Genetic Analysis of Bipolar Disorder and Alcohol Use Disorder

Shareefa Dalvie M.Sc. (Med)

Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the Division of Human Genetics, Department of Clinical Laboratory Sciences

UNIVERSITY OF CAPE TOWN

March 2015

Supervisors: Prof. Raj Ramesar and Prof. Dan J. Stein

Plagiarism Declaration

This study was performed from 2011-2015 under the supervision of Prof. Raj Ramesar and Prof. Dan Stein.

I hereby declare that this thesis is a presentation of my original research work and where collaborations were involved it has been clearly indicated. It is submitted for the Degree of Doctor of Philosophy (PhD) in Human Genetics at the University of Cape Town (UCT). I have used the *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* convention for citation and referencing.

Name: Shareefa Dalvie

Date: 16 March 2015

Abstract

Background: Mental health disorders represent a major public health problem in most countries around the world. In South Africa, the lifetime prevalence of psychiatric disorders is 30.3%, with substance-use disorders and mood disorders being the second and third most prevalent classes of lifetime disorders, respectively. Bipolar disorder (BD) has a lifetime prevalence of 1.4% and alcohol use disorder (AUD) a lifetime prevalence of 30.3%, and they are frequently comorbid. Both of these disorders have a relatively high heritability, yet the exact genetic basis of each remains unknown. Genetic variants within the hypothalamic-pituitary-adrenal (HPA)-axis and glutamatergic pathways have previously been implicated in both phenotypes. The aim of this project was to investigate the aetiology of BD and AUD, using high-throughput genomic technologies, bioinformatics, brain-imaging and environmental measures. An additional aim was to assess the genetic aetiology of BD-AUD comorbidity.

Methods: For the genetic analysis underlying BD, a South African „Afrikaner" family was investigated. Whole-genome sequencing (WGS) and whole-genome linkage analysis was performed for individuals with BD Type I (BDI) and unaffected family members using the Illumina HiSeq2000 and Affymetrix Axiom^{TM} Genome-wide CEU 1 Array, respectively. For the AUD analysis, two groups were investigated; a South African adolescent group comprising 80 individuals with AUD and 80 controls, and a group of 8123 individuals from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort. The South African group of adolescents were genotyped using the Illumina Infinium iSelect custom 6000 BeadChip, childhood trauma data was obtained and brain magnetic resonance images were collected for a subset of this group. Genotype data on HPA-axis genes were obtained from a previous study for the ALSPAC cohort. The fourth group of individuals investigated in this thesis comprised 233 individuals with BD-AUD comorbidity from the Systemic Treatment Enhancement Program for BD (STEP-BD). Genotype data for genes from the glutamatergic and HPA-axis pathways were obtained from a previous study conducted on these individuals.

Results: The chromosomal regions 6p25, 10p14-10p15.1, 11q23-11q25, and 13q21-22 scored the highest LOD scores for BD and the most over-represented pathway in the affected family members was the *T-cell receptor* signalling pathway. In the South African adolescent group, circadian rhythm genes were associated with AUD and childhood trauma predicted

alcohol use in adolescence. The gene-imaging analysis identified a SNP in the *glutamate receptor, ionotropic, N-methyl D-aspartate 2B* (*GRIN2B*) gene as being associated with brain volume in the left orbitofrontal cortex and posterior cingulate. HPA-axis genes did not show an association with AUD and no significant gene x environment interactions were detected for AUD in the ALSPAC cohort. Single variants in the glutamatergic genes and HPA-axis were not associated with BD-AUD comorbidity. However, from the gene-based analysis, the glutamatergic gene *PRKCI* was associated with BD-AUD comorbidity.

Conclusions: It appears that disruption in immune-related genes may contribute to the development of BD in an Afrikaner family. No significant gene x environment interactions were detected for adolescent AUD. The circadian pathway and childhood trauma may play a role in the development of adolescent AUD. Differential brain volume and BD-AUD comorbidity may be characterised by variation in the glutamatergic pathway. These pathways and the interactions between them should be further investigated in BD and AUD.

Preface

Investigating any single psychiatric disorder is a challenge, as revealed in the literature. In this exploratory study the candidate investigates two distinct but related (and often co-morbid) phenotypes (bipolar disorder and alcohol use disorder), using a range of methodologies. It was hoped that the candidates exposure to these psychiatric phenotypes, and examination of her own data generated through genomic analyses, and access to data from other international studies, provide her with skills, and access to resources for future work in the challenging area of psychiatric genetics/genomics.

This thesis consists of six chapters. Chapter 1 is an introduction, which includes descriptions and the epidemiology of the two psychiatric phenotypes under investigation, namely bipolar disorder and alcohol use disorder. Chapter 2 presents the investigation of the genetic basis of bipolar disorder in an Afrikaner family using whole-genome sequencing and whole-genome linkage analysis. Chapter 3 includes the investigation on alcohol use disorder in a local adolescent group using genetic, environmental and brain imaging measures. Brain imaging and gene-imaging analyses were performed in collaboration with Dr Samantha Brooks from the Department of Psychiatry and Mental Health at UCT. Chapter 4 provides an investigation of alcohol use in a European birth cohort, specifically investigating the HPA-axis. Chapter 5 includes the investigation of the glutamate and HPA-axis pathways in a bipolar disorder group with comorbid alcohol use disorder. Chapter 6 is a concluding chapter for the entire thesis.

The Guest House

This being human is a guest house.
Every morning a new arrival.

A joy, a depression, a meanness,
some momentary awareness comes
as an unexpected visitor.

Welcome and entertain them all!
Even if they're a crowd of sorrows,
who violently sweep your house
empty of its furniture,
still, treat each guest honorably.
He may be clearing you out
for some new delight.

The dark thought, the shame, the malice,
meet them at the door laughing,
and invite them in.

Be grateful for whoever comes,
because each has been sent
as a guide from beyond.

-Rumi

Acknowledgements

Thank you to my Creator, for guiding me through this challenging task.

The completion of this thesis would not have been possible without the contribution of the following individuals:

My supervisors, Prof. Raj Ramesar and Prof. Dan Stein, your mentorship, advice and support have been invaluable. I am very grateful to have had a place on this complex project.

Thank you to all the participants of this study. Without you this research would not have been possible.

Mr Gerrit Botha from CBIO for doing the whole genome sequence analysis.

Dr Morne du Plessis for your immense help with the bioinformatics analysis.

Prof. James Knowles for allowing me the opportunity to visit your lab and generate the whole genome sequences.

The CPGR and Jo McBride for running the Affymetrix array.

Dr Samantha Brooks for assistance with the brain imaging and gene-imaging aspect of the AUD study.

Prof. David Baldwin and EUSARNAD for providing me with the opportunity to visit the University of Bristol. Prof. Glyn Lewis, Dr Sarah Lewis and Mr Andrew Crawford, Assoc. Prof Fazil Aliev and Prof. Danielle Dick for your assistance with the analysis of the ALSPAC dataset.

Dr Chiara Fabbri and Prof. Alessandro Seretti for your help and guidance with the analysis of the STEP-BD dataset.

Prof Karestan Koenen for the funding of the Illumina microarray and advice on statistical analysis.

Sr Gameda Benefeld for your unfailing support.

Assoc/Prof Collet Dandara for your advice and mentorship.

Lindiwe Lamola, Tasneem Salie, Horacia Naidoo, Maryam-Bibi Rumaney, and Amy Roberts for all the laughs and support.

The Division of Human Genetics for having me as part of the family for the past 7years.

I would also like to thank my funders, the University of Cape Town and NRF-DAAD.

Lastly, and most importantly, thank you to my parents, my siblings, nieces and nephews for your unwavering support, patience and encouragement.

Table of Contents

List of Figures

List of Tables

Abbreviations

5-HTT	*Serotonin transporter*
5-HTTLPR	*Serotonin-transporter-linked polymorphic region*
µl	Microliter
µg	Microgram
AA	Alcohol abuse
AC	Adenyl cyclase
ACTH	Adrenocorticotrophin hormone
AD	Alcohol dependence
ADCY2	*Adenylate cyclase 2*
ADE	Affective Disorder Evaluation
ADHD	Attention deficit-hyperactivity disorder
AKAP2	*A kinase (PRKA) anchor protein 2*
AKAP5	*A kinase (PRKA) anchor protein 5*
AKR1C1	*Aldo-keto reductase family 1, member C1*
ALSPAC	Avon Longitudinal Study on Parents and Children
AMPA	α-amino-3-hydroxy-5-methyl-ioxyzole-4-propionic acid
ANK3	*Ankyrin 3*
APOER2	*Apolipoprotein E receptor 2*
ARNTL	*Aryl hydrocarbon receptor nuclear translocator-like*
ASD	Autism spectrum disorder
AUD	Alcohol use disorder
AUDIT	10-item Alcohol Use Disorders Identification Test
AVP	Arginine vasopressin
BAM	Binary alignment map
BBMRI-NL	Biobanking and Biomolecular Research Infrastructure-Netherlands
BDNF	*Brain-derived neurotrophic factor*
BD	Bipolar disorder
BDI	BD type I

BDII	BD type II
BDNOS	BD not otherwise specified
BHLHE41	*Basic helix-loop-helix family, member e41*
BMI	Body mass index
BQSR	Base quality score recalibration
BWA	Burrows-Wheeler aligner
CACNA1C	*Calcium channel, voltage-dependent, L type, alpha 1C subunit*
CACNG2	*Calcium channel, voltage-dependent, gamma subunit 2*
CBIO	Computational Biology Group
CD3	Cluster of differentiation 3
CHB	Han Chinese
CIDI	Composite International Diagnostic Interview
CLOCK	*Clock circadian regulator*
cM	Centimorgan
CNS	Central nervous system
CNV	Copy number variation
COGA	Collaborative Studies on Genetics of Alcoholism
COMT	*Catechol-O-methyltransferase*
CPGR	Centre for Proteomic and Genomic Research
CRF	Corticotropin releasing factor
CRH	Corticotropin-releasing hormone
CRHBP	*Corticotropin-releasing hormone binding protein*
CRHR1	*Corticotropin-releasing hormone receptor 1*
CRHR2	*Corticotropin-releasing hormone receptor 2*
CSF	Cerebrospinal fluid
CTQ	Childhood Trauma Questionnaire
CTQ-EA	CTQ- emotional abuse
CTQ-EN	CTQ- emotional neglect
CTQ-PA	CTQ- physical abuse

CTQ-PN	CTQ- physical neglect
CTQ-SA	CTQ- sexual abuse
CTQ-SF	CTQ-Short Form
CUBIC	Cape Universities Brain Imaging Centre
DA	Dopamine
DALY	Disability-adjusted life year
DGKH	*Diacylglycerol kinase, eta*
DISC1	*Disrupted in schizophrenia 1*
DLPFC	Dorsolateral prefrontal cortex
DNA	Deoxyribonucleic acid
DRD2	*Dopamine receptor D2*
DSM	Diagnostic and Statistical manual of Mental Disorders
DSM-IV	DSM, Fourth Edition
DSM-5	DSM, Fifth Edition
EDTA	Ethylenediaminetetraacetic acid
FARS2	*Phenylalanyl-tRNA synthetase 2, mitochondrial*
FAS	Foetal alcohol syndrome
FGGY	*FGGY carbohydrate kinase domain containing*
FIN	Finnish
FKBP5	*FK506 binding protein 5*
FWE	Family-wise error
GABA	Gamma-aminobutyric acid
GABRA2	*GABA A receptor, alpha 2*
GABRQ	*GABA A receptor, theta*
GAD	Generalised anxiety disorder
GADD45A	*Growth arrest and DNA-damage-inducible, alpha*
GATK	Genome Analysis Toolkit
GBR	British
GO	Gene ontology

GOLGB1	*Golgin B1*
GoNL	Genome of the Netherlands
GR	Glucocorticoid receptor
GRIA2	*Glutamate receptor, ionotropic, AMPA 2*
GRIA3	*Glutamate receptor, ionotropic, AMPA 3*
GRIK1	*Glutamate receptor, ionotropic, kainate 1*
GRIK4	*Glutamate receptor, ionotropic, kainate 4*
GRIN1	*Glutamate receptor, ionotropic, N-methyl D-aspartate 1*
GRIN2A	*Glutamate receptor, ionotropic, N-methyl D-aspartate 2A*
GRIN2B	*Glutamate receptor, ionotropic, N-methyl D-aspartate 2B*
GRIN3A	*Glutamate receptor, ionotropic, N-methyl D-aspartate 3A*
GRM3	*Glutamate receptor, metabotropic 3*
GRM7	*Glutamate receptor, metabotropic 7*
GRM8	*Glutamate receptor, metabotropic 8*
GWAS	Genome-wide association study
HC	Healthy controls
HPA	Hypothalamic-pituitary-adrenal
HREC	Human Research Ethics Committee
HWE	Hardy-Weinberg equilibrium
IBS	Identity by state
IBS	Iberian populations in Spain
Indel	Insertion/deletion
iPSC	Induced pluripotent stem cell
IQUB	*IQ motif and ubiquitin domain containing*
JMJD1C	*Jumonji domain containing 1C*
KCNH7	*Potassium channel, voltage gated eag related subfamily H, member 7*
KO	Knockout
K-SADS-PL	Schedule for Affective Disorders and Schizophrenia for School Aged Children (6–18 years) Lifetime Version
LD	Linkage disequilibrium

LOD	Logarithm of odds
LTP	Long term potentiation
MAF	Minor allele frequency
MAOA	*Monoamine oxidase A*
MAPK	Mitogen-activated protein kinase
MC2R	*Melanocortin 2 receptor*
MDD	Major depressive disorder
MDE	Major depressive episode
MDE-R	MDE-recurrent
MDE-S	MDE-single
MESDC2	*Mesoderm development candidate 2*
mGluR	Metabotropic G-protein coupled receptor
MHC	Major histocompatibility complex
MINI	MINI-International Neuropsychiatric Interview
miRNA	MicroRNA
MMN	Mismatch negativity
MNI	Montreal Neurological Institute
MR	Mineralocorticoid receptor
MRI	Magnetic resonance imaging
NaOH	Sodium hydroxide
NCAM1	*Neural cell adhesion molecule 1*
ncRNA	Non-coding ribonucleic acid
ng	Nanogram
NGS	Next generation sequencing
nM	Nanomolar
NMDA	N-methyl-D-aspartate
NR1D1	*Nuclear receptor subfamily 1, group D, member 1*
NR3C1	*Nuclear receptor subfamily 3, group C, member 1*
NR3C2	*Nuclear receptor subfamily 3, group C, member 2*

NRG1	*Neuregulin 1*
NTM	*Neurotrimin*
NXPE2	*Neurexophilin and PC-esterase domain family, member 2*
ODZ4	*Teneurin transmembrane protein 4*
OFC	Orbitofrontal cortex
OPRM1	*Opioid receptor, mu 1*
OZ-ALC	Australian twin-family study of alcohol use disorder
PCA	Principal component analysis
PCR	Polymerase chain reaction
PER1	*Period circadian clock 1*
PER2	*Period circadian clock 2*
PET	Positron emission tomography
PGC	Psychiatric Genomics Consortium
PI	Phosphoinositide
PKA	Protein kinase A
PKC	Protein kinase C
PLSCR5	*Phospholipid scramblase family, member 5*
POMC	*Proopiomelanocortin*
PRKCE	*Protein kinase C, epsilon*
PRKCI	*Protein kinase C, iota*
PRKCQ	*Protein kinase C, theta*
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus
QC	Quality control
RBC	Red blood cell
ROI	Region of interest
SABP	Schizoaffective bipolar disorder
SAGE	Study of Addiction: Genetics and Environment
SAM	Sequence alignment map

SASH	South African Stress and Health
SCID	Structured Clinical Interview for the DSM-IV
SCN	Suprachiasmatic nucleus
SCZ	Schizophrenia
SDQ	Strength and Difficulties questionnaire
SERPINA6	*Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6*
SFMBT2	*Scm-like with four mbt domains 2*
SNP	Single nucleotide polymorphism
SOD2	*Superoxide dismutase 2, mitochondrial*
STEP-BD	Systematic Treatment Enhancement Program for Bipolar Disorder
SUD	Substance Use Disorders
TCR	T-cell receptor
TDRD3	*Tudor domain containing 3*
TE	Tris-EDTA
TLFB	Timeline Followback
TRANK1	*Tetratricopeptide repeat and ankyrin repeat containing 1*
TSI	Toscani in Italy
UBAP2	*Ubiquitin associated protein 2*
UCN	*Urocortin*
UCN2	*Urocortin 2*
UCN3	*Urocortin 3*
UCT	University of Cape Town
UK	United Kingdom
UNODC	United Nations Office on drugs and crime
USA	United States of America
UTR	Untranslated region
VBM	Voxel-based morphometry
VCF	Variant call format
VQSR	Variant quality score recalibration

VRK2	*Vaccinia related kinase 2*
WES	Whole-exome sequencing
WGS	Whole-genome sequencing
WHO	World Health Organisation
WRNIP1	*Werner helicase interacting protein 1*
WT	Wild-type
YRI	Yoruba
ZNF259	*Zinc finger protein 259*

Chapter 1: Introduction

Background

South Africa has a history steeped in violence and oppression. For this reason, it is thought that this population group may be at higher risk of developing mental disorders [Williams et al., 2008]. Studies have shown the lifetime prevalence of psychiatric disorders is 30.3% in this country, with substance-use disorders (13.3%) and mood disorders (9.8%) being the second and third most prevalent classes of lifetime disorders, respectively [Herman et al., 2009]. It has been projected, that in 2030, depression will be one of the top three burdens of disease [Mathers and Loncar, 2006]. An emphasis needs to be placed on mental health research in South Africa, in order to improve the understanding of the biology and environmental factors contributing towards the development of psychiatric diagnoses which, in turn, may aid in the development of effective treatment and/or management strategies.

In this chapter the psychiatric phenotypes bipolar disorder and alcohol-use disorder, the foci of this study, are described in terms of the diagnostic classification and epidemiology of each disorder.

1.1 Epidemiology of Psychiatric Disorders: Worldwide and in South Africa

Mental health disorders represent a major public health problem in most countries around the world [Kessler et al., 2009]. In 2007, a study was conducted based on a cross-national epidemiological survey (Composite International Diagnostic Interview- CIDI) on 17 participating countries including South Africa, Belgium, Colombia, France, Germany, Israel, Italy, Japan, Lebanon, Mexico, Netherlands, New Zealand, Nigeria, China, Spain, Ukraine, and the United States of America (USA), to determine the age of onset, projected lifetime risk (an estimation of the proportion of the population group who will have the investigated disorder by the end of their life) and lifetime prevalence (an estimation of the proportion of the population group who have been diagnosed with the investigated disorder by the time of interview) of mental health disorders [Kessler et al., 2007]. For this survey, data on psychiatric illnesses for South Africa was obtained from the South African Stress and Health (SASH) study which is the largest population-based mental health epidemiological study conducted in South Africa, consisting of 4351 adults of all ethnic groups [Stein et al., 2008; Herman et al., 2009].

The results of the survey indicated that the lifetime prevalence for having one or more of the investigated classes of disorders varied considerably between countries. For example, the USA and Nigeria had a lifetime prevalence of 47.4% and 12.0%, respectively, for having one or more of the disorders. South Africa lies roughly in-between the two above-mentioned countries, with a reported lifetime prevalence of 30.3% for any mental disorder (investigated in the study). Anxiety disorders were the most prevalent diagnoses in ten of the 17 countries (including South Africa) and impulse control disorders were the least prevalent. Unlike lifetime prevalence estimates, the distributions of age of onset were similar across the surveyed countries. Impulse control disorders had the lowest age of onset for most countries, and mood disorders had a similar age of onset (i.e. 29-43 years) as generalised anxiety disorder (GAD), panic disorder and post-traumatic stress disorder (PTSD). South Africa has a projected lifetime risk of 47.5% for any disorder, as of the age of 75 years. Thus, nearly half of the South African population will develop a mental disorder in their lifetime. Countries with the highest rates of sectarian violence e.g. Israel, Nigeria and South Africa; had the highest risk-to-prevalence ratios of any mental disorder [Kessler et al., 2007].

