobiological dysfunctions specific for schizophrenia in each individual patient, with the hope to fill a key criteria to develop a biomarker. We targeted the regions related with first stages of visual processing involving mostly bottom-up mechanisms. This line of thinking will be also applied to explore other mental disorders: bipolar disease and depression.

**Methods:** We combined in a novel manner checkerboard pattern reversal visual-evoked potential with spectral resolution (sr-VEP), and electrophysiological neuroimage in patients (DSM-IV-R) drawn from in-patient and out-patient facilities and in matched healthy subjects to get individual statistical parametric maps. Individual patient's 3D-image with the intraindividual significant task-sensitive sources around 70 ms (N1), 100 ms (P1) and 145 ms (N2) were then compared (Z of Crawford) with the control group. Patients with other mental states were additionally compared, as before, with the schizophrenia group and to each other.

**Results:** In schizophrenia neurobiological dysfunctions were identified in the total of 100% of patients within a range that varied dynamically across the time of brain response. Affected areas visualized around 100 (P1) – 145 (N2) ms occurred in dorsal and/or ventral regions (extrastriate) and paralimbic/limbic structures in both hemispheres (Figure 1). The highest dorsal incidences were distributed in the cuneus, precuneus, superior parietal, posterior cingulum, and superior occipital, and ventrally, in parahippocampus and medial occipitotemporal areas. During the earliest component -70 (N1) ms - dysfunction adds to the previous one, frontal and basal structures including anterior cingulum, medial and lateral orbitofrontal, caudate, thalamus and nucleus accumbens, but with less frequency. None relationship of the extension of the neurobiological dysfunction (i.e. absolute number of affected areas per subject) with age, length of illness, age of onset, gender, and medication type was statistically significant. Preliminary, bipolar disorder and depression deviated from the control group with a similar picture as the schizophrenia, at least during P1, but at the same time, differ from schizophrenia, as was revealed by the comparison. The deficit preserves the topography but change the magnitude. Evaluations of the earliest (N1) and latest component (N2) are under way.

**Discussion:** Functional abnormalities found within dorsal and ventral cortex are quite consistent and stable across schizophrenia patients, but intra-individually are detected differentially throughout the time increasing the probability with the latency. This method allows to

detect short-lasting abnormalities that due to its brevity could be transparent for other techniques, as well as, let to track the dynamic of the deficit by each patient. In other words, we explore the visual system with an integrative vision. This result adds definitive evidences about the involvement of visual system through bottom-up mechanisms as core deficits of the disease. To our knowledge, this is the first visual system-based source study able to demonstrate brain abnormalities in all patients. In this work we visualized areas that, in addition to be sensitive to contrast, also share other networks (i.e. processes) that participate in visual information processing at same stages. Otherwise, the method seems to have at the same time some potential as state or trait marker. In regards to the other mental states, this appears to be able to dissociate between them with high accuracy, as well. Finally, the employed design demonstrates to be reliable enough to advance the move from the 'average' patient to the individual one in clinical praxis.

**Integrity of the Visual System at First Stages: Diagnostic Validation and Pathophysiological Implication in Schizophrenia: An Endophenotype?**

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**Background:** There is still a big gap between the advances at the neurobiological and electrophysiological level and our capacity to intervene therapeutically in the clinic in schizophrenia. As for diagnosis it remains the case that there are no generally accepted and validated biomarkers for any psychiatric condition that can be used clinically. In this context, the visual system as a target, is not the exception, because a huge amount of visual processes including both bottom-up and top-down mechanisms have been identified as abnormal in schizophrenia, but any of them are recognized and implemented as a diagnostic tool worldwide. In previous works carried out in our lab the combination of checkerboard pattern reversal evoked-potential with spectral resolution (sr-VEP), and electrophysiological neuroimage was suited to uncover neurobiological abnormalities during early stage of visual processing in all of the schizophrenia patients studied (n=43). Despite the success after comparison of each individual 3D-map with the intraindividual significant task-sensitive sources around 70 ms (N1), 100 ms (P1) and 145 ms (N2) with the control group (Z of Crawford) actually we didn't measure the efficacy of the instrument. Therefore, in this work we attempt to evaluate this design by testing the use of brain response from healthy subjects and schizophrenia patients as patterns to discriminate between mental states.

**Methods:** We built a set of patterns for N1, P1, and N2 components from two samples: controls (Pattern-I, [Figure-1]) and patients (Pattern-II). Then, we compared a mixed group of 60 schizophrenia patients and 54 control subjects against the two patterns for each component of the brain evoked-response, separately. To validate the instruments we used a Leaveoneout technique combining N1, P1, N2 brain sources and the mental states. The clinical precision was evaluated by using ROC curve for gold standard (DSM-IV-R). Predictive values were also calculated.

**Results:** We choose for the first analysis the brain sources at P1 component, because is the most reliable and has been extensively investigated in schizophrenia. First, we compared the whole group to Pattern-I and an area under the curve (AUC) of 0.7 was revealed. Second, we compared to Pattern-II and an AUC of 0.65 was revealed. Choosing a cutoff value of  $p \leq 0.001$  yields a positive predictive value of 100 % and a negative predictive value of 38,46 % to Pattern-I. The same threshold to Pattern-II showed a positive predictive value of 47,73 % and negative predictive value of 71,16 %. In other words, Pattern-I is very good in predicting patients while the second is better by predicting non-schizophrenic subjects. Unexpected, combination in a personalized manner of results from both Patterns allowed to classify correctly all individuals, reducing to zero the occurrence of false positive and false negative in the whole sample. The final result is an accuracy rate of 100% to dissociate both groups. Analysis of the brain sources N1 and N2 component are currently under way, as well as, a combination of them.

**Discussion:** Just the analysis of the sources in one of the component is enough to validate the instrument that showed a high accuracy as diagnostic tool. The combination of the patterns allowed to dissociate the subjects in different groups (like cluster) that at first sight appears to share the patterns. False positives with the Pattern-II (healthy subjects that shown the schizophrenia pattern) that reached about 20 % per cent of the non-patient sample, always behave as healthy according the Pattern-I (endophenotype). Conversely, false negatives (patients that do not shown the schizophrenia pattern) according Pattern-II, behave as non-healthy by the Pattern-I. It means that the groups are well delimited. Classification of first-degree relative (siblings) and the validity of the remaining component will be also presented. A pathophysiological view based on the result will be discussed.

### Cytochrome P450 Genotypes are not Associated with Refractoriness to Antipsychotic Treatment

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**Background:** The ultimate aim of pharmacogenetic research is to introduce pre-treatment testing that would allow personalization of therapies in terms of drug choice and drug dose, thereby improving clinical outcomes. Due to substantial progress in this field, it is now well-established that pharmacogenetics can provide information concerning the probability of a drug to be useful for a given patient and efforts are being made to create pharmacogenetics clinical practice guidelines. Variants in genes have been repeatedly associated with different response rates and treatment side effects. However, their predictive value is relatively small, with odds ratios for individual alleles or genotypes between 1.5 and 2.5. Possible exceptions are the polymorphisms that encode the cytochrome P450 (CYP) enzymes, for which the determination of the metabolic status can increase the efficacy of pharmacotherapy in 10-15% and reduce the incidence of side effects in 15-20%. Two CYP isoforms – the CYP2D6 and CYP2C19 – are particularly implicated in the metabolism of several psychotropic medications, including many antipsychotics commonly used in the treatment of schizophrenia, such as haloperidol, risperidone and aripiprazole, among others. Nonetheless, while for antidepressants it is already possible to make dose adjustments based on the CYP2D6 and CYP2C19 genotypes, evidence validating the impact of these genes in the response to antipsychotic treatment is still very scarce. The CYP enzymes activities are highly variable across individuals, with more than 90 allelic variations described, resulting in pronounced differences in plasma drug concentrations. Although these genetically based pharmacokinetic variations are well-known, some authors still debate whether they might change therapeutic outcomes. Interestingly in this regard, a recent report from the CATIE study examined a number of variants across the key CYP450 genes and found no strong relations with dosing, safety, or efficacy of the antipsychotic treatments used in the trial. In the present study, we evaluated a clinical sample of patients with schizophrenia to examine the hypothesis that there is a difference in the distribution of metabolic phenotypes between refractory and non-refractory individuals. We hypothesized that the refractory patients might have ended up in this condition due to a higher prevalence of CYP2D6 and CYP2C19 ultra-metabolizers among them.

**Methods:** 89 schizophrenia patients were enrolled and divided in two groups: 59 refractory patients according to IPAP algorithm (International Psychopharmacology Algorithm Project) and 30 non-refractory patients. We also genotyped 81 healthy individuals for CYP2D6 and CYP2C19 as control group. Polymorphisms in CYP2D6 and CYP2C19 genes were evaluated by allelic discrimination using TaqMan® system. The following alleles were investigated: CYP2D6\*1, \*2, \*3, \*4, \*5, \*6, \*9, \*10, \*15, \*17, \*29, \*35, \*39, \*40, \*41 beyond copy number variations and alleles CYP2C19\*1, \*2, \*3 and \*17. The phenotypes were classified in extensive metabolizers (EMs), poor metabolizers (PMs), intermediary metabolizers (IMs) and ultra-rapid metabolizers (UMs).

**Results:** Statistical analysis showed no significant difference between the distribution of the CYP2D6, CYP2C19 and healthy controls predicted phenotypes ( $p=0.256$  for CYP2D6 and  $p=0.893$  for CYP2C19) among refractory, non-refractory and healthy controls. The distribution of the CYP2D6 and CYP2C19 phenotypes were described in Table 1.

**Discussion:** Our hypothesis was that some of the patients would be refractory because they were ultra rapid metabolizers of antipsychotics. Therefore, they would not have responded to usual doses of these drugs and would be wrongly considered refractory. Some of them would be in use of clozapine with no need. Our findings do not reinforce the inclusion of genotyping of these genes as a tool in the clinical decision making in refractory schizophrenia.

### Behavioural Characterisation of the NRXN1 Knockout Mice: A Model for Neurodevelopmental Disorders

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**Background:** Copy number variations (CNVs) are emerging as an important genomic cause of several common neurodevelopmental disorders (NDDs; Sebat et al, 2007; Walsh et al, 2008; Mefford et al, 2008; Stefansson et al., 2008). Recently, deletions within the neurexin 1 gene (NRXN1; 2p16.3) have been found in cases with autism, mental retardation and schizophrenia (Kirov et al., 2008; Feng et al, 2006; Zahir et al, 2007). Furthermore, NRXN1 CNVs that disrupt exons were found to be significantly associated with schizophrenia with a high odds ratio (P = 0.0027; OR 8.97, 95 CI 1.8-51.9; Rujescu et al., 2008). A Neurexin 1 $\alpha$  knockout mouse (Nrnx1tm1Sud) already exists and has been used to analyse the role of neurexins in synapse formation and some behaviours (Etherton et al., 2009) within a mixed genetic background. The Nrnx1tm1Sud knockout is an ideal model of the human CNV as it disrupts exon one of the alpha-isoform, as do the majority of human disease-associated deletions.

**Methods:** We have backcrossed the Nrnx1tm1Su mice and performed behavioural phenotyping on a pure genetic background (C57BL/6J, backcross 8). 23 wildtype (12 male, 11 female), 29 heterozygote knockout (15 male, 14 female) and 18 homozygote knockout (9 male, 9 female) mice were tested using a battery of tasks that span the behavioural domains known to be affected in NDDs, such as anxiety, sociability, cognition, and learning and memory (Kas et al, 2007).

**Results:** Preliminary analysis of the data suggests that the homozygote knockout mice have a 26% reduction in their locomotor activity, 70% greater social preference, and 35% slower learning, compared to wildtype mice, indicating that there may be impairments in some traits related to NDDs.

**Discussion:** Such an approach with a diverse behavioural test battery will enable us to fully explore the behavioural consequence of such a deletion. The development of behavioural 'end-points' will also allow the use of this mouse as a model of neurodevelopmental disorders and a tool to study possible interventions and/or treatments. Further phenotypic investigation in the future will hopefully help to make the relationship between NRXN1 deletions and NDDs clearer.

### Set Based Analysis of Epigenetic-Related Genes in Genome-wide Association Data of Neuropsychiatric Phenotypes

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**Background:** Recent genome-wide association studies (GWAS) have identified or suggested genetic risk factors for genetically complex neuropsychiatric disorders, such as schizophrenia, bipolar disorder and major depressive disorder. Apart from genetic factors, environmental risk factors, probably often interacting with the individual genetic background, are thought to be involved in their pathogenesis. In this context, recent work has highlighted a role for dysregulation of epigenetic processes as susceptibility factors.

**Methods:** We used our GWAS data sets of German origin (n=1531 schizophrenia, n=1155 bipolar disorder, n=1505 major depressive disorder, n=2168 controls), and in order to reduce the complexity of the genome-wide data and increase power to detect small genetic effects focused on set-based analyses of genes involved in DNA-methylation and histone modification. We extracted SNP sets covering the pre-defined genes and analyzed them using set-based tests applying logistic regression.

**Results:** In the present study, we addressed the question if common genetic variability in genes that regulate epigenetic modifications plays a role in the etiology of schizophrenia, bipolar disorder or major depressive disorder. We detected a significant association (p<10<sup>-4</sup>) of the epigenetics gene set with schizophrenia while no significant effect was observed for bipolar disorder or major depressive disorder.

**Discussion:** Our results suggest that genetic variability in genes involved in epigenetic processes contributes to the pathogenesis of schizophrenia, but may be less relevant for affective disorders.

### Neuropsychological Intermediate Phenotypes as Tools for Genetic Studies in Schizophrenia

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**Background:** There is evidence for a strong genetic component in the etiology of schizophrenia, as demonstrated by family, twin and adoption studies. The risk of developing the disease increases with the genetic relatedness to an individual suffering from the disorder. Furthermore, schizophrenic patients, their unaffected relatives display alterations in various intermediate phenotypes.

**Methods:** We use complementary strategies to approach the pathobiology and genetics of schizophrenia including genetic association studies as well as animal and cell culture models.

**Results:** Furthermore, the use of schizophrenia-related intermediate phenotypes represents a complementary approach which has been used in this study. These comprise, among others, neuropsychological (e.g. working memory, attention/vigilance, verbal/visual learning and memory, speed of processing, and problem solving) intermediate phenotypes.

**Discussion:** We performed a genome-wide association study including over 2500 healthy controls and 550 patients with cognitive intermediate phenotypes. The poster will present these new results concentration on memory parameters.

### Trajectories Of Schizophrenia: Evaluation of Empirical Evidence and Their Use in Genetic Studies

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**Background:** Schizophrenia is a brain disorder that affects several behavioral domains. Since the beginning of schizophrenia research, emphasis has been placed on longitudinal aspects of the disorder. Individual long-term trajectories show considerable heterogeneity across patients. However, when trying to summarize empirical evidence to this end, we are faced with a number of differences across studies. Here, we therefore lay out a number of guidelines for studies along which we are able to abstract the large but inhomogeneous pool of literature on the long-term course of schizophrenia. Importantly, we have also identified key domains which are of particular interest to schizophrenia researchers. This systematic approach helps to identify relevant information for research networks aimed at elucidating both genetic and environmental risk factors associated with variable illness trajectories.

**Methods:** Guidelines: Schizophrenia diagnosis must be standardized by adhering to major classification systems (DSM-III / ICD-9 [or later versions of these] / Research Diagnostic Criteria). Also, other so-called schizophrenia-spectrum disorders (e.g. schizoaffective disorder) are not to be included. Studies have to be conceptualized as prospective with minimal retrospective data. There must be at least two points of assessment, separated by at least six months in time. Importantly, the same instruments/paradigms must be used at each assessment. At least one control group must be included in each study. The studies have to be observational in nature (no treatment intervention). All studies must be written in the English language and published in peer-review journals. Single case studies have to be excluded. Studies with adult subjects are of main interest.

**Results:** Domains of interest: Positive/Negative Symptoms, Cognition/Neuropsychology, Functional imaging (e.g. fMRI, EEG, NIRS), Structural imaging (fMRI), Psychosocial handicap, Biological markers and Animal models (not all guidelines for human studies do apply).

**Discussion:** Adhering to this framework, we will present an overview of trajectories of schizophrenia and how they can be integrated into the phenomic aspects of schizophrenia genetic research.

### The Genetic Basis of Co-Morbidity Between Schizophrenia and Autoimmune Diseases

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**Background:** Epidemiological studies indicate co-morbidity between schizophrenia and a number of autoimmune diseases (1, 2, 3). Additionally, it has recently been reported that treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) ease both positive and negative symptoms in schizophrenia (4) supporting the notion that inflammatory processes might be involved in the pathogenesis of schizophrenia. This observed co-morbidity might reflect that the genetic architecture predisposing to schizophrenia partly overlaps that of autoimmune diseases.

**Methods:** We have selected 633 at-risk variants associated with 23 different autoimmune diseases according to the NHGRI GWAS catalog. These were examined for association with schizophrenia in the SGENE+ GWAS sample (3765 cases and 14716 control subjects) using Cochran-Mantel-Haenszel exact test. Associated variants in the SGENE+ sample were then analyzed in the PGC-SCZ GWAS sample.

**Results:** 50 of the 633 at-risk variants examined were associated with schizophrenia ( $P = 0.0013-0.049$ , uncorrected) in the SGENE+ discovery sample. These 50 at-risk variants will be replicated in the PGC-SCZ GWAS sample.

**Discussion:** The preliminary results suggest that the observed co-morbidity between schizophrenia and autoimmune diseases is partially caused by shared genetic risk variants. References 1. Eaton WW., et al. Association of schizophrenia and autoimmune diseases: linkage of Danish national registers. *Am J Psychiatry* 2006;163(3):521-8. 2. Chen S. J., et al. Prevalence of autoimmune diseases in in-patients with schizophrenia: nationwide population-based study. *Br J Psychiatry* 2012; Mar 22 [Epub ahead of print]. 3. Benrose M. E., et al. Autoimmune diseases and severe infections as risk factors for schizophrenia: a 30-year population-based register study. *Am J Psychiatry* 2011; 168(12): 1303-10. 4. Sommer I. E., et al. Nonsteroidal anti-inflammatory drugs in schizophrenia: ready for practice or a good start? a meta-analysis. *J Clin Psychiatry* 2011; Dec 13 [Epub ahead of print].

### Using Polygenic and Pathway Analyses to Identify Sources of Shared Genetic Variance in Schizophrenia and Cognition

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**Background:** Cognitive performance in patients with schizophrenia is markedly impaired, typically between 0.5-2 standard deviations lower than controls across cognitive domains. Phenotypic associations between the severity of cognitive impairments, symptom severity and functional outcome are well documented, but less is known about their interactions on a genetic or pathway level. Schizophrenia and intelligence/cognitive ability share features of genetic architecture, notably that no single locus explains large proportions of the known genetic variance, but both are known to be substantially polygenic. The investigation of their shared genetic architecture using polygenic and set-based/pathway analysis may reveal common biological pathways. In this study, we investigated whether schizophrenia is predictive of cognitive ability using a polygenic approach. We also investigated whether cognitive phenotypes in cases and controls are enriched for genes in pathways associated with abnormal behavioural and cognition in mice.

**Methods:** The LD pruned PGC mega-analysis was our schizophrenia discovery set. Training sets were derived from association p-values  $< 0.5, 0.1, 0.01$  and  $0.001$ . A schizophrenia case/control cognitive sample was used as our target set. Polygenic scores were calculated using the Score function in Plink. These were regressed on cognitive scores for five tests (selected on the basis of schizophrenia deficits in their associated domains): WMS N-Back (working memory), WMS Verbal Memory, WMS DigitSpatial Span (working memory), WAIS Digit Symbol Coding (speed of processing) and WAIS IQ (general cognitive ability). Age, sex and a single principle component were used as covariates. We conducted this analysis separately for cases and controls. Separately, we tested 51 separate gene sets associated with behavioural and cognitive abnormalities in mice for enrichment against the five cognitive phenotypes described above for schizophrenia cases within the cognitive sample. We combined SNPs across genes in each set to create a single pathway to be tested for enrichment using set-based tests in Plink, correcting for LD and genomic inflation.

**Results:** Of 40 tests performed at four p-value thresholds across five cognitive domains in cases and controls, two emerged as nominally significant. SNPs associated with schizophrenia were a significant predictor of IQ for in cases ( $p=0.028$ ) using the PGC training set with SNPs  $p<0.001$ . Schizophrenia was a significant predictor of Digit Symbol Coding for SNPs in controls ( $p=0.036$ ) using the PGC training set with SNPs  $p<0.01$ . These are not significant after correction for multiple testing. Separately, preliminary results suggest none of the 51 behavioural or cognitive pathways are enriched for any of the five cognitive phenotypes tested.

**Discussion:** We were unable to identify evidence that schizophrenia is predictive of cognitive ability using a polygenic approach. Preliminary results suggest that genes in pathways associated with abnormal behavioural and cognitive phenotypes in mice are not enriched in cognitive phenotypes in schizophrenia. Our sample size may be underpowered to detect polygenic effects and pathways associated with schizophrenia and cognitive performance. We aim to repeat these analyses in a larger sample sizes thereby increasing power to detect any shared genetic variance.

### Association Study of Nogo-Related Genes with Schizophrenia in a Japanese Case-Control Sample

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**Background:** Multiple lines of evidence suggest that the function of myelin is disturbed in schizophrenia patients and that such abnormalities may be causally involved in the disease pathogenesis. Nogo (RTN4), myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMG), which are all myelin-associated proteins, that can bind to the common receptor, Nogo-66 receptor 1 (RTN4R). This axonal surface protein plays a crucial role in CNS axonal outgrowth inhibition, morphological changes of dendritic spines, and synaptic plasticity. Postmortem studies have implicated that these genes were associated with schizophrenia. Moreover, several linkage and association studies have suggested the involvement of these genes in schizophrenia. These association studies were undertaken using relatively small samples and a small number of polymorphisms. Also, there are inconsistencies among the studies, one of which probably results from the small sample sizes. Here, we examined the major Nogo-Nogo receptor pathway for genetic association with schizophrenia using a relatively large-scale cohort of 2120 subjects of Japanese descent.

**Methods:** We examined 68 single nucleotide polymorphisms (SNPs) (51 with genotyping and 17 with imputation analysis) from these four genes for genetic association with schizophrenia, using a 2,120 case-control sample from the Japanese population. The current study was approved by the Ethics Committees of RIKEN and Kanazawa University. All participants gave written informed consent to participate in the study.

**Results:** Allelic tests showed nominally significant association of two RTN4SNPs ( $P = 0.047$  and  $0.037$  for rs11894868 and rs2968804, respectively) and two MAGSNPs ( $P = 0.034$  and  $0.029$  for rs7249617 and rs16970218, respectively) with schizophrenia. The MAGSNP rs7249617 also showed nominal significance in a genotypic test ( $P = 0.017$ ). In haplotype analysis, the MAG haplotype block including rs7249617 and rs16970218 showed nominal significance ( $P = 0.008$ ). These associations did not remain significant after correction for multiple testing, possibly due to their small genetic effect. In the imputation analysis of RTN4, the untyped SNP rs2972090 showed nominally significant association ( $P = 0.032$ ) and several imputed SNPs showed marginal associations. Moreover, in silico analysis (PolyPhen) of a missense variant (rs11677099: Asp357Val), which is in strong linkage disequilibrium with rs11894868, predicted a deleterious effect on Nogo protein function.

**Discussion:** Despite a failure to detect robust associations in this Japanese cohort, our nominally positive signals, taken together with previously reported biological and genetic findings, add further support to the “disturbed myelin system theory of schizophrenia” across different populations.

### Genetic Determinants of Disease Severity and Treatment Outcome in Schizophrenia

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**Background:** Schizophrenia is a complex disease with heterogeneous symptomatology, course and treatment outcome. A subset of patients only experience one episode over their lifetime, others have a relapsing-remitting course, or psychotic episodes continuously from onset. The effectiveness of antipsychotic drugs and their side effects vary substantially between patients, and developing individualized treatments is an imprecise clinical process. Therefore, there is an emergent need for ways to understand and predict disease severity and drug response, which is the goal of the present study. We approach disease heterogeneity by assessing schizophrenia patients with a poor disease and treatment outcome, to search for genetic determinants.

**Methods:** We have collected a large case-control sample from a national epidemiological sampling frame in Sweden, and 57 of this sample has never been utilized for publication. All cases were identified via the Swedish Hospital Discharge Register, and had been hospitalized  $\geq 2$  times with a core schizophrenia discharge diagnosis. Controls were randomly sampled from the population and had never been hospitalized for schizophrenia or bipolar disorder. Genome-wide SNP and Illumina exome chip genotyping data are available for all subjects. Disease severity will be modeled based on data from Swedish population registers; the Patient register, Prescription Drug register, Swedish Population and Housing Census register, and Cause of death register. Specifically, our outcomes will be defined by (a) any clozapine use, since such prescriptions are given after failure of first-line treatment, (b) any clozapine use and/or several antipsychotics simultaneously, and (c) a comprehensive model of disease severity, taking into account treatment and hospitalization patterns as well as proxies for negative and affective symptoms, such as suicide attempts and early retirement pension.

**Results:** All genotyping is completed along with quality control. There are 11,580 subjects (5,192 cases and 6,391 controls) each with 1.36 million imputed or genotyped common SNP variants, copy number variation (CNV) calls, and 247K uncommon exon variants. These genetic data will be analyzed against the different disease severity phenotypes. About 17% had been prescribed clozapine. The potential genetic determinants of severity will be evaluated using schizophrenia risk profile scores, CNV burden, and pathway analysis.