Mental health disorders place a considerable burden on the economy due to direct costs such as hospitalisation and treatment, and indirect costs such as loss of income due to unemployment [Insel, 2008]. In South Africa, it has been estimated that the annual loss in income due to depression and anxiety disorders for individuals affected with these conditions, is approximately $3.6 billion [Lund et al., 2013]. This data highlights the importance of mental health research in South Africa to improve the understanding of the biology and environmental factors contributing towards the development of psychiatric diagnoses which, in turn, may aid in the development of effective management strategies, thus reducing the burden of disease.

1.2 Classes of Psychiatric Diagnoses

One of the most prominent classification systems in clinical and research practice is the Diagnostic and Statistical Manual of Mental Disorders (DSM) which is published by the American Psychiatric Association [American Psychiatric Association. Task Force on DSM-IV, 1994], the most recent being the fifth edition (i.e. DSM-5), which was released in May, 2013. For the purpose of this thesis, all psychiatric diagnoses will be based on the DSM-IV classification. The DSM-IV is a tool for psychiatrists to assess their patients based on a multiaxial classification system which consists of five axes [American Psychiatric Association. Task Force on DSM-IV, 1994]:

Axis I- Clinical Disorders: used for reporting on all conditions except Personality Disorders and Mental Retardation

Axis II: Personality Disorders and Mental Retardation

Axis III: General medical conditions which may be useful or relevant in the treatment of the individuals' mental illness

Axis IV: Psychosocial and environmental issues which could be useful in the understanding, treatment and prognosis of the individual with the mental illness

Axis V: Global functioning assessed by the clinician

1.3 Mood Disorders

Mood disorders are classified as Axis I disorders. In South Africa, mood disorders have a lifetime prevalence of 9.8% [Stein et al., 2008]. The development of effective therapies for mood disorders has been hampered by the lack of understanding of the pathophysiology of the disease and the mechanism by which current treatment strategies work [Sanacora et al., 2008]. One of the most severe and prevalent mood disorders is bipolar disorder (BD).

1.3.1 Bipolar Disorder– A Historical Perspective

The concept of BD was reportedly first recognised by the French Psychiatrist, Jean-Pierre Falret in 1851 who termed this disorder as "*folie circulaire*" or "circular madness' [Sedler, 1983]. He observed that this disorder was characterised by episodes of mania followed by melancholia with "symptom-free" periods in between the two mood states. Three years later, in 1854, another French psychiatrist, Jules Baillarger, described BD as "*folie à double forme*" or "dual form insanity'. In the 1890s, the renowned German psychiatrist Emil Kraeplin, described these symptoms as a "double attack" [Angst and Sellaro, 2000]. Today, the diagnosis of BD is based on the classification by the DSM-IV [American Psychiatric Association. Task Force on DSM-IV, 1994] criteria, or, the more recently revised DSM-5.

Based on different symptomatic criteria, there are several types of BD distinguishable in the population: i) BD type I (BDI), ii) BD type II (BDII), iii) Cyclothymia, and iv) "BD not otherwise specified" (BDNOS) [American Psychiatric Association. Task Force on DSM-IV, 1994]. The diagnostic criteria for BDI, the most severe type, are described below in greater detail.

1.3.2 Diagnostic Classification of BDI

According to the DSM-IV, BDI is a recurrent mood disorder, characterized by the presence of one or more manic or mixed episodes (see Table 1 for diagnostic criteria of each mood episode). Manic episodes are typified by a period of abnormally elevated (euphoric) or irritable mood, feelings of grandiosity, reduced need for sleep and increased participation in pleasurable activities. The occurrence of one or more major depressive episodes (MDE) may also be a feature. Depressive episodes, as the name suggests, are characterised by depressed mood and a lack of interest in pleasurable activities. The mania associated with BDI should not be due to a Substance-Induced Mood Disorder or Schizoaffective Disorder. During severe manic episodes, violent or abusive behaviour may be experienced. On average, untreated BDI patients experience four episodes each decade [American Psychiatric Association. Task Force on DSM-IV, 1994]. There are no differences in BDI prevalence across gender and ethnicity. However, it has been noted that females have a later onset of mania and have more depressive episodes than males [Robb et al., 1998]. Most individuals return to an improved or functional state between episodes [American Psychiatric Association. Task Force on DSM-IV, 1994].

1.3.1.2 Epidemiology

BD is estimated to have a lifetime prevalence of 1.4% [Merikangas et al., 2011]. The lifetime prevalence of BDI and BDII are estimated at 0.6%-1% and 0.4%, respectively [Merikangas et al., 2007; Merikangas et al., 2011]. BDI affects males and females at equal frequencies and disease onset is usually during early adulthood [Miller, 2006]. Approximately 25% of those affected with BDI have attempted suicide [Merikangas et al., 2011].

BD is associated with significant social impairment, such that affected individuals have greater difficulty in sustaining interpersonal relationships and have trouble maintaining employment compared to those without mood disorders: a total of 54% of affected individuals were dismissed from their jobs compared to 29% of a matched unaffected group [Calabrese et al., 2003]. Studies have also showed that individuals suffering from the disorder have difficulty abiding by the law and they have a significantly higher chance of being arrested for crimes [Calabrese et al., 2003; Daff and Thomas, 2014]. Violent behaviour is often observed during manic episodes [American Psychiatric Association. Task Force on DSM-IV, 1994]. In 1991, the total economic burden of BD in the USA was estimated at US$45 billion. This cost is attributed to medication, hospitalisation, misdiagnosis, and the estimated financial burden due to productivity loss [Hirschfeld and Vornik, 2005]. A more

recent study conducted in the United Kingdom (UK) estimated the total cost to the National Health Service, attributable to BD, at GB£342 million [Young et al., 2011].

BD is often accompanied by various other comorbidities. For example, approximately 65% of patients with BD have comorbidities such as an anxiety, substance abuse/use or eating disorder. Individuals with comorbidity usually have an earlier age of onset and a more severe phenotype [McElroy et al., 2001]. Yasseen et al. (2009) reported that 25% of BD patients also suffered alcohol abuse as a comorbidity. The United Nations Office on Drugs and Crime (UNODC) reported that an individual diagnosed with a mental disorder is three times more likely to be dependent on an illicit substance [http://www.unodc.org/unodc/en/data-and-analysis/WDR-2010.html, UNODC World Drug Report, 2010]. Large epidemiological studies in the USA have shown that, compared to other Axis I psychiatric disorders, BD had the highest association with alcohol and substance use [Salloum and Thase, 2000]. This may suggest common aetiological pathways and genes underlying BD and substance abuse or dependence.

1.4 Substance-Related Disorders

Substance-related disorders are classified as Axis I disorders. Substance use and abuse is a major public health problem worldwide and in South Africa. The lifetime prevalence of substance use disorders (SUD) is 13.4% (the second most prevalent class of disorders), and the median age of onset is 24 years in South Africa [Stein et al., 2008]. In 2010, mental disorders and SUDs accounted for approximately 7.4% of all disability-adjusted life years (DALYs) for the top ten causes of total disease burden, worldwide, and are the primary cause of years lived with disability. The DALYs differed by age whereby adolescents and young adults had the largest percentage of DALYs. Alcohol-use disorder (AUD) accounted for approximately 9.6% of the DALYs attributable to the class ‗mental disorders and SUDs' [Whiteford et al., 2013].

The World Health Organisation (WHO) states that alcohol is a ―psychoactive substance" with ―dependence–producing properties" which has been extensively used across many cultures for centuries [http://www.who.int/substance_abuse/publications/global_alcohol_report/en/]. Since the arrival of European settlers in South Africa, alcohol has played a major and often controversial role in the history of the country. Alcohol was the method of payment for cattle and labour from indigenous populations [Parry, 2005].

The Western Cape region has the highest reported prevalence of an overall community population of foetal alcohol syndrome (FAS) in the world [May et al., 2000]. Alcohol is the most abused substance at most speciality treatment centres across South Africa [Plüddemann et al., 2008]. Therefore, it is of great interest to investigate the intrinsic factors which place some individuals at greater odds of developing alcoholism than others, in a highly ~~"~~at risk" environment.

1.4.1.1 Diagnostic Classification of Alcohol Use Disorder

According to DSM-IV, AUD consists of alcohol abuse (AA) and alcohol dependence (AD). AUD is defined as the presence of three or more of the symptoms outlined in Table 2, in a 12 month period. Abuse is defined as the continued use of a substance resulting in adverse consequences. However, the diagnosis of _abuse' differs from _dependence' in that it does not include the criterion of _tolerance' (criterion 1), _withdrawal' (criterion 2) and _compulsive usage' (criterion 3) of the substance (DSM-IV).

1.4.1.2 Epidemiology

Alcoholic beverages are consumed by individuals worldwide. The psychoactive ingredient of alcohol is ethanol which has the ability to induce feelings of euphoria and relaxation. The effect of alcohol is characterised by a biphasic pattern whereby the initial ~~"~~positive effects" (euphoria and relaxation, etc.) are followed by hangover, exhaustion, depression and sometimes vomiting and loss of consciousness [Oscar-Berman and Marinković, 2007]. The WHO reported, in 2012, 3.3 million deaths (5.9%) per year globally, were as a result of the harmful effects of alcohol [http://www.who.int/substance_abuse/en/].

The lifetime prevalence of any AUD is approximately 30.3% [Hasin et al., 2007]. The WHO reports that the 12 month prevalence of AUD in South Africa in 2010 was 10% in males, 1.5% in females and 5.6% in both sexes [http://www.who.int/substance_abuse/publications/global_alcohol_report/en/]. AA has been shown to be one of the most prevalent mental disorders in South Africa with an annual prevalence of 4.5% [Williams et al., 2008] and lifetime prevalence of 11.4% [Herman et al., 2009]. AD has a lifetime prevalence of 2.6% across all age groups [Herman et al., 2009].

Table 1: Diagnostic criteria for mood episodes characteristic of BD [American Psychiatric Association. Task Force on DSM-IV, 1994]

Episode	DSM-IV Criteria
Major Depressive Episode (MDE)	a) Five or more of the following symptoms • Depressed or irritable (children and adolescents) mood • Decreased interest or pleasure in almost all activities • Significant changes in body weight • Insomnia or hypersomnia • Psychomotor agitation • Fatigue and decrease in energy • Feelings of worthlessness and excessive guilt • Diminished concentration and increased indecisiveness • Increased thoughts of death and suicidal ideation b) Symptoms do not meet criteria for mixed episode c) Symptoms cause functional impairment d) Symptoms not a result of physiological effect of substance e) Symptoms not as a result of bereavement and are present for longer than two months
Manic Episode	a) Period of abnormally elevated or irritable mood lasting at least one week b) Three or more of the following symptoms are present to a significant degree • Inflated self-esteem or grandiosity • Decreased need for sleep • Talkative • Racing thoughts • Easily distractible • Increased goal-directed activity or psychomotor agitation • Excessive participation in pleasurable activities with potential for painful consequences c) Symptoms do not meet criteria for mixed episode d) Symptoms cause functional impairment e) Symptoms not a result of physiological effect of substance
Mixed Episode	a) Criteria met for both Major Depressive Episode and Manic Episode nearly every day during a one week period b) Symptoms cause functional impairment c) Symptoms not a result of physiological effect of substance
Hypomanic Episode	a) Period of abnormally elevated or irritable mood lasting at least four days b) Three or more of the following symptoms are present to a significant degree • Inflated self-esteem or grandiosity • Decreased need for sleep • Talkative • Racing thoughts • Easily distractible • Increased goal-directed activity or psychomotor agitation • Excessive participation in pleasurable activities with potential for painful consequences c) Change in functioning d) Disturbance in mood observable by others e) Episode not severe enough to caused marked functional impairment f) Symptoms not a result of physiological effect of substance

Table 2: DSM-IV criteria for AUD diagnosis [American Psychiatric Association. Task Force on DSM-IV, 1994]

Criterion	DSM-IV Definition
1	**Tolerance:** The need for increasing volumes of the substance to reach intoxication or significant decrease of intoxication with continued use of the same volume
2a	**Withdrawal:** A maladaptive change in behaviour with physiological and cognitive features as a result of a decrease in tissue or blood concentrations of the substance, after extended and heavy usage of the substance
2b	When the individual maintains substance use to alleviate the symptoms of withdrawal
3	Compulsively taking the substance at high volumes or for a prolonged period of time
4	The individual expresses an intention to decrease or discontinue substance usage often with unsuccessful results
5	The individual spends a great portion of his/her time obtaining, using and recovering from the effects of the substance
6	The individual‘s daily routine revolves around the use of the substance, often resulting in not participating in important occupational, social or recreational activities
7	The individual continues to use the substance even though he/she is aware of the detrimental effects the substance has on the individuals mental and physiological health

In the Western Cape, the majority of the population is of mixed ancestry (ethnicity) and it is estimated that in this group, 3618 per 10 000 individuals have been treated at substance abuse centres. In comparison, the Black African, Caucasian, and Indian groups have 100, 504, and 1 per 10 000 individuals treated for substance abuse, respectively. In terms of the proportion of individuals treated for AA, 48% are of mixed ancestry (ethnicity), 45% are Caucasian, 7% are Black African and less than 1% are Asian or Indian [Plüddemann et al., 2008]. AD has been estimated to be the most expensive psychiatric disorders with costs exceeding US $225 x 10^9 per year [Sullivan et al., 2012]. According to the WHO, globally, the total number of deaths and DALYs due to alcohol-related diseases and injury are 5.1% [http://www.who.int/mediacentre/factsheets/fs349/en/]. In comparison, in South Africa, the total number of deaths and DALYs due to alcohol harm was estimated at 7.1% and 7.0%, respectively [Schneider et al., 2007].

1.4.1.3 Adolescent Alcohol Use Disorder

Adolescence is defined as the transitional period from childhood to adulthood, approximately between the ages of 12-18 years. During this developmental stage, exploration and experimentation is at its peak [Phillips, 2008] and increases in social and novelty-seeking/risk-taking behaviours are often observed [Spear, 2000] as well as an increased

occurrence of psychiatric diagnoses [Paus et al., 2008]. During this critical period of development, alcohol is one of the most used substances which in turn is associated with an increased likelihood of nicotine and drug use, academic failure, delinquency and pregnancy [Saraceno et al., 2009]. Studies have shown that alcohol use in adolescence is a predictor of AD in adulthood [Grant and Dawson, 1997; Grant et al., 2006]. An earlier age of onset (mean age = 18.7 years) for AD is associated with more severe symptoms, comorbidity and family history of AD, compared to individuals with a later age of onset (mean age= 31.9 years) [Chen et al., 2011].

A study involving high school learners from three sites in South Africa (Johannesburg, Durban and Cape Town) found that alcohol was the most abused substance, with cannabis being the most abused illicit substance [Parry et al., 2004]. Another study found that lifetime and current alcohol use was 57.8% and 37.7%, respectively, amongst female adolescents in the Western Cape [Phillips, 2008]. Alcohol usage has a detrimental effect on normal brain development during this time which may result in negative effects on cognition and emotional behaviour [Squeglia et al., 2009].

Typically, adolescents who abuse alcohol are more likely to be addicted to illicit substances in adulthood [Rohde et al., 2001]. Therefore, they are an ideal group to investigate in terms of alcohol use as they are the ideal target group for the development of preventative strategies [Zimmermann et al., 2007].

1.5 Overlap of Psychiatric Diagnoses

Most psychiatric disorders are found to cluster in families and have a relatively high heritability [Kendler, 2013]. In this study, the genetic aetiologies of two psychiatric phenotypes were investigated. It remains unknown to what extent the genetic aetiology is unique for a particular disorder or is shared with other psychiatric diagnoses, particularly since diagnoses are classified based on clinical symptoms and not pathophysiology (as this is still largely unknown) [Kendler, 2013]. A recent study by the Cross Disorder Group of the Psychiatric Genomics Consortium (PGC) investigated the genetic aetiology, using genome-wide SNP data, of the following psychiatric disorders: autism spectrum disorder (ASD), attention deficit-hyperactivity disorder (ADHD), BD, major depressive disorder (MDD) and schizophrenia (SCZ). The genetic correlation was high between SCZ and BD and moderate between SCZ and MDD; BD and MDD; ADHD and MDD [Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013a]. The same group also found that four genetic

polymorphisms shared an association across all five of the above-mentioned disorders. Two of the four polymorphisms are found in genes encoding subunits of L-type voltage-gated calcium channels [Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013b]. These studies suggest that there is considerable overlap in the genetic aetiologies (and therefore the underlying pathophysiology) of various psychiatric disorders.

In psychiatry, comorbidity between diagnoses is often observed [Merikangas et al., 1998a]. In an epidemiological study conducted in the USA, more than 40% of 12 month DSM-IV diagnoses had comorbidity, with comorbidity associated with disorder severity [Kessler et al., 2005]. Another study found that 37% of individuals with an AUD had mental disorder comorbidity [Regier et al., 1990]. Psychiatric comorbidity is defined as ~~"th~~he presence of a concurrent psychiatric syndrome in addition to the principal diagnosis…". Comorbidity is common in mental health disorders particularly in psychotic and major affective disorders. Much of the costs for treatment in mental health is attributed to comorbidity as comorbidity complicates diagnoses and hence treatment [Hirschfeld and Vornik, 2005].

1.5.1 Overlap in BD and AUD

BD has a high rate of comorbidity (65%) with other Axis I disorders [McElroy et al., 2001]. It has been established that BD and AUD are strongly comorbid [Hsieh et al., 2012; Schoepf and Heun, 2014]. Approximately half of patients diagnosed with BD have an AUD, and BD-affected individuals are three times more likely to have an AUD than someone from the general population [Hirschfeld and Vornik, 2005; Oquendo et al., 2010]. Individuals affected with both disorders have a more severe outcome, characterised by an earlier age of onset, overall poorer functioning and increased suicide attempts [Cardoso et al., 2008; Oquendo et al., 2010]. It has also been shown that there are gender differences in the prevalence of BD-AUD comorbidity, with BD-affected men more likely to have AUD comorbidity than females [Frye et al., 2003; Cardoso et al., 2008]. However, the direction of causality is as yet unknown. It has also been shown that individuals with a family history of a mood or AUD have three-fold higher prevalence of comorbidity than individuals with no family history of mental illness [Mantere et al., 2012]. It is also known that both of these psychiatric disorders have a high heritability [McGuffin et al., 2003; Epps and Holt, 2011]. Due to the substantial genetic component for both BD and AUD; and the high rate of comorbidity between the two disorders, it could be postulated that both BD and AUDs have common causative genetic variants. It has recently been proposed that investigating comorbid subtypes of BD, such as BPD-AUD, may be easier as these sub-phenotypes may be more genetically homogenous

[Kerner et al., 2011; Hu et al., 2013]. Indeed, BD with comorbid AUD has been shown to be a strong familial sub-phenotype [Saunders et al., 2008].

1.6 Project Aims and Objectives

The aim of this project, in its entirety, was to investigate the aetiology of two psychiatric phenotypes, namely, BD and AUD using bioinformatics, high-throughput genomic technologies, brain-imaging and environmental measures. An additional aim was to assess the genetic aetiology of BD-AUD comorbidity.

1.6.1 Objectives

The objectives of this entire study were as follows:

1) The genetic aetiology of BD was investigated using whole-genome sequencing and whole-genome linkage analysis in an Afrikaner family.