**Discussion:** Our large population-based sample provides a unique opportunity to study how genetic risk variants can predict outcome and correlate with measures of severity. We could find new etiological mechanisms by targeting potentially more homogeneous patient subsets, as well as reveal if patients with a more severe outcome have an increased load of common risk variants and CNVs.

### VAL66MET Polymorphism of Brain-Derived Neurotrophic Factor (BDNF) Gene is Associated with Depressive Symptoms in Schizophrenia in the Polish Population

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**Background:** Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin widely expressed in human brain. BDNF controls development of dopaminergic, serotonergic, GABA-ergic and glutaminergic neurons and exerts significant effect on neurogenesis and neuroplasticity. BDNF has been implicated in schizophrenia, affective disorders and suicidal behaviour. The aim of this study was to investigate association of the functional polymorphism Val66Met in BDNF gene with depressive symptoms in schizophrenia.

**Methods:** The study population consisted of 427 schizophrenic patients (207 males, mean age 30.4 and 220 females, mean age 32,3) and 625 control subjects (260 males, mean age 39,2 and 365 females, mean age 40,3). All subjects were recruited from Polish population and were Caucasians. Consensus diagnosis by two psychiatrists, according to DSM-IV and ICD-10 criteria, was made for each patient using a Structured Clinical Interview for DSM-IV Axis I Disorder (SCID). Presence of depressive symptoms was evaluated based on SCID and OPCRIT checklist. Presence of suicidal attempts, suicidal ideations, depression, irritable mood, dysphoria, relations between psychotic and affective symptoms were applied in statistical analyses. Val66Met (rs6265) polymorphisms of the BDNF gene was genotyped using PCR-RFLP method. The concordance with Hardy-Weinberg equilibrium was determined. The Pearson's chi-square ( $\chi^2$ ) and the Fisher's exact tests were applied to test differences in the genotypic and allelic (respectively) distribution between schizophrenic patients and controls.

**Results:** Genotype distributions were in Hardy-Weinberg equilibrium. We have found an association of Val/Val genotype ( $p=0.01$ ) with schizophrenia in the whole studied group. Association of the Val/Val genotype ( $p=0,009$ ) and Val allele ( $p=0,0045$ , OR=0,6017, CI 0,4213-0,8592) with irritable mood and trend toward association of Val/Val genotype ( $p=0,08$ ) as well as association of Val allele ( $p=0,034$ , OR=0,6987, CI 0,5019-0,9727) with dysphoria in schizophrenic patients have been found. No association with suicidal ideations and attempts has been found as well as depression and relations between psychotic and affective symptoms.

**Discussion:** Our study confirms involvement of Val66Met functional polymorphism of BDNF gene in pathogenesis of schizophrenia. The results show association of Val66Met polymorphism with mood disturbances in schizophrenic patients. This work was supported by MNiSW grant no NN40240733.

### DRD4 Gene is Associated with Age at Onset of Severe Mental Illnesses and it has an Active Estrogen Responsive Element

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**Background:** The 48bp variable number of tandem repeat (VNTR) polymorphism of DRD4 gene is proposed to be involved in the aetiology of schizophrenia and bipolar disorder. However, the results are inconsistent likely due to genetic and phenotypic heterogeneity. The use of phenotypes more directly influenced by genetic variations has been proposed. Age at onset (AAO) is considered an important alternate phenotype for genetic investigations of psychiatric disorders. In this work, it was our objective to investigate if the DRD4 VNTR 7 repeat allele (7R-allele) was associated with age at onset in schizophrenia and bipolar disorder subjects.

**Methods:** We have examined the effect of 7R-allele in 477 patients with major psychoses. Age at onset was defined as the age of first psychotic episode for schizophrenia and the age at appearance of first clinically recognized symptoms for the bipolar sample. Linear regression was performed with DRD4 genotypes (7R absent/ 7R present), gender and the interaction term as predictors, and age at onset as the dependent variable. The 7R-allele was analyzed considering a dominant model and we used  $-\log_{10}$  transformation of AAO to correct for non-normality. Student's T-test was performed to compare mean of  $\log_{10}$ AAO between groups. The chromatin immunoprecipitation-sequencing (ChIP-seq) assays on ER, FOXA1 and GATA3 were performed on MCF7, as described previously (Kong et al., 2011).

**Results:** Our results showed an interaction between sex and 7R-allele among schizophrenia subjects ( $n=203$ ,  $b=.213$ ,  $p=.017$ ). On comparing AAO between carriers and non-carriers of the 7R-allele, we observed that females with 7R-allele present had later onset ( $p=.021$ ). We have performed an in silico search for an estrogen receptor (ER) binding site close to DRD4 and we found a site approximately 1Kb 3' to the DRD4 stop codon. We have also identified binding sites for FOXA1 and GATA3, two ER associated transcription factors (TFs). In the bipolar disorder sample, we observed significant association between 7R-allele and AAO ( $n=274$ ,  $b=-.148$ ,  $p=.012$ ). The 7R-allele was associated with early onset of the bipolar illness ( $p=.028$ ). No interaction was observed between sex and genotypic groups of the bipolar sample.

**Discussion:** It was the first time that AAO was examined in schizophrenia considering DRD4 genotypes and males versus females. Our results suggest that 7R-allele may show a sex-dependent effect on AAO of SCZ. Our analysis in the SCZ sample showed that females bearing the 7R had later onset than males carrying the 7R-allele with a mean difference of 4.5 years. Another study has reported a sex-effect for DRD4 7R-allele for personality traits in males (Dmitrieva et al. 2011). The presence of estrogen receptor (ER) binding site close to 3' of DRD4 was first report in the present work. An estrogen responsive element (ERE) site has been described for DRD1 gene previously (Lee et al., 1999). DRD4 7R-allele was associated with earlier onset of symptoms in bipolar disorder. This was the first study to evaluate the effect of 7R-allele on early symptoms of bipolar disorder but other dopamine genes have also been implicated in AAO for bipolar disorder (Seeman and Van-Tol, 1994; Dmitrzak-Weglarz et al., 2006). Thus, our findings suggest that the 7R-allele is associated with AAO in both schizophrenia and bipolar disorders.

### Genome-wide Linkage Scan of Quantitative Traits Representing Symptom Dimensions in Multiplex Schizophrenia Families

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**Background:** The use of symptom dimensions of schizophrenia as quantitative phenotypes has been proposed as a mean to reduce the heterogeneity of schizophrenia and facilitate genetic research. This study aimed to identify loci influencing clinical feature of schizophrenia through genome scan of quantitative traits representing symptom dimensions of schizophrenia.

**Methods:** Clinical evaluations on all subjects were consistently performed by raters in a single research team, using the Korean version of the Diagnostic Interview for Genetic Studies, Krawiecka Rating Scale and Schedule for the Deficit Syndrome. Factor analysis identified prodromal impairment, negative symptom, auditory hallucination, Schneiderian first-rank symptom, paranoid, non-paranoid delusion, somatic preoccupation, and disorganization factor. We performed autosomal genome-wide multipoint non-parametric quantitative trait locus linkage analysis in fifty six Korean multiplex schizophrenia families, using these factor scores. Genome-wide empirical significance thresholds were derived from 1,000 gene-drop simulations.

**Results:** We observed seven regions yielding suggestive linkage signals, 15q26.1 for non-paranoid delusion factor, 2p24.3 and 7q31.1 for prodromal impairment factor, 1q32.1, 9p21.3, and 9q31.2 for negative symptom factor, and 10p13 for disorganization factor. 15q26.1 for non-paranoid delusion factor, showing the highest NPL Z score (NPL Z score = 3.49), almost reached threshold for significant linkage. Two loci, 2p24.3 and 1q32.1, in the current study overlapped with linkage peaks in our previous linkage studies using a dichotomous diagnosis of schizophrenia.

**Discussion:** These regions with linkage to clinical features of schizophrenia may inform functional hypotheses for further genetic studies for psychotic illness. Fine mappings and the detection of candidate genes within these regions are warranted.

### Cross-Phenotype Analysis for Schizophrenia Susceptibility Genes Based upon Type II Diabetes GWASS

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**Background:** Physical co-morbidity is more common in schizophrenia (SCZ) than in general population. For example, the prevalence of type 2 diabetes mellitus (T2D) in SCZ is higher compared to that in the general population, indicating the presence of shared genetic etiology between these disorders. In this study, we aim to examine whether the risk SNPs for T2D are associated with SCZ.

**Methods:** Fifty SNPs were selected based upon T2D genome-wide association studies (GWASS) including mega-analysis. We genotyped 1,000 unrelated subjects (500 SCZ and 500 controls) in the Japanese population.

**Results:** So far, we found no SNPs showed significant association with SCZ.

**Discussion:** As the sample size is limited, we will present the results of larger samples (by adding 1,000 subjects) at the conference.

### Genome-wide Association Study on IQ In Schizophrenia Patients and Unaffected Healthy Volunteers

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**Background:** Schizophrenia is a devastating brain disease with high heritability: A major challenge in medicine is to understand mechanisms underlying severe mental diseases including schizophrenia, affecting 0,5-1 of the population. It mostly presents with several episodes and tends to become chronic. Ca. 30 of patients require support throughout their lives.

**Methods:** There is evidence for a strong genetic component in the etiology of schizophrenia, as demonstrated by family, twin and adoption studies. The risk of developing the disease increases with the genetic relatedness to an individual suffering from the disorder. Furthermore, schizophrenic patients, their unaffected relatives display alterations in various intermediate phenotypes.

**Results:** The most selected suitable intermediate phenotypes include neuropsychological (e.g. working memory, attention/vigilance, verbal/visual learning and memory, speed of processing, and problem solving), neurophysiological (e.g. eye movement tasks, EEG gamma band activity, p300, p50) and brain imaging measures (DTI, structural and functional MRI).

**Discussion:** We performed a genome-wide association study on IQ including over 2500 healthy controls and 550 schizophrenia patients with several replication steps. The poster will present these new results on IQ.

### Definition and Refinement of the VIPR2 Duplication Region Associated with Schizophrenia

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**Background:** Schizophrenia is a severe disabling disorder of unknown etiology, and its pathophysiology remains elusive. Emerging evidence strongly supports the role for rare copy number variations (CNVs) as risk factors for schizophrenia. In a recent study, duplication at chromosome 7q36.3, encompassing VIPR2, was implicated in schizophrenia. Specifically, in a genome-wide association study of 8290 patients with schizophrenia, Vacic and colleagues found that 0.35% of patients carry rare CNVs in the chromosomal locus 7q36.3. In contrast, these microduplications were much less frequent (0.03%) among 7431 healthy controls. All variants overlap with VIPR2 or lie within the noncoding subtelomeric region, <89 kb from the transcriptional start site of VIPR2. This finding was supported by another study that points to CNVs at 7q36.3 as potential risk factors for schizophrenia. In our study, we explored the association between the CNVs at 7q36.3 and schizophrenia in a Japanese population.

**Methods:** Our samples were comprised of: (1) a screening set – 300 Japanese patients suffering from schizophrenia and (2) a confirmation set – 531 patients suffering from schizophrenia and 711 healthy controls. We used high-resolution NimbleGen comparative genomic hybridization (CGH) arrays for CNV typing of the screening set. The FASST2 Segmentation Algorithm, a Hidden Markov Model based approach, was used to make copy number calls. Duplications detected in our screening set were followed up in a confirmation set. Custom TaqMan copy number assays or CGH arrays were used to assess the copy numbers in the set. In TaqMan assays, experiments were carried out on four technical replicates.

**Results:** We detected a smaller (35kb) duplication (NCBI36/hg18, Chr7:158,658,128 - 158,693,128) within the critical region identified in Vacic and colleagues' study in three schizophrenic patients. The Duplications were followed up in larger independent confirmation set. The observed frequency of the CNVs was low (~2%) and we did not detect a statistically significant difference between patients and controls. Sixteen randomly selected duplication events were validated using custom NimbleGen CGH arrays with an average of one probe per 500bp. Head-to-tail orientation of the detected CNVs were confirmed by PCR, and fluorescence in situ hybridization revealed that the structural variant is duplicated in tandem and located on the telomeric region of chromosome 7.

**Discussion:** We identified a common (>1) duplication within the region associated with schizophrenia identified in Vacic and colleagues' study (VIPR2). In addition, the common duplication detected in the present study was not associated with schizophrenia. Our results suggest that the 35 kb region that harbors the common CNV should be excluded from the region of association peak in the schizophrenia group reported in the preceding study.

### Heritability and Familiality of Mental Dimensions in the Korean Psychotic Families

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**Background:** Schizophrenia is the most devastating mental illness that causes severe deterioration in social and occupational functioning. But the mystery for elucidating its causes is in line with brain's mystery. One possible mechanism for causing that syndrome may be the genetic aberrations in neurodevelopment and neurodegeneration. Categorical syndrome such as schizophrenia could be the complex of many continuous mental structure phenotypes including several personality development/degeneration dimensions. This is the study to search heritability and familiality of personality dimensions in the Korean schizophrenic LD(Linkage Disequilibrium) families.

**Methods:** We have recruited 422 probands(with psychosis) with their parents and siblings whenever possible. For best estimation of diagnosis, we have used medical records and a Korean version of DIGS & FIGS. We have used MMPI, SCL-90R, TCI, NEO questionnaires for measuring personality and symptomatic dimensions. Heritabilities of personality dimensions in total 1035 family members were estimated using Sequential Oligogenic Linkage Analysis Routines(SOLAR). Personality dimensions in total family members were compared with those in 336 healthy unrelated controls for measuring the familialities. Genetic/environmental correlations with symptomatic dimensions for significant personality dimensions aggregated in families were investigated.

**Results:** Four of the 10 MMPI variables, two of the 5 NEO variables, five of the 7 TCI variables were not significantly heritable and were excluded from subsequent analyses. The three groups (control, unaffected 1st degree relative, case) were found to be significantly different and with the expected order of average group scores for five of the MMPI scales, three of the NEO scales, and two of the TCI scales. Genetic/environmental correlations with symptomatic dimensions for significant personality dimensions aggregated in families will be suggested.

**Discussion:** Our results show that the aberrations in several personality dimensions could form the complexity of schizophrenic syndrome as a result of genetic-environment coactions or interactions in spite of some limitations(recruited family, phenotyping). These will be the base as important coefficients of so mysterious equations forming schizophrenia. But still, most areas in positional genetic variations and environmental factors as loaded variables of equations for causing that syndrome remain doubtful.

### Whole Genome Sequencing of Schizophrenia in a Founder Population

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**Background:** Increasing attention in psychiatric genetics has been paid to the identification of rare (<1%) variants with relatively high penetrance. Microarrays have provided support for the rare variant hypothesis, with the identification and replication of several high-penetrance copy number variants associated with schizophrenia, autism, and other neuropsychiatric disorders. The recent advent of affordable next-generation sequencing provides the opportunity to identify a broader range of rare variants, but interpretation is hindered by the large number of mutations observed in healthy genomes. To reduce the "needle-in-the-haystack" problem, we utilized a two-part strategy. First, we examined cases and controls in a homogeneous founder cohort: the Ashkenazi Jewish population. Second, we utilized an identity-by-descent analysis of DNA microarray data to narrow the search space. Specifically, regions shared identical by descent amongst >6 cases, and zero controls, were prioritized for investigation.

**Methods:** Whole genome sequencing (mean depth > 40x across >96% of the genome) was performed by Complete Genomics, Inc. Subjects were n=40 cases with schizophrenia and n=137 healthy controls, drawn from a larger cohort on the basis of identity-by-descent (IBD) data (GERMLINE analyses). Candidate regions containing strong case-specific IBD sharing encompassing genes expressed at the synapse were examined for potential loss of function variants. The genomewide landscapes of both cases and controls were also characterized. Ashkenazi Genome Consortium.

**Results:** Analyses are ongoing, and detailed results will be presented at the meeting.

**Discussion:** Because the range of rare of variation in the human genome is immense, strategies for increasing signal-to-noise are required to more rapidly identify functional variants associated with psychiatric disease.

### Impact of the Functional Coding Variant ASN107ILE of the Neuropeptide S Receptor Gene (NPSR1) on Schizophrenia and Related Endophenotypes

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**Background:** Neuropeptide S (NPS) acts on a G-protein-coupled receptor (NPSR1) widely expressed across the entire brain and elicits an anxiolytic-like and stress reducing effect in experimental animal studies (Reinscheid, 2008). Moreover, injections of NPS also specifically enhance memory consolidation while acquisition is unaffected (Okamura et al., 2011). Regarding the NPSR1 gene, an amino acid exchange Asn to Ile at position 107 (Asn107Ile, rs324981) with 10-fold increased agonist potency in the Ile107 variant has been identified (Reinscheid et al., 2005). Recently, an excess of the T-allele encoding for the Ile107 variant was replicated in patients with panic disorder (Domschke et al., 2011). A role of NPS in schizophrenia was suggested based on the finding that NPS blocks NMDA antagonist induced prepulse inhibition (PPI) deficits (Okamura et al., 2010). Thus, we aimed at examining the role of the NPSR1 genotype in schizophrenia, verbal memory, and the acoustic startle response.

**Methods:** A case-control sample consisting of 778 schizophrenia patients diagnosed according to DSM-IV and 713 healthy control subjects was genotyped for the NPSR1 variant Asn107Ile. Verbal declarative memory as measured by the auditory verbal learning test and the acoustic startle response were analyzed in 199 and 71 of these schizophrenia patients, respectively.

**Results:** The case-control comparison revealed that the low-functioning NPSR1 Asn107 variant was significantly associated with schizophrenia (OR = 1.19 [CI = 1.03-1.38], p = 0.01685). Moreover, specifically decreased verbal memory consolidation was found in homozygous Asn107 carriers compared to Ile107-carriers (both p < .05) while memory acquisition was unaffected by the NPSR1 genotype (figure 1a). The schizophrenia patients carrying the Ile107 variant demonstrated significantly reduced startle amplitudes compared to patients with an Asn107/Asn107 genotype (figure 1b). Contrary to our expectations and recent findings reported by Okamura et al. (2010), PPI was not affected by the Asn107Ile variant.

**Discussion:** The schizophrenia patients in our sample were less likely to carry the high functioning Ile107 variant of the NPSR1 gene suggesting a lower NPS tone. Moreover, in agreement with an animal study by Okamura et al. (2011) homozygosity for the Asn107 variant was specifically related to a worse memory consolidation. Other experimental animal studies demonstrated anxiolytic effects of NPS on the acoustic startle reactivity and other anxiety-related phenotypes (Fendt et al., 2010; Reinscheid, 2008), an effect confirmed by our finding that schizophrenia patients carrying the high functioning Ile107 variant showed significantly reduced startle responses. Since injections of NPS were comparable to clozapine with regard to their potency to recover NMDA-antagonist induced PPI deficits, Okamura et al. (2010) suggested that NPS may also have antipsychotic effects. Based on this finding and on our results indicating a lower NPS tone due to genetic variation of the NPSR1 gene in schizophrenia patients, NPS can be regarded as an interesting target for future pharmacological drug development with potential antipsychotic effects (Lennertz et al., in press).

### Meta-analysis of the Association Between Single Nucleotide Polymorphisms in Neuregulin-1 (NRG1) and Schizophrenia

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**Background:** The neuregulin-1 (NRG1) gene is involved in neuronal myelination. Systematic fine mapping using extended Icelandic pedigrees identified an associated haplotype in the 5' end region of the gene (HAPICE), which was described to double the risk for schizophrenia in an Icelandic population. However, a number of subsequent independent studies showed that replication of this haplotypic association is a failure. In fact, combination of other SNPs was found significantly associated with schizophrenia.

**Methods:** To reconcile these inconclusive findings, we investigated three SNPs (rs764059, rs2954041 and rs3924999) using 417 cases and 429 controls consisted of three major ethnic groups (Malay, Chinese and Indian) in Malaysia. These SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-FRLP) and Taqman® SNP Assays. Allelic and genotype frequency differences between patients and controls were analysed using the chi-square ( $\chi^2$ ) test. We also performed meta-analyses by combining our findings and published population-based association studies up to 2012.

**Results:** Out of the three analysed SNPs, only rs2954041 and rs3924999 were found polymorphic and we found no evidence for an association of these two polymorphisms with schizophrenia. Our meta-analyses also did not provide sufficient evidence for association of these two SNPs as being significant in the association with schizophrenia.

**Discussion:** The study reveals that rs2954041 and rs3924999 of the NRG1 do not contribute significantly to schizophrenia. Our meta-analyses indicate that the effect of population stratification is minimal due to low number of previous publications regarding these two SNPs. In future, haplotype blocks consisted of other polymorphisms with much larger sample sizes can be considered for genetic association analysis on schizophrenia.

### Weak Positive Association of the AKT1 Gene with Schizophrenia: Evidence from Haplotype and Meta Analyses

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**Background:** V-akt murine thymoma viral oncogene homolog 1 (AKT1) is vital in signal transduction pathway that regulates cellular functions. The AKT1 gene is implicated in the etiology of schizophrenia in patients of European, Japanese, Iranian, and Chinese origin, but not in the Koreans. The present study aimed to replicate these findings in Malaysians.

**Methods:** Six singlenucleotide polymorphisms (SNPs) of AKT1 (rs3803300, rs2498784, rs1130214, rs2494732, rs3803304, and rs2498804) were genotyped in a sample of 417 subjects with schizophrenia and 429 controls. In addition, haplotype and meta-analyses were carried out for the six SNPs.

**Results:** No significant difference in allele and genotype frequencies of the six SNPs in the AKT1 gene was observed between controls and patients. Haplotype analysis showed evidence for weak association ( $p=0.0352$ ) in the six-marker haplotype (TGGGCG). Weak associations ( $p=0.0478$ ,  $p=0.0461$ ,  $p=0.0404$ ) were also found in three five-marker haplotypes (TCGGT, GCGTC, TGGGC). Meta-analysis of rs3803300, rs1130214 and rs2498804 showed no association ( $p=0.631$ ,  $p=0.546$ ,  $p=0.481$ ) between these SNPs with schizophrenia while positive association ( $p=0.018$ ) was found across eight published association studies for rs2494732.

**Discussion:** This study provides weak support for the hypothesis that AKT1 is a susceptibility gene for schizophrenia.

### Targeted Resequencing of the NKAPL Gene in Schizophrenia

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**Background:** The most robust signal observed in genome-wide association studies (GWAS) of schizophrenia is in a region between 27 and 32 Mbp on chromosome 6p22. Although these signals are due to common variants in the population, sequencing GWAS genes or intervals to identify lower frequency, functional and potentially deleterious variation in cases is increasingly important in corroborating associations in specific genes and has great potential to identify specific loci within a region of more extended signal such as observed on chromosome 6p22. We included part of this region (chr 6: 27.4-28.4 Mb) in the 4 Mb target selection for a pilot sequence study of schizophrenia, with the goal of generating initial sequence read data for the construction of our analysis pipeline and variant data for validation on our recently acquired Ion Torrent PGM. Subsequently, an independent GWAS in a Han Chinese sample implicated the NFkB activating protein-like (NKAPL) gene as a schizophrenia common variant locus. We detected 8 variants in NKAPL in the 6 samples sequenced and this gene was chosen to pilot both our variant validation and additional discovery sequencing approaches. Through resequencing NKAPL in Irish schizophrenia cases and controls we demonstrate validation of variants identified in high-throughput sequencing and novel functional variant detection in a schizophrenia GWAS locus.

**Methods:** We sequenced 48 cases from the Irish Case-Control Study of Schizophrenia and 44 Irish controls from the Trinity Biobank. We amplified a 10kb region containing the NKAPL locus using long range PCR. Fragments were sonicated using the Bioruptor UCD-200. We then performed end repair followed by ligation of custom adapters for multiplexed sequencing. Indexed libraries were pooled before size selection for increased library preparation efficiency. Libraries were initially sequenced with 100bp read lengths, with the final 20 samples sequenced at 200bp read lengths when these became available. We aligned reads to the reference genome using TMAP v0.2.3 and called variants using SAMtools v1.18 with parameters optimized for Ion Torrent data. We compared known variant calls for 6 cases to the variant calls from PGM sequencing.

**Results:** We validated single nucleotide variants from the 6 known samples and discovered more novel variants. We observed a validation rate of 87% for the 23 variants contained in the 10kb amplicon from the original six cases' targeted sequence data. There were five novel singleton variants observed upstream and downstream of NKAPL and one novel missense singleton variant observed in NKAPL. Five singleton variants were observed in case samples including the novel missense variant. Two singleton variants were observed in control samples. The associated functional SNP rs1635 from the study in the Han Chinese population in NKAPL is rarer in the Irish population, with a 4.2% allele frequency in cases and 0% allele frequency in controls. One additional annotated (rs114643216) singleton variant observed in a schizophrenia case sample was located in the 3' untranslated region of NKAPL. The two novel rare variants were observed more frequently in cases than controls with the first observed in 3.1% cases vs. 0% controls and the second observed in 7.3% case vs. 3.4% controls.

**Discussion:** We demonstrated validation and discovery of novel variants in a potential schizophrenia risk loci. There is an emerging pattern of rare variation in the form of singleton variants occurring more frequently in schizophrenia cases and novel rare variants having higher case allele frequencies in the NKAPL locus. We are currently sequencing more samples to increase statistical power. We will sequence a total of 1000 Irish cases and 1000 ethnically matched Irish controls. We will employ rare variant statistical testing such as the C-alpha test statistic. Discovering excess functional rare variation in NKAPL would add independent evidence implicating this gene as a schizophrenia risk locus containing both common and rare variation contributing to risk.

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### Familial Aggregation of Schizophrenia in the Cuban Population

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**Background:** Schizophrenia is a common complex disorder with a substantial genetic contribution. Family and twin studies have been essential in defining the genetic epidemiology of schizophrenia over the last several decades. To study this in the Cuban population, data from 553 extended Cuban families and 145 twin probands with schizophrenia was examined. The sample was ascertained in 10 out of 14 provinces of the country.

**Methods:** The authors included 553 schizophrenia probands together with 1627 of their first-degree relatives and a matched group of control probands including their first-degree relatives. This sample was ascertained through the national registry of families with common disorders available at the medical genetic services at the community level. 114 twin pairs (46 monozygotic and 68 dizygotic) with one or both members affected, 145 affected individuals in total, were also ascertained. Diagnosis of schizophrenia was made according to DSM IV criteria. We tested for association between family history and disorder in the extended family sample. We also compared the probandwise concordance in MZ with DZ twins and calculated heritability.

**Results:** The estimated odds ratio (OR) from the study of familial aggregation was 6.6 (95% CI 5.85; 7.39). In twins, 48% of the monozygotic and 13% of the dizygotic pairs were concordant for schizophrenia. Heritability was 83%. Depression, anxiety disorders and alcohol abuse/dependence showed patterns of familial co-aggregation with schizophrenia.

**Discussion:** This is the largest study of familial aggregation of schizophrenia reported in the Cuban population. Our results show that first-degree relatives of affected individuals have an excess risk of schizophrenia of the same order of magnitude as reported in previous studies from other countries.

### Case Report of a Patient with Schizophrenia and a Mutation in the Insulin Receptor Substrate-4 Gene

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**Background:** This report concerns a 50 year-old female patient, diagnosed with paranoid schizophrenia according to the DSM-IV criteria and on long-term treatment with perphenazine, who participated in a recent study regarding the insulin receptor substrate-4 (IRS-4) gene and schizophrenia (1).

**Methods:** The patient gave her written informed consent to participate in this case report. She was interviewed by a psychiatrist about her mental and physical health and a venous blood sample was taken in an EDTA-containing tube and stored at -20°C until preparation of DNA. Then, DNA-sequencing of the whole IRS-4 gene on the chromosome Xq.22.3 was performed.

**Results:** The DNA sequence of the IRS-4 gene from the patient was compared to the reference sequence of the gene (<http://www.ensembl.org/release/46>) and it was found that the patient had what is referred to as a mutation: the G/A genotype instead of G/G, G/C or C/C at gene position 107863596, resulting in a change in amino acid coding from histidine to tyrosine at amino acid position 879.

**Discussion:** Since the IRS-4 protein may be involved in neuronal growth and function in several areas of the brain, it is possible that this IRS-4 gene mutation underlies this patient's schizophrenia development. It also seems that this patient belongs to the group of schizophrenia patients who have multiple individually rare mutations, impacting genes in pathways of the brain important for neurodevelopment and/ or neurotransmission, thereby contributing to schizophrenia (2). References 1. Melkersson K., Persson B., Hongslo T. The insulin receptor substrate-4 (IRS-4) gene and schizophrenia: no evidence for a main genetic factor, however one report of a single schizophrenia patient with a mutation. *Neuroendocrinology Letters* 2011;32:51-58. 2. Walsh T., McClellan J. M., McCarthy S. E., Addington A. M., Pierce S. B., Cooper M., et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* 2008;320:539-543.

### Effects of Glutamate Receptor DELTA 1 (GRID1) Genetic Variation on Brain Structure in Schizophrenia: A VBM Study

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**Background:** Common genetic variation in the promoter region of the glutamate receptor delta 1 (GRID1) gene has recently been shown to confer increased risk for schizophrenia in several independent large samples. In this study, we aimed to link the single SNP rs3814614 (located in the GRID1 promoter region), which was most significant in a recent study of GRID1 in a German schizophrenia population (Treutlein et al., 2009).

**Methods:** We analysed high-resolution magnetic resonance imaging (MRI) data (1x1x1mm resolution, 1.5T) from 62 patients with schizophrenia and 54 healthy controls using voxel-based morphometry (VBM) to assess the effect of single nucleotide polymorphism rs3814614 (located in the GRID1 promoter region), of which the T allele was identified as a risk factor in a previous association study. MRI data were obtained on a 1.5 Tesla scanner (Magnetom Vision plus, Siemens, Erlangen, Germany) using a T1-weighted 3D FLASH sequence (TR 15 ms, TE 5 ms, flip angle 30°, field-of-view 256mm, 192 slices, voxel dimensions (1x1x1) mm<sup>3</sup>). For analysis of MRI data, we used the VBM5 package, a freely available tool-box (<http://dbm.neuro.uni-jena.de/vbm/vbm5-for-spm5/>) implemented in SPM5 software. We also applied a threshold during VBM analysis to remove voxels with a low probability of GM (or WM, resp.), which was set to 0.2 (i.e. voxels with less than 0.2 probability of being classified as GM, or WM, resp.). We analyzed both grey and white matter density across the entire brain. In addition, we assessed genotype effects on total brain grey and white matter (derived from voxel counts). For all main statistical analyses of VBM data, we used a uniform threshold of  $p < 0.001$  (uncorrected). The applied general linear model included group (schizophrenia; healthy control) and GRID1 rs3814614 genotype (CC; CT; TT), as well as the nuisance variables age and gender.

**Results:** There were no effects of genotype or group X genotype interactions on total brain grey matter or white matter, but on regional gray matter. In healthy subjects, we identified a significant effect of rs3814614 genotype in the anterior thalamus (bilaterally), superior prefrontal cortex, and orbitofrontal cortex – in all cases with the homozygous risk genotype TT resulting in higher grey matter density. We did not find this association within the schizophrenia sample, where rs3814614 variation was only associated with grey matter reduction in TT homozygous subjects in medial parietal cortex and increased grey matter in right medial cerebellum. For white matter, we did not find significant genotype effects in healthy controls, and only minor effects within schizophrenia patients in the posterior temporal lobe white matter.

**Discussion:** In this study, we provide an analysis of the effects of single SNP marker (rs3814614) in a recently identified candidate gene coding for a delta glutamate receptor on brain structure in healthy controls and patients with schizophrenia. Our results suggest that variation within this marker of a schizophrenia-associated gene has effects on prefrontal and anterior thalamic regions, with a dissociation of effects between healthy and schizophrenia-affected populations. Our finding of a disease effect on anterior thalamus voxels contrasts with a genotype effect only in healthy controls but not patients, suggesting a differential effect related to either the expression of the

schizophrenia disease phenotype or genetic liability. This could be related to several factors. An effect on other gene products might explain the lack of a significant direct effect of GRID1 (rs3814614) on brain structure in schizophrenia in our sample. Given the interaction of several pathways and epistatic effects between schizophrenia risk genes, the effect on brain structure could thus differ between diagnostic groups. Hence, even though the T risk allele might be overrepresented in schizophrenia, it might not be sufficient on its own to interact in the emerging pathology leading to disease manifestation. Also we need to consider that even if the risk gene affects brain structure, its effects might be obscured by those of other genes or factors – either related or unrelated to this particular gene or its products. Therefore, the effect of other genes, developmental pathology, or effects related to the onset of schizophrenia (as well as confounding factors) acting on thalamic morphology might in fact have contributed to the lack of significant impact of this GRID1 genotype in the patient population.

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### Convergent Functional Genomics of Schizophrenia: From Comprehensive Understanding to Genetic Risk Prediction

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**Background:** Schizophrenia is a devastating disorder affecting approximately 1% of the population. While there is clear evidence for roles for both genes and environment, a comprehensive biological understanding of the disorder has been elusive so far. Most notably, there has been until recently a lack of concerted integration across functional and genetic studies, and across human and animal model studies, resulting in missed opportunities to see the whole picture.

**Methods:** As part of a translational Convergent Functional Genomics (CFG) approach, developed by us over the last decade, and expanding upon our earlier work on identifying genes for schizophrenia and biomarkers for psychosis, we set out to comprehensively identify candidate genes, pathways and mechanisms for schizophrenia, integrating the available evidence in the field to date. We have used data from published genome-wide association studies (GWAS) datasets for schizophrenia. We integrated those data with gene expression data - human postmortem brain gene expression data, human iPSC-derived neuronal cells, and human blood gene expression data published by others and us, as well as with relevant animal model brain and blood gene expression data generated by our group and others. In addition, we have integrated as part of this comprehensive approach other genetic data- human genetic data (linkage, CNV or association) for schizophrenia, as well as relevant mouse model genetic evidence. Animal model data provides sensitivity of detection, and human data provides specificity for the illness. Together, they help identify and prioritize candidate genes for the illness using a polyevidence CFG score, resulting in essence in a de facto field-wide integration putting together the best available data to date. Once that is done, biological pathway analyses can be conducted and mechanistic models can be constructed. An obvious next step is developing a way of applying that knowledge to genetic testing of individuals to determine risk for the disorder. Based on our comprehensive identification of top candidate genes described in this paper, we have chosen the nominally significant SNPs inside those genes in the GWAS dataset used for discovery (ISC), and assembled a Genetic Risk Prediction (GRP) panel out of those SNPs. We then developed a Genetic Risk Prediction Score (GRPS) for schizophrenia based on the presence or absence of the alleles of the SNPs associated with the illness in ISC, and tested the GRPS in independent cohorts (GAIN EA, GAIN AA, nonGAIN EA, nonGAIN AA) for which we had both genotypic and clinical data available, comparing the schizophrenia subjects to normal controls.

**Results:** Using this polyevidence scoring and pathway analyses, we identify top genes (DISC1, TCF4, MBP, MOBP, NCAM1, NRCAM, NDUFV2, RAB18, as well as ADCYAP1, BDNF, CNR1, COMT, DRD2, DTNBP1, GAD1, GRIA1, GRIN2B, HTR2A, NRG1, RELN, SNAP-25, TNIK), brain development, myelination, cell adhesion, glutamate receptor signaling, G-protein coupled receptor signaling and cAMP- mediated signaling as key to pathophysiology and as targets for therapeutic intervention. Overall, the data is consistent with a model of disrupted connectivity in schizophrenia, resulting from the effects of neurodevelopmental environmental stress on a background of genetic vulnerability. In addition, we show how the top candidate genes identified by CFG can be used to generate a genetic risk prediction score (GRPS) to aid schizophrenia diagnostics, with predictive ability in independent cohorts. The GRPS also differentiates classic age of onset schizophrenia from early onset and late-onset disease. We also show, in three independent cohorts, two European-American (EA) and

one African-American (AA), increasing overlap, reproducibility and consistency of findings from SNPs to genes, then genes prioritized by CFG, and ultimately at the level of biological pathways and mechanisms. Lastly, we compared our top candidate genes for schizophrenia from this analysis with top candidate genes for bipolar disorder and anxiety disorders from previous CFG analyses conducted by us, as well as findings from the fields of autism and Alzheimer.

**Discussion:** Overall, our work maps the genomic and biological landscape for schizophrenia, providing leads towards a better understanding of illness, diagnostics, and therapeutics. It also reveals the significant genetic overlap with other major psychiatric disorder domains, suggesting the need for improved nosology.

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### Association Analysis of NCAN Genotype (RS 1064395) with Schizophrenia Phenotype in Sub-population of Bosnia and Herzegovina

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**Background:** Recent findings of genetic association between NCAN polymorphism rs 1064395 with BPAD involving Bosnian BPAD patient subset (Cichon et al. 2011) and schizophrenia (Muhleisen et al. 2012) confirmatory contributed to the hypothesis of shared ethiopathology between two major psychoses (ISC 2009).

**Methods:** Total of 86 individuals were genotyped for rs 1064395 using direct sequencing method. A case-control analysis of common genetic polymorphism within NCAN gene and schizophrenia status (in a prospectively sampled patient cohort) have been done using Fisher-exact test with odds-ratio calculation.

**Results:** No statistically significant allele and genotype association with disease status was found ( $p=0.361$ , OR-2.2).

**Discussion:** Our results do not confirm the positive association of tested NCAN polymorphism as common ethiology factor in major psychoses although strongly associated with schizophrenia in large scale metapopulation studies. NCAN variation at rs 1064395 was significantly associated with BPAD in Bosnian and Herzegovinian population subset as observed previously (Cichon et al 2011) but not in a schizophrenia sample. Our finding supports the fact that large-scale genetic association studies with harmed methodological approach need to be employed when exploring the variants with small additive effect in phenotypes with complex ethiology.

### Peripheral Blood Expression of Neurotransmitter Receptors and Regulators Genes in First-Episode Patients

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**Background:** Psychosis is a severe condition and is clinically and genetically complex. Therefore, there is a need to find biomarkers that will help to better understand the biological pathways underlying psychotic disorders and also to discover novel anti-psychotic drugs. One potential biomarker is peripheral blood-based expression, since brain tissue is not readily accessible. Moreover, peripheral gene expression can be examined at multiple time points to assess disease progression or treatment effect. In our pilot study, we aimed to select neurotransmitter receptors and regulators genes from the Human Neurotransmitter Receptors and Regulators RT2 Profiler™ PCR Array System, excluding those with undetected expression, in whole blood of first-episode psychosis (FEP) patients and healthy controls for a further analysis in a larger sample.

**Methods:** A total of eight first-episode psychosis (FEP) patients was evaluated at two times (T0 - at admission; and T1 - after two months of treatment) by a trained psychiatrist using Structured Clinical Interview for DSM-IV (SCID). The control group was composed of seven healthy controls, matched by age and sex, with no psychiatric diagnosis or family history of psychiatric illness. Whole blood samples from patients, at T0 and T1, and controls were collected in PAXgene® RNA tubes, then, RNA was isolated and reverse transcribed. Gene expression was assessed with the Human Neurotransmitter Receptors and Regulators RT2 Profiler™ PCR Array System, which interrogates 84 neurotransmitter receptors and regulators genes. Data analysis was performed using the manufacturer's integrated web-based software package for the PCR Array System. Each of the 84 neurotransmitter receptors and regulator genes were divided by expression level in four groups: I – high expression (Ct<25); II – medium expression (25≤Ct<30); III – low expression (30≤Ct<35); and IV – no expression (Ct≥35 or undetected expression).

**Results:** In the control group, 1.19% of the 84 genes were found to have high expression levels (level I), 15.48% of the genes presented medium expression levels (level II) and 71.43% had low expression levels (level III). In the FEP group, 1.19% of the genes were in level I, 16.67% in level II and 71.43% in level III. For the patients after treatment, 1.19%, 14.29% and 71.43% of the genes were in level I, II and III, respectively. As we focused on detecting those genes with undetected expression (level IV), to exclude from our larger study, the genes that fall in this category are described below. Genes with undetected expression in the three groups (control, FEP and after treatment) included: GABRG1, GABRP, NPY1R and SSTR. Genes not expressed in two groups were: DRD2, QRFPR and NPY2R (control and FEP); and GABRB2, NPFFR2 and HTR2A (control and after treatment). For GABRE; for GABRA2, GABRA3 and PROKR1; and for GRIA1, expression levels were undetected in FEP, after treatment and control groups, respectively.

**Discussion:** Though 9.52-13.10% of the genes were not expressed in whole blood, we have to consider that some of these genes, such as DRD2 and HTR2A, have been consistently associated to psychosis and anti-psychotic treatment and were reported to be differentially expressed in blood samples. Therefore, in our further analysis, we will investigate the expression of the genes with high to low expression levels and also DRD2 and HTR2A in larger samples, verifying the effect of psychosis and treatment on the expression levels of the selected genes.

### Expression of Neurotransmitter Receptor and Regulator Genes in the Prefrontal Cortex and Nucleus Accumbens of a New Schizophrenia Animal Model

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**Background:** Schizophrenia (SCZ) is a complex mental illness that genetic and environmental factors interact to develop the disease. Neurotransmission (NT) problems involving Nucleus accumbens (NAC) are responsible for positive symptoms, while the negative and cognitive symptoms are mainly related to prefrontal cortex (PFC) deficits. Recently, our group suggested that the SHR (Spontaneously Hypertensive Rat) strain – mainly based on its behavioral - could be a useful animal model to study several aspects of schizophrenia. In this respect, the aim of the present study was to characterize gene expression profiles of neurotransmitter receptors and regulators in the PFC and NAC in two SHR groups, treated with saline (SS) and treated with risperidone (SR), and a control group with Wistar rats treated with saline (WS).

**Methods:** Each group was composed of 8 adult male rats (6 months). After the brain dissection, RNA was extracted and converted to cDNA. To perform the gene expression analysis we used the PCRarray technology, which verifies the expression of 84 genes related to NT plus five housekeeping genes simultaneously. We utilized the Student's t-test to investigate the significance of each gene. It was considered significant p-values lower than 0.05.

**Results:** In the comparison SS x WS we found 9 genes differentially expressed and that have already been associated with schizophrenia: CPF downregulated genes in SHR group – Chat (p = 0.033), Gabrg2 (p = 0.026), Gabrb4 (p = 0.001), Gad2 (p = 0.003), Maoa (p = 0.024), Slc5a7 (p = 0.0001) and Tacr3 (p = 0.019); NAC upregulated genes – Gabra2 (p = 0.047), Tacr3 (p = 0.05); NAC downregulated genes - Gabrr2 (p = 0.006). In the comparison SS x SR, 5 genes showed significant differential expression, analyzing the three tissues, but just Tyrosine Hydroxylase (Th) gene (p = 0.023) seems to be related with schizophrenia and antipsychotic treatment.

**Discussion:** CHAT activity seems to be reduced in NAC and pontine tegmentum of patients and also correlated significantly with measures of cognitive performance in the disorder. The Tachyinin Receptor 3 (Tacr3) is present in dopamine neurons, and when activated it increases the dopaminergic cell firing, increasing dopamine. A clinical study showed that Tacr3 antagonists displayed antipsychotic effects, because they have the potential to attenuate both cortical hypofunctionality and mesolimbic hyperactivity of dopamine system, and, hence, improving cognitive and positive symptoms of SCZ patients. Interestingly, the expression of Tacr3 in SS compared to WS was reduced in the PFC and increased in the NAC. Regarding Gad2, a study showed that knockout mice had prepulse inhibition (PPI) deficits characteristic of schizophrenia patients, as well, another study showed a reduced expression level in the PFC of SCZ patients, concordant to our results in SHR group. The expression levels of Maoa found in PFC could influence the bioavailability of many neurotransmitters since MAOA has an important role in the catabolism of them, such as dopamine and serotonin. Those pathways altered in PFC and NAC of SHR group could be the cause of their deficits in behavior tests like impairment of cognition, social interaction, and PPI found previously by our group. In conclusion, our data show that SHR presented alteration in gene expression profiles in the PFC and NAC that have been related to schizophrenia. In this way, these results, coupled with our previous studies reinforce this strain as an animal model to study several aspects of schizophrenia.

### A First Protein-protein Interaction Network for Mood Disorders and Schizophrenia

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**Background:** Here, we present the generation of the first comprehensive protein-protein interaction (PPI) networks for mood disorders and schizophrenia using high-throughput yeast two-hybrid (Y2H) interaction screening technologies and bioinformatics. The results obtained with automated Y2H assays were confirmed in mammalian cells using a modified version of the LUMIER method (Barrios-Rodiles et al, 2005, Science). Using 46 proteins as baits a PPI map connecting 1103 proteins via 1923 interactions was created.

**Methods:** The quality of individual Y2H PPIs was assessed on the basis of multiple independent features that have previously been used to assign confidence scores. These features include properties like the network neighborhood or the physiological relevance of protein interaction pairs. To further assess the quality of high confidence Y2H interactions, a modified version of the LUMIER method was applied, successfully confirming 108 (84,3%) of 128-tested high confidence PPIs in mammalian cells.

**Results:** Recently, genome wide association studies revealed that the protein ZNF804A is a very important risk factor for bipolar disorder and schizophrenia (Michael C. O'Donovan et al. Nature Genetics 2008). Currently, the function of this protein is unknown and it remains unclear how ZNF804A influences the pathogenesis of schizophrenia. Thus, we decided to specifically focus on ZNF804A and to generate a high quality PPI map for this important disease protein. A focused PPI network linking 88 proteins to ZNF804A was generated and 55 of these interactions were validated with LUMIER assays. Furthermore, a gene-based analysis of the ZNF804A interaction partners was conducted using genome-wide genotyping data from German schizophrenia patients and controls. Finally, the Biological Networks Gene Ontology (BiNGO) plugin of the Cytoscape program was used to assess the overrepresentation of GO categories in the ZNF804A PPI data set.

**Discussion:** Bioinformatic analysis revealed that proteins involved in RNA splicing are significantly ( $p$ -value  $5.51 \times 10^{-5}$ ) overrepresented in the PPI data set of ZNF804A compared to the human genome, suggesting that this process is critical for disease pathogenesis in schizophrenia.

### Analysis of Compound Heterozygous Mutation in Schizophrenia

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**Background:** Whole exome sequencing data was analysed in 180 parent-offspring trios diagnosed with schizophrenia. Here we focus on the identification of rare, nonsynonymous variants in the compound heterozygous state. As these mutations affect both gene copies they are likely to have a highly damaging effect on protein structure and function. This study aimed to estimate the rates of compound heterozygosity in probands. As controls we used the rate among parents. We wanted to identify specific genes preferentially hit by compound heterozygous mutation in probands, and to test whether the nucleotide sequence affected by these mutations is more evolutionary conserved in probands when compared with parents.

**Methods:** Variants detected by whole exome sequencing were annotated using ANNOVAR software for exonic function (synonymous/nonsynonymous), frequency in the 1000 genomes project, and overlap with segmental duplications (SD). Further annotation of variant frequency was conducted using data from the Exome Variant Server (~5000 samples). Variants were subsequently selected for potential involvement in compound heterozygosity if they were rare (<1% frequency), predicted to be nonsynonymous, and did not overlap with segmental duplications. Genes affected by compound heterozygous mutation in probands were identified if they were found to harbour variants inherited from both parents. Genes affected by compound heterozygous mutation in parents were identified if they contained two (or more) variants, but only one of them was transmitted to the proband, therefore suggesting the remaining variant is found on the other chromosome. The evolutionary conservation of nucleotides found in compound heterozygous state was compared using GERP/PhyloP scores. A Kolmogorov-Smirnoff test was performed to compare the distribution of these scores between probands and parents.

**Results:** We identified a total of 303 rare and non-synonymous compound heterozygous mutations in probands and 543 in parents, a rate of 1.7 and 1.5, respectively. Several genes were found to be hit multiple times in probands, and eight genes were found to be hit twice in probands and never in parents. The nucleotides affected by these mutations were under greater evolutionary constraint in probands compared to parents, as shown when comparing the cumulative distribution of their GERP ( $P = 0.03$ ) and PhyloP ( $P = 0.04$ ) scores.

**Discussion:** The rate of compound heterozygosity was marginally higher in probands than parents, although this was not significant. The rate is comparable with that found in a recent Autism study (Sanders et al, 2012). Several genes were hit twice in probands and never in parents, although none of these genes have been previously associated with schizophrenia and none were obvious candidates based on their functional annotation. However, compound heterozygous mutations in probands are more likely to be damaging compared to those in parents as they affect more highly conserved sequence, and therefore could potentially be responsible for an increased risk of schizophrenia. We aim to continue this study using whole exome sequencing data from a further ~400 trios.

**MIR-183 as a Biomarker for Schizophrenia and Cancer**

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**Background:** Reports have suggested that a reduced risk of cancer is associated with schizophrenia. Genes that are involved in cell cycle regulation, it seems that they have additional functions in post mitotic neurons related to neuronal migration and synaptic plasticity. MicroRNAs have a dominant role in the regulatory mechanisms of gene expression in the central nervous system (CNS). Their implications in a big number of multiple pathways in CNS make them an interesting investigating field, having an important diagnostic, prognostic and therapeutic value.

**Methods:** In the present study we investigated the probable association between cancer and schizophrenia in two samples of patients. We analyzed a big number of mir-RNA's in a sample of 6 schizophrenic patients (control group) and in a sample of 8 schizophrenic patients with a solid cancer (study group). All experiments were conducted at Exiqon Services, Denmark. The quality of the total RNA was verified by an Agilent 2100 Bioanalyzer profile.

**Results:** When performing the analysis, comparing the two groups, we found that only has-miR-183 was differentially expressed between the control and the study group, after correction for multiple testing.

**Discussion:** Although our sample was small and it is rather difficult to generalize our results, it seems that miR-183 could play an important role, through regulation of expression of other genes involved in onco-suppressor activity. Our results are in agreement with the theory that patients with schizophrenia may have a tumor suppressor gene or enhanced neuronal apoptotic activities. Further studies are warranted in order to establish the role of micro-RNA's and specifically the suppressor role of miR-183 in the neurobiology paths of schizophrenic disorder.