2) The aetiology of AUD was investigated by integrating high-throughput genotyping data with brain-imaging and environmental measures in a South African adolescent group and in a European birth cohort.

3) The genetic aetiology of BD-AUD comorbidity was assessed by investigating the glutamatergic pathway and hypothalamic-pituitary-adrenal (HPA)-axis using publically-available genetic data on a well-established BD cohort.

Chapter 2: An Investigation of the Genetics Underlying Bipolar Disorder

Abstract

Family, adoption and twin studies strongly suggest that BD has a genetic component, with heritability estimates as high as 85%. The aim of this study was to identify BD susceptibility variants using whole-genome technologies in a large Afrikaner family affected with BD. Whole-genome sequencing was performed on four related individuals with BDI from a large Afrikaner family, using the Illumina HiSeq2000^{TM} Sequencer. Additional family members were genotyped using the Axiom^{TM} Genome-wide CEU 1 Array. Quality control of sequencing data was performed using FastQC, mapping to the reference sequence using the Burrows Wheeler Aligner program and post-alignment processing and variant calling was performed using SAMtools and the Genome Analysis Toolkit. Whole-genome linkage analysis was performed using the program Superlink Online SNP 1.1. The chromosomal regions 6p25, 10p14-10p15.1, 11q23-11q25, and 13q21-22 showed the highest LOD scores, and these genomic regions containing immune–related genes and pathways were the most over-represented in BD. These findings need to be validated in other Afrikaner families (as well as other BD cohorts) and the exact disease-causing variants identified. Whole-genome sequencing data may contribute to understanding which genetic variants underlie complex disorders such as BD.

2.1 Introduction

BD is a severe psychiatric illness, characterised by extremes in mood with affected individuals experiencing episodes of mania and depression. The understanding of the aetiology of this devastating disorder has been hampered by clinical and genetic heterogeneity. With the advancement of next generation sequencing (NGS) technologies, it is anticipated that details of the underlying pathophysiology of BD will become clearer.

2.1.1 Genetic Basis of BD

Family, adoption and twin studies strongly suggest that BD has a genetic component [Smoller and Finn, 2003]. First degree relatives and half-siblings of an affected individual are at a higher risk of developing the disorder [Müller-Oerlinghausen et al., 2002; Lichtenstein et al., 2009]. Heritability estimates (a measure of the phenotypic variance attributable to the additive genetic variance) from twin studies have been as high as 85% [McGuffin et al.,

2003]. A recent study showed that the offspring of individuals with BD also have a higher rate of other mood (~35%) and anxiety (~43%) disorders [Vandeleur et al., 2012].

Despite the high heritability of BD, the underlying genetic aetiology is still unknown. Linkage analysis, candidate gene association and genome-wide association studies (GWAS) have been conducted for BD in various populations, the details of which are outlined below. However, it is evident from the results of these past studies, due to the lack of a replicable genetic signal, that it is unlikely that just a single gene or single variant will confer a predisposition towards developing BD. The onset of BD is likely to be determined by common variants of small effect within numerous genes [Purcell et al., 2009], possibly interacting with the environment [Uher, 2014].

2.1.1.1 Linkage Studies

Linkage analysis is used to identify chromosomal regions which contain disease genes. This technique measures the recombination fraction between genetic markers and is based on the observation that genes which are physically close to each other co-segregate after meiosis. The measure of linkage is the logarithm of odds (LOD) score which is defined as the logarithm of the odds that the loci are linked divided by the odds that the loci are unlinked [Pulst, 1999]. Once the chromosomal region has been identified, fine mapping is used to identify the disease-causing gene/s.

Regions on almost every chromosome have been found to be linked to BD i.e. the strongest evidence of linkage for BD has been shown for the following chromosomal regions: 1p34-p36 [Schumacher et al., 2005]; 3p14 [Etain et al., 2006]; 4q31-q32 [Ekholm et al., 2003; Schumacher et al., 2005]; 6q [Dick et al., 2003; Middleton et al., 2004; Pato et al., 2004; Schumacher et al., 2005]; 8q24 [Cichon et al., 2001]; 10p12 [Cheng et al., 2006]; 10q25 [Cheng et al., 2006]; 12q24.3 [Ewald et al., 2002]; 13q32 [Detera-Wadleigh et al., 1999]; 16q24 [Cheng et al., 2006]; 16p13 [Cheng et al., 2006]; 16p12 [Ekholm et al., 2003; Cheng et al., 2006]; 17q [Dick et al., 2003]; 18p [Stine et al., 1995]; 20p11.2 [Radhakrishna et al., 2001]; 22q13 [Kelsoe et al., 2001]. There has not been much consistency in terms of the top regions identified across studies, which could possibly be due to false positive and negative findings. False negatives occur because of under-powered studies [Segurado et al., 2003] and 5% of all reported significant linkage findings are likely false positives [Lander and Kruglyak, 1995]. In an attempt to overcome this, meta-analyses have been used to identify the most significant regions from a group of similar linkage studies [Segurado et al., 2003].

Several meta-analyses of whole-genome linkage scans for BD have been conducted and have shown the most significant regions of the genome to be 6q [McQueen et al., 2005], 8q [McQueen et al., 2005], 13q [Badner and Gershon, 2002] and 22q [Badner and Gershon, 2002]. Segurado et al. (2003) found nominal significance in the regions 9p and 18p-q. Again, there is not much consistency across these findings suggesting that alternate approaches to linkage be utilised or to combine linkage analysis with other techniques and technologies, such as NGS.

2.1.1.2 Candidate Gene and Genome-Wide Association Studies

The candidate gene approach investigates genes which have been reported to be linked to disease in previous research, or focuses on loci based on their purported role in the biology of the disorder. Numerous candidate gene association studies have been conducted for BD, however as with many other psychiatric disorders, results from these studies have been inconclusive [Sullivan et al., 2012]. Most association signals have not been able to be replicated and much of the missing heritability is still unexplained. The greatest disadvantage of association studies is that they are highly influenced by confounding factors such as ethnicity, which may result in findings of spurious significance [Risch, 2000].

More recently, instead of candidate gene analysis, researchers have adopted more high-throughput approaches such as GWASs. Candidate gene based association studies are more cost-effective than GWAS but require a hypothesis regarding the relationship of the gene to disease [Hirschhorn and Daly, 2005]. GWAS is an *a priori* association method which utilises high-throughput genotyping methods, on very large cohorts of subjects/patients and controls, to determine whether genetic variants, such as single nucleotide polymorphisms (SNPs) spread across the genome, have an association with disease status compared to equally large numbers of controls [Hirschhorn and Daly, 2005].

The first GWAS for any disorder was published in 2005 and since then, as many as 1000 associations have been identified for approximately 200 genetic traits [Olfson and Bierut, 2012]. Initially, GWASs have had limited success in identifying causative loci for psychiatric disorders. However, with considerably large increases in sample size, replicable signals have been identified. For example, a recent SCZ GWAS consisting of approximately 13 000 cases and 18 000 controls (with a replication sample of 7 413 cases; 19 762 controls and 581 parent-offspring trios), found 22 loci with genome-wide significance [Ripke et al., 2013]. Another such study for SCZ, conducted on 36 989 cases and 113 075 controls, found 108 loci

which met genome-wide significance, 83 of which were novel loci. A large proportion of these significant genes are expressed in tissues involved in the immune system [Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014].

To date, approximately 38 GWASs, with a minimum of 100 000 SNPs assayed, have been conducted for BD [http://www.genome.gov/gwastudies/]. Results from each of these studies have been variable, with few of the findings being replicable. Most BD GWAS studies were unable to find genome-wide significant hits, but instead report on findings below threshold that have biological plausibility. Most GWASs have been conducted on Caucasian and European population groups. Several BD GWASs comprise individuals from large consortia such as the PGC. The aim of the PGC, which was started in 2007, was to conduct meta-analyses for psychiatric GWASs. This consortium, which is one of the biggest in psychiatry, consists of more than 80 institutions from 25 countries. The main focus of the PGC is to investigate the genetics underlying ASD, ADHD, BD, MDD and SCZ [https://pgc.unc.edu/index.php].

Some of the many interesting findings from BD GWAS over the last several years include *ankyrin* 3 (*ANK3*); *calcium channel, voltage-dependent, L type, alpha 1C subunit* (*CACNA1C*), and *diacylglycerol kinase, eta* (*DGKH*) [Sklar et al., 2008; Ferreira et al., 2008; Liu et al., 2011b; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013b; Mühleisen et al., 2014; Ruderfer et al., 2014]. A list of the top findings of each of the 38 BD GWASs is listed in Table 3 (several of these GWASs have overlapping datasets). The largest BD GWAS to date, which was a meta-analysis conducted exclusively on BD, investigated 2.3 million SNPs in 9 747 BD affected individuals and 14 278 controls. This study identified two new loci associated with BD, adenylate cyclase 2 (*ADCY2*) located at 5p15.31 and the region 6q16.1, and was able to replicate previous associations found in the genes *ANK3*, *odd Oz/ten-m homolog 4* (*ODZ4)* and *tetratricopeptide repeat and ankyrin repeat containing 1* (*TRANK1*) [Mühleisen et al., 2014].

As results from linkage and association studies have been inconclusive regarding the BD phenotype, recent studies have focused on stratifying affected individuals by comorbidity [Kerner et al., 2011], symptom dimensions [Labbe et al., 2012], or clinical subtypes [Greenwood et al., 2013]. A recent study has shown a strong association between BD

symptom dimension of ~~–n~~egative mood delusions" and the chromosomal region 3q26.1 [Meier et al., 2012].

Table 3: List of GWASs conducted for BD

Study Title	Reference	Phenotype	Original Sample Size	Ethnicity/ Ancestry	Genotyping Platform	Genome-wide Significant/Top Findings
Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls	[Stratton, 2007]	BD	2000 cases, 3000 controls	Caucasian	Affymetrix GeneChip 500K	16p12
A genome-wide association study implicates *diacylglycerol kinase eta* (*DGKH*) and several other genes in the etiology of bipolar disorder	[Baum et al., 2008]	BDI	1233 cases, 1439 controls	Caucasian	Illumina HumanHap550	*DGKH* (13q14.11)
Collaborative genome-wide association analysis supports a role for *ANK3* and *CACNA1C* in bipolar disorder[1]	[Ferreira et al., 2008]	BDI, BDII, SABP*	4387 cases, 6209 controls	Caucasian	Affymetrix GeneChip 500K	*ANK3* (10q21) *CACNA1C* (12p13.3)
Whole-genome association study of bipolar disorder	[Sklar et al., 2008]	BDI	1461 cases, 2008 controls	Caucasian	Affymetrix GeneChip 500K	*MYO5B* (18q21) *TSPAN8* (12q14.1)
Preliminary genome-wide association study of bipolar disorder in the Japanese population	[Hattori et al., 2009]	BDI	107 cases, 107 controls	Japanese	Affymetrix GeneChip 500K	*DEC1* (9q32), *IRF2*(4q34.1), *VEPH1*(3q24), *EXTL1*(1p36.1), *PCDH15* (10q21.1), *MYT1L* (2p25.3), *CSMD1*(8p23.2), *DYNC1I1* (7q21.3), *PCSK5* (9q21.3), *OLFM3* (1p22), *PLXNA2* (1q32.2), *KCNMB2* (3q26.2), *INTS9* (8p21.1), *AUTS2* (3q25), *NUP107*(12q15)

A genomewide association study of response to lithium for prevention of recurrence in bipolar disorder	[Perlis et al., 2009]	BDI, BDII-subset treated with lithium	1177 cases	Caucasian	Affymetrix GeneChip 500K	rs10795189 (10p15)
Genome-wide association and meta-analysis of bipolar disorder in individuals of European ancestry[1]	[Scott et al., 2009]	BDI	2076 cases, 1676 controls	Caucasian	Illumina HumanHap550	1p31.1, 3p21 (*ITIH1, GNL3 NEK4, ITIH3*), 5q15(*MCTP1*),
Genome-wide association study of bipolar disorder in European American and African American individuals[1]	[Smith et al., 2009]	BDI, SABP BDI, SABP	1001 cases, 1033 controls 345 cases, 670 controls	Caucasian African American	Affymetrix SNP 6.0	Xq27.1, *NAP5* *DPY19L3*, *NTRK2*
Singleton deletions throughout the genome increase risk of bipolar disorder	[Zhang et al., 2008]	BDI, SABP	1001 cases, 1033 controls	Caucasian	Affymetrix SNP 6.0	Singleton deletions increased in BD
A genome-wide association study of bipolar disorder in Norwegian individuals, followed by replication in Icelandic sample	[Djurovic et al., 2010]	BD	194 cases, 336 controls	Caucasian	Affymetrix SNP 6.0	*DLEU2, GUCY1B2, PKIA, CCL2, CNTNAP5, DPP10,* and *FBN1*
A genome-wide meta-analysis identifies novel loci associated with schizophrenia and bipolar disorder[1]	[Wang et al., 2010a]	BD, SCZ	653 cases, 1034 controls	Caucasian	Affymetrix SNP 6.0	rs11789399 (9q33.1)
Genome-wide Association Study Identifies Genetic Variation in Neurocan as a Susceptibility Factor for Bipolar Disorder[1]	[Cichon et al., 2011]	BD	682 cases, 1300 controls	European	Illumina HumanHap550v3	*NCAN*- rs1064395

Case-case genome wide association analysis reveals markers differentially associated with schizophrenia and bipolar disorder and implicates calcium channel genes	[Curtis et al., 2011]	BD, SCZ	506 cases, 523 SCZ, 505 controls	Caucasian	Affymetrix GeneChip 500K, Affymetrix SNP 5.0	*CACNG5*-rs17645023
Propensity Score-Based Nonparametric Test Revealing Genetic Variants Underlying Bipolar Disorder	[Jiang and Zhang, 2011]	BD	1998 cases, 3004 controls	Caucasian	Affymetrix GeneChip 500K	rs420259, rs9378249, rs12938916
Genome-Wide Association Study in Bipolar Patients Stratified by Co-Morbidity	[Kerner et al., 2011]	BD with comorbidity	1000 cases, 1034 controls	European	Affymetrix SNP 6.0	rs1039002 near *PDE10A* (6q27)
Genome-wide association study of bipolar I disorder in the Han Chinese population	[Lee et al., 2011]	BD	1000 cases, 1000 controls	Hans Chinese	Illumina HumanHap550-Duo	Near to *ST8SIA2*- rs2709736, rs8040009, *KCTD12*- rs2073831, *CACNB2*- rs11013860
Meta-Analysis of Genome-Wide Association Data of Bipolar Disorder and Major Depressive Disorder[1]	[Liu et al., 2011b]	BD, MDD	4387 cases, 6209 controls	Caucasian	Affymetrix GeneChip 500K	*CACNA1C*-rs1006737, rs7297582
Genome-wide association analysis of age at onset and psychotic symptoms in bipolar disorder[1]	[Belmonte Mahon et al., 2011]	BD with psychosis	2836 cases, 2744 controls	Caucasian	Affymetrix SNP 6.0, Illumina HumanHap550	-

Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4[1]	[Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011]	BD	7481 cases, 9250 controls	European-American	Affymetrix 500K, Affymetrix 5.0, Affymetrix 6.0 and Illumina HumanHap 500	*CACNA1C, ODZ4*
Genome-Wide Association of Bipolar Disorder Suggests an Enrichment of Replicable Associations in Regions near Genes	[Smith et al., 2011a]	BD	2191 cases, 1434 controls	European	Affymetrix SNP 6.0	Near *CACNA2D*- rs2367911
Genome-wide association study on bipolar disorder in the Bulgarian population	[Yosifova et al., 2011]	BD	188 cases, 376 controls	European	Illumina HumanHap550	*GRIK5*-rs8099939, *PARD6B*-rs6122972 *CTSH*- rs2289700
Genome-wide association study in a Swedish population yields support for greater CNV and MHC involvement in schizophrenia compared with bipolar disorder[1]	[Bergen et al., 2012]	BD, SCZ	2343 cases, 2093 controls	European	Affymetrix SNP 6.0, Affymetrix SNP 5.0	MHC region for SCZ
Genome-wide association of mood-incongruent psychotic bipolar disorder[1]	[Goes et al., 2012]	BD with psychotic features	2196 cases, 8148 controls	European	Affymetrix SNP 6.0, Affymetrix SNP 5.0	*PRSS35/SNAP91*-rs1171113 (6q14.2), *TRANK/LBA1*- rs9834970 (3p22.2), *NUMB* -rs2333194 (14q24.2)
Genome-wide Association Study of Temperament in Bipolar Disorder Reveals Significant Associations To Three Novel Loci	[Greenwood et al., 2012]	BD with temperament score	1263 cases, 1434 controls	European	Affymetrix SNP 6.0	*DTL* (1q32.3)- rs17018426

Genome-wide significant association between a „negative mood delusions' dimension in bipolar disorder and genetic variation on chromosome 3q26.1	[Meier et al., 2012]	BD with clinical symptom dimensions	927 cases, 2168 controls	European	Illumina HumanHap550v3, Illumina Human610-Quad, Illumina Human660Quad	rs9875793 (3q26.1)
A genome-wide association study of attempted suicide[1]	[Willour et al., 2012]	BD with suicide attempters	1201 cases, 1497 controls	Caucasian, European	Affymetrix SNP 6.0, Illumina HumanHap550	rs300774 (2p25)
Genome-wide association study meta-analysis of European and Asian-ancestry samples identifies three novel loci associated with bipolar disorder[1]	[Chen et al., 2013a]	BD and schizoaffective disorder	6658 cases, 8187 controls	European, Asian	Affymetrix SNP 6.0, Illumina HumanHap550	near *TRANK1*-rs9834970
Genome-Wide Association Study of Irritable vs. Elated Mania Suggests Genetic Differences between Clinical Subtypes of Bipolar Disorder	[Greenwood et al., 2013]	BD with clinical subtypes	2191 cases, 1434 controls	European	Affymetrix SNP 6.0	33 SNPs (13q31)
A genome-wide association study of seasonal pattern mania identifies NF1A as a possible susceptibility gene for bipolar disorder	[Lee et al., 2013]	BD with seasonal patterned mania	1001 cases, 1033 controls	Caucasian	Affymetrix SNP 6.0	*NF1A*- rs41350144

Genetic Predictors of Risk and Resilience in Psychiatric Disorders: A Cross-Disorder Genome-wide Association Study of Functional Impairment in Major Depressive Disorder, Bipolar Disorder, and Schizophrenia	[McGrath et al., 2013]	Functional impairment in BD	765 cases	European American	Affymetrix GeneChip Human Mapping 500K, Affymetrix SNP 5.0	*ADAMTS16*
GWAS meta-analysis identifies TSNARE1 as a novel Schizophrenia / Bipolar susceptibility locus[1]	[Sleiman et al., 2013]	BD, SCZ, SABP	13394 cases, 34676 controls	Mixed Ancestry	Illumina 1MDuoV3, Illumina HumanHap550 or 610 Quad	TSNARE1- chr8q24.3
Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis[1]	[Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013b]	BD	6990 cases, 4820 controls	European	Multiple platforms	3p21, 10q24, *CACNA1C* and *CACNB2*
Cross-disorder genomewide analysis of schizophrenia, bipolar disorder, and depression[1]	[Huang et al., 2014]	BDI, BDII	1575 cases, 1204 controls	Caucasian	Affymetrix GeneChip 500K	*ADM*-rs6484218
Identification of novel loci for bipolar I disorder in a multi-stage genome-wide association study	[Kuo et al., 2014]	BD	200 cases, 200 controls	Taiwanese	Illumina HumanOmni1-Quad	rs7619173
Genome-wide association study reveals two new risk loci for bipolar disorder[1]	[Mühleisen et al., 2014]	BD	9747 cases, 14278 controls	Caucasian	Illumina Infinium Human660W-Quad, HumanOmni1-Quad, Human610-Quad, HumanHap550, Human660Q-Quad, HumanHap300	*ANK3, ODZ4, TRANK1, ADCY2*

Genetic Relationships Between Suicide Attempts, Suicidal Ideation and Major Psychiatric Disorders: A Genome-Wide Association and Polygenic Scoring Study[1]	[Mullins et al., 2014]	Depression and BD with suicide attempt	426 cases, 2844 controls	European	Illumina Human-Hap550-Quad, Illumina Human610-Quad	-
Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia[1]	[Ruderfer et al., 2014]	BD and SCZ	19779 cases, 19423 controls	Caucasian	Affymetrix 500K, Perlegen 164K, Affymetrix 5.0, Affymetrix 6.0, Illumina 550K, Illumina Human 610-Quad, Illumina 300K, Illumina 650	*CACNA1C*, *IFI44L*, *MHC*, *TRANK1*, *MAD1L1*, *PIK3C2A*
Genome-wide association study of bipolar disorder accounting for effect of body mass index identifies a new risk allele in TCF7L2	[Winham et al., 2014]	BD with BMI	388 cases, 1020 controls	European American	Affymetrix SNP 6.0	*TCF7L2*- rs12772424
Genome-wide association study of bipolar disorder in Canadian and UK populations corroborates disease loci including SYNE1 and CSMD1	[Xu et al., 2014]	BD	950 cases, 950 controls	Caucasian	Illumina-Sentrix Human Hap550 BeadChip, Affymetrix 5.0	*SYNE1*, *PPP2R2C, ZNF659, CNTNAP5, CDH13*

[1] Indicates a meta-analysis. BD- bipolar disorder, BDI- bipolar disorder type I, BDII- bipolar disorder type II, BMI- body mass index, MDD- major depressive disorder, SABP- schizoaffective bipolar disorder, SCZ- schizophrenia

2.1.2 Next Generation Sequencing Technologies and Complex Disorders

Since its discovery in 1977 [Sanger et al., 1977], Sanger sequencing, also known as *first generation* sequencing, has been the gold-standard in deoxyribonucleic acid (DNA) sequencing [Tsiatis et al., 2010]. The automation of Sanger sequencing led to the completion of the sequenced human reference genome [Metzker, 2010] at a cost of approximately $1 billion [Bentley, 2006]. However, over the years a demand arose for faster and cheaper sequencing methods which ushered in the era of *second generation* or NGS technologies. Most NGS platforms amplify isolated DNA and have removed the bacterial cloning step characteristic of traditional Sanger sequencing [von Bubnoff, 2008]. Since 2005, the use of this technological advancement has become widespread and offers an approximately four times reduction in cost compared to traditional Sanger sequencing [Bamshad et al., 2011]. NGS technologies have been relatively successful in identifying rare causal variants in ‗simple' Mendelian disorders [Ku et al., 2011]. In addition, NGS ―success" stories include the rapid diagnosis of genetic diseases (approximately 50 hours) in neonates [Saunders et al., 2012], and the use of whole genome sequencing (WGS) has allowed the identification of a locus at chromosomal region 8q24 as having an association with prostate cancer in individuals of Icelandic origin [Gudmundsson et al., 2012].

In terms of the applicability of NGS in psychiatric disorders, using whole-exome sequencing (WES) (includes only coding regions of the genome), several *de novo* mutations in sporadic cases of SCZ were detected. Non-synonymous, protein altering mutations were more common in SCZ cases than controls [Xu et al., 2011]. Similarly, the use of WES of individuals with ASD have identified *de novo* and inherited mutations of large effect size [Sanders et al., 2012; Yu et al., 2013]. To date, only a handful of studies have interrogated the genetic aetiology of BD using NGS. One of these studies employed WES in a Caucasian familial cohort with BD, to determine whether rare variants predispose individuals to having a good response to lithium, a BD sub-phenotype. The most notable finding was that a missense variant in the gene *zinc finger protein 259* (*ZNF259*) segregated completely with affected family members in one of the families [Cruceanu et al., 2013]. Another WES study which examined BD with comorbid anxiety spectrum disorder in a Caucasian family found that rare heterozygous variants in the genes *IQ motif and ubiquitin domain containing* (*IQUB*), *jumonji domain containing 1C* (*JMJD1C*), *growth arrest and DNA-damage-inducible, alpha* (*GADD45A*), *golgin B1* (*GOLGB1*), *phospholipid scramblase family, member 5* (*PLSCR5*), *vaccinia related kinase 2* (*VRK2*), *mesoderm development candidate 2*

(*MESDC2*), and *FGGY carbohydrate kinase domain containing* (FGGY) were exclusively in the affected family members but not in the unaffected members or 200 additional controls. These genes all play a role in various intracellular-signalling pathways important for neuronal and synaptic plasticity, cognition, and response to chronic stress [Kerner et al., 2013]. The mitogen-activated protein kinase (MAPK) signalling pathway was associated with BD in a separate WES study [Chen et al., 2013b]. A WES study conducted on Old Order Amish families found that a missense variant (c.1181G>A) in the *potassium channel, voltage gated eag related subfamily H, member 7* (*KCNH7*) gene had an association with BD (Strauss et al., 2014). Another study investigating Old Order Amish families combined WGS data with whole-genome linkage analysis and family-based association analysis in a group of 18 parent-child trios [Georgi et al., 2014]. This study showed several linkage peaks, which included chromosomal regions 7q21 and 18p11. However, the combination of the results of the three types of analyses in the affected families was not able to identify convergent risk loci or pathways for BD. Another WGS study was conducted on 99 individuals affected with BD in a European population group. Variants within the *ANK3* and *CACNA1C* genes with significantly different allele frequencies between cases and 1000 Genomes Project data were validated in a separate BD case-control cohort. From this study, the most significant SNPs identified were *CACNA1C* rs79398153 and *ANK3* rs139972937 [Fiorentino et al., 2014]. It can be noted that there is not much overlap between each of the above-mentioned NGS studies. However, since the studies were generally conducted on families, it is possible that the identified variants or regions were family or population specific. Therefore, in terms of NGS analysis, family-based study designs with large pedigrees may be ideal in detecting possible candidate genes for BD in specific population groups.

Although NGS offers many interesting and exciting avenues of exploration for researchers, there are still several challenges associated with the use of this technology. One of these challenges is the generation of short sequence reads which is a characteristic of several platforms including Illumina. Short sequence reads complicate sequence assembly and mapping to a reference sequence particularly in ‚repeat-rich' regions. Another challenge is that certain areas of the genome are more difficult to sequence (for e.g. GC-rich regions) which results in low coverage in those particular regions. Each of the NGS platforms has an associated error rate which presents another challenge in the data analysis [Hui, 2014]. Besides the technical difficulties related to sequencing, the bioinformatics analysis poses another hurdle in NGS projects. One of the many challenges of NGS technologies is the

identification of novel disease-causing genes or variants from the many thousands of benign variants and sequencing errors [Bamshad et al., 2011]. One method of controlling for this is by the use of *discrete filtering*. In most instances the "novelty" of a gene or variant is assessed by filtering against databases of known variants such as dbSNP and 1000 Genomes Project or unaffected members of the population. This method is based on the premise that the disease-causing variant/s are not present in the "filter set" [Bamshad et al., 2011].

2.1.2.1 Illumina NGS Platform

Illumina manufactures one of the most used NGS platforms [Metzker, 2010]. The first step in sequencing on most NGS platforms is library preparation. Genomic DNA is randomly fragmented and ligated to common adapters [Shendure and Ji, 2008]. Most sequencing technologies are incapable of detecting single fluorescent molecules, therefore all fragments of interest need to be amplified before sequencing can occur. The Illumina WGS platform makes use of a solid phase amplification system known as bridge amplification or "clustering". This is achieved by the amplification of target DNA by forward and reverse primers covalently attached to a glass slide. The single molecule target DNA is firstly primed and extended, thereafter bridge amplification occurs, resulting in approximately 1000 copies of a single DNA molecule [Shendure and Ji, 2008; Metzker, 2010].

2.1.3 Aims and Objectives

The aim of this investigation was to identify susceptibility genes and variants for BD in a large Afrikaner family.

2.1.3.1 Objectives

The objectives of this investigation were as follows:

1) To identify novel BD variants and genes present in BDI affected family members using WGS.

2) To filter the variants and genes identified using whole-genome linkage analysis.

3) To identify over-represented pathways in BDI.

2.2 Materials and Methods

2.2.1 Research Group

The research group consisted of members of an Afrikaner family who were affected with BDI and their unaffected family members. Additionally, publically-available genetic data for the Dutch background population were used as controls. Details regarding each of these groups are outlined below.

2.2.1.1 Family 30

The BD research project, based in the Division of Human Genetics at the University of Cape Town (UCT) was first granted institutional ethics approval on the 30th of May 1996. The focus was on investigating the molecular basis of BD. To date, a total of 919 individuals from 221 families have been recruited from all over South Africa. These individuals are of Caucasian (British, Afrikaner, Portuguese, Greek, and Ashkenazi Jewish), mixed ancestry, Indian and African ethnicity. Each individual has provided blood or saliva samples for DNA studies and undergone numerous neuropsychological tests, including the Structured Clinical Interview for the DSM-IV (SCID) [First et al., 1996]. As the name suggests, SCID is a semi-structured questionnaire which allows for the diagnosis of DSM-IV axis disorders. The most prevalent diagnoses present in the archive includes mood disorders (BDI, BDII, MDE) (with BDI being the primary diagnosis), borderline personality disorder, PTSD and AA. For the purposes of this study, only one family from the BD genetics repository was investigated.

Family 30 is one of the largest families in the BD research project, consisting of 74 individuals. A subset of this family tree (consisting of two family branches) can be found in Appendix 1 (Family tree). A total of five individuals have been diagnosed with BDI, one individual with BDNOS, 17 individuals with recurrent MDE, and 11 with single episode MDE. The rest of the family members do not have a psychiatric diagnosis. This family is relatively well characterised and of Afrikaner ancestry. The Afrikaner population group of South Africa are considered to be genetically distinct from other groups in the country. This population group are mainly the descendants of Dutch settlers (approximately 1000-2000) who arrived in the Western Cape Province from 1652, onwards. European immigration to South Africa continued in the 17th Century by the French, and in the 18th and 19th centuries by the Dutch, German and British [Botha and Beighton, 1983]. Subsequently, the Afrikaners moved inland where they formed and maintained geographically isolated communities [Karayiorgou et al., 2004]. Today, the Afrikaner population group consists of approximately

three million individuals, and due to language barriers, lack of immigration, and endogamy in the earlier generations, have remained relatively genetically isolated [Abecasis et al., 2004]. Afrikaner families have a high prevalence of rare Mendelian disorders such as porphyria variegata, Fanconi anaemia and Huntington Disorder [Greeff, 2007] and have been shown to have conserved haplotypes around disease-causing genes, suggesting strong genetic _founder' effects. Due to these factors, individuals of Afrikaner descent have often been described as ideal candidates for molecular studies [Abecasis et al., 2004].

For the purposes of this study, for which updated ethical approval was obtained (UCT Human Research Ethics Committee (HREC) reference number 023/2012), five individuals with BDI and 15 family members without a BDI diagnosis were investigated. One of the BDI affected family members had committed suicide prior to blood collection; however permission was obtained for genetic analysis from the surviving family members.

2.2.1.2 Control Group

The Genome of the Netherlands (GoNL) forms part of the Biobanking and Biomolecular Research Infrastructure-Netherlands (BBMRI-NL) which is a national network of biobanks in the Netherlands and a node of the bigger European biobanking initiative. The aim of the GoNL was to characterise DNA sequence variation in a Dutch population group by the use of WGS. The group consisted of 250 family trios which were sequenced with the Illumina HiSeq2000 at approximately 13x coverage [Boomsma et al., 2014; Genome of the Netherlands Consortium, 2014]. The summary counts of the SNVs and small indels (<20bp) for all chromosomes (release 5) for the parents (n=498) of the GoNL was accessed on the 22nd of August 2014 [https://molgenis26.target.rug.nl/downloads/gonl_public/variants/release5/]. Variants from this dataset were called using the Genome Analysis Toolkit (GATK) *UnifiedGenotyper* v1.4, using the data from all individuals together.

2.2.2 Genetic Analysis

Blood samples were obtained from members of Family 30 with the appropriate informed consent (see Appendix 1- Example of Patient Consent Form) and DNA was isolated using the Gentra Puregene^{TM} DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). The protocol involved the following steps: the lysis of red blood cells (RBCs) and nucleated cells, protein precipitation, DNA precipitation, recovery, and rehydration. Following rehydration in DNA hydration solution, the isolated DNA was stored at either -20°C (for short-term storage)

or -80°C (for long term storage). A more detailed protocol is included in Appendix 1 (DNA Isolation Protocol). Integrity of the DNA was verified by spectrophotometric quantification using the Nanodrop 1000 spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE, USA) and 1% agarose gel electrophoresis (Appendix 1-DNA Integrity Check). All DNA samples were deemed to be of sufficient concentration and integrity for downstream genetic analysis.

2.2.2.1 Whole-Genome Sequencing

WGS for four of the BDI affected members in Family 30 was carried out at the University of Southern California using the Illumina HiSeq2000 (San Diego, California, USA) system. This technology is based on a proprietary reversible terminator-based method which is able to detect single nucleotides as they are incorporated into growing DNA strands. The WGS protocol consisted of the following steps: i) library creation; ii) clustering; and iii) sequencing. Briefly, these steps are described below and a more detailed protocol can be found in Appendix 1 (WGS Protocol).

2.2.2.1.1 Library Creation

A total of 1 microgram (µg) of genomic DNA in Tris-ethylenediaminetetraacetic acid (EDTA) (TE) buffer was used for the library creation. DNA was randomly sheared with the Covaris Focused Ultrasonicator (Woburn, Massachusetts, USA) yielding double-stranded fragments of approximately 300-400bp in length, with 3‘ and 5‘ overhangs. This process occurred at a temperature between 3-4°C for a duration of 40 seconds. The Covaris Focused Ultrasonicator is able to shear DNA by the process of Adaptive Focused Acoustics™. This process generates bursts of high frequency ultrasonic acoustic energy which is able to be focused on a sample immersed in a water bath [http://covarisinc.com/technology/how-it-works/]. End repair was performed using the Illumina End Repair mix (Illumina, Inc., San Diego, California, USA) which removes 3‘ and 5‘ overhangs to create fragments with blunt ends. The blunt-ended fragments were then cleaned with the Agencourt AMPure XP polymerase chain reaction (PCR) purification system (Beckman Coulter Genomics, Danvers, Massachusetts, USA). The 3‘ ends of the fragments were then adenylated with a single adenine nucleotide to prevent the fragments from binding to one another at the adapter ligation step. The adenine nucleotide binds to the complementary thymine nucleotide found on the 3‘ end of the multiple-indexing adapter which allows for fragment-adapter binding. The adapter-bound fragments were then cleaned with the Agencourt AMPure XP PCR purification system (Beckman Coulter Genomics, Danvers, Massachusetts, USA). The

fragments were then further purified on an Invitrogen™ E-Gel® (Thermo Fisher Scientific, Waltham, Massachusetts, USA), which allows for the removal of un-ligated adapters, adapters bound to one another, and to size select fragments of the appropriate length for clustering. The adapter-bound fragments of the correct size were then selectively amplified by PCR to increase the amount of DNA. This PCR contained primers which specifically annealed to the adapters. The PCR products were then cleaned with the Agencourt AMPure XP PCR purification system (Beckman Coulter Genomics, Danvers, Massachusetts, USA). The concentration, size, and molar concentration of the purified PCR products were then determined by spectrophotometric quantification using the Nanodrop 1000 spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE, USA) and the Agilent 2100 Bioanalyser (Santa Clara, California, USA). The purpose of this process was to ensure high quality DNA libraries. All of the samples were at the required concentrations and fragment size. For cluster generation a starting molar concentration of 10 nanomolar (nM) was required.

2.2.2.1.2 Clustering

The aim of this step was to clonally amplify the fragments by a process of bridge amplification, resulting in millions of clusters. This was achieved by the hybridisation of the DNA fragments to oligonucleotides attached on a flow cell, which are complementary to the adapter sequences. Firstly, the fragments were denatured using 2N Sodium hydroxide (NaOH) and incubated at room temperature for five minutes. A serial dilution was performed and the PhiX viral control genome was added to each tube. The samples were then placed on the Illumina cBot (San Diego, California, USA) where cluster generation occurred.

2.2.2.1.3 Sequencing

The paired-end sequencing reaction was performed on the Illumina HiSeq 2000 which is based on the sequencing-by-synthesis chemistry. This entails the use of four reversible terminator nucleotides, each with a different, cleavable, fluorescent dye and chemically blocked hydroxyl group. Primers were hybridised to the amplified, covalently bound DNA templates on the flow cell and the labelled nucleotides and DNA polymerase were washed over the flow cell. Thereafter, chain elongation occurs whereby the fluorescently-labelled nucleotides compete for incorporation into the growing DNA strand. Once a complementary nucleotide was incorporated, chain termination occurred. The incorporated fluorescent nucleotide was excited by a laser, displaying a characteristic fluorescence which was detected and recorded in an imaging step by a charge-coupled device. The fluorescent dye was then cleaved and the 3‘ hydroxyl block chemically reversed, allowing chain elongation to

continue. This process was repeated several times, the result of which were a series of images which were interpreted by the HiSeq control system and Realtime Analyser software [Mardis, 2008; Liu et al., 2012]. As each reaction contained all four fluorescently-labelled terminator-bound nucleotides, there was no incorporation bias.

2.2.2.2 Microarray Genotyping

A total of 18 family members (three with BDI and 15 unaffected) from Family 30 were genotyped using the Axiom™ Genome-wide CEU 1 Array Plate (Affymetrix, Santa Clara, California, USA) at the Centre for Proteomic and Genomic Research (CPGR) (Cape Town, South Africa). This array consists of 587 353 markers which cover both common and rare variants specifically designed for European populations. These variants were selected from the dbSNP [http://www.ncbi.nlm.nih.gov/SNP/], 1000 Genomes Project [1000 Genomes Project Consortium, 2012] and the Axiom Genomic databases [http://www.affymetrix.com/support/technical/sample_data/axiom_db/axiomdb_data.affx].

A total of 50 nanograms (ng) of DNA sample was used in the assay. Each sample was processed in accordance with the Affymetrix Axiom™ protocol. The target preparation included the following workflow: DNA amplification, fragmentation and precipitation, sample drying, re-suspension and quality control (QC). QC was performed using a Nanodrop spectrophotometer and gel electrophoresis with a 4% gel. All samples met the QC criteria for hybridization to the array which required a total sample yield of greater than 1000µg and DNA fragments between 25 and 125bp. The fragmented DNA sample was loaded onto the GeneTitan® Multi-Channel instrument for denaturation, hybridisation to the array, ligation, staining and scanning.

Following scanning, the output files generated by Affymetrix GeneChip® Command Console® were analysed with the Affymetrix® Genotyping Console version 4.2.0.26. All the samples met the default QC thresholds, which were a dish QC (sample quality metric) greater than or equal to 0.82 and a call rate greater than 97%.