**Stratification of Individuals with Psychiatric Disorders Based on Nation-wide Health Registries**

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**Background:** Our understanding of the underlying biology of psychiatric disorders is still elusive. However, the findings of genetic variants associated with psychiatric disorders have in recent years been steadily increasing. Notably, several high-risk copy number variants (CNVs) have been identified. These findings provide a novel and unique starting point for gaining insights into the underlying biological processes implicated in the pathology of mental illness. One entry point for such an understanding is to investigate in which way, if any, carriers of such genetic variants are set apart from non-carriers affected by the same disorders. This can be done by comparison of quantified and structured parameters such as co-morbidities, age of onset, severity, number of hospitalizations, duration of hospitalization etc. An abundance of co-morbidities of psychiatric disorders are well known and documented. The co-morbidities have for the majority been discovered in a clinical or scientific setting, using observation of patient cohorts or by going through the medical history of individual patients as reported in the health record at non central locations i.e. regular doctor/clinic, local hospital or institution. Such cohorts will usually be limited both in number and in their duration. The Danish national health register including the central psychiatric registry provides a different approach for obtaining such information on individuals across the various entry points to the health system and over an extended period of time. The aim of this project is to utilize the Danish registries of individuals with psychiatric disorders and reveal their similarity particularly somatic and psychiatric co-morbidities. Furthermore, to evaluate to what degree carriers of genetic variants associated with psychiatric illness will map differently onto these findings compared to non-carriers.

**Methods:**

With focus on disorders that have been associated with (pleiotropic) CNVs (Schizophrenia, Bipolar disorder, Autism, ADHD or Mental retardation) and the study will be divided into two phases:

- 1) Clustering of people affected with psychiatric disorders.
- 2) Mapping of patients with known CNV carrier status to the clusters established under 1.

First, on the basis of the Danish health registry data obtained for people diagnosed with either schizophrenia, bipolar disorder, autism, ADHD or mental retardation we will derive a general image of the medical characteristics of these cases. The participants will be identified through the Central Danish Psychiatric Registry and will be described by a vector in the n-dimensional space created from the available parameters of the structured health registry information. Comparisons of these vectors will make it possible to identify clusters within this space. The dimensions characterizing these clusters should hopefully confirm already known co-morbidities supporting the use of the method, and may possibly reveal new correlations. Secondly, 700 participants selected from the Danish Psychiatric Bio-bank housed at Mental Health Center Sct. Hans will be mapped onto the clusters obtained above. The participants are all psychiatric patients, both CNV carriers and non-carriers, identified using targeted MAQ assays or Illumina SNP arrays for CNV detection. Health registry data of the selected individuals is accessible due to their status as members of the Bio-bank. By subsequently mapping these patients on the system formed by the general approach it will be evident to which degree their CNV carrier status introduces a basis for stratification of the patients. This will provide valuable information for diagnostics, treatment or prevention.

**Results:** Study is ongoing and results will be disclosed at the meeting.

**Discussion:** The information in health registries is prone to introduce some degree of bias in a setting as above. This is due to (additional) diagnoses only being reported when deemed relevant for the specific hospitalization. In addition the reimbursement of funds to hospitals based on the ICD10 classification is known (although sparsely quantified) to introduce bias into the registries, and thus especially relevant in regards to somatic comorbidities.

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### **Metalloproteinase-9 (MMP9) in Schizophrenia and Depression: A Common Susceptibility Factor?**

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**Background:** Recently it has been reported that the protein expression of the metalloproteinase-9 (MMP9), an extracellular protease, is similarly increased in patients suffering from schizophrenia and depression. Given the long discussed hypothesis that schizophrenia and affective disorder follow a hierarchical disease model and might have overlapping pathophysiological dysfunction processes MMP9 might mirror a biological link between schizophrenia and affective disorder. Therefore, within this pilot study the blood concentration of MMP9 and its inhibitor TIMP1 (Tissue Inhibitors of Metalloproteinases) were examined in schizophrenic and depressed patients, as well as in healthy controls. Additionally a possible relation to the functional -1562C/T polymorphism in the MMP9 gene and to the 372C/T (F124F) variant in the TIMP1 gene were analyzed.

**Methods:** 37 patients with a DSM-IV diagnosis of a schizophrenia spectrum disorder, 78 patients with a DSM-IV diagnosis of a major depression and 38 healthy controls were included into the study. The Positive and Negative Syndrome Scale (PANSS) as well as the Hamilton Depression Rating Scale (HAMD-17) were applied. Blood draws and clinical interviews were performed at baseline and every two weeks within the six weeks study period. Plasma concentrations of MMP9 and TIMP1 were measured by Elisa. The SNPs rs3918242 (-1562C/T) in the MMP9 gene and rs4849 (F124F; 372C/T) in the TIMP1 gene were genotyped applying the TaqMan® technology (Assay-on-Demand). ANOVA and univariate tests were calculated using the statistical program R 2.11.1.

**Results:** The MMP9 concentration differed significantly between the patients and healthy controls (schizophrenia 143 ± 101 ng/ml, depression 135 ± 141 ng/ml, healthy controls 54 ± 48 ng/ml; ANOVA  $F=7,06$ ,  $p=0.001$ ). A significant positive correlation was found between illness severity of the depressed patients via the HAMD and the MMP9 concentration both at study entry (Pearson 0.234;  $p=0.040$ ) and also at endpoint (Pearson 0.302;  $p=0.0012$ ). Also, in the schizophrenia patients a significant positive association was found in terms of the severity of depressive symptoms measured via the depression item (Pearson 0.50;  $p=0.0111$ ) and the PANSS depression subscore (Pearson 0.44;  $p=0.0289$ ) at baseline. We could not detect any significant relation between the MMP9 and TIMP1 concentrations or between the diagnosis and the genotypic, respectively the allelic distribution of the investigated polymorphisms.

**Discussion:** These are the first preliminary results of MMP9 in the blood of patients with schizophrenia and depression. This suggests that both diseases might share more biological underpinnings than thought before. The analyzed genetic variants in MMP9 and TIMP1 seem not to influence these findings. Future studies are warranted to replicate present results.

## Recruitment

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**Background:** Denmark has extensive use of national registers, which has provided unique opportunities for epidemiological research. These complete registers allow us to do targeted recruitment into genetic studies. Thus, recruit individuals with or without known risk factors, e.g. family history of psychiatric disorders. We have explored the feasibility of targeted letter recruitment, for identifying the frequency of known pathological Copy Number Variations (CNV) and new putative pathological CNV. Target: Some characteristics seems to emerge from the publications on CNV which increases the risk of developing schizophrenia: 1) Pleiotropic effect: The same CNV are found in individuals diagnosed with autism, ADHD, mental retardation and epilepsy, 2) Early onset: carriers seems to have an earlier debut of disease and 3) the CNV seems to be under negative selection. This project focus on a population of individuals admitted to the Child and Adolescent Psychiatry (CAP) with three matched population controls, for whom there will be performed targeted recruitment and indentified carriers with known pathological CNV and CNV under putative negative selection.

**Methods:** The population cohort consist of all individuals who has been admitted to a CAP department from 1969 to 2004 (n>62.000), and three population controls, matched on gender, birth -year, -month and -county. For all individuals, including first degree relatives, a complete medical history until 2009 was collected. The pilot recruitment strategy is based on randomly selected individuals (proband and controls born in 1964, 1974, 1977 and 1984). These all received a pamphlet with information about the project called "inheritance and environment - impact on mental illness". Resending the pamphlet to non-responders. Positive responders were given informed consent by phone. Subsequently, saliva collectors where collected by mail for genetic analysis.

**Results:** Here were focus on the individuals previously admitted to CAP born in 1964, 1974, 1977 and 1984: a) 5244 individuals admitted, b) 2826 who could be contacted (low numbers due to research protection, emigration, death), c) 2735 with a valid address, d) 647 responded positively, and e) 568 (88 %) provided a saliva sample. Thus a participants rate of 21 % (568 saliva samples/2735 possible participants): a) 64 % (n=366) responded by first letter, b) 36 % (n=202) responded after a second letter, and c) skewed gender distribution (30 % men and 70 % women). The quality of saliva samples for DNA extraction is almost equivalent to whole blood samples, as the subsequent genetic analysis. The initial genetic analysis gives rise to CNV-carrier frequency of 5%.

**Discussion:** The pilot study shows a lower response rate than reported in other studies of saliva samples sent, but it is also expected since a large proportion of the former patients have severe mental illness. The preliminary analysis of the DNA quality resembles that of whole blood sample; therefore, this approach is useful for recruiting large groups.

## Replication of Schizophrenia GWAS Results in a Large Population from Indonesia

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**Background:** Schizophrenia is a complex genetic disorder with an estimated heritability of up to 80. We hypothesize that genes conferring risk for schizophrenia are present in all populations and can therefore be detected in populations with different ethnic background. However, population specific susceptibility alleles may exist. Identification of these alleles may aid in gene identification in schizophrenia as well as in characterization of the reported differences in course and outcome between developing and developed countries.

**Methods:** We have consecutively ascertained a sample of 1081 individuals with schizophrenia and 1111 non-psychiatric controls in five Mental State Hospitals in the area of Jakarta, Indonesia. Diagnosis was made according to the DSMIV criteria by psychiatrists, trained in the use of a structured interview (DIP) in Bahasa Indonesia translation. In addition, from previous studies we have a sample of 124 families from Indonesia with 2 or 3 affected siblings with parents (318 affected, 686 individuals in total) available. We followed up on findings from GWAS studies in Caucasian and Asian samples, i.e. transcription factor 4 (TCF4), chr18q21.2, zink finger gene 804A (ZNF804A), chr2q32.1, micro RNA 137 (MIR137), chr 1p23.3, and CUB Sushi and multiple domains 1 gene (CSMD1), chr 8p23.2, and DNA variants located in the HLA-region on chromosome 6p using Taqman genotyping technology. We characterized all samples in respect to sampling error and genotyping quality, using a DNA test panel with 374 evenly spaced SNPs (Illumina). For assessment of ethnicity we used the program STRUCTURE 3.2.2 and Eigenstrat.

**Results:** No evidence for stratification of our case-control sample was observed. A genomic inflation factor of 1.02 indicated homogeneity. Evidence for association was obtained for DNA-variants located in the ZNF804A gene region, the CUB and Sushi multiple domains 1 (CSMD1) gene region and the HLA region. Interestingly, statistically significant association was observed for DNA-variants located in the ZNF804A gene and the CSMD1 gene in the case – control sample as well as in the family sample. DNA-variants located in the HLA-region were only significantly associated in the more powerful case-control sample.

**Discussion:** Our case control sample is reasonably powered in order to detect a genotypic relative risk of 1.4 and higher. We would like to point out that replication in the family sample might be underpowered. However, analyses in this sample are not subject to stratification since parents are available, thus allowing the application of an association test with internal control (transmission disequilibrium test). Our results support a potential role of ZNF804A, CSMD1 and the HLA region in the etiology of schizophrenia and may confirm that the associated susceptibility genes are not specific for the Caucasian populations.

### The Effect of Clozapine on MRNA Expression for Genes Encoding G Protein Coupled Receptors and the Protein Components of Clathrin Mediated Endocytosis

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**Background:** Clathrin-mediated endocytosis (CME) is an intracellular trafficking mechanism for packaging cargo, including G-protein coupled receptors (GPCRs), into clathrin-coated vesicles (CCVs). The antipsychotic chlorpromazine inhibits CCV assembly of adapter protein AP2 while clozapine increases serotonin 2A receptor internalization. We hypothesized that clozapine alters expression of CME genes modulating vesicle turnover and GPCR internalization.

**Methods:** SH-SY5Y human neuroblastoma cells were incubated with clozapine (1-20  $\mu$ M) for 24-72 hours. GPCR and CME-related gene mRNA expression was measured with RT-PCR. We quantified changes in the same genes using expression data from a microarray study of mice brains after 12 weeks treatment with 12 mg/kg/day clozapine.

**Results:** Expression of genes encoding adaptor and clathrin-assembly proteins, AP2A2, AP2B1, AP180, CLINT1, HIP1, ITSN2, and PICALM, increased relative to control in SH-SY5Y cells incubated with 5-10  $\mu$ M clozapine for 24-72 hours. The microarray study showed significantly altered expression of the above CME-related genes, with a striking 641- and 17-fold increase in AP180 and the serotonin1A GPCR respectively. Expression of three serotonergic receptor and lysophosphatidic acid receptor2 (EDG4) GPCR genes were up-regulated in SH-SY5Y cells incubated with 5  $\mu$ M clozapine for 24 hours. EDG4 expression was also increased with 10-20  $\mu$ M clozapine treatment at 48-72 hours. Clozapine significantly decreased the expression of beta-arrestin, involved in GPCR desensitization, both in vitro and vivo.

**Discussion:** The changes we report in CME and GPCR mRNAs implicates CCV-mediated internalization of GPCRs and the serotonergic system in clozapine's mechanism of action, which may be useful in the design of more effective less toxic antipsychotic therapies.

### BDNF VAL66MET Polymorphism and Polydipsia in Schizophrenia

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**Background:** Polydipsia, not explained by medically-induced polyuria, may be present in more than 20 of chronic psychiatric inpatients, notably in patients with schizophrenia. Polydipsia may lead to hyponatremic symptoms including vomiting, seizures, disturbance of consciousness and even death. To date, the underlying pathophysiology of polydipsia is poorly understood. Dopaminergic neuronal systems in lateral hypothalamus are involved in the control of water intake. Brain-derived neurotrophic factor (BDNF) may play a role in modulating dopamine neurotransmission and synaptic connections in lateral hypothalamus.

**Methods:** We examined the association between BDNF Val66Met polymorphism and polydipsia in a sample of schizophrenia with and without polydipsia. The allele-specific TaqMan method was used for SNP genotyping. This study was approved by the Ethics Committee of the University of Occupational and Environmental Health and informed consent was obtained from all subjects.

**Results:** Although our preliminary data does not suggest a significant association, analysis with complete data set is currently undergoing to clarify the relationship between BDNF gene and polydipsia in schizophrenia.

**Discussion:** Further studies with larger samples are warranted to establish pharmacogenetics of polydipsia in schizophrenia.

### Characterization of Schizophrenia Patients with Copy Number Variations

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**Background:** A number of copy number variants (CNVs) associated with moderate to high risk for schizophrenia have been identified in the past few years. However, there is still little knowledge on the clinical manifestation of these pathogenic CNVs. A pilot study conducted by our group has indicated that schizophrenia patients with CNVs associated with schizophrenia have an earlier age of onset, are hospitalized longer and seem to have a poorer effect of antipsychotic treatment. To test these findings, we conducted a matched case-control study on the extensive public registry data available in Denmark.

**Methods:** The study is a case-only study of patients suffering from schizophrenia. CNV carriers are identified using Illumina SNP arrays or targeted MAQ assays developed for detection of known pathogenic CNVs. The study design includes two different matching strategies for the CNV carriers. First, each carrier of a pathogenic CNV will be matched to 3 known CNV non-carriers on age, gender and diagnosis among patients recruited to the Danish Psychiatric Biobank from different clinical departments. Second, each CNV carrier will be matched to 3 patients with schizophrenia selected randomly from the Central Danish Psychiatric Register. This two-fold strategy is applied in order to estimate the potential effect of ascertainment bias, which may arise as a consequence of recruitment from different departments. Data on age of onset, all hospitalizations (from 1969) and outpatient contacts (since 1995), housing, employment and pharmacological treatment will be obtained from Danish Psychiatric Central Register, Danish Accommodation Database, Integrated Database for Labor Market Research and the National Prescription Database for all cases in the 3 groups; the CNV carriers, the CNV-non carriers and the randomly selected patients.

#### Results:

The genetic screening for pathogenic CNVs is ongoing and has currently topped the identification of hundred CNV carriers. We expect to be able to provide a comprehensive description of CNV carriers suffering from schizophrenia.

**Discussion:** The findings of confident genetic risk factors for schizophrenia provide a novel and unique entry point for understanding of the disease pathology. One of the first steps for gaining such insights is to map phenotypic characteristics (other than the disease itself) that are common clinical manifestations of these risk factors.

### Detecting Copy Number Variants Using Exome Genotyping Chips in 11,000 Schizophrenia Cases and Controls from Sweden

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**Background:** Large samples are essential for progress for any human complex trait including many debilitating psychiatric disorders. For large-scale genetic studies, microarrays are a cost-effective method to capture common SNPs and a mainstay platform for CNV discovery. Several large, rare CNVs have been shown to increase risk for schizophrenia, autism, ADHD, and epilepsy using microarray data. With recent development in sequencing, array-based genotyping platforms have been developed to capture an extensive list of exon variants (e.g. the Illumina Infinium HumanExome BeadChip and the Affymetrix Axiom Exome Array Plate). These exome chips offer an excellent opportunity to interrogate uncommon and rare SNPs in very large samples. For example, the Illumina exome chip may be used in as many as 1 million subjects. Although designed for a different purpose, it is possible that these chips could also be used to detect CNVs given their content, 250,000 probe sets targeting exons. In this work, we set out to evaluate the capability of Illumina exome chip for detecting CNVs, especially the schizophrenia associated large, rare CNVs.

**Methods:** All subjects from the Swedish Schizophrenia Study were born in Sweden and the schizophrenia (SCZ) cases were identified via the Swedish Hospital Discharge Register (Sullivan, submitted). DNA was extracted from whole blood and was genotyped at the Broad Institute using Affymetrix 6.0 and Illumina exome chips. After quality control, 5001 SCZ cases and 6500 controls were available for analysis of CNVs for the exome chip. To detect CNVs from the exome chip, we used the intensity data from all SNPs and applied both PennCNV and the median Z score approach. Unlike previous GWAS chips, >80% SNPs assayed on the exome chip have <1% allele frequency, requiring adjustment in detection methods used for GWAS chips. Therefore, we developed a set of customized parameters for CNV detection and quality control, and visually inspected selected regions.

**Results:** Approximately 78.4% of human genes had  $\geq 1$  SNP from the Illumina exome chip, comparable to the Affymetrix 6.0 chip with 81.8% gene coverage. As proof-of-concept, using Affymetrix 6.0 data, 13 subjects had deletions (1q21.1, 3q29, 15q13.3, or 22q11.21) or duplications (16p11.2) known to be associated with SCZ. Using Illumina exome chip data, we detected all of these 13 SCZ CNVs with high confidence. Additional large rare CNVs were observed in excess in SCZ cases and will be experimentally validated. Analyses of CNV association including both locus-specific test and gene-focused burden test are in progress. We are now applying our analysis pipeline to the entire cohort of ~11,000 Swedish cases plus controls as well as large samples genotyped for other diseases.

**Discussion:** These results strongly suggest that Illumina exome chip data can also be used to genotype large CNVs. This track record combined with its low cost suggests that the exome chip provides an exciting opportunity for surveying important psychiatric CNVs in very large samples as well as gene-focused CNV calling.

### Family Based Linkage and Sequence Analysis of Thought Disorder in Schizophrenia

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**Background:** Disorganization of thoughts is one of the main symptoms of schizophrenia, it constitutes a major component in what the surroundings perceive as psychosis and delusion, and what the patients themselves experience as frustration and suffering. As for schizophrenia, a strong genetic component is suggested to underlie thought disorders [Levy, et al., 2010] [Gambini et al., 1997], but consistent genetic findings are yet to emerge. An explanation for this might lie in the difficulties in assessing thought disturbances easily and reliably, as its subjective nature makes it difficult to measure and quantify, and therefore few studies have collected enough samples to provide the basis for a genetic analysis with sufficient power. Possibly the most extensive and elaborate tool to capture thought disorder is the Thought Disorder Index (TDI) original made by Johnston and Holzman and later revised by Solovay [Solovay et al., 1986]. Which provide an extensive framework for scoring and qualifying thought disorders on a continuous scale. Here we present a study based on meticulous examinations of thought disorder in extended Danish pedigrees, using the combined strength of family-based linkage analysis with the precision of whole genome sequencing, to detect novel genetic variants implicated in thought disorder.

**Methods:** The Copenhagen Schizophrenia Linkage Study is based on six unrelated schizophrenia probands ascertained from the preceding Copenhagen High-Risk Project (1962-86) and their extended multi-generational pedigrees, characterized by a high frequency of schizophrenia and other psychiatric disorders. Detailed diagnostic and phenotypic information from the families was collected between 1989-1999. Genotyping of 258 individuals was performed using both the Illumina Human610-quad SNP microarray and a microsatellites panel of 530 markers. DeCode genetics Disease Miner software was used to perform linkage study and subsequent whole genome sequencing was also processed at their facility.

**Results:** Family based linkage analysis using micro satellite markers resulted in a linkage peak with a LOD score of 3.8. Subsequent analysis of the peak narrowed the signal to a specific haplotype 6 – 8 Mb in length, segregating within one of the pedigrees. 39 individuals (33 with assessed TDI) was found to carry this haplotype, a majority of them had high TDI scores, as suggested in the Solomon et al., study. A significant difference in TDI scores ( $P = 0.0047$ ) was found between haplotype carriers (mean = 11.8) and non-carriers (mean = 7.5) from the same pedigree, this furthered our interest in the particular haplotype. By using the available SNP data the specific boundaries of the haplotype were determined and subsequently 3 carriers were selected for whole genome sequencing, enabling a high resolution search for any causative mutation within the specified region. Results from the linkage analysis and the ongoing sequence analysis will be presented at the symposium.

**Discussion:** Since thought disorder on its own make up a complex phenotype, not much would be expected for it as an endophenotype, even if it is an intrinsic feature of the schizophrenia disorders. However, the findings of this study suggest a common genetic susceptibility between thought disorder and schizophrenia. This result may renew hope for complex endophenotypes as long as an accurate method and statistical power is available.

### Nationwide 22Q11 Deletion Syndrome Survey: Estimating Incidence Rates and Clinical Profiles using Danish Health Registers and Biobanks

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**Background:** The 22q11 deletion is associated with an elevated risk of a number of severe somatic conditions and mental disorders collectively called the 22q11 deletion syndrome (22q11DS). Approximately 30% of the patients with 22q11DS develop psychotic symptoms and s22q11 deletion carriers account for approximately 1–2% of sporadic cases of schizophrenia why this chromosomal anomaly represents the largest known genetic risk factor for schizophrenia. The causal factors as well as the resulting patterns of biological perturbations underlying the seemingly stochastic constellation of co-morbidities observed in individuals carrying 22q11 deletions have escaped discovery. A possible reason for the lack of insight may stem from the selected nature of the cohorts of 22q11 deletion carriers that traditionally are being investigated, leaving the estimates of incidence and prevalence rates of 22q11DS in the population uncertain. The project aims to determine the 22q11 deletion frequency in Denmark and provide a multidimensional register profile for 22q11 deletion carriers.

**Methods:** 22q11DS frequency estimates will be extracted from the Danish National Cyto-Genetic register, which holds information on all Danish citizens that have been given a diagnose of 22q11DS based on genetic testing and thus represents a unselected population sample. The register data will be compared to estimates derived from genetic screening of 1) Neonatal blood spots obtained from the Danish Neonatal and DNBC biobanks, which contain samples from all newborn Danes since 1980 ( $n > 1.9$  mill), 2) the prospective Danish National Birth Cohort study on mother-child duos ( $n = 100,000$ ; 35% of all pregnancies in 1997-2002) and 3) 22q11 disease carriers known through collaborating clinical genetic and pediatric departments. Data from the Danish national health registers (The Danish National Cyto-Genetic register, The Danish National Patient register and the Psychiatric Central Register) will be used in combination with matrix based algorithms to determine multidimensional disease profiles of patients with 22q11DS. The analyses may include disease register profiles from first-degree relatives.

**Results:** The project initiated in January 2012 and will report frequencies of 22q11 deletion de novo events among children delivered pre-term and children with atypical teeth development as well as national incidence rates of 22q11DS in clinical settings. We expect to be able to provide global clinical and epidemiological characteristics of 22q11 deletion carriers, including familial predispositions to disease.

**Discussion:** This survey contrast other studies in the field and is unique in providing comprehensive comorbidity data on the nationwide population of 22q11 deletion carriers in Denmark.

### Predicting Risk for Schizophrenia using Genetic and Environmental Factors

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**Background:** Genetic epidemiology shows unequivocally that schizophrenia is predominantly a genetic disorder: heritability estimates are around 80 and disease risk is increased approximately 10-fold in first degree relatives. It is becoming clear that the mode of transmission is complex such that genetic disease predisposition is the result of many common polymorphisms, of small effect, and rare copy number variants (CNVs) of larger effect. Genome-Wide Association Studies (GWAS) have identified a number of specific risk loci (SNPs and CNVs) and reported evidence for a polygenic contribution to schizophrenia. The recent advances in the field of genetic research and their wide publicity have created a rising demand for genetic counselling regarding schizophrenia; clinicians are increasingly confronted with questions from patients and families regarding risks of disorders for themselves and their children. Currently, the morbid risks of schizophrenia remain an empirical estimation based on the morbidity of other members of the family and the closeness of the relationship. Attempts to quantify the risk of schizophrenia using only genetic data would not be fruitful as only 6 of the variance of schizophrenia is currently explained by the polygenic model, consisting of thousands of genetic markers.

**Methods:** Epidemiological research has identified environmental factors, including obstetric complications, cannabis use, migration, upbringing in an urban environment and paternal age, which also have a significant role in the development of the disorder, and which have a large effect size. We hypothesise that combining known genetic and environmental risk factors would improve the prediction of developing schizophrenia in asymptomatic individuals or individuals with at risk mental state in comparison with genetic or environmental factors alone.

**Results:** In this talk we will present a model calculating schizophrenia risk based on an individual's age, family history, environmental factors and genotypic data, with the flexibility to incorporate new risk factors as further evidence arises. A prospective likelihood for schizophrenia risk  $P(D | G, E, FHx)$  is constructed to estimate risk of developing the disease schizophrenia (D) for a specific combination of genetic (G), environmental (E) and family history (FHx) risk factors. The risk conferred by common SNP alleles contributing to a multifactorial genetic predisposition for schizophrenia, is assumed independent to the environmental factors. Family history risk (assessed by the sibling, parental and offspring relative risks) is adjusted to account for the proportion of familiarity accounted for by SNPs included in the model. Inclusion of CNVs in the model is more challenging, as due to the higher relative risk and the rarity of CNVs it is difficult to evaluate whether they act synergistically with other environmental risk factors. The predictive properties of the model will be validated in clinical samples from South London.