2.2.3 Statistical and Bioinformatic Analyses

2.2.3.1 Bioinformatic Analysis of WGS Data

In order to derive useful information from the WGS data, the appropriate bioinformatic analysis was performed by the Computational Biology (CBIO) Group (UCT). Computations

were performed using facilities provided by UCT's ICTS High Performance Computing team [http://hpc.uct.ac.za]. Outlined below is the workflow and programs used in the analysis:

2.2.3.1.1 Quality Control

The raw sequence reads were obtained from the sequencer in _fastq' format. The files were divided in terms of lane number or run number (each sample comprised 5 runs), direction (forward and reverse), and read number (each lane and direction file consisted of 26 or 27 reads). Therefore, each individual sample consisted of a minimum of 260 raw sequence files. The program FastQC [http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/] was used to ensure that the quality of the raw sequencing data was sufficient for downstream analysis. This program allows for the visual inspection of the read quality and statistics. Included in the output are the following:

- Basic statistics: contains details about the file, the Illumina system used, the total and filtered number of sequences, the sequence length and percentage GC content
- Per base sequence quality: quality score for each of the bases
- Per sequence quality scores: a plot of the quality scores for each of the sequences
- Per base sequence content: base content across all sequences
- Per base GC content: percentage GC content across all bases
- Sequence GC content: plot of percentage GC content across all sequences
- Per base N content: percentage N content across all bases
- Sequence length distribution: distribution of sequence length across all sequences
- Sequence duplication levels: percentage duplicate compared to unique sequences
- Over-represented sequences: presence of over-represented sequences
- K-mer content: relative enrichment of K-mers across read length

2.2.3.1.2 Alignment to Reference Genome

The Burrows-Wheeler Aligner (BWA) (bwa- 0.5.9) [Li and Durbin, 2009] tool was used to align the short sequence reads to the reference human genome sequence (hg19 assembly). The BWA-backtrack algorithm was implemented as this algorithm is able to align relatively short sequence reads to a long reference sequence. Alignments were run on each read for each sample and subsequently combined, creating a sequence alignment map (SAM) file.

2.2.3.1.3 Post-Alignment Processing and Variant Calling

SAMtools [Li et al., 2009], Picard [http://broadinstitute.github.io/picard/] and GATK [McKenna et al., 2010] were used for post-alignment processing and for variant calling. Post-processing includes the sorting and indexing of the binary alignment map (BAM) file.

Firstly, the combined aligned reads were sorted using SAMtools (samtools-0.1.17), which created a binary SAM file called a BAM file. Statistics for the sorted BAM file were obtained, duplicates were removed, and the BAM file was indexed. Picard (picard-tools-1.51) is a java–based program with command line functionality allowing for the manipulation of SAM and BAM files. This program was used to obtain various metrics regarding the aligned sequence, such as an alignment summary and quality score distribution. Qualimap [Garcia-Alcalde et al., 2012] was used to determine the sequence coverage, percentage GC content, percentage of the reads which were successfully mapped and the read length. Indel realignment was performed using GATK (gatk v1.2). Insertion/deletion (indel) realignment consists of two steps, firstly the identification of target intervals, and secondly, the actual realignment process using the *IndelRealigner* utility in GATK. To ensure that all mate-pair information was correct across reads, the *FixMateInformation* utility was used in Picard (picard-tools-1.51).

At this point of the data analysis, the quality scores of each of the bases sequenced were recalibrated from the aligned BAM file using GATK. This process is performed since the quality scores assigned by various sequencers are often inaccurate and biased. Thus the base quality score recalibration (BQSR) process assigns quality scores based on the actual probability that the base mismatches the reference genome. Also, this process is able to improve the variation in quality with machine cycle and sequence context. This is achieved by examining the covariation of the base based on the following features [http://www.broadinstitute.org/gatk/guide/article?id=44]:

i) Reported quality score

ii) Position within the read

iii) Preceding and current nucleotide

In order to call variants from the aligned sequence, the *UnifiedGenotyper* in GATK was used. Once the variants were called, variant quality score recalibration (VQSR) was performed. This process allows for the assignment of well calibrated probability scores to each of the

variants (known and novel) in a variant call list [http://www.broadinstitute.org/gatk/guide/article?id=39]. It is achieved by the development of a continuous, covarying estimate of the relationship between SNP call annotations and the probability that a variant is truly a variant as opposed to a sequencing or data analysis error. This is a two-step process whereby the following two options are used: *VariantRecalibrator* and *ApplyRecalibration*. The former generates a recalibration file based on a Gaussian mixture model which assesses the annotation values over a high quality subset of the input call set and then evaluates all input variants. This model is based on data from ~~"k~~known sites" or ~~"t~~rue sites training resource" such as HapMap 3.3 [Gibbs et al., 2003], dbSNP 132 and the Omni 2.5 SNP human microarray. The HapMap resource is a SNP call set with a very high degree of confidence assigned to each of the variants. GATK will select the variants in this resource as representative of true sites and will use these variants to train the recalibration model [http://www.broadinstitute.org/gatk/guide/article?id=1259]. The result of this is a variant call format (VCF) file which is a text file containing a list of the variants called. Thereafter, the variant list was filtered to only include variants with a high quality and high coverage.

2.2.3.1.4 Variant Annotation

The latest version of ANNOVAR [Wang et al., 2010b] was used to annotate the variant list. Variants were assigned to their respective genes and were classified according to their respective locations (intronic, exonic, intergenic, upstream, downstream, 3' untranslated region (UTR), 5' UTR, etc.). The hg19 sequence build was used as a reference and the refGene database [Pruitt et al., 2005] was used to assign genes.

2.2.3.2 Whole-Genome Linkage Analysis

Linkage analysis is commonly used to identify rare susceptibility variants, for a particular disorder, in an affected family. In this study, a narrow definition of the phenotype was used whereby only individuals with BDI were considered affected. Any individuals with a psychiatric diagnosis other than BDI were coded as unaffected. Genotype data was available for 587 352 variants (SNPs and insertions/deletions (indels)). Using the Affymetrix® Genotyping Console™, the variants were filtered by including only those variants with a 95% call rate, a minor allele frequency (MAF) of greater than 0.1, and any variants on the X-chromosome were excluded. All of the short indels and any variants without a dbSNP identifier were removed. After filtering, the variant list consisted of 293 047 SNPs. A two-point linkage analysis was carried out using the online program Superlink Online SNP 1.1

[Silberstein et al., 2013]. Chromosomes which obtained LOD scores greater than the genome-wide maximum LOD, score as calculated by the program were filtered by 2 centimorgan (cM) to reduce linkage disequilibrium (LD) [Smith et al., 2011b]. These chromosomes were also ‗deaned', which is a process of removing erroneous markers, Mendelian errors, genotyping errors and uninformative markers. The two-point linkage analysis was now repeated on these regions of interest with the penetrance set at 0.8 and the disease allele frequency at 0.01 [Cheng et al., 2006]. As the genetic model of BD is unknown, the LOD scores were calculated for a recessive and dominant model. The base pair positions of these chromosomal locations were identified using NCBI dbVar Genome Browser [http://www.ncbi.nlm.nih.gov/dbvar/browse/] (Homo sapiens: GRCh38).

2.2.3.3 Principal Component Analysis

Principal component analysis (PCA) is used to reduce the dimensions in a large dataset. In this study, PCA was used to determine whether the Dutch population group would be a suitable control for the affected Afrikaner individuals, based on genome-wide genotype data. PCA was performed using the BD WGS data for the four affected individuals together with GWAS data for a Dutch control population group and data from the 1000 Genomes Project [1000 Genomes Project Consortium, 2012] as reference populations. The Dutch GWAS data was obtained from PGC for SCZ which consisted of 637 control individuals from the Netherlands genotyped with the Illumina 550K microarray. The 1000 Genomes Project data (phase 3) for 26 different populations for all chromosomes was accessed on the 22 August 2014 [ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502]. This dataset consisted of SNPs, indels, complex short substitutions and structural variant classes. The following six population groups were selected for the analysis: Han Chinese (CHB), Yoruba (YRI), British (GBR), Finnish (FIN), Toscani in Italy (TSI) and Iberian populations in Spain (IBS). The genotype data from a total of 615 unrelated individuals from the above-mentioned population groups were used in downstream analysis.

Prior to the PCA analysis, the variants were filtered using Plink (version 1.9) [https://www.cog-genomics.org/plink2/] [Purcell et al., 2007] whereby the overlapping variants (number of variants= 148 788, number of individuals from the combined datasets= 1 256) between the datasets were identified and subsequently used. PCA was conducted using the package *SNPRelate* [Zheng et al., 2012] implemented in the statistical environment R 3.0.0 [http://www.r-project.org/]. Prior to this; variants in LD were pruned at an LD threshold of 0.2. Also, variants with a MAF of less than 0.05 and with a ‗missingness' rates of greater

than 5% were discarded. The PCA plots were subsequently viewed and edited using the software Genesis 0.3 (http://www.bioinf.wits.ac.za/software/genesis/downloads.html).

2.2.3.4 Comparison of Case with Control Samples

For the BD samples, a list of 870 875 variants was obtained for which each of the BD affected individuals shared the same genotype (either heterozygous or homozygous for the alternate allele). A total of 625 965 of these variants were present in the GoNL dataset, thus leaving a list of 221 703 variants which were present exclusively in the BD group (23 207 variants were located in the mitochondrial genome or were labelled as part of the random chromosomes). This list was further filtered to include only those regions of interest as identified from the linkage analysis (i.e. only variants in the chromosomal regions 6p25, 10p14-10p15.1, 11q23-11q25, and 13q21-22 were retained for analysis).

To determine whether there were any pathogenic variants in the list, ANNOVAR [Wang et al., 2010b] was used to generate SIFT and Polyphen2 scores, with the hg19 human genome build. Sift predicts whether an amino acid substitution is tolerant or intolerant to the respective protein, based on sequence homology [Ng and Henikoff, 2001]. Polyphen2 is similar to SIFT but differs in that it uses a structure-based approach in addition to the sequence homology to determine whether a missense mutation has a pathogenic effect [Adzhubei et al., 2010]. The web-based program ConsensusPathDB [Kamburov et al., 2009] was used to determine which pathways were over-represented in BD. Within this program, enrichment analysis was performed using the pathway-based, gene ontology (GO)-based and enriched protein complex-based sets. The minimum overlap for each set analysis was set at two genes and the p-value cut-off was set at 0.01. For the protein complex-based set analysis, the minimum complex size was set at two.

2.3 Results

2.3.1 Research Group

2.3.1.1 Family 30

A total of 20 family members were genetically analysed. Four of the affected individuals were males and the other was female. The average age of the affected group was 65 years (SD=16.3 years) and the mean age of symptom onset was 25 years (SD=16.7 years). Table 4 describes the number of depressive episodes, as well as number of hospitalisations for the five affected individuals. In the unaffected group, nine of the family members were female

and six were male. Two of the individuals in the unaffected group had MDE (one with recurrent MDE and the other with a single episode). The average age of the unaffected family members was 63 years (SD=16.2 years), and the youngest and oldest members were 43 and 84, respectively. As all of the unaffected family members were older than the mean age of onset for the affected family members, it was thought unlikely that any of these individuals would develop BDI later in life.

Table 4: Phenotype characteristics of BDI individuals

Individual ID	Age of symptom onset	Number of depressive episodes	Number of hospitalisations depression	Number of hospitalisations mania
30_8	19	unknown	0	2
30_10	55	unknown	0	2
30_29	16	unknown	0	2
30_30	16	unknown	0	3
30_50	21	2	0	7

2.3.2 Statistical and Bioinformatic Analyses

2.3.2.1 Bioinformatic Analysis of WGS Data

2.3.2.1.1 Quality Control

Based on the results of FastQC, overall all four samples had a passable quality in terms of per base sequence quality, per sequence quality scores, per base sequence content, per base GC content, per sequence GC content, per base N content, sequence length distribution, sequence duplication levels, over-represented sequences, and kmer content. As an example, Appendix 1 (FastQC Results) has the FastQC results for one of the lanes for one of the samples.

2.3.2.1.2 Post-Alignment Processing and Variant Calling

The four BDI samples had an average coverage of approximately 29X. The average percentage of reads mapped to the reference was approximately 94%. The mean GC percentage across all four samples was approximately 39% and the mean read length was 101. Table 5 outlines the values for the above-mentioned variables per sample.

Table 5: Per sample alignment and variant characteristics

Sample ID	GC (%)	Coverage	Mapped reads (%)	Read mean length (bases)	Total number of variants	Number of indels
30_10	39.73	25.59	93.49	101	4 759 614	669 438
30_29	38.8	36.66	93.57	101	4 746 223	686 403
30_30	39.4	23.76	93.65	101	4 824 753	666 561
30_50	39.44	30.04	93.83	101	4 872 249	688 696

2.3.2.1.3 Variant Annotation

The average number of variants called across all four samples was 4 800 710 (SNPs and short indels). The average number of intronic and exonic variants was 1 649 268 and 22 420, respectively. The number of intronic, exonic and regulatory variants for each of the samples is listed in Table 6.

2.3.2.2 Whole-Genome Linkage Analysis

Eighteen of the family members were genotyped with the genome-wide microarray (three of these individuals were affected with BDI). To include the two other BDI affected individuals in the linkage analysis, genotype data for the microarray variants were obtained from the WGS data. Therefore, 20 family members with genetic data were included in the linkage analysis. None of the chromosomal regions obtained a LOD score of greater than three, the traditional threshold for significant linkage, indicating that the odds that the loci are linked is 1000 times greater that the odds that the loci are not linked [Nyholt, 2000]. A total of four chromosomal regions, 6p25, 10p14-10p15.1, 11q23-11q25, and 13q21-22, reached the maximum LOD score (marked with a green line) as indicated by the figures below (Figures 1-8). The SNPs corresponding to these regions with the respective LOD scores are reported in Table 7.

Table 6: Per sample variant characteristics

Sample ID	**Total number of variants**	**3'UTR**	**5'UTR**	**Intronic**	**Exonic**	**Intergenic**	**Other** (upstream, downstream, non-ncRNA, splicing)
30_10	4 759 614	31 518	4 782	1 796 177	22 238	2 822 732	82 167
30_29	4 746 223	31 399	4 484	1 585 912	21 906	2 806 951	295 571
30_30	4 824 753	31 710	4 392	1 602 586	22 401	2 863 885	299 779
30_50	4 872 249	32 398	4 723	1 612 396	23 134	2 899 020	300 578
Total	19 202 839	127 025	18 381	6 597 071	89 679	11 392 588	978 095

ncRNA- non-coding ribonucleic acid

Table 7: Top LOD scores across four regions of interest

Chromosome Region	**Marker**	**LOD score**	**Gene**	**Genetic Model**
6p25.1	rs9392683	0.9866	*FARS2*	Recessive
6p25.2	rs160701	1.2062	*WRNIP1*	Dominant
10p15-p14	rs2961611	1.0950	*AKR1C1*	Dominant
10p14	rs570825	0.9957/1.3340	Intergenic	Recessive and dominant
11q25	rs2027784	1.3146	*NTM*	Dominant
11q23.3	rs1712789	0.7236	Intergenic	Recessive
13q21.2	rs9538758	1.4009/1.4062	Intergenic	Recessive and dominant
13q22.1	rs1536399	1.0196	Intergenic	Recessive

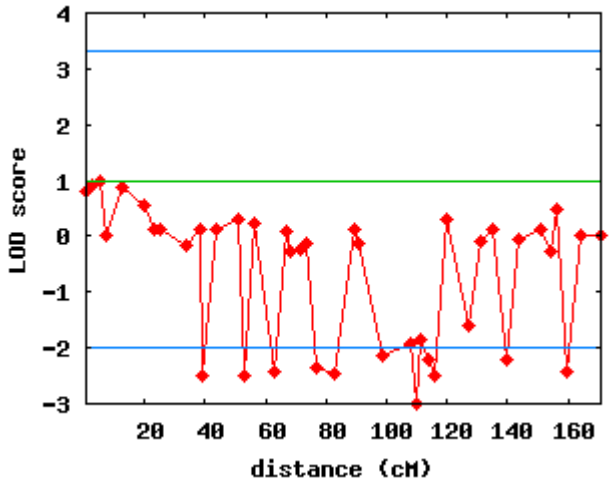

Figure 1: Two-point linkage results for chromosome 6 with a recessive model.

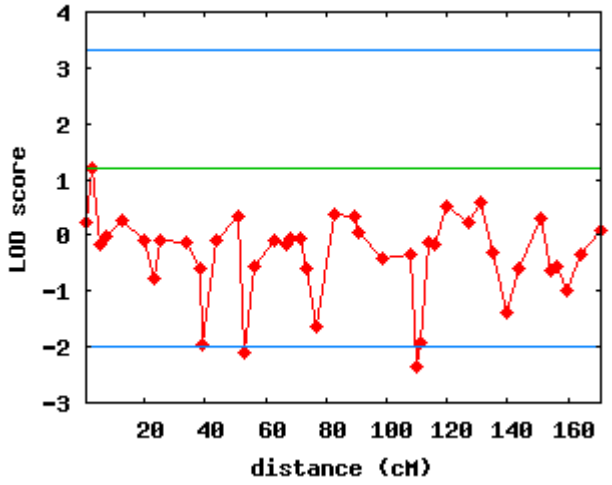

Figure 2: Two-point linkage results for chromosome 6 with a dominant model.

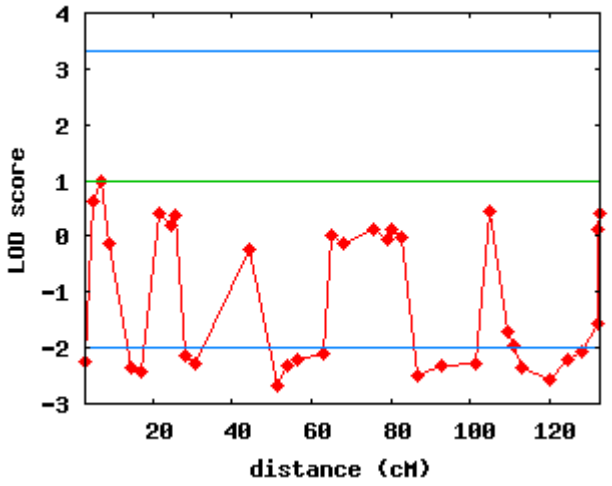

Figure 3: Two-point linkage results for chromosome 10 with a recessive model.

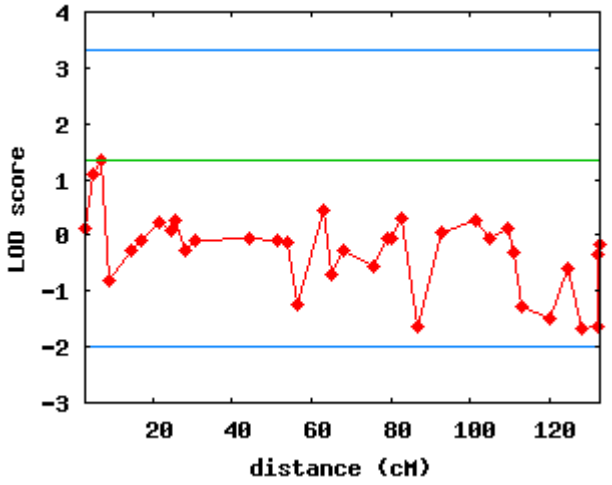

Figure 4: Two-point linkage results for chromosome 10 with a dominant model.

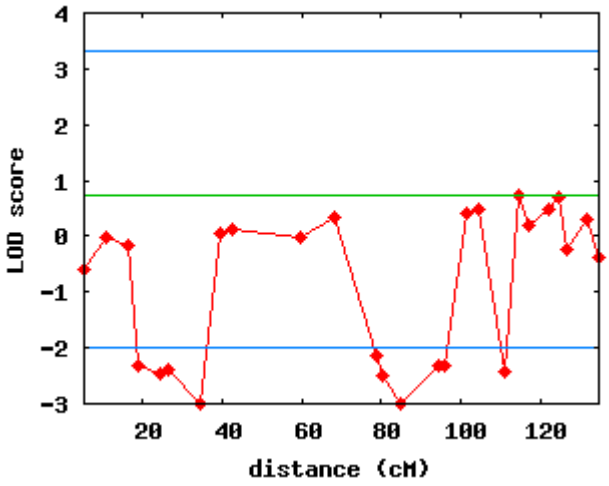

Figure 5: Two-point linkage results for chromosome 11 with a recessive model.