**Discussion:** The aim of this study is to provide a tool for more accurate risk estimation of schizophrenia which will be valuable to genetic counseling, will facilitate prevention targeted on high risk individuals (e.g. descendants from families with genetic loading for schizophrenia) and with potentially clinical utility in individuals with prodromal symptoms.

### Ohnologs are Overrepresented in Pathogenic Copy Number Mutations

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**Background:** A number of rare copy number variants (CNVs), including both deletions and duplications, have been associated with developmental disorders, including schizophrenia, autism, intellectual disability, and epilepsy. These CNVs are pathogenic, presumably because one or more genes within each locus is dosage sensitive. In order to understand pathophysiology, and thus develop targeted interventions, the gene(s) with each copy-number locus that give rise to specific disease phenotypes need to be identified. In the present study we tested the hypothesis that ohnologs (genes retained after ancestral whole genome duplication events which are known to be dosage sensitive) are overrepresented in pathogenic copy number loci.

**Methods:** We selected three set of genes implicated in copy number pathogenicity: i) genes mapping within rare disease-associated CNVs; ii) genes within de novo CNVs under negative genetic selection; and iii) genes identified by clinical array comparative genome hybridization studies as potentially pathogenic. We analysed these gene sets for the presence of ohnologs and compared the frequency to 'control' genes mapping to CNVs not known to be disease associated (copy number polymorphisms).

**Results:** We found that ohnologs are overrepresented in all three sets of genes mapping to pathogenic CNVs, with over 90% containing an ohnolog compared to about 30% of control CNVs over 100Kb. There was also a significant greater density of ohnologs in genes mapping to pathogenic CNVs (both  $P < 1 \times 10^{-5}$ ). We also found that ohnologs in pathogenic CNVs are significantly enriched for OMIM disease genes ( $P = < 2 \times 10^{-15}$ ), and are more frequently members of protein complexes (20.9% vs 10.4%), although the latter was not statistically significant. In some CNVs, such as del15p11.2 (CYFIP1), del17p12 (Lhx1), and 16p13.11 (Nde1) the most plausible prior candidate gene was also an ohnolog.

**Discussion:** We found that ohnologs are enriched in pathogenic CNVs, and that specific ohnolog genes have a plausible connection with the observed phenotypes. This supports the hypothesis that these genes are critical dosage sensitive elements of the genome and may be responsible for some of the deleterious phenotypes observed for pathogenic CNVs.

### Label Free Quantitative Proteomic Analysis Reveals Dysfunction of Complement Pathway in Peripheral Blood of Schizophrenia Patients: Evidence for Immune Hypothesis of Schizophrenia

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**Background:** Schizophrenia is a complex mental disease caused by a combination of serial alterations in genetic and environmental factors. Although the brain is usually considered as the most relevant organ in schizophrenia, accumulated evidence suggests that peripheral tissue also contributes to this disease. In particular, abnormalities of the immune system have been identified in the peripheral blood of schizophrenia patients.

**Methods:** To screen aberrant expression of low abundance proteins in the serum of schizophrenia patients, we conducted nano-LC-MS/MS analysis, a shotgun proteomic method, of serum samples from patients and normal controls. High abundance proteins were eliminated by immunoaffinity before LC-MS/MS analyses.

**Results:** The multivariate statistical test partial least squares-discriminant analysis (PLS-DA) was applied to build models for screening out the variable important plot (VIP) and 27 proteins were identified to be differentially expressed. Pathway analysis revealed that complement and coagulation cascades was the most significant involved pathway. ELISA-based activity analyses confirmed that the alternative complement pathway was suppressed in schizophrenia patients. Ingenuity Pathways Analysis was used to conduct the interaction network of the altered proteins. The network exhibited common features of schizophrenia such as "Nervous System Development and Function, Humoral Immune Response and Inflammatory Response" and highlighted some proteins with important roles in immune system as hub nodes.

**Discussion:** Our findings indicate that dysregulation of the alternative complement pathway is involved in the pathogenesis of schizophrenia. The protein interaction network enhances the interpretation of proteomic data and provides evidence that the immune system may be an important component of the pathology in schizophrenia.

### NPY Gene and Suicidal Behaviour

August Wang<sup>1</sup>, Pernille Koefoed<sup>1</sup>, Anne Sophie Jacoby<sup>1</sup>, Henrik B. Rasmussen<sup>1</sup>, Sally Timm<sup>1</sup>, Merete Nordentoft<sup>1</sup>, Gesche Jürgens<sup>1</sup>, Holger J. Sørensen<sup>1</sup>, Ole Garsdal<sup>1</sup>, Thomas Werge<sup>1</sup>

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**Background:** Suicidal behaviour is frequent among persons with a variety of psychiatric disorders. A stress-diathesis model has been described. Psychiatric disorders ongoing for years, such as schizophrenia, can indeed be seen as continuous stress (Mann & Currier 2006). Some studies have found some indications that Neuropeptide Y (NPY) may be related to suicidal behaviour, including reduced NPY concentration in suicide brain, correlation between plasma-neuropeptide and suicidal patients versus controls, alteration of NPY plasma levels in mood disorder patients with a recent suicide attempt, NPY receptors Y1 and Y2 expression in prefrontal cortex of psychiatric patients and relation between Y2 subtype to suicidal behaviour and cerebrospinal NPY variations in suicide attempters during long-term antidepressant treatment (Olsson et al., 2004; Westrin et al., 1999; Westrin et al., 1998; Widdowson et al., 1992).

**Methods:** The design used was a case-only study. The cases were schizophrenics with history of suicidal behaviour and the controls schizophrenics without history of suicidal behaviour. Suicidal behaviour was divided into 3 categories: None (group 1), suicidal behaviour of non-determinant type (group 2), suicidal behaviour of determinant type (including suicide), called group 3. The psychiatric services in Copenhagen, Denmark have, together with the Research department of Sct. Hans Hospital, Copenhagen, established the Danish Psychiatric Biobank. It is thus possible to relate biological and genetic findings to clinical characteristics from patient interviews and records. As most patients have been living the whole life in the Copenhagen area with one public psychiatric and general hospital service, it is possible to get almost comprehensive life-time information, including suicidal behaviour. 630 patients were included. We studied several SNPs on the NPY gene as well as on the NPY receptors Y1 and Y2.

**Results:** For distribution of genotypes variation was in Hardy-Weinberg equilibrium across the suicidality types. Numbers was very low, and there was no indication of any differences. For SNPs, we calculated Chi-square for group 2 plus group 3 versus group 1 and group 3 versus group 1; no significant results were found. Haplotype construction rendered no new results. The frequencies of the SNP variation in the NPY1R gene were almost zero. For the NPY5R gene we calculated chi-square. Neither group 2 plus group 3 versus group 1, nor group 3 versus group 1 did show any significant results.

**Discussion:** The variations in SNP frequency existed mostly in small numbers. Even if a greater sample would give a definite picture, possible significant variations would have little impact in the population. Our study of the SNPs mentioned, the first study of schizophrenic patients, NPY gene and suicidality, did not support any important involvement of the NPY gene or the two receptors in suicidal behaviour among schizophrenic patients.

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### NPY and Suicidal Behaviour

August Wang<sup>1</sup>, Pernille Koefoed<sup>1</sup>, Anne Sophie Jacoby<sup>1</sup>, David Woldbye<sup>1</sup>, Henrik B. Rasmussen<sup>1</sup>, Sally Timm<sup>1</sup>, Henrik Dam<sup>1</sup>, Merete Nordentoft<sup>1</sup>, Gesche Jurgens<sup>1</sup>, Holger J. Sørensen<sup>1</sup>, Ole Garsdal<sup>1</sup>  
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**Background:** Neuropeptide Y (NPY) is a peptide neurotransmitter in the CNS. It has been associated with regulation of many functions, such as balance and memory. Some studies have linked cerebrospinal NPY to suicidal behaviour. So far no study has been published about NPY genetic variations and suicidality. We aimed to study NPY gene on chromosome 7 as well as Npy receptors Y1 and Y2 on chromosome 5 in schizophrenia patients with and without suicidal behaviour.

**Methods:** The design was a case-only study. The cases were schizophrenia patients. Thus cases and controls had the background. From records and interview the history of suicidal behaviour was recorded and classified into categories: no suicidal behaviour, suicidal behaviour on non-determinant type, of determinant type (including suicide). From Danish Psychiatric Biobank 650 patients participated. We studied from the NPY gene several SNPs, including two functional variants: -399 C>T (rs16147) and leu7Pro (rs16139). The receptors genes we also analysed several SNPs, including 1278 G>A (rs11946004).

**Results:** The groups were comparable according to gender, age at onset and duration of illness. Frequencies of some variants were small. We did not find any variations related to any suicidal behaviour. Nor did haplotype calculations.

**Discussion:** Our study could not confirm a genetic impact from NPY on suicidal behaviour among schizophrenic patients.

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### Plasma MicroRNA Profiling Reveals Altered Mir-150 and Mir-486-3P in Paranoid Schizophrenia

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**Background:** Schizophrenia is a mental disorder characterized by poor emotional responsiveness and thought disorder. A combination of genetic and environmental factors play a role in the development of schizophrenia. microRNA (miRNA) is a short non-coding RNA molecule that is involved in post-transcriptional regulation. Recent studies have indicated that microRNA in plasma can serve as biomarkers of cancer and other diseases. In this study, we examined the plasma microRNA expression profiling in the patients with schizophrenia.

**Methods:** 1. Total RNA was extracted from mixing plasma samples of patients with schizophrenia and age- and sex-matched controls. RNA sequencing (solexa) data revealed a draft profiling. 2. TaqMan small RNA assays were used to exclude false positive results in a small cohort (n=50). 3. Results were confirmed in the entire sample (n=200).

**Results:** Quantitative PCR assessment revealed lower plasma levels of miR-150 (p=0.049) and miR-486-3p (p=0.003) in schizophrenia.

**Discussion:** Our data demonstrate that plasma miRNA profiles are different between schizophrenia patients and health subjects.

### Genetic Associations between Schizophrenia and Cognition

Laura Whitlow<sup>1</sup>, Gerwyn Davies<sup>1</sup>, Sarah Dwyer<sup>2</sup>, Hywel Williams<sup>2</sup>, Elaine Green<sup>2</sup>, Michael Owen<sup>2</sup>, Michael O'Donovan<sup>2</sup>, James Walters<sup>2</sup>  
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**Background:** Schizophrenia is a highly heritable major psychiatric disorder characterised by a range of symptoms such as perceptual hallucinations, delusions, disorganised thinking and cognitive deficits. Even with its high level of heritability (~80), the genetic underpinnings of the disorder are still relatively unknown. Yet with the introduction of Genome-wide Association studies (GWAS) it has been possible to identify several new loci associated with schizophrenia. Most recently the schizophrenia Psychiatric Genetics Consortium (PGC) GWAS discovered five new loci with genome wide significant associations with schizophrenia (1p21.3, 2q32.3, 8p23.2, 8q21.3 and 10q24.32-q24.33). The functions of these variants must be associated with observable phenotypes in people with schizophrenia in order to determine the possible biological pathways involved between these variants and behaviour. Cognitive deficits are a core symptom associated with schizophrenia but are easier to quantify than other symptoms making it a preferable phenotype from which to test for genetic associations. However, efforts to detect genetic associations between schizophrenia and cognitive phenotypes have been limited and this is an important relationship to understand as highlighting biological pathways involved in cognition could potentially help inform future treatments. In this study we investigated whether there was an association between SNPs located at the five new loci identified by the PGC GWAS and cognitive performance in a schizophrenia sample.

**Methods:** Using a sample of patients identified as having a DSM-IV diagnosis of Schizophrenia (n=261) we tested whether there was an association between any of the 5 SNPs (rs1625579, rs17662626, rs10503253, rs7004633 and rs1119580) located at the new loci identified through the PGC GWAS paper and cognitive performance as measured by the MATRICS Consensus Cognitive Battery (MCCB). The MCCB was chosen as it measures cognitive domains known to be impaired in schizophrenia speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving and social cognition. Linear regression was performed using PLINK with age and sex included as covariates.

**Results:** We selected SNPs that were highlighted by the PGC GWAS. Any SNPs that were not available were substituted for perfect proxies ( $r^2=1$ ), rs13269120 for rs10503253 and rs12220375 for rs1119580. The reported p-values are unadjusted but if corrected for number of SNPs analysed 5 out of 14 would survive (please see Table 1). Correction for number of tests was not performed due to the low power of this study and the high correlation between tests. After performing a binomial test on the number of test and domain outcomes it was found schizophrenia risk alleles at these SNPs were associated with poorer performance at a greater level than would be expected by chance ( $p > 2.709 \times 10^{-7}$ ).

**Discussion:** In conclusion our results suggest that possessing the schizophrenia risk allele at each of these SNPs resulted in significantly poorer cognitive performance within these tests and domains. This expands the PGC GWAS results which found an association between these loci and schizophrenia and additionally previous results which have found significant associations with GWS SNPs within the MHC region and cognition. A limitation with this study however is the small sample size and so in order to validate these results a larger case sample will need to be tested as well as replication in a larger control sample.

### Whole Exome Sequencing of 600 Schizophrenia Trio Samples: Analysis of Functional Point Mutations on Chromosome X

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**Background:** We have performed whole exome sequencing on 600 trio samples where the proband has a DSM-IV diagnosis of schizophrenia or schizoaffective disorder. The rate and severity of schizophrenia is elevated in males when compared to females leading to the suggestion that the X-linked genetic factors may play a role in the disorder. For other neurodevelopmental disorders such as intellectual disability and autism there is strong evidence for an enrichment of causative loci on the X chromosome and again there is an elevated ratio of male to female patients. Recent work has highlighted the role of de novo CNVs and point mutations in the aetiology of schizophrenia but a systematic analysis of the X chromosome has not been reported.

**Methods:** We aim to study the rate of de novo and inherited functional point mutations on the X chromosome and to specifically look for cases with potentially damaging mutations in genes previously shown to be involved in neurodevelopmental disorders. We will also determine whether there is an enrichment of functional point mutations in males who are haploid for this chromosome compared with female probands and to look for any parent of origin effects.

**Results:** This work is part of an ongoing research program, results will be presented following the completion of sequencing and annotation of variants.

**Discussion:** The relevance of these results will be discussed in the context of on going schizophrenia research.

### Schizophrenia Associated Polymorphism Regulates Ptptra Transcript Expression in Lymphoblastoid Cell Lines

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**Background:** Receptor protein tyrosine phosphatase  $\alpha$  (RPTP $\alpha$ , encoded by PTPRA) is known to be a signaling molecule that regulates a variety of cellular processes including neurodevelopment, oligodendrocyte differentiation, radial cortical migration, and synaptic plasticity. Loss of Ptptra function in mice has mimicked neurobehavioral endophenotypes of schizophrenia, and also has shown decreased mRNA levels of multiple myelination genes. We detected association between PTPRA single nucleotide polymorphisms (SNPs) and schizophrenia with rs1016753. In the present study, we conducted PTPRA transcripts expression analysis of lymphoblastoid cell lines (LCLs) derived from patients with schizophrenia and healthy controls, using exon specific probes and evaluated the relevance of PTPRA single nucleotide polymorphisms (SNPs) genotype effect on alternative splicing variant.

**Methods:** Peripheral blood lymphocytes derived from 28 patients with schizophrenia and 20 healthy controls were transformed to immortalized lymphoblastoid cell lines (LCLs) by the widely used Epstein-Barr virus method, and total RNA was then isolated from the LCLs. Quantitative polymerase chain reaction (qPCR) using TaqMan gene expression assays designed to detect 3 PTPRA alternative splicing variants were performed. SNP genotyping was performed using the Affymetrix 5.0 Array.

**Results:** We surveyed PTPRA expression and the choice among alternative splicing events. We performed qPCR on LCLs derived from 48 subjects (43 CC carriers, 5 CG carriers), using primers directed against exons specific to each of the 3 PTPRA transcripts described by the National Center for Biotechnology Information (NCBI). This revealed significantly increased expression of the NM\_080840.2 transcript, but not of the NM\_002836.3 and NM\_080841.2 transcripts, in CG compared with CC carriers.

**Discussion:** The carrier of the risk allele (G) for schizophrenia showed altered PTPRA transcripts expression. However, the effect of rs1016753 allelic status and altered NM\_080840.2 expression on the balance between two RPTP isoforms with known differences in biological activity is unclear. Further replication studies and functional analysis at protein levels are needed to elucidate whether alternative splicing regulated by schizophrenia risk allele can contribute to molecular pathology.

### Genetic Studies of Consanguineous Pakistani Multiplex Pedigrees with Schizophrenia

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**Background:** Pakistan has the highest rate of endogamous/consanguineous marriage in the world. The potential influence of such a long history of consanguineous unions on overall levels of homozygosity genome-wide and on genetic disease profiles remains largely under-investigated, especially for more common and complex diseases. In the past few years, with the cooperation from local clinicians, we have identified and characterized 17 large multiplex pedigrees aggregated with schizophrenia, schizoaffective disorder and bipolar disorder from Sindh, Pakistan.

**Methods:** Full family studies were carried out in the field and at local clinics by the trained geneticist, psychiatrist, and psychologist; and further validated by two independent psychiatrists. Standard instruments, i.e. DIGS/FIGS, were used in each participating individual. A high density SNP array genome scan was performed in six selected SCZ/SAF, and BPD pedigrees. Whole exome sequencing was performed in all affected individuals from two selected pedigrees.

**Results:** In total, ten SCZ/SAF/BPD pedigrees have been fully collected, clinically characterized and genetically verified. Genomewide linkage analyses, both two-point and multipoint linkage analyses, as well as parametric and non-parametric analyses, have been performed on six selected SCZ/SAF/BPD pedigrees and have identified potential candidate regions in some single pedigrees and in pedigrees combined. Joint whole-exome sequencing data and linkage candidate regions are currently underway.

**Discussion:** Preliminary linkage analyses strongly suggest a major gene effect in most of these large consanguineous pedigrees aggregated with SCZ/SAF/BPD, with potential candidate regions identified, harboring high-penetrant risk variants.

### Altered Postmortem Gaba-Ergic Gene Expression in the Anterior Cingulate Cortex in Schizophrenia - Effects of Medication

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**Background:** Schizophrenia occurs in up to 1 of the population worldwide, and is a severely debilitating mental illness which is highly heritable and poorly understood. Schizophrenia is characterized by disordered thought, memory and emotion. Numerous studies indicate that reduced activity of the gamma-aminobutyric acid (GABA) system occurs in the prefrontal cortex of schizophrenia patients. GABA receptor function has not been characterized in the anterior cingulate cortex (ACC) in schizophrenia, even though this region is considered to play an important role in the regulation of cognition and mood. Therefore we have tested the hypothesis that the expression of GABAergic genes in the ACC is disrupted in schizophrenia, by comparing the abundance of several GABAergic transcripts in the ACC of postmortem schizophrenia patients with control subjects.

**Methods:** Candidate genes within the GABAergic system were selected for analysis on the basis of previous association with schizophrenia in animal and human studies. These candidates included the transcripts of the GABA-A receptor subunits  $\alpha 1$ -  $\alpha 3$ ,  $\alpha 5$ ,  $\beta 1$ ,  $\beta 2$ ,  $\gamma 1$ - $\gamma 3$ ,  $\delta$ ,  $\epsilon$ , the GABA transporter GAT2, and the enzyme glutamic acid decarboxylase-67 (GAD67). Total RNA was extracted from frozen gray matter of the postmortem ACC of 35 schizophrenia cases and 34 control subjects. Of the patients, 9 were unmedicated for more than 4 weeks before death, while the rest were medicated at the time of death. Measurements of RNA integrity were conducted and gene expression was measured using quantitative polymerase chain reaction (QPCR) for thirteen candidate GABAergic genes and three housekeeping genes (cyclophyllin A, glyceraldehyde-3-phosphate dehydrogenase and beta-glucuronidase). Data were normalized to the geometric mean of the expression of the housekeeping genes and were analyzed using the relative standard curve method.

**Results:** Statistically significant interactions of gender and diagnosis were detected when the expression of the GABA $\alpha 3$  and GABA $\epsilon$  subunits were analyzed in schizophrenia patients relative to controls (F=6.55, df=1, 55, p=0.02; F=3.98, df=1, 54, p=0.05). Altered expression was not detected in schizophrenia for any of the other GABAergic genes tested. When the medication status of the patients was considered, significant increases in GABA $\alpha 2$ , GABA $\alpha 3$ , GABA $\alpha 5$  and GAD67 but not GABA $\epsilon$  expression levels were detected in medicated patients compared with patients off medication (p<0.03). When schizophrenia patients off medication were compared with controls, only GABA $\alpha 3$  expression was significantly reduced in the patients (F=5.47, df=1, 34, p=0.02).

**Discussion:** GABA $\alpha 3$  and GABA $\epsilon$  were found to have reduced expression in the ACC in schizophrenia. GABA $\epsilon$  expression was not altered by medication status of the patients. While GABA $\alpha 3$  expression appeared to be increased in patients on medication, significantly reduced GABA $\alpha 3$  expression was detected in patients off medication relative to the controls. This apparent reversal of GABA $\alpha 3$  expression levels by medication indicates that this specific subunit could be a therapeutic target for antipsychotic drug development. Both genes encoding GABA $\alpha 3$  and GABA $\epsilon$  are located at Xq28, which is a region linked with mood disorders, mental retardation, autism and

malformations of the cerebral cortex. GABA $\alpha 3$  and GABA $\epsilon$  genes are considered to be important for brain development, which is thought to be impaired in schizophrenia. The interaction of gender with diagnosis with GABA $\epsilon$  expression indicated a possible X-linked genetic effect, while medication effects on GABA $\alpha 3$  expression may have masked the differences between the genders. In normal populations, females have a slightly larger ACC than males, but in schizophrenia, this trend is reversed. Identification of predictors of these developmental differences between male and female patients could help elucidate some of the pathophysiological mechanisms giving rise to schizophrenia. Our ongoing studies aim to consolidate these findings.

### Associations of DRD2 and COMT Genes with Theory of Mind in Schizophrenia

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**Background:** Deficits in social cognition are promising endophenotypes that can advance the understanding of schizophrenia neurobiology. Recently a number of dopamine-related genes have been implicated in different aspects of social cognition and social behavior. The present study investigated associations between theory of mind (TOM) domain of social cognition and dopaminergic genes in schizophrenia.

**Methods:** Eighty nine ICD-10 schizophrenic patients and 152 controls participated. Participants were genotyped for DRD2 TaqIA, COMT Val158Met and DRD4 VNTR polymorphisms. They completed three second-order false belief (FB) tasks and three faux pas (FP) tasks and were divided into bad and good FB/FP mentalizers. We used a logistic regression analysis to examine DRD2, COMT and DRD4 genotypes as predictors of TOM, separately for FB and FP understanding abilities. Sex, age, and group served as covariates.

**Results:** There were significantly more bad FB and FP mentalizers among patients than controls.

**Discussion:** We conclude that genetic variation in dopaminergic neurotransmission may be implicated in schizophrenics' deficit of the ability to make inferences about others' cognitive mental states. These COMT and DRD2 influences on false belief tasks performance may be explained both in terms of different neurobiological pathways to social cognition phenotypes and in terms of a common factor such as dopamine receptor sensitivity or transcriptional control of these genes by ZNF804a. This work was generously supported by grant 12-06-00040-a from the Russian Foundation for Basic Research.

### The Association Study of IL-1BETA -511C/T and IL-1RA Polymorphisms with Schizophrenia

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**Background:** There is accumulating evidence that alterations in kinurenine metabolism could be involved in the pathophysiology of schizophrenia and major depressive disorder. Interleukine-1beta (IL-1beta), a pro-inflammatory cytokine, shown to be increased in schizophrenic and depressed patients, induced an upregulation of the enzymes that regulate the kynurenine pathway thus playing a critical role in regulating neurogenesis whereas affecting the availability of tryptophan and the production of enzymes conducive to toxic metabolites. The association between increased IL-1beta levels and PANSS negative symptoms was recently reported (Note et al. 2011). An anti-inflammatory cytokine IL-1RA antagonist is thought to be involved in the pathophysiology of schizophrenia as well. Previous studies have reported that functional polymorphisms -511C/T in IL-1beta and (86bp)(n) repeats in IL-1RA are associated with schizophrenia. The aim of the present investigation was to extend previous studies on the association of these polymorphisms with schizophrenia and its symptoms.

**Methods:** We studied -511C/T (rs16944) and IL-1RA (rs380092) polymorphisms in 824 patients (471 men and 353 women, mean age 39.1 (14.2) years, mean age at disease onset 24.6 (9.5) years) with ICD-10 diagnosis of schizophrenia and 236 matched controls from the Russian population.

**Results:** Allele frequencies for -511C/T IL-1beta were 0.66 for allele C and 0.34 for allele T in patients with schizophrenia and 0.68 and 0.32, respectively, in the control group. The frequency of the CC genotype compared to the CT genotype was lower in the group of patients ( $\chi^2=7.0$ ;  $p=0.01$ ). No between-group differences in the distribution of IL-1RA alleles and genotypes were found. The IL-1RA polymorphism was associated with negative symptoms of schizophrenia measured with the PANSS. Patients homozygous for allele 2 had lower scores compared to those with 1/1 and 1/2 genotypes (26.7 vs. 28.9 and 28.6, respectively ( $p=0.03$ )). No additive effect of two polymorphisms on PANSS scores was found.

**Discussion:** The results are in line with the previous study (Mata et al 2006) argued that this polymorphism may be a useful predictor of negative symptom improvement in schizophrenic patients treated with antipsychotic drugs. In this study, the genotype 2/2 was associated with the better improvement. In conclusion, these findings lend some support to the role of IL-1beta in the pathogenesis of schizophrenia. This work was partially supported by RFBR grant №12-04-00108.

### Characterization of an Ultra Rare DISC1 Human Variant R338Q Found in Bipolar Disorder Regarding Its Interaction with TNIK

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**Background:** Disrupted in Schizophrenia 1 (DISC1) is one of the strongest risk factors for schizophrenia and other neuropsychiatric disorders. DISC1 interacts with another emerging psychiatric risk kinase, Traf2- and Nck-interacting kinase (TNIK), and modulates its kinase activity. An ultra rare DISC1 human variant R338Q was found in 1 of 1,008 bipolar disorder patients but not in 1,152 controls in the same cohort and not in 10,000 controls screened and combined from other studies, suggesting this mutation might contribute to the etiology of bipolar disorder. Since R338 is located in the binding site of TNIK on DISC1 (aa335-348 on DISC1), we characterized the R338Q DISC1 mutant regarding its interaction with TNIK in cells.

**Methods:** Site-directed mutagenesis, co-immunoprecipitation and immunofluorescent staining were used to characterize the mutant DISC1.

**Results:** The R338Q mutation was introduced into the DISC1 mammalian expression construct and interactions of DISC1WT/R338Q and TNIK were tested by co-immunoprecipitation, which showed the R338Q mutation did not affect the interaction between DISC1 and TNIK in cells. TNIK colocalized with both DISC1WT and DISC1R338Q in cells as shown by immunofluorescent staining. In addition DISC1WT and DISC1R338Q showed a similar aggressive-like staining in cells and cultured hippocampal neurons. The Tat fusion peptide derived from the TNIK binding site on DISC1 containing the R338Q mutation has been synthesized and its effects on TNIK kinase activity in vitro and at the level of synaptic proteins are being tested.

**Discussion:** In summary, we have not seen significant difference between DISC1 WT and R338Q regarding their interaction with TNIK.

### A Genome-wide Association Study of Psychosis - Results from the Psychosis Endophenotypes And WTCC2 Consortia

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**Background:** Psychotic disorders including schizophrenia, bipolar and schizo-affective disorders affect approximately 2% of the general population. Epidemiological data show they are heritable and it is believed thousands of genes of small effect interacting with environmental risk factors are involved. Through genome-wide association studies (GWAS) a number of promising loci are being identified for schizophrenia and bipolar disorder and there is growing evidence for a shared genetic architecture between diverse mental disorders. We set out to perform a GWAS looking at a broad phenotype of psychosis in a multi-centre sample from Europe and Australia.

**Methods:** All participants, including controls, underwent a structured clinical interview with the SADS, SCID or SCAN to ascertain (or rule out) a DSM-IV diagnosis of a psychosis spectrum disorder. Participants were excluded if they had a history of neurological disease or head injury resulting in loss of consciousness. Quality control of DNA and genotyping with Affymetrix 6.0 were coordinated by the Wellcome Trust Sanger Institute. A final sample of 4,835 comprising 1,239 cases, 857 of their unaffected relatives and 2,739 healthy controls passed quality control and were genotyped successfully. Finally, 695,193 SNPs were included. Analyses were conducted using UNPHASED, which allows a combined case control and family based association analysis, thus maximising the statistical power of this sample. We included 3 ancestry-informative principal components as covariates. In silico replication was conducted in independent samples from the Psychiatric GWAS and the SGENE+ Consortia.

**Results:** Primary study: We identified no SNPs reaching genome-wide significance levels for association with psychosis as a broad phenotype. The strongest evidence for association was at two SNPs located in the integrin alpha 9 gene (ITGA9: rs981057,  $p=1.17 \times 10^{-6}$ , C allele odds ratio= 0.76, MAF=0.40; rs743393,  $p=1.23 \times 10^{-6}$ , T allele odds ratio= 0.76, MAF=0.35). Integrins are membrane glycoproteins that mediate cell-cell and cell-matrix adhesion. Two further SNPs on chromosome 1 showed some evidence of association and included rs335559 ( $p=1.84 \times 10^{-6}$ , A allele odds ratio= 1.29, MAF=0.46) and rs335555 ( $p=1.97 \times 10^{-6}$ , T allele odds ratio=1.29, MAF=0.46). The nearest gene to these SNPs is centromere protein F (CENPF), which encodes a protein thought to play a role in chromosome segregation during mitosis. Replication: We performed a replication study of most significant findings in the PGC and SGENE+ cohorts giving a two-study independent replication meta-analysis with 10,352 patients and 24,474 controls in total. None of these SNPs was significantly associated, after adjusting for multiple testing. By adding our primary study data, we also conducted a three-study meta-analysis with a total of 11,540 patients, 833 of their unaffected relatives and 26,571 controls and of the 63 SNPs tested none was replicated at genome wide significance level. Using our dataset we performed a more focused analysis, testing for replication at SNPs included in the catalogue of published Genome-wide association studies). Of the published SNPs associated with schizophrenia ( $n=29$ ) and bipolar disorder ( $n=51$ ), none showed evidence of association in our study after multiple testing adjustments.

Finally, we performed a polygenic score analysis, using the SNPs associated with schizophrenia in the Psychiatric GWAS Consortium schizophrenia study. Logistic regression analyses showed that the PGC's panel of SNPs significantly predicted case-control status in our study ( $p < 1 \times 10^{-8}$ ) and explained approximately 2.6% of the variance in risk for the disease.

**Discussion:** Given the size of this sample it is not surprising that no SNPs reached genome-wide significance in a case-control analysis. However the polygenic score analysis indicates that the PGC-derived panel of SNPs conveying risk for schizophrenia is also predictive of the disease in our sample. Our sample is characterised for a wealth of endophenotypes of brain structure and function (cognitive, structural MRI and EEG biomarkers) and these may increase the power to detect new interesting associations for psychotic disorders.

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### Functional Analysis of the Mutations Identified in Neurogranin Gene in Schizophrenic Patients

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**Background:** Schizophrenia is a highly heritable disorder, but many aspects of its etiology and pathophysiology remain poorly understood. Recently, a SNP rs12807809 located upstream of the Neurogranin (NRGN) gene achieved genome-wide significance in this disorder.

**Methods:** In order to find the causal variants of NRGN gene in schizophrenia, we searched for genetic variants in the promoter region and all the exons (including both UTR ends and rs12807809) using direct sequencing in a sample of patients with schizophrenia (n=346) and non-psychotic controls (n=345), both being Han Chinese from Taiwan, and conducted an association and functional study.

**Results:** We identified 7 common polymorphisms in the NRGN gene. SNP and haplotyped based analyses displayed no associations with schizophrenia. Additionally, we identified 5 rare variants in 6 out of 346 patients, including 3 rare variants located at the promoter region (g.-620A>G, g.-578C>G, and g.-344G>A) and 2 rare variants located at 5' UTR (c.-74C>G, and c.-41G>A). No rare variants were found in the control subjects. The results of the reporter gene assay demonstrated that the regulatory activity of construct containing g.-620G, g.-578G, g.-344A, c.-74G, and c.-41A was significantly lower as compared to the wild type construct ( $p < 0.01$  for g.-578G;  $p < 0.001$  for the other constructs). In silico analysis also demonstrated their influences on the regulatory function of NRGN gene.

**Discussion:** Our study lends support to the hypothesis of multiple rare mutations in schizophrenia, and provides genetic clues to indicate the involvement of NRGN in this disorder.

## Gene-Based Analysis in PGC Schizophrenia Study

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**Background:** Gene-based analyses of GWAS datasets, or more generally, set-based tests, combine information from several single nucleotide polymorphisms (SNP) and estimate significance of the set as a whole, rather than at every marker individually. Since genes differ by their length and linkage disequilibrium (LD) correlations between the markers, to make the overall gene-based p-value comparable across genes, the analysis has to take into account these differences. We suggested and evaluated [1] an alternate approach to calculate the significance of a set of SNPs based on theoretical approximation [2] of Fisher's statistics, which requires only the list of p-values for each SNP and knowledge of correlations between SNPs. The latter can be calculated from the data directly, or for those without access to the raw data, they can be estimated using publically available data. We evaluate this approach on the datasets where the individual genotypes were available, and apply the method to the Psychiatric Genetic Consortium (PGC) schizophrenia (SZ) data set, using only summary statistics.

**Methods:** To evaluate the approximate method, we compared set-based analyses results with a permutation-based product of p-values method using individual genotypes generated in GWAS studies of SZ, one restricted to a UK sample [3] and the other on a more complex data set reported by the International Schizophrenia Consortium [4]. We have applied this method to summary statistics for PGC SZ data set [5] imputed with HapMap3 reference panel. We inferred the LD correlation structure from the HapMap3 and obtained gene-wide p-values for 17661 genes. Furthermore we have tested whether the genes, which are expressed in brain are likely to show smaller p-values as compared to those which are not expressed in brain. Linear regression analysis was employed to assess the relationship between the logarithms of gene-wide p-values and the binary variable 1/0 (expressed/not expressed in brain) while controlling for the length of genes. To identify the genes expressed in brain we used Johnson et al [6] and BrainSpan (<http://www.brainspan.org/home.html>) datasets.

**Results:** The correlation between the p-values obtained for each gene using the permutation-based product of p-values and the approximate methods was 0.996 in the UK SZ dataset and 0.990 in the ISC SZ dataset. The approximate method was consistently slightly conservative for significant results, and anticonservative for non-significant results as compared to the product of p-values. We have observed that in similar populations, reference datasets of markers are an appropriate substrate for deriving marker-marker LD, thus the access to individual level genotypes can be avoided for set-based analyses. The PGC gene-based p-values show an excess of significant (at the thresholds 0.05, 0.01 and 0.001) genes as compared to the null distribution (overrepresentation p-values were smaller than  $10^{-6}$  assuming independence of genes). The genes which are expressed in brain, are more likely to be large (logistic regression  $B=0.0005$ ,  $p<10^{-16}$ ). Logarithms of gene-wide p-values are slightly positively correlated with the gene length ( $cor=0.03$ ,  $p=6.4 \times 10^{-5}$ ), possibly due to the cumulative effect of overestimation of the correlation coefficients between markers (as the reference panel sample size is much smaller than the PGC sample size), resulting in conservative p-values for larger

genes. The gene-wide p-values are significantly smaller for the brain expressed genes. Regression analysis of the logarithms of p-values has shown significant association of small p-values with brain expressed genes (over and above the number of SNPs):  $B=-0.11$ ,  $SE=0.019$ ,  $p=4.8 \times 10^{-14}$ ).

**Discussion:** We confirmed that large gene size is a significant predictor of expression in brain. We have also shown that association with SZ on a gene level is significantly stronger for brain expressed genes. Furthermore, brain expression and gene size are independent predictors of SZ association gene-wide p-values calculated with the Brown's approximate method. References 1 Moskvina et al (2011) *Genet Epidemiol* 35(8):861-66 2 Brown M. B. (1975) *Biometrics* 31:978-92 3 O'Donovan M. C. et al (2008) *Nat Genet* 40:1053-55. 4 ISC (2009) *Nature* 460:748-52 5 Ripke et al (2011) *Nat Genet* 43:969-76 6 Johnson et al (2009) *Neuron* 62(4):494-509

## Substance Abuse

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#### Genetic Variation in Alcohol Consumption and Binge Drinking of Adolescents

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**Background:** Monoaminergic networks within the reward system play a key role in the development of substance use disorder. Variations in candidate genes coding for receptors, transporters and metabolizing enzymes involved in these pathways have been implicated for association with alcohol use.

**Methods:** A sample of N=320 high school students aged 16-18 was phenotypically characterized in detail for their drinking habits such as frequency, intensity, problem use and binge drinking. A total of 32 SNPs were genotyped on the Applied Biosystems Open Array Real-Time PCR Platform in. The studied genetic variations included SNPs in cholinergic receptors (CHRNA3/4/5, CHRN3), dopaminergic (DRD2, DRD4), serotonergic, opioid, cannabinoid and GABAergic receptors, transporters and metabolizing enzymes, alcohol and aldehyde dehydrogenase genes, as well as some HPA axis gene variants..

**Results:** Nominally significant genetic effects were observed within the groups. The FKBP5 rs3800737 SNP remained significant after Bonferroni correction for multiple testing with the TT being the risk genotype associated with frequency of alcohol use, frequency of drunk episodes and binge drinking over the last month. Gene-gene epistasis analysis revealed interaction between FKBP5 and ALDH1B1 rs2073478.

**Discussion:** FKBP5 (Caldesmon1) is involved in the HPA axis. It functions as a co-chaperone regulating the cortisol-binding affinity and nuclear translocation of the glucocorticoid receptor. Polymorphisms have been associated with increased recurrence risk of depressive episodes, suicidal ideation, rapid response to antidepressant treatment and PTSD. These results might prove useful in understanding development of alcohol use disorder in healthy adolescents and young adults. The European Union and the European Social Fund has provided financial support under the grant agreement No.: TAMOP 4.2.1./B-09/1/KMR-2010-0003. The study was co-funded by National Grants: OTKA F-46788 and KAB-KT-10-0016.

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#### Variation In The Cannabinoid Receptor CNR1 Gene Modulates The Effect Of Trait Impulsivity On Number Of Marijuana Problems

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**Background:** Impulsivity has been associated with increased marijuana use and subsequent marijuana-related problems among marijuana users. In addition, single nucleotide polymorphisms (SNPs) in the Cannabinoid Receptor (CNR1) and Fatty acid amide hydrolase (FAAH) genes have been associated with cannabis-related phenotypes. This exploratory study tested whether the association between different aspects of impulsivity and number of marijuana related problems among users is explicated by variation in these putative cannabinoid-related genes.

**Methods:** 151 young adult regular marijuana smokers provided a DNA sample and completed measures of trait (the Barratt Impulsiveness Scale) and behavioral impulsivity (including the Stop signal task and Delay Discounting Questionnaire), as well as self-report of marijuana-related problems. Three CNR1 and five FAAH SNPs were genotyped and tested for haplotype blocks..

**Results:** Variation in the CNR1 gene significantly moderated the association between trait, but not behavioral measures of impulsivity, and marijuana-related problems ( $B = .54$ ,  $SE = .20$ ,  $p = .007$ ), such that the combination of higher trait impulsivity and CNR1 genetic risk was associated with greater number of marijuana problems. In addition, there was a main effect of FAAH on marijuana problems ( $B = .32$ ,  $SE = .15$ ,  $p = .03$ ), but no significant FAAH by impulsivity interactions.

**Discussion:** These findings support a role for CNR1 and FAAH genes in predicting risk for marijuana problems among regular users. In particular, CNR1 variation emerged as a moderator of the relationship between trait impulsivity and marijuana problems, suggesting that marijuana users with CNR1 genetic risk and high levels of trait impulsivity are at higher risk for developing marijuana related problems.

### The AKT1 (RS2494732) Genotype Modifies the Risk of Psychotic Disorders in Cannabis Users

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**Background:** Cannabis use is associated with an increased risk of psychosis. One study has suggested that genetic variation in the AKT1 gene might influence this effect.

**Methods:** In a case-control study of 489 first episode psychosis patients and 278 controls, we investigated the interaction between variation at the AKT1 rs 2494732 single nucleotide polymorphism (SNP) and cannabis use in increasing the risk of psychosis.

**Results:** The rs 2494732 locus was not associated with an increased risk neither of a psychotic disorder, nor with lifetime cannabis use, or with frequency of use. We did however find that the effect of lifetime cannabis use on risk of psychosis was significantly influenced by the rs 2494732 locus (Likelihood ratio statistic for the Interaction=8.54;  $p=0.014$ ). Carriers of the C/C genotype with a history of cannabis use showed a greater than two-fold increased likelihood of a psychotic disorder (OR=2.18 [95% CI: 1.12, 4.31]) when compared to T/T carriers. Moreover, the interaction between the rs 2494732 genotype and frequency of use was also significant at the 5% level (LR=13.39;  $p=0.010$ ). Among daily users C/C carriers demonstrated a seven-fold increase in the odds of psychosis compared to T/T carriers (OR=7.23 [95% CI: 1.37, 38.12]).

**Discussion:** Our findings provide strong support for the initial report that genetic variation at rs 2494732 of AKT1 influences the risk of developing a psychotic disorder in cannabis users.

### Genome-wide Association of Behavioral Disinhibition

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**Background:** Behavioral disinhibition (BD) is a construct encompassing substance use, antisocial, and other risky and impulsive behaviors. It is strongly heritable, perhaps even more so than its component phenotypes. Although GWAS have been conducted on phenotypes that fall under the umbrella of BD (such as alcohol dependence and conduct disorder), few studies have searched genome-wide for genetic influences on the overall BD construct. The Center for Antisocial Drug Dependence (CADD) provides a unique sample of adolescents over-selected for high BD characteristics, such as substance problems.

**Methods:** The current sample included 1673 unrelated adolescents drawn from the ongoing studies within the CADD. BD was evaluated at the individuals' first assessment, between ages 13 and 19, as a composite score of age- and sex-corrected substance abuse/dependence, conduct disorder, and novelty seeking measures. Individuals were selected for genotyping based on demonstrating average to high levels of BD. The final sample was 26% female, with 50% of participants drawn from clinical samples. The sample was highly diverse, with 25% of participants reporting Hispanic ethnicity and 32% reporting non-Caucasian ancestry. All individuals were genotyped on the Affymetrix 6.0 array and genotype calls were refined using BeagleCall, resulting in a total of 754531 autosomal SNPs called. Linear regression was conducted to estimate the association of each SNP with the BD phenotype, including ancestry principle components as covariates.

**Results:** SNPs were identified as significantly associated with BD if their p-values fell below  $5e-8$ , with p association.

**Discussion:** Previous research has demonstrated that BD is more heritable than its component phenotypes, and GWAS of individual phenotypes have failed to identify many SNPs significantly associated with these BD components. Examining genetic effects on the higher-order BD phenotype can inform our understanding of specific BD-related behaviors, and vice versa.

### Shared Genetic Risk Between Methamphetamine-induced Psychosis and Schizophrenia

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**Background:** Several epidemiological studies suggested that the shared genetic risk between Methamphetamine (METH) use disorder and schizophrenia. Also it is well-known that METH can provoke psychotic reactions requiring immediate treatment, called METH-induced psychosis. The distinction between METH induced and primary psychosis is critical for understanding clinical course and planning appropriate yet we do not fully understand why only some of METH abusers develop schizophrenia like psychosis.

**Methods:** To clarify genetic convergences between METH-induced psychosis and schizophrenia, we conducted Genome-Wide Association studies targeting METH (241 METH-abuse/dependence. Among them, 193 METH-induced psychosis and 41 patients without psychosis) and schizophrenia (560 schizophrenics and 548 controls) in the Japanese population.

**Results:** We detected large number of SNPs selected as ‘risk’ alleles defined by comparison between METH-induced psychosis and non-psychosis samples are enriched in individuals with schizophrenia (Pbest=.0090).

**Discussion:** This supports previous epidemiologic and neurobiologic evidence connecting METH-induced psychosis and schizophrenia.

### Pilot Association Study of Heroin and Amphetamine Addiction, Neurocognitive Impulsivity, and Genes Related to Brain Reward and Anti-Reward Systems

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**Background:** Impulsivity and drug addiction are polygenic and characterized by often overlapping genetic mechanisms. Neurocognitive aspects of impulsivity are among the most substantiated candidate endophenotypes for addiction and have been shown to be more sensitive to the effects of genetic variation than self-report measures of impulsivity. Neurocognitive impulsivity is multidimensional and most commonly classified as reward-based/“cognitive impulsivity” or rapid-response/“motor impulsivity”, which have been shown to be mediated by different brain networks. The goal of this study is to determine which neurocognitive dimensions of impulsivity would show the greatest utility for being used as endophenotypes for genetic research on drug addiction. To this end, in a pilot case-control study we explore the associations of core addiction phenotypes (heroin and amphetamine dependence) with candidate genes most consistently implicated in impulsivity and addiction, and neurocognitive performance on various laboratory-based measures of impulsivity.

**Methods:** We used seven well-delineated neurocognitive tasks that fractionate neurocognitive impulsivity into distinct and quantifiable domains. Cognitive Impulsivity was assessed with tasks involving various risk and reward contingencies, such as: (1) the Iowa Gambling Task; (2) the Cambridge Gambling Task; (3) the Delayed Reward Discounting Task and (4) the Balloon Analog Risk Task. Motor Impulsivity was indexed by response inhibition tasks, such as: (1) the Stop Signal Task; (2) the Go/No-Go Task; and the (3) Immediate Memory Task. Participants included 25 heroin addicts, 20 amphetamine addicts, and 43 controls with no history of substance dependence. All addicted participants met DSM-IV criteria for substance dependence and most were in protracted abstinence. Polymorphisms in the OPRM1, DRD2, CRH and TPH2 genes were selected based on their implication in the brain reward and anti-reward systems. Genomic DNA was isolated and genotyping was performed using TaqMan assays (Applied Biosystems). Statistical analysis was carried out using PLINK toolset, v1.07.

**Results:** The analysis was performed in groups according to drug of abuse and for the two addicted groups compared to controls. No significant associations were found after corrections for multiple comparisons. However, nominally significant results and interesting trends were observed for several markers: (1) The intron 3 marker of OPRM1 rs648893 was associated with amphetamine (p=0.02), but not with heroin dependence. The rare C allele was observed at lower frequency in the amphetamine users, compared to the controls. Similarly, OPRM1 rs648893 was associated with a variety of neurocognitive indices of motor and cognitive impulsivity in amphetamine but not in heroin users. (2) The G allele of rs6984397 in the CRH gene was more frequent among both addicted groups relative to controls (p=0.015). It was also associated with motor impulsivity in amphetamine users but not in heroin users or controls. (3) The C allele of rs1872824 in the TPH2 gene and the C containing genotypes

were more frequently found among heroin addicted ( $p=0.03$ ) and the combined addicted group ( $p=0.016$ ) compared to controls. They were associated with cognitive impulsivity in controls but not in the addicted groups.

**Discussion:** Polymorphisms in the OPRM1, CRH and TPH2 genes show trends for association with addiction and putative differential associations with amphetamine versus heroin addiction. Results contribute to the growing literature on differences between different drug classes and the involvement of reward and anti-reward systems in protracted abstinence and the recovery from addiction. However due to the small sample size, results should be treated as preliminary and followed-up by future analyses with larger samples. Supported by R01DA02421 to Jasmin Vassileva from the National Institute on Drug Abuse and the Fogarty International Center at NIH.

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### Association of KIAA1324L and GRM3 With Alcohol Dependence in the Irish Affected Sib Pair Study Of Alcohol Dependence

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**Background:** The genomewide association study (GWAS) has provided the opportunity for unbiased assessment of genetic association across the genome. We previously conducted a pooled GWAS in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) case-control sample (unpublished data). The pooled GWAS of alcohol dependence (AD) identified a cluster of 9 significantly associated SNPs on human chromosome 7. The 9 SNPs map within and just upstream of KIAA1324L, a gene encoding a one-pass transmembrane protein that is evolutionarily conserved and widely expressed. The SNPs are also in linkage disequilibrium (LD) with markers in the metabotropic glutamate receptor 3 (GRM3) gene. Both genes lie within an AD linkage region in humans and an alcohol consumption QTL in a syntenic region from the rat. We therefore sought to examine the cluster of LD-tagging single nucleotide polymorphisms (SNPs) at the individual genotyping level to confirm the results observed in the pooled study. 40 SNPs were genotyped in and around KIAA1324L and GRM3 in the IASPSAD case-control sample and tested for association with AD and AD symptom count. Finally, we sought to replicate this association in another ascertained sample, the Collaborative Study on the Genetics of Alcoholism (COGA).

**Methods:** A total of 40 SNPs were genotyped in the independent IASPSAD case-control samples (562 genetically independent AD cases and 569 screened controls). These SNPs included 9 SNPs with evidence from the pooled GWAS, 23 SNPs tagging common variation across the ~660kb region on chromosome 7 (86039131-86699249), and 8 additional SNPs for consistency with the higher density GWAS data from the COGA sample. SNPSpD was used to determine the number of independent tests for multiple testing correction. For replication, genotypes for analysis were extracted from the COGA Illumina 1M case-control GWAS dataset and identical association analyses run (when the same SNPs were not available in COGA for direct replication of the IASPSAD data, proxy SNPs were chosen in the COGA dataset). All analyses were done in PLINK.

**Results:** All 9 SNPs identified in the pooled GWAS remained nominally associated with AD in the IASPSAD sample. 31 additional SNPs were genotyped in the IASPSAD sample and multiple SNPs were nominally associated with AD. Analysis with SNPSpD identified 17 independent SNPs, yielding an adjusted threshold of  $p < 0.0030$ . After this multiple testing correction, rs802467 and rs1859122 remained significantly associated with AD ( $p=0.0025$  and  $0.0012$ , respectively) and rs1635037 ( $p=0.0031$ ) was borderline significant. rs802467 and rs1635037 are each located in introns of GRM3 and KIAA1324L, and are only in LD with SNPs within each respective gene. rs1859122 maps ~44.5 kb upstream of KIAA1324L and is in weak LD ( $r^2$ ).