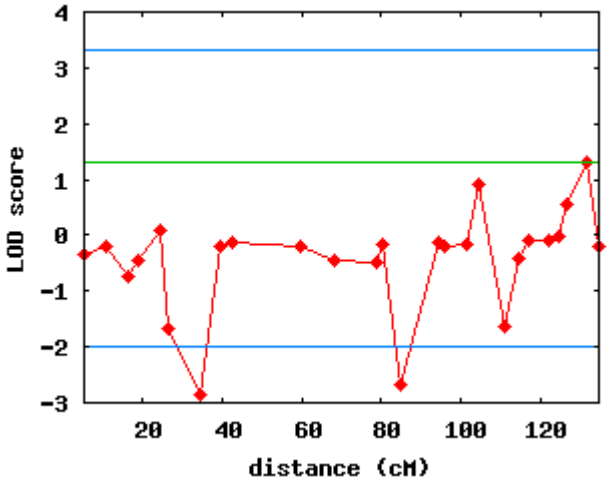

Figure 6: Two-point linkage results for chromosome 11 with a dominant model.

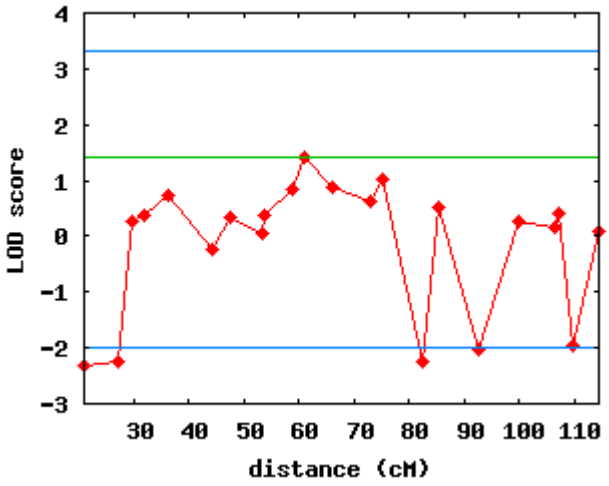

Figure 7: Two-point linkage results for chromosome 13 with a recessive model.

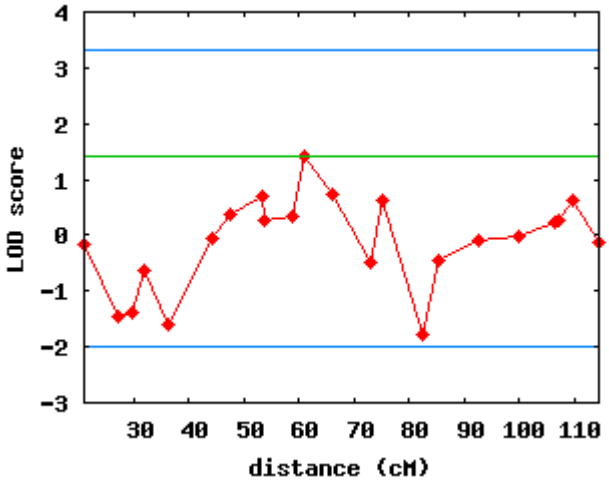

Figure 8: Two-point linkage results for chromosome 13 with a dominant model.

2.3.2.3 Principal Component Analysis

Figure 9 is a graphical plot of PCA 1 and PCA2 for the BD, Dutch and all the population groups from the 1000 Genomes Project dataset, which includes the CHB and YRI. The CHB and YRI cluster separately from the other groups illustrating that these groups are genetically distinct from the European population groups. Figure 10 is a is a graphical plot of PCA 1 and PCA2 for the BD, Dutch and only the European population groups from the 1000 Genomes Project dataset. It can be seen that the BD individuals (red circles) cluster more closely with the Dutch individuals (green circles) and the GBR individuals (blue triangles) than any of the other European population groups. This suggests that the BD affected individuals are genetically closer to the Dutch and British populations than any of the other European groups included in this analysis.

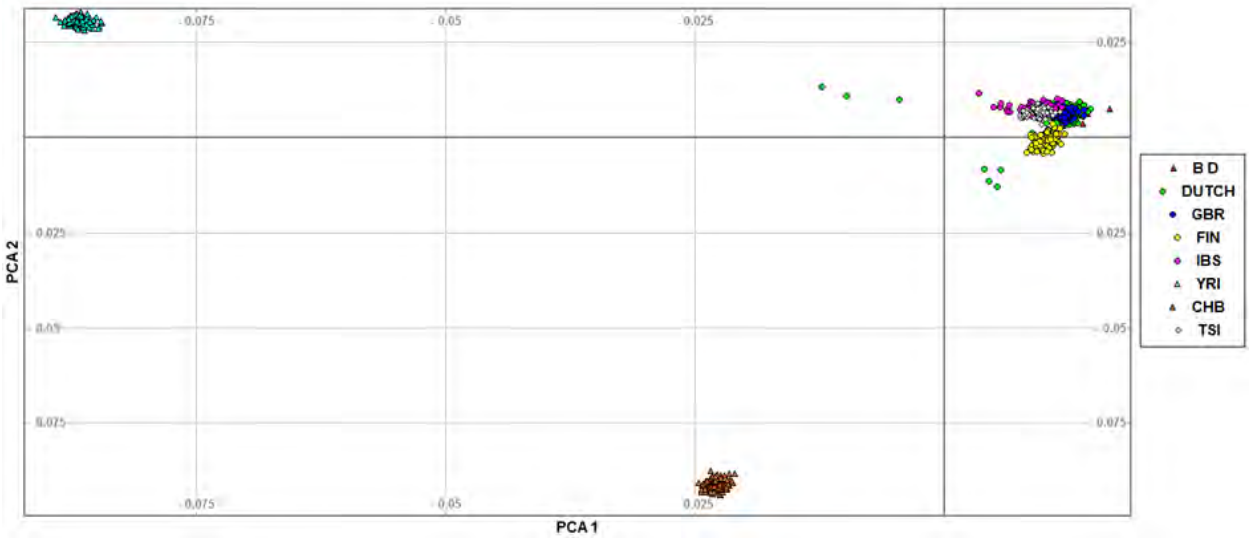

Figure 9: Population group structure for autosomal variants for PCA1 and PCA2. BD-bipolar disorder, CHB- Han Chinese from Beijing, GBR- British in England and Scotland, FIN- Finnish in Finland, IBS- Iberian populations in Spain, TSI-Toscani in Italy, YRI-Yoruban from Nigeria.

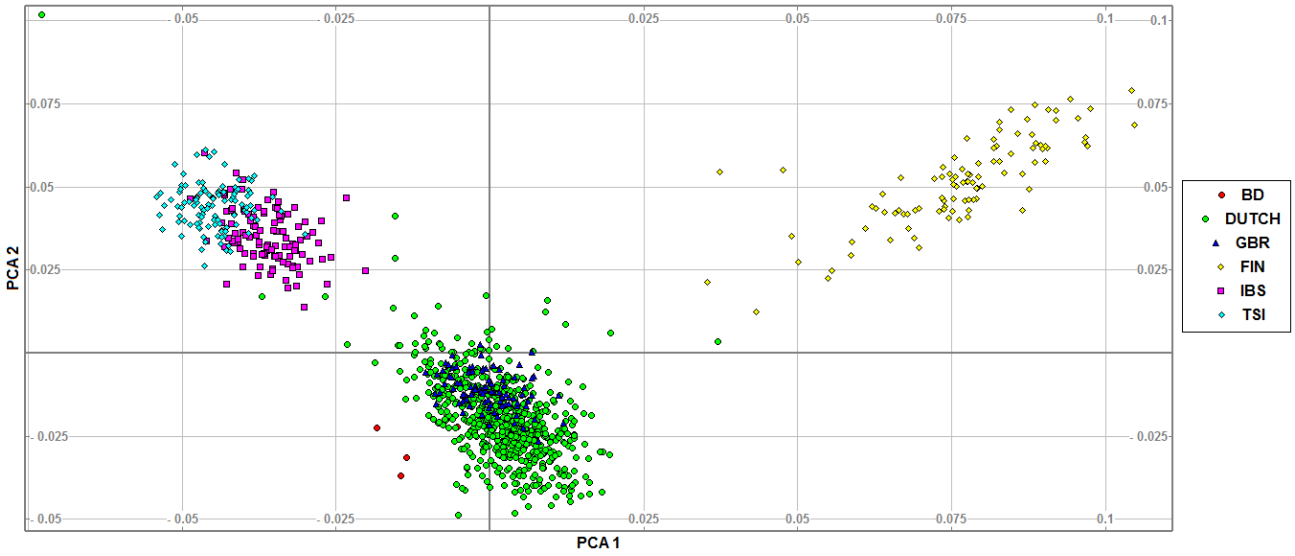

Figure 10: European and Afrikaner population group structure for autosomal variants for PCA1 and PCA2. BD-bipolar disorder, GBR- British in England and Scotland, FIN- Finnish in Finland, IBS- Iberian populations in Spain, TSI-Toscani in Italy.

2.3.2.4 Comparison of Case with Control Samples

A list of WGS variants which were present in all four affected individuals and not the Dutch control group was generated. This list was then filtered to only include variants which are located in the regions identified by the whole-genome linkage analysis (6p25, 10p14-10p15.1, 11q23-11q25, and 13q21-22). This list consisted of 5386 variants and around 279 genes. A total of 18 of the 5386 variants were exonic and seven of these were non-synonymous. Table 8 lists the details of the non-synonymous polymorphisms, most of which are located on chromosome 11. None of these SNPs were predicted to be deleterious or intolerant by either SIFT or Polyphen2 (Table 8). A SIFT score of less than 0.05 is indicative of a *deleterious* variant. Polyphen2 scores of greater than or equal to 0.957 is indicative of a *probably damaging* variant and a score greater than or equal to 0.453 and less than or equal to 0.956 denotes a *possibly damaging* variant.

For the enrichment analysis, 238 out of the 279 genes were mapped. From the pathway-based enrichment analysis, 49.6% of the input genes were able to be mapped to at least one pathway and the most significant pathway was the *T-cell receptor (TCR) signalling pathway* (q-value=0.148, p-value=0.0004, pathway source= Wikipathways), with six of the genes forming part of this pathway (pathway consists of 92 genes). From the GO-based enrichment analysis 79.4% of the genes formed part of at least one of the GO categories and the most significant GO term was *interleukin-4 production* (q-value=0.0356, p-value=0.0002). The

second and third most significant GO terms were also involved in interleukin production. A total of 41.6% of the genes are present in at least one protein complex. The most significant protein complex was *CD3 epsilon: CD3 gamma with phosphorylated ITAM* (p-value=0.0002, q-value=0.003, complex source= Reactome). Other protein complexes include *TCR/CD3/MHC II/CD4/LCK/ZAP-70/CBL/SLAP-2/Ubiquitin* and *HTR3:5HT*. Appendix 1 (Enrichment Analysis Results) lists the results for all the significantly enriched pathways, GO terms and protein complexes and Figure 11 is a visual representation of the interactions between the significantly enriched pathways.

Of the 5 386 variants, 216 were novel, i.e. not reported in dbSNP. None of the novel variants were located in exonic regions and were therefore not predicted to be pathogenic. A full list of the novel variants and the genes they are located within are reported in Appendix 1 (Novel Variants).

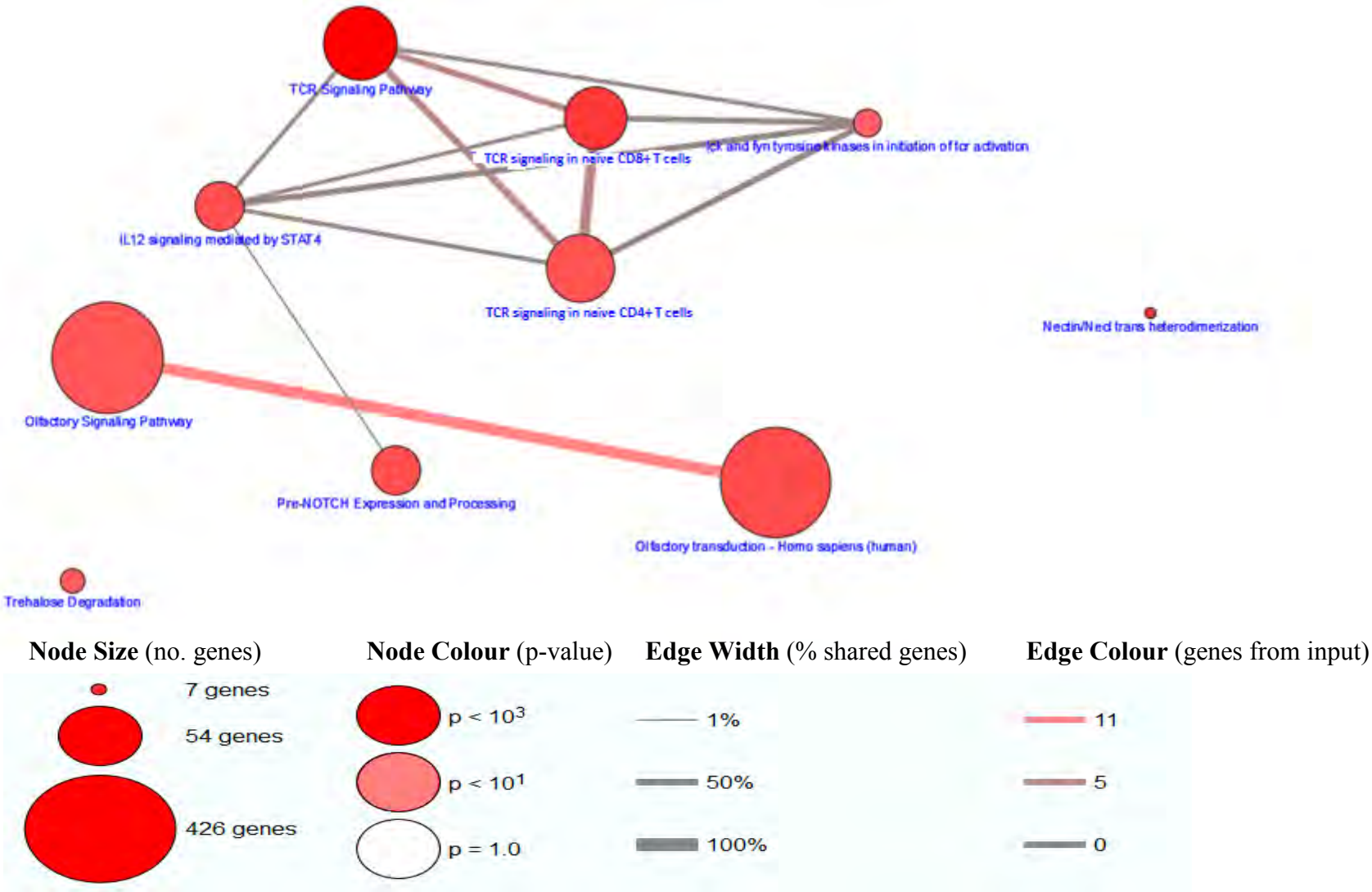

Figure 11: ConsensusPathDB output for the over-representation analysis (enriched pathway-based set).

Table 8: Non-synonymous polymorphisms in BD1 affected individuals

Chromosome	**Chromosomal Position** (GRCh37.p13)	**Gene**	**SNP**	**Nucleotide change**	**Protein Change**	**Sift prediction score and category**	**Polyphen2 prediction score and category**
10	7763661	*ITIH2*	rs7075296	c.A788G	p.N263S	0.27-Tolerated	0.04-Benign
11	112088499	*BCO2*	rs2217401	c.A1423C	p.I475L	1.0-Tolerated	0.0- Benign
11	117160347	*BACE1*	rs539765	c.T1366C	p.C456R	0.4-Tolerated	0.0- Benign
11	121437819	*SORL1*	rs1699107	c.C3220G	p.Q1074E	1.0-Tolerated	0.0- Benign
11	123754844	*TMEM225*	rs10893099	c.A401G	p.N134S	1.0-Tolerated	0.0- Benign
11	130780225	*SNX19*	rs681982	c.G1854T	p.L618F	1.0-Tolerated	0.0- Benign
11	134182375	*GLB1L3*	rs2509062	c.G1420A	p.V474M	0.08-Tolerated	0.002- Benign

2.4 Discussion

The aim of this chapter was to identify susceptibility variants for BD in a large Afrikaner family using whole-genome technology. Although no chromosomal regions obtained a significant LOD score, the four chromosomal regions which obtained the highest LOD scores, namely 6p25, 10p14-10p15.1, 11q23-11q25, and 13q21-22 were further investigated. Interrogation of WGS data, filtering for these four regions, identified variants and pathways which may a play a role in the aetiology of BD. The TCR signalling pathway was the most enriched pathway in individuals with BD.

The four chromosomal regions obtained the highest LOD scores in both the dominant and recessive model analyses. This has been observed in a previous linkage analysis for BD, where positive LOD scores were detected for chromosome 5 using a dominant and recessive model [Homer et al., 1997]. The hypothesis is that if the difference in the maximum LOD scores between the two models is greater than 1.5, then the higher LOD score of the two inheritance patterns is the true method [Greenberg and Berger, 1994]. In this study, a difference of greater than 1.5 was not observed for any of the four chromosomal regions, therefore, the above hypothesis does not hold true for this particular study. It may be speculated that the true mode of inheritance could be determined by the genotyping of successive generations as biological material is not available for the first parental generation.

Previous linkage studies have not found evidence for the region 6p25 for the BD diagnosis. However, this region has been suggestively linked to the psychosis sub-phenotype in individuals affected with BD [Cheng et al., 2006]. In this study, the SNPs which obtained the highest LOD score in this region were rs9392683 and rs160701. Neither these SNPs nor the genes in which they located in, namely, *phenylalanyl-tRNA synthetase 2*, *mitochondrial* (*FARS2*) and *Werner helicase interacting protein 1* (*WRNIP1*), respectively have previously been implicated in BD. However, these genes should not be discounted in terms of playing a role in the pathophysiology of BD as other SNPs within *FARS2* and *WRNIP1* have previously been associated with cognitive deficit in other psychiatric disorders, i.e. SCZ and ADHD, respectively [Jablensky et al., 2012; Franke et al., 2009].

Chromosome 10 is thought to harbour susceptibility variants for psychiatric disorders such as BD and SCZ [Wildenauer et al., 1999]. The region 10p14 has been linked to BD and suggestively linked to ADHD features in subjects with BD [Lambert et al., 2005; Joo et al., 2010]. In this study, the SNPs which obtained the highest LOD score in this region were

rs2961611 and rs570825. The SNP, rs2961611, is located within the gene *aldo-keto reductase family 1*, *member C1* (*AKR1C1*). The SNP, rs570825, is located in an intergenic region close to the genes *protein kinase C, theta* (*PRKCQ*) and *Scm-like with four mbt domains 2* (*SFMBT2*). PRKCQ forms part of glutamatergic signalling, a pathway previously implicated in BD [McCullumsmith and Meador-Woodruff, 2002; Hashimoto et al., 2007; McCullumsmith et al., 2007]. Also, this gene has been shown to have reduced expression in the temporal cortex of patients with MDD [Aston et al., 2005]. Mice treated with the mood-stabilising drug, valproate, showed increased expression of *SFMBT2* in the brain, suggesting a role for this gene in the pathophysiology of BD [Chetcuti et al., 2006].