**Discussion:** Two clinically ascertained samples show association with the same region on chromosome 7, encompassing both GRM3 and KIAA1324L. GRM3 encodes a Group II metabotropic glutamate receptor. KIAA1324L has recently been implicated in bone morphogenetic protein (BMP) signaling. We are currently assessing association with this region in a population sample, the controls from

the Molecular Genetics on Schizophrenia (MGS) sample. We also plan to examine GRM3 and KIAA1324L mRNA expression levels in 41 chronic alcoholic and 41 control post mortem brain samples from the Sydney Brain Bank. This further research is warranted to better understand the nature of the association signal in this region and the possible mode of action of these loci in human alcoholism or related phenotypes.

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### A Genome-wide Association Study (GWAS) of Alcohol Dependence (AD) and Related Traits Using A Hybrid Design

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**Background:** The powerful, systematic, and unbiased GWAS has been successful in identifying replicated susceptibility variants for numerous complex diseases. Compared to several other psychiatric disorders, such as schizophrenia and autism, the total sample size for genome-wide studies of AD is more modest with individual samples in the range of 500-2500 individuals. We report here results from a comparably sized sample (total N=2567) consisting of related cases and unrelated, lightly screened controls.

**Methods:** The cases are from the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) and were diagnosed using DSM-IV criteria. Genotyping was conducted using the Affymetrix V6.0 array by three separate genotyping core facilities. Because artifacts are a known issue when combining samples genotyped at multiple sites, genotypes were called using BeagleCall, which considers both allele signal intensities and LD information. After quality control filtering, the sample contained 717 cases, 1850 controls, and 686,646 SNPs for analysis. The within-site and cross-site duplicate error rates were 0.0043 and 0.0054, respectively. While power calculations for related case/unrelated control designs are complex, power for a likelihood-based approach implemented in the software package LAMP can be approximated from a 1-df genotypic test. Using an FDR of 0.1, our sample has 80 power to detect effect sizes of 1.5 with minor allele frequencies of 0.3. Methods for analysis using the hybrid design are not widely implemented and the optimal analysis approach is being investigated. Here we present results from an analysis of AD symptom count (ADsx, range=3-7) within cases. The analysis was conducted using a 1-df genotypic score test implemented in the R package GenABEL; this test uses a kinship matrix to correct for the non-independence of siblings.

**Results:** While no SNP reached genome-wide significance ( $p < 10^{-3}$  in an association analysis of AD in European Americans (Edenberg et al., 2010). Of these 5 SNPs, 4 were either in our analysis or proxies at an  $r^2 > 0.8$ . In our sample, the p-values for these 4 SNPs ranged from 0.02-0.07, which may indicate that there are independent regions within TMEM132C that alter AD risk.

**Discussion:** We have identified loci associated with alcohol dependence symptom count that should be followed-up in independent samples. Additionally, as part of the Alcohol GWAS Consortium, our data will provide the opportunity to identify additional associations within the context of a mega-analysis.

### TPH2, HTR, and HTT Gene Polymorphisms and Alcohol-Related Suicide

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**Background:** Substantial evidence from family, twin, and adoption studies corroborates implication of genetic and environmental factors, as well as their interactions, on suicidal behavior and alcoholism risk. Serotonin (5-HT) is an important neurotransmitter with wide-ranging functions. Its dysfunction in the central nervous system seems to play an important role in many psychiatric disorders and suicidal behavior, as well as it seems to be involved in the pathophysiology of substance abuse. Recent studies of the genes comprising the serotonergic system showed mild or no association with suicide and alcohol-related suicide. In our study we tested several polymorphisms in different serotonin receptor genes (HTR): HTR1A (polymorphism -1019C>G), HTR1B (polymorphisms 861G>C and -161A>T), HTR1F (polymorphism -78C>T) and HTR2A (polymorphism -1420C>T), serotonin transporter gene (5-HTT) (polymorphism LPR in promoter and VNTR in the second intron), and tryptophan hydroxylase 2 gene (TPH2) (five single nucleotide polymorphisms (SNPs), one functional (p.Arg441His), two in intron 5 (Rs1843809, Rs1386493), and two in the 5' regulatory promoter region (Rs4131348, Rs11178997)) and completed alcohol-related suicide, as well as between alcohol-dependent suicide victims. The genotyping was performed on a population with one of the highest suicide rates in the world. Namely, Slovenia ranked 7th in the year 2011 with suicide rate of 30.9 suicide victims per 100,000 citizens.

**Methods:** The study subjects were 388 Slovenian suicide victims and 227 controls autopsied in the years 2002 through 2005. During autopsy venous blood was drawn, and afterwards DNA extraction and alcoholimetric analysis were performed. Relatives of 79 suicide victims were interviewed using a semi-structured questionnaire designed according to Slovenian cultural and economic conditions. They provided information about the alcohol abuse of the suicide victims. Amongst the suicide victims were 25 alcohol misusers and 54 non-misusers. We performed PCR/RFLP and qRT-PCR (Real-Time Polymerase Chain Reaction) genotyping analysis of SNPs.

**Results:** For the gene of TPH 2 we determined some associations. The results showed association between suicide ( $P(\chi^2) = 0.043$ ) and alcohol-related suicide ( $P(\chi^2) = 0.021$ ) for SNP Rs1843809. A tendency for association was determined also for polymorphism Rs1386493 ( $P(\chi^2) = 0.055$ ) and alcohol-related suicide. Data acquired from psychological autopsies in a subsample of suicide victims (n = 79) determined more impulsive behavior ( $P(\chi^2) = 0.016$ ) and verbal aggressive behavior ( $P(\chi^2) = 0.025$ ) in the subgroup with alcohol misuse or dependency. Association between polymorphisms in the selected serotonin receptor genes, transporter gene and completed alcohol-related suicide, as well as between alcohol-dependent suicide victims was not established.

**Discussion:** Our results suggest implication of polymorphisms in suicide and alcohol-related suicide, but further studies are needed to clarify the interplay among serotonergic system dysfunction, suicide, alcohol dependence, impulsivity and the role of TPH2 enzyme. Results also suggest that selected polymorphisms of the 5-HT receptor genes and transporter gene are not involved in genetic susceptibility to completed suicide under acute influence of alcohol or among alcohol-dependent individuals, but further studies in a larger sample are needed. Taken together, our results could represent important guideline for further study design, since they were obtained on a population with one of the highest suicide rates in the world.

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### Validation of CNVs and De Novo Mutations in Schizophrenia Cases Using a Droplet-based Approach to Digital PCR

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**Background:** In the study of a complex genetic disease such as schizophrenia (SCZ), accurate measurement and characterization of genome variation including rare de novo single nucleotide variants (SNVs) as well as copy number variants (CNVs) is critical. The detection of these forms of variation through next generation sequencing array-based data must be validated in order to rule out the possibility that a detected event arose from systematic or experimental error. Traditionally quantitative PCR (qPCR) has been utilized to validate many genetic alterations. However, the precision of qPCR is limited by variation in the kinetics of PCR reactions and by the limits of precision of fluorescence measurements. This can be particularly troublesome when typing CNVs, for which the magnitude of concentration differences is highly important for accurate assay calibration.

**Methods:** We describe a method based on performing digital PCR in thousands of nanoliter-sized reaction droplets. We have used droplet digital PCR (ddPCR) to validate rare de novo mutations seen in exome sequencing studies as well as screen for CNVs present in cases of SCZ. The technology adapts the qPCR assay into a digital environment by encapsulating reaction components into ~20,000 monodisperse droplets. Each droplet can be counted as its own qPCR reaction, which is then read through an automated droplet flow-cytometer and counted as positive or negative based on fluorescence of sequence-specific probes. The counts for each probe are then used to calculate highly accurate absolute concentrations based on a Poisson model.

**Results:** The digitization of the qPCR reaction has allowed for highly enhanced accuracy when typing CNVs and SNVs due to massively parallel readings for each reaction mixture. We have found ddPCR to be more accurate, precise, and robust than traditional qPCR techniques in CNV detection. The technology has also been used to efficiently validate de novo SNPs, as well as characterize somatic mosaicism.

**Discussion:** The ddPCR technology has provided a scalable and more sensitive method for the validation and interrogation of specific genetic abnormalities than traditional fluorescence based qPCR methods.

### Deep Re-Sequencing of ST8SIA2 In Bipolar Disorder: A Generalized Susceptibility Gene

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**Background:** Alpha-2,8-sialyltransferase 2 (ST8SIA2) is an enzyme that modulates the glycosignaling of key proteins involved in neural development. ST8SIA2 lies within a bipolar disorder linkage peak on chromosome 15q26.11, and has been associated with bipolar disorder, schizophrenia and autism. High expression of ST8SIA2 early in brain development, and expression of its product, polysialylated-NCAM (PSA-NCAM) in regions of neurogenesis in the adult brain suggest that aberrant temporal or spatial expression of ST8SIA2 may disrupt the highly coordinated early development of brain connectivity and affect plasticity of established neuronal networks.

**Methods:** To discover potentially deleterious sequence variation in ST8SIA2, we have conducted a targeted deep re-sequencing study of a ~100kb genomic region including ST8SIA2 in 48 Caucasian Australian cases with bipolar disorder. We developed an analysis pipeline incorporating the Genome Analysis Toolkit (GATK) for SNP calling and genotyping, and have used Galaxy, a web-based platform, to make our data and methodology easily accessible to other researchers for use on their data sets.

**Results:** We identified 380 SNPs in the 48 bipolar disorder cases, including over 50 putative novel SNPs (MAF=0.09±0.10) not described in analysis of Caucasian Europeans (n=176) from phase 1 of the 1000 Genomes project. A number of known SNPs (n=55) have allele frequency differences > 5% between our bipolar case population and 1000 Genomes Caucasians. 221 SNPs lie within a 54 kb region containing a 6-SNP haplotype we recently identified as associated with bipolar disorder. Interestingly, a SNP included in the associated haplotype (rs4777974, MAF=0.5) lies within a DNase I hypersensitivity hotspot present in neuronal cell lines, suggesting this variant may impact transcriptional regulation in the brain. A previously identified SNP near the boundary of exon 5 (rs11637874, MAF=0.094) is predicted to affect splice donor site efficiency. This finding is consistent with the presence of a novel Δ5 splicing isoform which we have identified in the dorsolateral prefrontal cortex from neonatal and adult post-mortem brain samples. In addition to potential effects on ST8SIA2 transcription, 12 SNPs are transcribed within ST8SIA2 mRNA (11 in the 3'UTR and a synonymous coding SNP in exon 5) and thus may influence post-transcriptional regulation.

**Discussion:** We have identified a large number of variants that potentially affect the expression of ST8SIA2 and may modulate its function during human development and disease. We are currently performing integrative analyses using genomic, mRNA expression and bioinformatic prediction data to identify candidate functional variants likely to affect transcription, mRNA splicing, or post-transcriptional regulation of ST8SIA2.

### The Neural Correlates of Psychosocial Stress Processing

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**Background:** Stress has been described to influence both the onset as well as the course of various psychiatric disorders as depression, alcoholism and posttraumatic stress disorder. Those disorders have also been linked to a dysregulation of the human stress response and one of its main components, the hypothalamus-pituitary-adrenal (HPA) axis. Furthermore several genetic variants associated with the regulation of the HPA axis have been implied in the etiology of stress related psychiatric disorders. Central nervous processes play a major role in the regulation of the stress response. Functional magnetic resonance imaging (fMRI) has proven to be a valuable tool to investigate intermediate phenotypes that mediate the association between genetic variation and clinical phenotype.

**Methods:** To study the neuronal mechanisms underlying the acute stress response, we sought to design a paradigm that would be (a) feasible to serve as a psycho-social stressor inducing robust HPA axis responses and (b) suitable for scanner environments. Based on the psychological characteristics uncontrollability and social-evaluative-threat our paradigm consists of two tasks with adaptively varied speed and difficulty that have to be solved under time pressure. A scientist panel that is presented by live video stream monitors the subject's responses and gives continuous visual feedback during the tasks and social-evaluative verbal feedback between both sequences. The paradigm is presented as block design with four stress and four control blocks. The paradigm was evaluated in a fMRI study. For measurement of HPA axis hormones saliva and blood samples were collected prior to, during and after the fMRI session. Additionally, heart rate was continuously recorded and mood ratings were assessed.

**Results:** Subjects showed an increase both in ACTH and Cortisol levels. Under stress a significant increase of neural activation was observed in areas related to completion of cognitive tasks and in stress relevant regions as the thalamus, hypothalamus and limbic structures. Significant deactivation was observed in the perigenual anterior cingulate cortex, a region involved in emotion regulation. Currently we analyze sex differences in the neuronal activation patterns under stress as well as the association of HPA axis reactivity, subjective ratings and heart rate responses with the neuronal activation. Results of these analyses will be reported at the conference.

**Discussion:** Our data suggest that we developed a stress paradigm that is capable of inducing stress related changes on the endocrine and neuronal level. We propose that the developed paradigm can be applied to address questions considering the neuronal basis of the human stress response. We intend to apply the paradigm to investigate the neural processes underlying stress related disorders. Therefore we will analyze the modulation of the stress response by corresponding genetic polymorphisms to investigate how the neural response is altered by risk variants. Promising candidate genes are genes involved in the regulation of the HPA axis [GR, FKBP5, CRHR1, BDNF, 5HTTLPR] and genes associated with stress related psychiatric disorders in genome-wide or candidate studies [HOMER1, NPY].

### Genetic Markers that are Informative for Allelic Non-Disjunction in Down Syndrome-Related Mental Retardation

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**Background:** Mental retardation (MR) in Down syndrome (DS) is genetically complex and phenotypically variable, even among individuals with partial trisomy-21. Genes that are located in the 21q22.1 critical region, implicated in DS neuropathology and mediating functions such as learning and memory may therefore confer susceptibility to DS-related MR via allelic non-disjunction. Accordingly, we elected to genotype several single nucleotide polymorphisms and microsatellite markers in the kainate subtype glutamate receptor subunit (GluR5 / GRIK1) and in GARS-AIRS-GART that is involved in de novo purine biosynthesis.

**Methods:** Down patient families (trios and duos) were recruited for the study from the Outpatient Department of Manovikas Kendra after obtaining written informed consent from all participants and prior approval from institutional Human Ethics Committee. All cases were evaluated by a clinical psychologist and psychiatrist for referral. Peripheral blood samples from patients, parents and volunteer controls were used for genetic analysis. Genomic DNA from lymphocytes was isolated by the salting out procedure of Miller et al., 1988. PCR amplification of target polymorphisms was followed by either RFLP, gel electrophoresis, automated sequencing or SNaPshot™ assays, as appropriate for genotyping. Statistical analyses were carried out as per published protocols and publicly available software..

**Results:** PCR-RFLP-based genotyping of three GluR5 markers [522(A/C)-rs363538; 1173(C/T)-rs363430 and 2705(T/C)-rs363504] indicates that the distribution of allele frequencies is in Hardy-Weinberg equilibrium. Moderate heterozygosity (0.339) and a major allele frequency of 0.78 render the 1173(C/T) marker informative. The estimated ratio of meiosis-I to meiosis-II errors arising from allelic non-disjunction of 1173(C/T) is 4;1 in maternal cases and 2;1 in paternal cases. Pair-wise comparisons reveal that 522(A/C)-1173(C/T) [ $\chi^2=31.2$ ,  $df=1$ ,  $p=0.0001$ ,  $D'=0.42$ ] and 1173(C/T)-2705(T/C) [ $\chi^2=18.3$ ,  $df=1$ ,  $p=0.0001$ ,  $D'=0.34$ ] are in significant linkage disequilibrium of weak magnitude. Though a robust risk haplotype was not predicted, the maternal origin and non-disjunction in meiosis-I was independently verified for A-522, C-1173 and T-2705 alleles. Microsatellite markers were genotyped by means of gel electrophoresis and automated DNA sequencing of PCR amplicons. Relative to the reference D21S2055-(GATA)<sub>n</sub> marker, the GluR5-(AGTA)<sub>n</sub> polymorphism has a lower heterozygosity [ $H_o=0.286$ ]. However, a high power of discrimination [ $PD=0.977$ ] and low probability of matching [ $pM=0.023$ ] indicates utility of this marker in distinguishing between two unrelated individuals. The parental origin of non-disjunction was discerned in 34 / 72 families and a 1:1:1 tri-allelic inheritance was detected in 1 / 72 cases. SNaPshot™ assay-based quantitative multiplex genotyping reveals that 16614470(A/G)-rs363484 is monomorphic, whereas, 16586604(A/G)-rs363506 of GluR5 has moderate heterozygosity [ $H_o=0.21$ ] and a major allele frequency (0.88) higher than similar estimates for the 34901423(A/G)-rs2834235 and 34877070(A/G)-rs7283354 polymorphisms of GARS-AIRS-GART. There is also evidence of a meiotic recombination event between the GluR5-rs363506 and GARS-AIRS-GART-rs2834235 markers on chromosome-21.

**Discussion:** Systematic studies with additional marker polymorphisms and patient families may help define chromosomal region(s) prone to non-disjunction and aid the identification of marker haplotypes with diagnostic value in DS-related MR.

### Evidence of an Inflammatory Pathway Leading to Psychosis in Bipolar Disorder

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**Background:** Family history is the strongest risk factor for bipolar disorder. Yet it has been difficult to identify susceptibility gene variants. An alternative approach to unearth causal genetic mutations is to focus on biomarkers, i.e., measurable key components in biological pathways between genotype and disease. We therefore conducted a genome wide association study (GWAS) of kynurenic acid (KYNA) in cerebrospinal fluid (CSF), based on that elevation of KYNA in brain is a consistently found biochemical aberration in psychotic disorders. We then studied the genetic finding in relation to psychotic symptoms, cognition, and brain gray matter volume. Lastly, we used an in vitro model to test if IL-1b is the link between the genetic variant and elevated CSF levels of KYNA.

**Methods:** CSF was collected from patients with bipolar disorder (N=76) and a genome-wide association study in relation to CSF KYNA was conducted. Patients underwent magnetic resonance imaging (MRI) scans of the brain and a neurocognitive test battery. Human cortical astrocytes were cultured and stimulated with recombinant human IL-1b (10ng/ml). Analysis of KYNA was performed using an isocratic reversed-phase high-performance liquid chromatography (HPLC) system.

**Results:** One SNP located on chromosome 1 reached genome-wide statistical significance in relation to CSF KYNA (rs10158645,  $b=1.05$ ,  $P=3.85 \times 10^{-8}$ ,  $MAF=0.15$ ). The minor allele of rs10158645 was associated with increased risk of psychotic features ( $n=76$ ,  $OR=4.0$ ,  $95\%CI:1.4-12$ ), increased verbal working memory ( $n=108$ ,  $b=1.8$ ,  $P=0.019$ ) and increased gray matter thickness in corresponding brain regions ( $n=138$ ,  $P=0.02$ , voxel-level FWE-corrected). The in vitro results showed that IL-1b increases KYNA levels in human cortical astrocytes by inducing the rate-limiting enzyme TDO2.

**Discussion:** The minor allele of rs10158645 has previously been coupled to a decreased expression of sortin nexin 7 (SNX7), which in turn activates caspase-8 that increases IL-1 $\beta$ . Here we found that IL-1 $\beta$  stimulates KYNA, known to be increased in psychotic disorders. This raises the possibility that SNX7-induced IL-1b dependent activation of the kynurenine pathway is a molecular pathway underlying psychosis in bipolar disorder.

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**A Statistical Framework for the Evaluation of De Novo Variation in Psychiatric Disease**

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**Background:** With the increasing number of exome sequencing studies focused on de novo mutation discovery, it has become critical to have a rigorous method to evaluate the de novo findings. Particularly, it is important to determine the potential significance of genes that harbor multiple events, especially as the number of sequenced individuals rises. The need for this statistical framework was recently highlighted by four exome sequencing studies of autism spectrum disorders that found an apparent excess of genes that had two loss-of-function (LoF) mutations (Neale et al. 2012; Sanders et al. 2012; O’Roak et al. 2012; Iossifov et al. 2012). While rough estimations were used to determine significance, we propose here a systematic approach to estimating individual gene significances as well as the significance of experiment-wide excesses.

**Methods:** To properly evaluate the probability of mutation, it is key to precisely estimate the expected mutation rate. To do so, we applied a mutational model based on intergenic SNPs identified by the 1000 Genomes Project to each gene region in the genome. This mutational model incorporates local sequence information to determine the probability of each possible base change for every genomic base. We defined the target bases of mutation as those in coding sequence or in the conserved splice sites. We then combined the individual probabilities of each possible substitution to determine the overall probabilities of a synonymous, missense, nonsense, splice site, frameshift, and inframe mutation per gene per trio sequenced. These probabilities can be used directly to evaluate individual gene observations, as well as to simulate efficiently large sequencing studies similar to the ones recently conducted for autism spectrum disorders. ARRA Autism Sequencing Collaboration.

**Results:** The synonymous mutations identified in our exome sequencing study, as well as mutations seen in 1000 Genomes trios and unaffected individuals in autism pedigrees, were used to validate our model. Using the validated model, we create a gene-specific probability for different types of mutations for all genes in the human genome. We use this to evaluate the combined results of four recent autism exome sequencing projects to determine a revised significance of each gene and an appropriate threshold for genome-wide significance. We were also able to model the expected number of genes containing two or more de novo LoF mutations and experiment-wide significance for this observation in individual and combined studies, confirming a significant excess of genes with two or more LoF mutations. To validate top ranking genes, we assessed evidence for association in a large, independent case-control exome sequencing data set.

**Discussion:** While this method was applied to exome sequencing for autism spectrum disorders, it can also be applied more generally. It not only takes into account the targeted sequence context, but also the coverage found in our studies and the number of trios sequenced. These probabilities, therefore, can be scaled and applied to other exome sequencing studies to assess the significance of potentially promising candidate genes for further evaluation.

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**Testing the Role of Circadian Genes in Conferring Risk to Mood Disorders**

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**Background:** Disruption in circadian rhythms and perceived changes in sleep quality are common features of mood disorders. Insomnia or hypersomnia are criteria for diagnosis of a major depressive episode, while reduced need for sleep is a prodromal marker for manic episodes in bipolar patients. Studies show that 60-85% of patients with MDD report experiencing insomnia. Similarly in bipolar disorder, disturbed circadian rhythms, hypersomnia and insomnia are common. The association between disturbed circadian rhythms and mood disorders has led many to investigate the role of circadian “Clock” genes in conferring risk to mood disorders. Using summary data from the Psychiatric GWAS Consortium, we sought to examine the evidence that variants in circadian genes increase risk to bipolar disorder onset, and to test whether carrying risk alleles made people more susceptible to sleep disruption in the general population.

**Methods:** Summary statistics for more than 1.2 million SNPs from a large collaborative genome-wide association study of bipolar disorder were provided by the Psychiatric GWAS Consortium. VEGAS, a freely-available program that computes a gene-based test of association based on all of the SNPs in a gene was used to analyse the summary statistics. Initially, gene-based and single p-values were analysed for 21 core circadian genes. Subsequently, all genes that have been linked to sleep regulation in humans or animal models were analysed. To assess whether there is evidence for genetic overlap between mood disorders and sleep disruption, an independent Australian sample from the Australian Twin Registry who had answered a questionnaire about usual sleep habits was analysed. A genetic profile score with each SNP weighted by the estimated odds ratio from the PGC analyses was generated for each individual, and the scores were regressed against three self-reported sleep phenotypes. Psychiatric Genetics Consortium

**Results:** After correcting for multiple comparisons, none of the circadian genes were significantly associated with bipolar disorders. Several genes previously implicated in the etiology of mood disorders such as CLOCK and TIMELESS, harboured no SNPs significant at the nominal level of  $p < 0.05$ . There was no evidence of an enrichment of associations in circadian genes. After expanding the search to genes previously associated with sleep or circadian rhythms, the MAPK1 gene showed strong evidence in both the single SNP and gene-based analysis. This gene is expressed in a circadian fashion in the suprachiasmatic nucleus, where the central clock is located. The profile scores did not explain a significant proportion of the variation of any of the three sleep phenotypes - sleep quality, sleep latency and sleep timing.

**Discussion:** Our results suggest that genes encoding components of the molecular clock are not good candidates for harbouring common variants that increase risk to bipolar disorder. Further studies that attempt to find associations with mood disorders should focus on SNPs that approach significance in the PGC analysis or on other genes thought to influence sleep rather than on circadian genes. This finding is only true for common variants found on current SNP chips. It is possible that rare variants in clock genes do increase risk. Several studies have reported associations between clock gene variants and age-at-onset and response to treatment in bipolar disorder, however our study could not test for association with those phenotypes. No evidence was found that bipolar risk variants increase the risk of sleep disruption or are associated with sleep timing in the general population. This may have been due to a lack of power and studies with larger sample sizes are needed. Furthermore, our study relied on self-report sleep phenotypes that were not collected from a standardised questionnaire and hence may be subject to perceptual or cognitive biases.

### Stress-Induced DNA Methylation Changes in Rat Brain

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**Background:** Stressful events are known risk factors for a number of psychiatric conditions including depression and post-traumatic stress disorder. There is growing interest in understanding the molecular mechanisms in the brain underlying stress-related learning and memory processes, and how these can influence long-term neurobiological pathology. Emerging evidence suggests that epigenetic mechanisms underlying transcriptional regulation are capable of biologically embedding stressful experiences, and hence contribute to long-term phenotypic development.

**Methods:** We used a validated rodent model of stress, forced swimming, to examine stress-associated DNA methylation changes in the promoter regulatory regions of five candidate genes (c-fos, Fgf1, Gadd45beta, Nr3c1 (glucocorticoid receptor) and Bdnf). DNA was extracted from the dentate gyrus, rest of hippocampus and neocortex from stressed and control rats. DNA methylation was quantified across multiple CpG sites in each promoter region using bisulfite pyrosequencing.

**Results:** We observed tissue-specific significant differences in DNA methylation following the forced swim test in several CpG sites located in the promoter regulatory region of specific Bdnf isoforms.

**Discussion:** These data further our understanding about the molecular mechanisms underlying stress-related learning and memory, and further support a role for Bdnf in relation to stress and neuropsychiatric disease. As epigenetic modifications are potentially reversible, understanding how such mechanisms can be environmentally influenced may lead to novel, potentially more effective therapeutic interventions.

### Epigenetic Alteration of the Dopamine Transporter Gene in Alcohol Dependent Patients is Associated With Age

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**Background:** Chronic alcohol abuse and dependence are associated with dysfunctional dopaminergic neurotransmission in mesocorticolimbic circuits. Genetic and environmental factors have been shown to modulate susceptibility to alcohol dependence, and both may act through epigenetic mechanisms that can modulate gene expression, e.g. DNA methylation at CpG sites. Recent studies have suggested that DNA methylation patterns may change over time. However, few data are available concerning the rate of these changes in specific genes. A recent study found that hypermethylation of the promoter of the dopamine transporter (DAT) gene was positively correlated with alcohol dependence, and negatively correlated with alcohol craving. The aim of the present study was to replicate these findings in a larger sample of alcohol dependent patients and population-based controls matched for age and sex.

**Methods:** The patient sample comprised 100 alcohol dependent individuals hospitalized for alcohol detoxification treatment. A diagnosis of alcohol dependence was assigned according to DSM-IV criteria. Alcohol craving was assessed using the Obsessive Compulsive Drinking Scale. 100 population-based controls matched for age and sex were recruited within the German National Genome Research Network (NGFN) from the area of Bonn, Germany. Controls were screened for excessive drinking using the Alcohol Use Disorders Identification Test. Blood samples were taken from all patients on admission after an overnight fast. After 14 days of detoxification, a repeat fasting blood sample was taken from 85 patients. Blood samples from control individuals was also taken after an overnight fast. Methylation status of the DAT promoter was analysed applying a pyrosequencing approach. Statistical analyses of the pyrosequencing results were conducted using R Version 2.7.2 (<http://www.r-project.org>). The methylation level of each subject was defined as the average percentage of methylated cytosins from all of the assessed CpG sites.

**Results:** No difference in methylation level was observed between patients and controls, and no difference in methylation level was observed before and after alcohol withdrawal in patients. However, patients with more severe craving showed a trend towards lower DAT methylation levels ( $p=0.07$ ), which is consistent with previous findings. Furthermore, in our overall sample, DAT methylation levels increased with age. Interestingly, a separate analysis of patients suggested that this finding was mainly driven by the patient group.

**Discussion:** Although the present data do not clarify whether chronic alcohol abuse is responsible for this phenomenon or merely enhances an aging specific process, our findings suggest that hypermethylation in alcohol dependent patients is a consequence, rather than a cause, of the disorder.

### The Histidine Decarboxylase Gene (HDC) is Associated with Gilles De La Tourette Syndrome in a Large Sample of Trios

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**Background:** Gilles de la Tourette Syndrome (GTS) is a childhood onset neuropsychiatric disorder defined by persistent motor and vocal tics. Its etiological background is complex, with multiple genes interacting with environmental factors to lead to the onset of symptoms. GTS prevalence is estimated between 0.3 and 1%. Recently, analysis of linkage in a two-generation pedigree led to the identification of a rare functional mutation in the HDC gene encoding L-histidine decarboxylase, the rate-limiting enzyme in histamine biosynthesis. However, the participation of this gene in GTS etiology, has not yet been studied extensively, and is, up until now, considered rare.

**Methods:** We present here our results on the investigation of the HDC gene in association to GTS in a large multi-centered study of eight populations. This is the largest study of the HDC gene in relation to HDC reported to date. A total of 521 trios with GTS were studied, originating from Canada, Germany and Spain, as well as the TSGeneSEE consortium (Greece, Poland, Hungary, Albania, Italy, supported by the Tourette Syndrome Association, USA and COST Action BM0905). In order to cover variation across the HDC gene, 12 tagging Single Nucleotide Polymorphisms (SNPs) were selected from the HapMap database, using the CEPH Europeans as reference. The transmission test for linkage disequilibrium (TDT) for single markers and haplotypes was run as implemented in HAPLOVIEW.

**Results:** Joint analysis of all 521 trios yields significant association results that withstand correction for multiple testing (highest single marker association found at P value =0.002, corrected P value=0.02 after 1000 permutations of the data). Strong association is also found with a 2-site haplotype (P value=0.0003, corrected P value=0.002 after 1,000 permutations of the data). It should be noted that, individual population analysis reveals some heterogeneity among observed LD patterns.

**Discussion:** Our results indicate that the HDC gene may play a role in GTS etiology. Interestingly, the patterns of association may vary in different populations. Our findings warrant further investigation of the intriguing histaminergic pathway hypothesis in relation to GTS.

### Pitt-Hopkins Syndrome-Associated Mutations in TCF4 Lead to Variable Impairment of the Transcription Factor Function Ranging from Hypomorphic to Dominant-Negative Effects

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**Background:** TCF4 is located on chromosome 18q21.2 and encodes a basic helix–loop–helix (bHLH) transcription factor TCF4 also known as ITF2 (immunoglobulin transcription factor 2), SEF2 (murine leukaemia virus SL3-3 enhancer factor 2) and E2-2. TCF4 gene has been associated with several mental disorders: (1) common variant SNPs in TCF4 confer risk of schizophrenia; (2) in context of large 18q deletions, TCF4 hemizygoty is responsible for the absent cognitive and motor development beyond the milestones normally acquired by one year of age and increased risk of autistic-like behaviours; and (3) missense, nonsense, frame-shift and splice-site mutations as well as translocations and large deletions encompassing TCF4 gene cause Pitt-Hopkins syndrome (PHS), an autosomal dominant disorder of severe motor and mental retardation. Regardless of mutation type, haploinsufficiency has been proposed as an underlying mechanism of PHS, but extensive functional analyses to validate this concept are lacking. We have recently shown that human TCF4 gene is transcribed using numerous 5' exons. Here, we re-evaluated the impact of all the published PHS-associated mutations, taking into account the diversity of TCF4 isoforms. In addition, we analysed the effects of a series of reading frame extending and missense mutations on TCF4 stability, subcellular localization and ability to heterodimerize, to bind DNA and to transactivate E-box-controlled transcription.

**Methods:** The functions of wild-type and mutant TCF4 proteins were studied in vitro, in human embryonic kidney cells (HEK293) and in rat primary cortical and hippocampal neurons using western blotting, immunocytochemical staining, quantitative PCR, electrophoretic mobility shift and reporter assays.

**Results:** Our analyses revealed that not all deletions and truncating mutations in TCF4 result in complete loss-of-function and the impact of reading frame elongating and missense mutations ranges from subtle deficiencies to dominant-negative effects. We show that (1) missense mutations in TCF4 bHLH domain and a reading frame extending mutation damage DNA-binding and transactivation ability in a manner dependent on dimer context (homodimer versus heterodimer with ASCL1 or NEUROD2); (2) the reading frame extending mutation and the missense mutation at the dimer interface of the HLH domain destabilize the protein; and (3) missense mutations outside of the bHLH domain cause no major functional deficiencies.

**Discussion:** We conclude that different PHS-associated mutations impair the functions of TCF4 by diverse mechanisms and to a varying extent, possibly contributing to the phenotypic variability of PHS patients. Functionally unequal mutations are found among deletions and truncating mutations as well as missense mutations in TCF4. This provides a starting point for making PHS phenotype–genotype correlations that would not be based on mutation type solely, but would take into account the functional impacts of the mutations.

### Association Study of 60 Candidate Genes with Antipsychotic-induced Weight Gain in Korean Schizophrenia Patients

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**Background:** Schizophrenia patients treated with antipsychotic medication often develop clinically significant weight gain. Inter-individual variability and known genetic contributions to obesity suggest that antipsychotic-induced weight gain (AIWG) may be influenced by genetic variation. This study was designed to investigate the association of hitherto-suggested candidate genes with AIWG.

**Methods:** A total of 211 single nucleotide polymorphisms (SNPs) within 60 candidate genes were genotyped using the Sequenom MassARRAY system in 84 Korean schizophrenia patients treated with risperidone, olanzapine, haloperidol, amisulpride, or quetiapine for up to 8 weeks. BMI changes from baseline across subsequent weeks were analyzed by a linear mixed model for repeated measures. Additionally, we assessed appetite change during risperidone or olanzapine treatment in another 46 schizophrenia patients and tested for association with the genetic variations showing significance in the AIWG analysis.

**Results:** 13 SNPs of GHRL, ADRA2A, GRIN2B, ADRA2B, DRD2, SEC16B, NEGR1 and DRD1 present P value < 0.05. No SNP reached the level of significance after correction for multiple testing. Most significant SNP was rs696217 in GHRL (P = 0.0013). Among them, only rs696217 in GHRL showed nominally significant association with appetite change (P = 0.024). Patients carried T allele of rs696217 in GHRL exhibited lower increase rate of BMI and degree of appetite increase than those carried GG genotype.

**Discussion:** Our findings suggest the involvement of a GHRL (ghrelin gene) polymorphism in weight gain, especially caused by increased appetite, during antipsychotic treatment in schizophrenia patients.

### A Genome-wide Gene Expression Study Suggests New Candidate Genes for Lithium Response in Bipolar Patients of Sardinian Ancestry

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**Background:** Bipolar disorder (BD) is a chronic and severe psychiatric illness affecting 0.7-6% of the general population. Among mood stabilizers, lithium is the mainstay for the treatment of BD, with ~30% of patients showing a complete clinical response and the remaining 70% responding partially or not responding. While a large number of evidence supports a key role for genetic predisposition, the molecular underpinnings of lithium response are still not completely understood. Given these assumptions, we carried out a genome wide gene expression (GWGE) analysis in lymphoblastoid cell lines (LCLs) from 10 BD patients full-responders (FR) and 10 BD patients non-responders (NR) to lithium, with the aim of identifying new molecular determinants of lithium response. In order to investigate whether lithium treatment in vitro differentially influenced gene expression in FR and NR, each cell line was divided into 2 lines: one was treated with LiCl while the other was cultured in regular medium.

**Methods:** BD patients were selected from an existing clinical sample recruited at the Lithium Clinic of the Clinical Psychopharmacology Centre of the University Hospital of Cagliari, Italy. Diagnoses were made according to RDC and DSM-IV criteria using personal semi-structured interviews (SADS-L). Lithium response was evaluated with the retrospective scale described in Grof et al., 2002. LCLs of the selected subjects were thawed from liquid nitrogen and re-grown following standard protocols. After reaching the required cell count, each LCLs was divided into two lines and cultured in medium with or without LiCl 1mM for 7 days. Gene expression was measured with GeneChip Human Gene 1.0 ST Arrays. Statistical analysis were carried out using R and Bioconductor framework. Differentially expressed genes were identified using linear models/ANOVA fitted at gene level using the Limma package. Pathways and Gene Ontology analysis were performed based on the hypergeometric test for over-representation of specific GO/KEGG terms in the selected list of genes compared to the whole set of genes considered. The difference between FR and NR in the effect of LiCl in vitro (interaction) was evaluated by means of two-way repeated measures ANOVA. Genes to be validated by real-time PCR were selected from the list of genes differentially expressed between FR and NR at the basal level using a FDR ≤ 0.05 and a Fold Change (FC) ≥ 1.2 or ≤ 0.83.

**Results:** Endoplasmatic reticulum, inositol metabolism and B-cell receptor signalling were the most significantly over-represented pathways. A total of 3142 genes were differentially expressed between FR and NR. In the interaction analysis 678 genes showed a significantly different effect of LiCl between FR and NR. Among the 3141 genes, the 10 most significantly altered were selected for validation. Five genes were selected from those showing significant interaction effect. The validation experiments will be concluded in one week from the time this abstract is submitted. Among the most significantly altered genes were: PIK3CG (FC = 2.52; p < 1x10<sup>-4</sup>), a kinases responsible for the phosphorylation of phosphoinositides,

HSPA1A (FC = 0.56;  $p = p < 1 \times 10^{-4}$ ), a heat shock protein that stabilizes and repairs defective proteins and IL2RA (FC = 2.47;  $p = 6 \times 10^{-3}$ ), an interleukin receptor involved in neuronal differentiation and survival. The expression of these genes was not influenced by LiCl, as suggested by the similar expression levels in treated and non-treated LCLs. LILRA3 (FC = 1.34 ;  $p < 1 \times 10^{-4}$ ), an immunoreceptor expressed predominantly on B cells, and BIRC3 (FC = 0.78;  $p < 1 \times 10^{-4}$ ), a protein that inhibits apoptosis by binding to tumour necrosis factor receptors, showed the most significant  $p$  values in the interaction analysis, suggesting that LiCl differentially influenced their expression level in FR and NR.

**Discussion:** Our study highlighted a number of genes potentially involved in lithium response. These genes belong to pathways previously suggested to play a role in bipolar disorder or in the mechanism of action of lithium as well as to pathways not previously involved. To the best of our knowledge, ours is the first GWGE study on bipolar patients with different response to lithium treatment. Pending validation with real-time PCR, our data may provide useful and relevant information for a better understanding of the molecular underpinnings of lithium response. To verify our findings, the validation of the selected genes will be also extended to another sample of 6 FR and 6 NR BD patients and the data will be presented at the conference. In conclusion, our study may provide new insights in the pharmacogenomics of lithium response.

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### Differential Expression of MicroRNAs in Cerebrospinal Fluid in Schizophrenia Patients

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**Background:** MicroRNAs are small non-coding RNA molecules that are involved in post-transcriptional regulation of messenger RNAs. Recent studies have started to link alterations in miRNA expression to schizophrenia and other psychiatric disorders. However, most of these studies have used post-mortem brain tissue or whole blood as the source of transcript. By contrast, examination of microRNAs in cerebrospinal fluid (CSF) might provide an in vivo biomarker more directly reflecting functional changes in the brain. For this report, we focused on 9 miRNAs that have been associated with schizophrenia and/or neurodevelopment in prior studies (mir-9, mir-34a, mir-125a, mir-132, mir-137, mir-174, mir-195, mir-328 and mir-346).

**Methods:** Four patients with schizophrenia-spectrum disorders and four healthy volunteers underwent lumbar puncture. For each subject, total RNA was separately extracted from 3-5ml of CSF. Expression of 381 validated microRNAs was assessed from CSF for each of the subjects with the Taqman Human MicroRNA A array (Applied Biosystems), which uses real-time RT-PCR to quantify the number of amplification cycles (Ct) required to reach a given threshold. Mean Ct values were normalized for each sample using mammalian U6 snRNA levels, due to its abundant detection in all CSF samples. Of those 381 miRNAs examined using microarray analysis, 9 were selected for bivariate analysis using t-tests to examine differential expression between schizophrenia patients and healthy controls. Quantitative PCR analyses were conducted to validate findings from some of those miRNAs.

**Results:** A mean of 180.5 (SD=11.2) miRNAs were detected in patients with schizophrenia (male: 100%, mean age: 41, black: 75%) and a mean of 136.5 (SD=11) miRNAs were obtained from CSF in healthy volunteers (male: 100%, mean age: 38.8, black: 75%). Approximately one-third of all expressed microRNAs demonstrated robust levels of expression (Ct<30). 58 (23.4%) of the miRNAs were expressed in one or more CSF samples of patients with schizophrenia but not in CSF samples from healthy controls. Out of the 9 miRNAs selected for this analysis, four miRNAs were significantly down regulated in schizophrenia patients compared to healthy controls: mir-9 ( $p=0.005$ ), mir-125b ( $p=0.01$ ), mir-328 ( $p=0.001$ ) and mir-346 ( $p=0.003$ ). Furthermore, mir-137 was detected in 3 CSF samples from patients but not in healthy controls. However, for this particular miRNA, qPCR analysis did not detect any expression in any of the CSF samples from patients or controls. No significant differences between groups were detected in levels of mir-34a, mir-132, mir-174, and mir-195. Quantitative PCR analyses validated findings for mir-328 ( $p=0.003$ ) but not for mir-9 ( $p=0.09$ ).

**Discussion:** Some miRNAs are differentially expressed in CSF in patients with schizophrenia compared to healthy controls. Therefore, the investigation of these miRNAs may help establish an illness-specific miRNA signature that could help with a better classification and understanding of schizophrenia-spectrum disorders.

### Characterization of Transcriptional and Protein Variations in Major Isoforms of NCAM1, a Pivotal Regulator of Neural Development, in Schizophrenia

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**Background:** Neural Cell Adhesion Molecule 1 (NCAM1), a member of immunoglobulin (Ig) superfamily, is widely expressed in central nervous system. A pivotal regulator of neural development, NCAM1 has a role in neuroblast migration, neurite growth, axonal guidance, fasciculation and synaptic plasticity. Alternative splicing results in three major protein isoforms at 120kDa, 140kDa, and 180kDa. The membrane bound 140kDa and 180kDa isoforms are predominant carriers of polysialic acid and their expression declines postnatally. Whereas, the 120kDa isoform which is membrane-free and characteristic isoform of oligodendrocytes and myelin sheaths, remains polysialic acid free and its expression increases postnatally. Inclusion of a Variable Alternative Spliced Exon (VASE) between exon 7-8 has been reported to repress neurite outgrowth and alter NCAM mediated cell-cell interactions. Addition of polysialic acid chain on 5th Ig like domain results in formation PSA-NCAM (Polysialylated NCAM), which plays a key role in maintaining neural plasticity by increasing intracellular spaces. Various studies have found links between alterations in NCAM1 mRNA and protein levels and schizophrenia. For example, increased NCAM1-180 mRNA expression in BA46, decreased 140/180kDa ratio in BA10, elevated NCAM1-VASE and 120kDa isoforms in cerebrospinal fluid, and reductions in PSA-NCAM in the hippocampus have been reported.

**Methods:** Expression levels of 5 mRNA transcripts were quantified via RT-qPCR (NCAM1-120, NCAM1-140, NCAM1-180, NCAM1+VASE, NCAM1-VASE), using ABI 7900HT RT-PCR system. cDNA was synthesized from RNA extracted from postmortem brain tissues (dorso-lateral pre-frontal cortex) of 37 individuals with schizophrenia and 37 matched controls from Tissue Resource Center (Australian Caucasian cohort). NCAM1 isoform expression was normalized to the geometric mean of four housekeepers (GAPDH, ACTB, TBP, and UBC).

Western blots were performed using protein extracted from the same postmortem brain tissues (described above). Results were analysed using Quantity One 1D software (BioRad). Raw data values of each isoform were normalized to average control values per gel. Data distribution was tested by Kolmogorov-Smirnov test, and then analysed using parametric statistics. T-tests, one-way and factorial ANOVAs and ANCOVAs were performed, followed by post hoc Fisher LSD test. Any correlations between mRNA and protein level of interest and demographic variables [post mortem interval, age, brain pH, RNA integrity, age of onset, anti-depressant history, and antipsychotic (chlorpromazine) use] were also analysed. All analyses were carried out using Statistica Software (v 7.1, Statsoft, USA).

**Results:** An increase in NCAM1-180 mRNA expression was observed in right hemisphere of schizophrenics [ANCOVA  $F(1,63) = 4.43$ ,  $p=0.039$ ; pH and RIN as covariates] compared to right hemisphere of normal controls ( $p=0.005$ ,  $df=63$ ), left hemisphere of schizophrenics ( $p=0.002$ ,  $df=63$ ), and left hemisphere of controls ( $p=0.018$ ,  $df=63$ ). Also, a decrease in ratio of NCAM1-140/180 mRNA was observed in right hemisphere of schizophrenics compared to right hemisphere of controls ( $p=0.039$ ,  $df=64$ ), this result is consistent with an already published finding by another group. In protein quantification studies, an increase of the 120kDa isoform was observed in schizophrenics

compared to controls ( $p=0.04$ ,  $df=72$ ,  $t=2.04$ ). A decrease in PSA-NCAM expression was observed in schizophrenic females [ $F(1,68) = 7.26$ ,  $p=0.008$ ; PMI and pH as covariates] and a correspondent decrease in total polysialylatable form of NCAM (140+180kDa) in schizophrenic females ( $F(1,68)=4.30$ ,  $p=0.041$ ).

**Discussion:** Our data reveals analogy with some previously published results, showing isoform specific alterations in NCAM1 at both the mRNA and protein level in the DLFPC in schizophrenia.

### Common Variants On XQ28 Conferring Risk of Schizophrenia in Han Chinese

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**Background:** Schizophrenia is a highly heritable, severe psychiatric disorder affecting approximately 1% of the world population. A substantial portion of heritability is still unexplained and the pathophysiology of schizophrenia remains to be elucidated. Genetic variants in the X-chromosome have also not been well studied for their association to schizophrenia.

**Methods:** To identify more schizophrenia susceptibility loci in the Han Chinese population, we performed a genome-wide association study (GWAS) and a follow-up study with a total sample size of 1,577 Han Chinese patients with schizophrenia and 3,131 controls. In the follow-up study, we included 384 single nucleotide polymorphisms (SNPs) which were selected from the top hits in our GWAS and from previously implicated loci for schizophrenia based on Schizophrenia Research Forum database (SZGene database), NHGRI GWAS Catalog, CNV studies, GWAS meta-analysis results from the international Psychiatric Genomics Consortium (PGC) and candidate genes from plausible biological pathways.

**Results:** Within the chromosomal region Xq28, SNP rs2269372 in RENBP achieved genome-wide significance with a combined p-value of (OR of allele A = 1.31). SNPs with suggestive p-values were identified within two genes that have been previously implicated in schizophrenia, MECP2 (rs2734647, p<sub>combined</sub> = , OR=1.28; rs2239464, p<sub>combined</sub> = , OR=1.26) and ARHGAP4 (rs2269368, p<sub>combined</sub> = , OR=1.25). In addition, the patient sample in our follow-up study has showed a significantly greater burden for pre-defined risk alleles than the controls. This further supports ultragenic inheritance in schizophrenia.

**Discussion:** Our findings identified a new schizophrenia susceptibility locus on Xq28, which harbor the genes RENBP, MECP2 and ARHGAP4. Our follow-up study, which is characterized by the incorporation of evidence for schizophrenia from other external research resources, gives better insight into SNP prioritization.

### Housing Conditions Modulate the Cognitive Performance in Transgenic Mice Overexpressing the Schizophrenia Susceptibility Gene TCF4

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**Background:** The basic helix-loop-helix (bHLH) transcription factor TCF4 was confirmed in the combined analysis of several large genome-wide association studies (GWAS) as one of the most significant schizophrenia (SZ) susceptibility genes [Stefansson et al., 2009; Li et al., 2012; Steinberg et al., 2011; Ripke et al., 2011]. *TCF4* influences verbal learning and memory in humans [Lennertz et al., 2012] and modulates sensorimotor gating in SZ patients [Quednow et al., 2011]. We showed recently that transgenic mice overexpressing *Tcf4* in forebrain (*Tcf4*tg) display profound deficits in fear memory and sensorimotor gating [Brzózka et al., 2010]. Environmental factors interacting with genetic vulnerability (G x E interactions) play one of the pivotal roles in development of schizophrenia [Myin-Germeys and van Os, 2007] and physical exercise can counteract some symptoms of disease in patients [Pajonk et al., 2010].

**Methods:** We investigated the influence of different environmental conditions on the behavior and cognitive performance of *Tcf4*tg mice by exposing 4 weeks old animals for 4 weeks to single cage housing (SH) or to group housing in enriched environment (EE). In SH, animals were housed under poor conditions without any enrichment. In contrast, EE cages contained two separate compartments: the first with one-way entrance and one-way exit for drinking/feeding, and the second compartment with tubes and a running wheel enabling physical exercise. Animals underwent a profound analysis addressing basic behavior and cognitive performance.

**Results:** Different housing conditions altered anxiety and curiosity behavior of *Tcf4*tg mice in open field and hole board. This contrasts with the phenotype of these mice upon standard group housing [Brzózka et al., 2010]. Housing in EE rescued the cognitive impairment of recent and remote memory in *Tcf4*tg mice assessed in fear conditioning paradigm. Moreover, SH under poor environment worsened the cognitive performance of transgenic animals in water maze. Poor performance of SH *Tcf4*tg mice in the reversal water maze task and in delay matching to place paradigm indicates impairment of flexibility learning which may be of particular relevance for SZ [Crider, 1997].

**Discussion:** In the present study, we demonstrated that SH (isolation stress) increases whereas housing in EE (combination of social support with physical exercise) rescues the cognitive impairment of *Tcf4*tg mice. We provided evidence that the manifestation of the phenotype of *Tcf4*tg mice depends strongly on environmental factors. This substantiates the importance of GxE interactions in the manifestation of schizophrenic phenotypes. The observed phenotype of *Tcf4*tg mice may resemble the situation in patients where symptoms can be elicited by the “second hit” (social stress) [Myin-Germeys and van Os, 2007] and can be ameliorated by social support or physiotherapy [Pajonk et al., 2010].

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