Copy number variation (CNV) has been observed in the chromosomal region, 11q23-11q25, in individuals with BD and major depression [Baysal et al., 2002; Böhm et al., 2006; Reardon et al., 2007; Ye et al., 2012]. Also, this region has been shown to interact with the chromosomal region 2p15-12 in families with BD [Fullerton et al., 2010]. The SNPs which obtained the highest LOD score in this region were rs2027784 and rs1712789. The SNP, rs2027784, is located within the gene *neurotrimin* (*NTM*) which encodes a protein that stimulates neurite outgrowth and cell adhesion [Gil et al., 1998]. *NTM*, has not previously been associated with BD, but variation within this gene has been associated with IQ [Pan et al., 2011], which in turn has been shown to be associated with BD [Mancuso et al., 2015]. The other SNP, rs1712789, is located in an intergenic region close to the gene, *neurexophilin and PC-esterase domain family, member 2* (*NXPE2*). This gene has not been associated with BD before.

The 13q region has previously been linked to BD, including in a meta-analysis of whole genome linkage scans [Stine et al., 1997; Badner and Gershon, 2002; Liu et al., 2003; Potash et al., 2003; Cheng et al., 2006]. Specifically, 13q21 has been shown to be linked to psychotic features in families with BD [Goes et al., 2007]. Also, an epistatic relationship between 13q21 and 20p12 was observed in families with BD [McInnis et al., 2003]. The polymorphisms which obtained the highest LOD score in this region were the intergenic SNPs rs9538758 and rs1536399, neither of which has shown an association to BD or any other psychiatric disorder, previously. The SNP, rs9538758, is located close to the gene, *tudor domain-containing 3* (*TDRD3*). This gene has previously been associated with variation in size of the mid-saggital area of the corpus collosum, an area implicated in BD [Newbury and Rosen, 2012].

From the enrichment analysis, the most over-represented pathway was the *TCR signalling pathway*, the most over-represented GO-term was *interleukin production* and the most over-represented protein complex was (cluster of differentiation 3) *CD3 epsilon*. Each of these entities are involved in the body‘s immune system, suggesting a role of this system in the pathophysiology of BD. Psychiatric disorders such as SCZ have previously been significantly associated with disruption in immune function [Maes et al., 1995; Theodoropoulou et al., 2001; Steiner et al., 2010].

The immune system comprises two parts: the innate and adaptive response. The innate immune system provides an immediate response (considered as the ―first line of defence" to a pathogen) and includes physical, chemical and microbiological barriers [Parkin and Cohen, 2001; Strous and Shoenfeld, 2006]. The cellular component of the innate response includes monocytes/macrophages, granulocytes and natural killer cells. The humoral part of the innate system consists of acute phase proteins and the complement system [Strous and Shoenfeld, 2006]. The adaptive immune system provides a slower response to infection but has a higher degree of specificity compared to the innate system. The adaptive system is characterised by a cellular component consisting of antigen-specific receptors present on the T-lymphocytes (T-cells) and B-lymphocytes (B-cells) and a humoral component consisting of antibodies [Parkin and Cohen, 2001; Strous and Shoenfeld, 2006]. The TCRs bind to CD3, a T-cell co-receptor consisting of four subunits (which includes CD3 *epsilon*), to form the TCR/CD3 complex, involved in antigen/major histocompatibility complex (MHC) recognition [San José et al., 1998]. Upon presentation of an antigen, the TCR complex elicits a cytotoxic or inflammatory response [Parkin and Cohen, 2001]. Another integral part of the immune system is the cytokine component, which comprise of small molecular weight compounds released from a particular cell to change the actions/functions of either the cell itself, or that of other cells. Interleukins are a type of cytokine which are secreted from white blood cells and mainly affect the behaviour of other white blood cells [Parkin and Cohen, 2001].

Disruption in the immune system in terms of BD has been described previously [Breunis et al., 2003; Ortiz-Domínguez et al., 2007; Brietzke et al., 2009; Drexhage et al., 2011; Wieck et al., 2013; Barbosa et al., 2014]. Studies have found increased levels of activated T-cells in the blood of BD patients compared to healthy controls [Breunis et al., 2003; Drexhage et al., 2011; Wieck et al., 2013]. However, findings are sometimes conflicting as another study found reduced T-cells in BD affected individuals [Barbosa et al., 2014]. During manic phases an increase in certain interleukins, which are pro-inflammatory cytokines, have been

observed in the serum of patients with BD [Brietzke et al., 2009]. The inverse interleukin levels were shown during the depressive phase [Ortiz-Domínguez et al., 2007]. The administration of the cytokine interferon-alpha, used to treat hepatitis C and certain cancers, has been shown to result in neuropsychiatric side effects, including mania and depression [Capuron and Ravaud, 1999; Constant et al., 2005]. There has also been genetic evidence for a link between the immune system and BD. Association between polymorphisms and differential expression patterns of immune-related genes have been reported in BD [Papiol et al., 2004; Padmos et al., 2008; Dieset et al., 2014; Yoon and Kim, 2012; de Baumont et al., 2014]. Also, drugs with anti-inflammatory effects are used to treat BD [Dean et al., 2012].

There is a close, bi-directional relationship between the immune system and the central nervous system (CNS) [Wrona, 2006]. In the brain, the microglial cells are the macrophages of the CNS [Hanisch and Kettenmann, 2007]. The exact mechanism as to how a disruption of the immune system results in an altered mental state is still unknown, however it is thought to possibly occur via several pathways, including the HPA-axis and various neurotransmitter pathways [Dantzer et al., 2008].

Although this investigation was able to identify several interesting candidate genetic regions and pathways for BD, this study had several limitations. As illustrated by the PCA plot from this study, the Afrikaner individuals clustered with the Dutch control group as well as individuals of British descent. The use of the GoNL dataset was not ideal as Afrikaner individuals are not purely of Dutch descent, but are of British, French and German ancestry [Botha and Beighton, 1983]. However, at the time of analysis, the researchers of this study only had access to the WGS data for the Dutch group. Future studies should consider including other European population groups, such as the French, British and Germans, and determine whether these individuals also cluster with the Afrikaner family members. WGS data should have been obtained for the unaffected family members as these individuals would have served as the ideal controls in this type of analysis.

Another limitation of this study is that CNVs and novel variants were excluded from the linkage analysis. This was done so that the input files met the requirements of the linkage program. However, it is possible that these regions harboured disease-causing variants which were excluded. Also, the list of potential susceptibility variants identified was present in the affected individuals but not in the Dutch control individuals. Thus, susceptibility variants occurring at a low frequency in the control group were potentially overlooked. Another

limiting factor was that two of the unaffected family members had an MDE diagnosis. Depression is a hallmark of BD and the inclusion of these two relatives could have biased the linkage results. However, the aim was to determine variants which were segregating with BDI, therefore a narrow diagnostic model was employed in the linkage analysis. It should also be noted that disruption in the immune system could be unrelated to the BD phenotype and may be due to the presence of immune-related diseases present in this family. BD affected family members should be further assessed in terms of immune function.

This study identified four regions which may be linked to BD and suggests that the immune system may possibly underlie the aetiology of BD in an Afrikaner family. These findings need to be validated in other Afrikaner families (as well as other BD cohorts) and the exact disease-causing variants identified. If validated, immunotherapy may be a key to an effective treatment regime for BD.

The following chapter of this thesis investigates the aetiology of AUD in a South African adolescent group.

Chapter 3: An Investigation of AUD in a Group of South African Adolescents

Abstract

In South Africa, AUD is a serious mental and public health issue. AUD has a considerable genetic influence and the heritability is estimated to be between 40-60%. However, the genetic basis remains unknown. Endophenotypes, such as differential brain volume, may be useful in determining the genetic aetiology of AUD. Besides biological factors, environmental risk factors can also determine whether an individual will be more at risk for an AUD. The aim of this investigation was to determine whether variants previously implicated in other psychiatric disorders have an association with adolescent AUD and differential brain volume in AUD. An additional aim was to determine whether these genetic factors interact with childhood trauma to result in adolescent AUD. Genotyping was done using the Illumina Infinium iSelect custom 6000 BeadChip for 80 adolescents with AUD and 80 matched controls. Statistical analysis was performed using SPSS, Plink and SPM8. No single SNP was associated with adolescent AUD, after correction for multiple testing. The circadian rhythm genes *nuclear receptor subfamily 1, group D, member 1* (*NR1D1*) and *basic helix-loop-helix family, member e41* (*BHLHE41*) were associated with AUD in a gene-based analysis. Childhood trauma was shown to be higher in adolescents with AUD compared to matched controls. No significant gene-environment interactions were found for lifetime alcohol units. The gene-imaging analysis identified a SNP in the *glutamate receptor, ionotropic, N-methyl D-aspartate 2B* (*GRIN2B*) gene as being associated with brain volume in the left orbitofrontal cortex (uncorrected p-value < 0.05) and posterior cingulate (uncorrected p-value < 0.05). Findings from this study imply that the onset of AUD in adolescents may be as a result of dysfunction in the circadian and glutamatergic pathways. Both of these pathways have previously been implicated in BD and suggest a common pathophysiology between AUD and BD.

3.1 Introduction

AUD, consisting of AA and AD, is a serious mental and public health issue, worldwide and in South Africa. Of particular concern is the growing number of adolescents in the country who consume alcohol excessively and who eventually develop an AUD. It is important to

identify the biological and environmental risk factors which predispose individuals to an AUD and to understand the possibly complex way in which these factors interact. This may ultimately aid in the identification of ~~–at~~ risk" individuals and enable the tailoring of effective interventions and treatment strategies.

3.1.1 Addiction and the Neurobiology Underlying Alcohol Use

Addiction is the ~~–l~~oss of control over drug use" [Nestler, 2005]. Each drug of abuse has its own chemistry and mechanism of action, resulting in distinct behavioural and physiological outcomes. Despite this, after acute and chronic administration, most drugs of abuse have a common effect whereby they are acutely rewarding, resulting in repeated use and eventually, in some individuals, to addiction [Nestler, 2005]. It has been established that most drugs of abuse exert their effects on the brain limbic system [Nestler, 2005]. The limbic system consists of the following brain structures: the amygdala, hippocampus, hypothalamus, anterior thalamic nuclei, parts of the basal ganglia, epithalamus and septal nuclei [Maclean, 1954; Ganong, 1995]. Most addictive substances are known to increase the extracellular amount of the neurotransmitter, dopamine (DA), in the limbic system of the brain. This increase in DA is associated with the reinforcing properties of the addictive substance [Wise, 1996; Volkow et al., 2009].

Alcohol is a simple organic molecule (CH_3CH_2OH) which, upon consumption, is able to enter nearly all biological compartments within the body [Costin and Miles, 2014] through absorption from the stomach and intestine. Alcohol is able to cross the blood-brain-barrier where it exerts its effects on neurotransmitters, receptors, enzymes and various other molecules [Most et al., 2014]. Human and animal studies have not shown a specific effect of alcohol on the brain but rather that it affects a variety of brain systems [Palmer et al., 2012], including the reward and stress response systems via several neurotransmitter pathways. These include, but are not limited to, the DA, opioid, gamma-aminobutyric acid (GABA), glutamate, serotonin, and corticotropin-releasing hormone (CRH) systems.

As shown in studies conducted on rats and humans, alcohol exerts some of its effects on the brain by increasing the secretion of DA and serotonin in the nucleus accumbens [Boileau et al., 2003]. Furthermore, upon withdrawal of alcohol, the levels of these neurotransmitters (DA and serotonin) are reduced [Weiss et al., 1996]. The opposite effect is true for the neurotransmitter, glutamate. At increasing concentrations, alcohol inhibits glutamate signalling [Lovinger et al., 1989] and after acute withdrawal an increase in glutamate

neurotransmission is observed [Tsai et al., 1998]. Alcohol also affects GABAergic signalling, with a post-mortem study showing increased GABA receptors in the brains of individuals with AD [Tran et al., 1981]. Also, alcohol stimulates the release of opioid peptides in the nucleus accumbens [Olive et al., 2001] and opioid receptor antagonists such as naltrexone have been shown to be effective in reducing alcohol intake [Davidson et al., 1999; Morris et al., 2001]. Alcohol also has an effect on the CRH as consumption of alcohol increases expression of the CRH receptors in the hypothalamus [Lee and Rivier, 1997] and it has been shown that ethanol-preferring rats have dyrsegulated secretion of CRH in the amygdala compared to control rats [Richter et al., 2000]. The interaction of alcohol with the above-mentioned systems results in the acute reinforcing effects of this substance, and after chronic exposure, results in sensitization, tolerance, withdrawal and dependence [Gilpin and Koob, 2008].

Alcohol use is characterised by both positive and negative reinforcement [Cooper et al., 1995]. Reinforcement is defined as a response or behaviour which is governed by a previous experience. Positive reinforcement, mediated by the reward network (DA, GABA and opioid peptides), is when the probability of a certain response is strengthened (e.g. alcohol–seeking behaviour) by a rewarding stimulus or experience such as alcohol-induced euphoria. Negative reinforcement, mediated by the stress system (CRH and norepinephrine), is characterised by an increase in the probability of a particular response (e.g. alcohol-seeking behaviour) if this response allows the individual to avoid or alleviate an aversive stimulus (e.g. alcohol withdrawal). Positive reinforcement is usually observed in the early stages of alcohol use and negative reinforcement during the transition of use to dependence, or when the individual has a psychiatric comorbidity [Gilpin and Koob, 2008]. This principle is illustrated by Figure 12 which shows the systems which alcohol acts upon during positive and negative reinforcement, and the differences between non-dependence and dependence.

3.1.1.1 Genetic Basis of AUD

Certain individuals are more at risk of developing an AUD than others. The use of twin studies have been extremely useful in showing that AUD has a considerable genetic influence [Prescott and Kendler, 1999], whereby the heritability is estimated to be between 40-60% [Prescott and Kendler, 1999; Epps and Holt, 2011]. In the USA, a total of 22% of adults have at least one biological parent with an AUD [Yoon et al., 2013]. Children of an individual with AUD are more likely to develop AUD themselves; although this could be due to both genetic and/or environmental risk factors [Enoch, 2006]. A study has shown that individuals with one

parent affected with an AUD have a 2.5 fold increased lifetime odds of developing an AUD themselves [Yoon et al., 2013]. In addition, those individuals with two affected parents have a 4.4 fold increase in the odds of developing a lifetime AUD, and females are more vulnerable (than males) to the effect of a parent with an AUD [Yoon et al., 2013]. With the use of family-based linkage analysis, candidate gene-based association and GWASs, previous research has implicated many genes and variants in the predisposition to AUD (reviewed below).

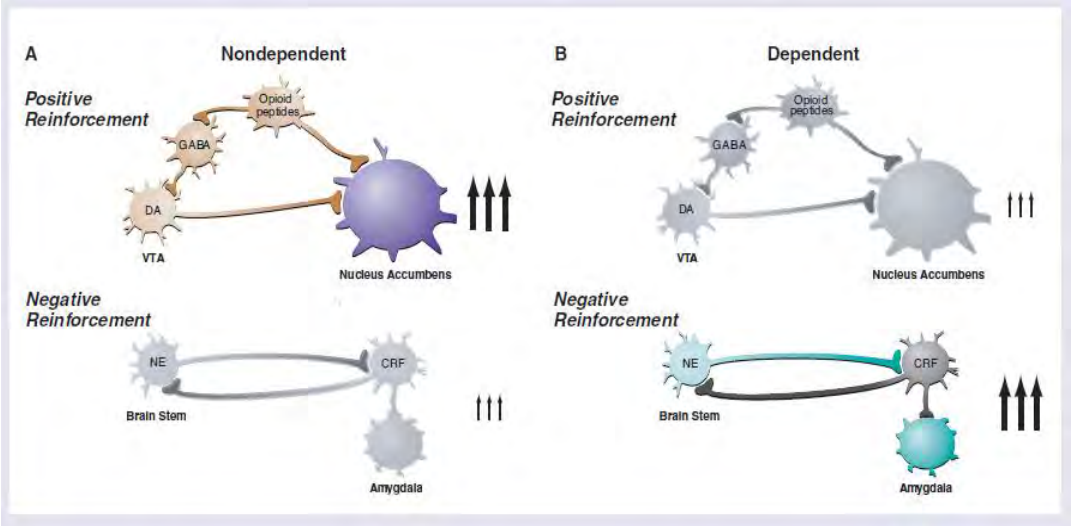

Figure 12: A simplified representation of the transition of the brain reward system from alcohol non-dependence to dependence [Gilpin and Koob, 2008].

3.1.1.1.1 Linkage Studies

In Chapter 2, the aim of a linkage study is described. Over the years, numerous linkage studies have been conducted for AUD. As with BD, findings have been variable, with a region on nearly every chromosome being implicated: chromosome 1 [Reich et al., 1998; Turecki et al., 1999; Foroud et al., 2000; Nurnberger Jr et al., 2001; Dick et al., 2002], 2 [Turecki et al., 1999]; 4p [Long et al., 1998], 4q22-32 [Prescott et al., 2006], 7 [Reich et al., 1998; Foroud et al., 2000]; 8 [Turecki et al., 1999]; 9q [Bergen et al., 2003], 10q23 [Gelernter et al., 2009], 11p [Long et al., 1998], 16 [Foroud et al., 2000], 19 [Turecki et al., 1999], 21 [Turecki et al., 1999]. Results from linkage analyses indicate that it is possible that multiple chromosomal regions are responsible for individuals developing AUD. Thus instead of single genomic regions, gene networks and relevant pathways should be considered and investigated in terms of AUD.

3.1.1.1.2 Candidate Gene and Genome-Wide Association Studies

Many candidate gene association studies have been performed to determine the aetiology of AUD. Table 9 lists some of the most commonly associated genes in AUD. Each of the listed genes is involved in either neurotransmission or alcohol metabolism. However, as with BD, findings have been conflicting and in most instances non-replicable. Therefore, more recent studies have performed GWASs with relatively large sample sizes.

Approximately 26 GWASs have been published for the diagnosis of AD and related phenotypes [http://www.genome.gov/gwastudies/]. Table 10 lists the details of these GWASs and the main findings. Many of the reported GWASs were conducted on samples from large consortia such as the Collaborative Studies on Genetics of Alcoholism (COGA), Study of Addiction: Genetics and Environment (SAGE), and the Australian twin-family study of alcohol use disorder (OZ-ALC). COGA was first initiated in 1989, and has collected data on more than 300 extended families with alcoholism [Edenberg et al., 2005]. SAGE consists of unrelated individuals of European-American and African-American descent, a subset of which are from the COGA consortium [Bierut et al., 2010]. OZ-ALC consists of Australian twins and their extended families [Heath et al., 2011]. The aims of each of these consortia are to determine the genetic factors predisposing to AUD.

The first GWAS for AD, conducted on the COGA cohort, was published by Johnson et al. (2006). They identified SNPs involved in cell signalling, gene regulation, cellular adhesion, development and those involved in Mendelian disorders, associated with AD, and concluded that this phenotype is a result of polygenic inheritance [Johnson et al., 2006]. Similarly, the conclusions from subsequent GWASs were that AD is likely to be as a result of the combined small effects of hundreds of variants [Heath et al., 2011; Yang et al., 2014]. Using a process of genome partitioning, a recent GWAS on a group of African-Americans with AD, found that variants within 10-20kb of all genes, on all chromosomes, have an effect on the AD phenotype [Yang et al., 2014]. Considering all of the best results from the GWASs, no obvious chromosomal region, gene or variant seems to be common in all of the studies. Therefore, instead of examining genetics in isolation, it may be necessary to consider the aetiology of AUD in a more holistic manner by examining other biological factors or endophenotypes implicated in alcohol use, and the environment.

3.1.1.2 Brain Volume as Endophenotype for AUD

Differential brain volume, as measured by various imaging techniques, is a prime example of an intermediate phenotype or endophenotype for AUD. It has been postulated that the power to detect aetiological factors of complex disorders, such as AUD, would be to investigate endophenotypes which represent ―mediating factors of behaviour" and which are likely to be affected by variations in overlapping sets of genes [Lesch, 2005]. Several studies have had success in identifying candidate genes for AD by the genetic investigation of endophenotypes such as brain oscillations and subjective level of response to alcohol [Edenberg et al., 2004; Wang et al., 2004; Wall et al., 2005]. A particular trait needs to meet the following criteria to be considered as an endophenotype: it should (i) be associated with a disorder in the population; (ii) be heritable; (iii) be evident in individuals whether the disorder is active or not; (iv) co-occur with disease within families; and v) manifest in non-affected family members of affected individuals at a higher frequency than in the general population [Gottesman and Gould, 2003].

Brain-imaging studies have shown that various brain regions are altered in individuals with AD. For example, individuals who are alcohol dependent are more likely to have smaller prefrontal cortical volumes [Bellis et al., 2005; Medina et al., 2008], smaller right and left hippocampal volumes [Agartz et al., 1999; De Bellis et al., 2000; Nagel et al., 2005], smaller grey and white matter volumes [Gazdzinski et al., 2005] and reductions in brain regions, such as the amygdala, involved in the reward network, [Makris et al., 2008], compared to healthy controls. Previous research, conducted by our group on adolescents with alcohol exposure, found that the pattern of grey matter density differences was limited to regions in the left lateral frontal, parietal and temporal lobes [Fein et al., 2013].

Brain development is influenced by genetic factors, and twin studies have shown that brain structural differences have considerable heritability [Posthuma et al., 2000; Baaré et al., 2001; Thompson et al., 2001]. However, exactly which genes are responsible for brain volume variation is largely unknown. In this regard, examining specific mental disorders may help to elucidate the association between genes and disordered phenotypes. Several gene-imaging studies have focussed on AD and alcohol-related phenotypes in adults. For example, a missense mutation in the *superoxide dismutase 2* (*SOD2*) gene had an association with grey matter loss in AD individuals [Srivastava et al., 2009]. A study by Ramchandani et al. (2010), using positron emission tomography (PET), reported that a functional polymorphism within the *μ-opioid receptor* (*OPRM1*) gene influences striatal dopamine release after alcohol

administration in social drinkers. Several gene-imaging studies have focussed on adolescents, either with AUD, or who were at a high risk for alcoholism. One such study investigated the effects of variation within the *corticotrophin-releasing hormone receptor 1* (*CRHR1*) gene and found that individuals homozygous for the rs110402 had greater activation in the right ventrolateral prefrontal cortex in adolescents and young adults at high risk for alcoholism [Glaser et al., 2014]. Other such studies have found that variations within the *serotonin transporter* (*5-HTT*), *gamma-aminobutyric acid A receptor, alpha 2* (*GABRA2*), *brain-derived neurotrophic factor* (*BDNF*), *catechol-O-methyltransferase* (*COMT*), and *dopamine receptor D2* (*DRD2*) genes had an association with brain structure and function [Hill et al., 2009; Hill et al., 2011; Hill et al., 2013].

3.1.1.3 Environmental Measures

Besides biological factors, studies have shown that early environmental risk factors can determine whether an individual will be more susceptible to psychiatric disorders later on in life [Schmidt, 2007]. In this regard, some studies have shown that marital status has an influence as to whether individuals consume more or less alcohol. Some reports observed that unmarried individuals are heavier alcohol drinkers compared to their married counterparts [Black and Markides, 1993; Bobak et al., 1999] and another study found the opposite to be true [Moore et al., 2005]. Religion, education level, alcohol availability, parental control and peer influences have also been shown to play an important role in the level of alcohol consumption [Dielman et al., 1987; Black and Markides, 1993; Loveland-Cherry et al., 1996; Kendler et al., 1997; Bobak et al., 1999; Moore et al., 2005; Enoch, 2006; Latvala et al., 2011].

Some of the more severe environmental factors influencing alcohol use include parental emotional and physical abuse, experience/exposure to violence and sexual abuse [Brietzke et al., 2012]. Individuals who have experienced childhood abuse and neglect are at an elevated risk for substance use and AD [Duncan et al., 1996; Felitti et al., 1998; Dube et al., 2006; Khoury et al., 2010; Nelson et al., 2010; Copeland et al., 2011; Sartor et al., 2013]. Childhood abuse and neglect are interactions with a child which are considered abnormal which results in the actual or potential harm of the child. The abuse and neglect has a negative effect on the child‘s development and (sometimes) survival [Glaser, 2000].

Table 9: Candidate gene studies for AUD

Gene	Polymorphism	Description	Sample number	Reference
DRD2 (11q23)	-141C	Ins/Del allele was significantly different between alcoholic and control subjects	130 cases, 251 controls	[Konishi et al., 2004]
	rs1800497	A1 allele higher in early onset alcoholics	100 cases, 93 controls	[Kono et al., 1997]
DRD4 (11p15.5)	Exon III	7-repeat allele positively associate with binge drinking	233 cases (binge drinking)	[Vaughn et al., 2009]
COMT (22q11.21)	rs4680 (p.val158met)	p.Met158 allele associated with higher drinking activity when there was less parental supervision	285 adolescents	[Laucht et al., 2012]
		p.Met158 allele associated with an increased risk of suicide in male alcoholics	393 cases with alcohol dependence, 609 controls	[Nedic et al., 2011]
BDNF (11p13)	rs6265 (p.val66met)	p.Met66 allele associated with alcohol use in older men	68 cases, 232 controls	[Shin et al., 2010]
		p.Met66 allele protective effect for substance-related disorders	meta-analysis	[Gratacòs et al., 2007]
ADH1B (4q23)	rs2066702 (p.arg370cys)	haplotype associated with habitual and heavy drinking	2299 controls	[Matsuo et al., 2007]
	rs1229984 (p.arg48his)	SNP associated with alcohol dependence	meta-analysis-9638 cases, 9517 controls	[Li et al., 2011]
		SNP associated with flushing, alcohol consumption and alcohol dependence	4597 subjects from 2618 families	[Macgregor et al., 2009]
		haplotype associated with habitual and heavy drinking	2299 controls	[Matsuo et al., 2007]

ADH1C (4q23)	rs1693482 (p.arg272gln)	SNP associated with decreased alcohol metabolic rates	250 participants	[Martínez et al., 2010]
		haplotype associated with habitual and heavy drinking	2299 controls	[Matsuo et al., 2007]
	rs698 (p.ile350val)	SNP associated with decreased alcohol metabolic rates	250 participants	[Martínez et al., 2010]
		haplotype associated with habitual and heavy drinking	2299 controls	[Matsuo et al., 2007]
5-HTT (17q11.2)	*5-HTTLPR*	short allele preferentially transmitted to alcoholics	92 triads	[Lichtermann et al., 2000]
		short allele predicted a greater alcohol consumption over time	202 non-regular adolescent drinkers	[van der Zwaluw et al., 2010]
		predicted alcohol consumption	200 participants	[Nilsson et al., 2005]
		short allele preferentially transmitted to alcoholics	130 cases, 251 controls	[Konishi et al., 2004]
ADH4 (4q22)	rs3762894	SNP associated with alcohol dependence	COGA	[Edenberg et al., 2006]
		SNP associated with alcohol dependence	226 cases, 110 controls	[Liu et al., 2011a]
		SNP suggestively associated with alcohol consumption	4597 subjects from 2618 families	[Macgregor et al., 2009]
GABRA2 (4p12)	rs9291283	Associated with alcohol dependence	291 cases, 295 controls	[Soyka et al., 2008]
	rs279858, rs279826	SNPs associated with alcohol dependence	173 families	[Villafuerte et al., 2012]

Table 10: GWASs conducted for AUD

Study Title	Reference	Phenotype	Initial[1] Sample Size (does not include replication sample size)	Ethnicity/Ancestry	Platform	Genome-wide Significant/Top Findings
Pooled association genome scanning for alcohol dependence using 104,268 SNPs: validation and use to identify alcoholism vulnerability loci in unrelated individuals from the collaborative study on the genetics of alcoholism	[Johnson et al., 2006]	AD	Cases: 120 Controls: 160	European American (COGA)	Affymetrix ~100k"	51 chromosomal regions
Genome-wide Association Study of Alcohol Dependence	[Treutlein et al., 2009]	AD	Cases: 487 Controls: 1358	European	Illumina Human-Hap 550	121 SNPs
A Genome-wide Association Study of Alcohol Dependence	[Bierut et al., 2010]	AD	Cases: 1897 Controls: 1932	European American and African American (COGA and SAGE)	Illumina Human 1M	15 SNPs
Genome-Wide Association Study of Alcohol Dependence Implicates a Region on Chromosome 11	[Edenberg et al., 2010]	AD	Cases: 1192 Controls: 692	European American and African American (COGA)	Illumina HumanHap1M	*SLC22A18, PHLDA2, NAP1L4, SNORA54, CARS, OSBPL5* (chr11)
A genome-wide association study of nicotine and alcohol dependence in Australian and Dutch populations[1]	[Lind et al., 2010]	AD	Cases: 1124 Controls: 1162	Australian	Illumina Infinium 1M	CTBP2- rs12761801 (chr 10q26)
Genome-wide association studies identify genetic loci related to alcohol consumption in Korean men	[Baik et al., 2011]	Alcohol Consumption	Cases: 1721	Korean	Affymetrix Human SNP array 5.0	12 SNPs on chr 12q24

A quantitative-trait genome-wide association study of alcoholism risk in the community: findings and implications	[Heath et al., 2011]	AD, factor score and heaviness of drinking factor score	Cases: 2062 Controls: 6692	Australian	Illumina 370K	*TMEM108, ANKS1A*
Association Analysis of Symptoms of Alcohol Dependence in the Molecular Genetics of Schizophrenia (MGS2) Control Sample	[Kendler et al., 2011a]	Symptoms of AD	Cases: 3169	European American and African American	Affymetrix 6.0	*KCNMA1, AKAP9, PIGG, CEACAM6*
Confirmation of prior evidence of genetic susceptibility to alcoholism in a genome-wide association study of comorbid alcoholism and bipolar disorder	[Lydall et al., 2011]	Comorbid alcoholism and BD	Cases: 143 Controls: 510	European	Affymetrix Gene Chip Human Mapping 500K	*CDH11, COL11A2, NMUR2, XPO1, SEMA5A*
Genome-wide association and genetic functional studies identify autism susceptibility candidate 2 gene (AUTS2) in the regulation of alcohol consumption[1]	[Schumann et al., 2011]	Alcohol Consumption	Cases: 26 316	European	Affymetrix 500K, Illumina HumanHap 300, Illumina 317K, Illumina 370K, Perlegen 600K	*AUTS2*-rs6943555 (chr 7q11)
A meta-analysis of two genome-wide association studies identifies 3 new loci for alcohol dependence[1]	[Wang et al., 2011]	AD	Cases: 1283 Controls: 1416	Caucasian (COGA, SAGE, OZALC)	Illumina Human 1M, Illumina HumanCNV370v1	131 SNPs
Genome-wide significant association between alcohol dependence and a variant in the *ADH* gene cluster	[Frank et al., 2012]	AD	Cases: 1333 Controls: 2168	European	Illumina Human610Quad, Illumina Human660w Quad	rs1789891 (between *ADH1B* and *ADH1C*-chr 4q23)
Genome-wide association study identifies 5q21 and 9p24.1 (KDM4C) loci associated with alcohol withdrawal symptoms	[Wang et al., 2012]	AD with withdrawal symptoms	Cases: 461 Controls: 408	Caucasian (COGA)	Illumina Human 1M	51 SNPs

Genome-Wide Association Study of Alcohol Dependence Implicates KIAA0040 on Chromosome 1q[1]	[Zuo et al., 2012a]	AD	Cases: 2090 Controls: 2026	European American and African American (COGA and SAGE)	Illumina Human 1M	*KIAA0040*
Genome-wide search for replicable risk gene regions in alcohol and nicotine co-dependence[1]	[Zuo et al., 2012b]	Alcohol and nicotine co-dependence	Cases: 1267 Controls: 1876	European American and African American (SAGE)	Illumina Human 1M	*SH3BP5-NR2C2*
A meta-analysis of two genome-wide association studies to identify novel loci for maximum number of alcoholic drinks[1]	[Kapoor et al., 2013]	Excessive alcohol consumption	Cases: 4915	European American (COGA,SAGE)	Illumina Human OmniExpress array 12.VI, Illumina Human 1 M	rs9523562(13q31.1), rs67666182 (chr8), *LMO1, PLCL1*
A Genome-Wide Association Study of Behavioral Disinhibition	[McGue et al., 2013]	Alcohol consumption and dependence	Cases: 7188	Caucasian	Illumina Human 660W-Quad	13 SNPs
Genome-wide association studies of maximum number of drinks[1]	[Pan et al., 2013]	Maximum number of drinks	Cases: 5824	Caucasian (COGA, SAGE, OZALC)	Illumina Human 1 M, ILLUMINA Human CNV370v1	rs11128951 (3p24.3)
Extended genetic effects of ADH cluster genes on the risk of alcohol dependence: from GWAS to replication	[Park et al., 2013]	AD	Cases: 117 Controls: 279	Korean	Illumina Human660 W	*ADH* gene cluster, *ALDH*
A genome-wide association study of alcohol-dependence symptom counts in extended pedigrees identifies C15orf53	[Wang et al., 2013]	AD, AD symptom count	Cases: 2322	Caucasian (COGA)	Illumina Human OmniExpress array 12.VI	C15orf53
NKAIN1-SERINC2 is a functional, replicable and genome-wide significant risk gene region specific for alcohol dependence in subjects of European descent	[Zuo et al., 2013b]	AD	Cases: 1409 Controls: 1518	European American (COGA,SAGE)	Illumina Human 1M	*NKAIN1-SERINC2*

Genome-wide significant association signals in IPO11-HTR1A region specific for alcohol and nicotine co-dependence	[Zuo et al., 2013c]	Alcohol and nicotine co-dependence	Cases: 818 Controls: 1396	European American (COGA,SAGE)	Illumina Human 1M	*IPO11, HTR1A*
Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci	[Gelernter et al., 2014]	AD	Cases and Controls: 5697	European American and African American (SAGE)	Illumina HumanOmni1-Quad v1.0	*ADH1B, ADH1C*
ALDH2 Is Associated to Alcohol Dependence and Is the Major Genetic Determinant of ―Daily Maximum Drinks" in a GWAS Study of an Isolated Rural Chinese Sample	[Quillen et al., 2014]	AD related phenotypes: flushing response and maximum drinks	Cases: 595	Chinese	Illumina Cyto12	*ALDH2*
Family-Based Association Analysis of Alcohol Dependence Criteria and Severity	[Wetherill et al., 2014]	Individual DSM-IV criteria for AD	Cases: 2322	European American (COGA)	Illumina Human OmniExpress array 12.VI	*OR51L1*
Exploring the genetic architecture of alcohol dependence in African-Americans via analysis of a genome-wide set of common variants	[Yang et al., 2014]	AD	Cases: 1719 Controls: 1156	African American	Illumina Omni 1-Quad	chr4

[1] Indicates a meta-analysis

3.1.1.4 Gene x Environment Interactions

Environment together with genetics plays an important role in the development of AD [Lesch, 2005]. Classical genetic studies, which include GWASs, assume that the effects of the identified risk gene/variant is the same across changing environmental conditions and that environmental risk factors have the same effect across different genotypes [Sher et al., 2010]. A gene x environment interaction occurs when the above assumptions are not met [Duncan and Keller, 2011]. In other words, the effects of the genes and environment on the phenotype are dependent on each other or when genes and environment "act together" [Dick and Kendler, 2012]. Gene x environment interactions are particularly pertinent in adolescent alcohol use, as there is still a certain degree of environmental control by the parent or adult guardian. Also, for an AUD to occur there has to be an initial exposure to alcohol. If alcohol is unavailable or restricted in a particular environment, any genotypic effects on the phenotype will be minimal or non-existent [Dick and Kendler, 2012].

Gene x environment interactions can be investigated in 2 ways: i) via latent genetic effects, based on a "genome" approach. This type of approach is exemplified by twin or relative studies, ii) molecular or biological perspective via a specific molecular variant. The advantage of the latter approach is that it provides an understanding of the biological aetiology of the phenotype in question [Kendler et al., 2011b].

In terms of the molecular perspective, there are three types of gene x environment effects: i) an **additive** effect is one whereby there is no significant interaction between the genes and environment of interest. In other words, there is a main effect of genes on the phenotype and a main effect of the environment on the phenotype but no interaction between the two. The effects of the genes and environment add together. This type of gene x environment effect is exemplified by Panel A in Figure 13, ii) a **fan-shaped** interaction is one whereby the effect of the genes and environment on the phenotype is dependent on each other. This type of interaction (Panel B in Figure 13) is characterised by the fact that genes have more of an effect on the outcome or the phenotype of interest in more adverse environments than in "normal" environments, and iii) a **cross-over** interaction is one in which the order of the genetic effect changes as a function of the environmental effect i.e. individuals at the lowest genetic risk in an benign environment will be at the highest genetic risk in the most adverse environment (Panel C in Figure 13) [Dick and Kendler, 2012; Kendler et al., 2011b].

One of the first few gene x environment interaction type studies, conducted by [Caspi et al., 2002] found that a polymorphism within the *monoamine oxidase A* (*MAOA*) gene interacted with childhood maltreatment to determine anti-social behaviour during adulthood. A subsequent study by the same authors found that the level of depression was moderated by an interaction between the *5-HTT* gene and stressful events [Caspi et al., 2003]. In the last couple of years, there has been a dramatic increase in the number of gene x environment (usually candidate genes) interaction-type studies, as opposed to the classical candidate-gene association studies. Kaufman et al. (2007) demonstrated that abused or neglected children are seven times more likely to abuse alcohol compared to those not abused during childhood. Also, that childhood maltreatment together with the *5-HTT* variant (*5-HTTLPR*) predicted risk of early alcohol use [Kaufman et al., 2007]. In another study, examining the circadian gene *PER2*, carriers of the G-allele of the SNP rs56013859 interacted with stressful life events to result in lower alcohol consumption than those individuals who were homozygous for the A-allele [Blomeyer et al., 2013]. Many other studies have also found significant gene x environment interactions for alcohol consumption [Nilsson et al., 2005; Covault et al., 2007; Ducci et al., 2007; Nilsson et al., 2007; Nelson et al., 2010; Miranda et al., 2013; Sartor et al., 2014; Kim et al., 2015].

One of the disadvantages of gene x environment interactions is that the power to detect an interaction is significantly less than detecting a main effect [Duncan and Keller, 2011]. Also, statistical interactions are not the same as biological interactions [Dick and Kendler, 2012]. Gene x environment interactions have been met with a lot of scepticism in the psychiatric genetic community as most of the previous significant findings had not been successfully replicated [Duncan et al., 2014]. One of the recommendations for future gene x environment interaction type studies is to employ a genome-wide by environment interaction approach- instead of investigating one or two candidate genes, and to investigate multiple variants spread across the genome [Duncan et al., 2014].

3.1.2 Aims and Objectives

AUD is a multifactorial disorder with unknown causative factors. The aim of this investigation was to determine whether variants previously implicated in other psychiatric disorders have an association with adolescent AUD and differential brain volume in AUD. An additional aim was to determine whether these genetic factors interact with childhood trauma to result in adolescent AUD.

3.1.2.1 Objectives

The objectives of this investigation were as follows:

1) Using Illumina BeadChip technology, determine whether variants previously associated with other psychiatric disorders, have an association with AUD.

2) Determine whether differential brain volumes predict AUD and whether the selected genetic variants have an association with brain volumes.

3) Determine whether there are interactions between the selected genetic variants and childhood trauma predicting adolescent AUD.

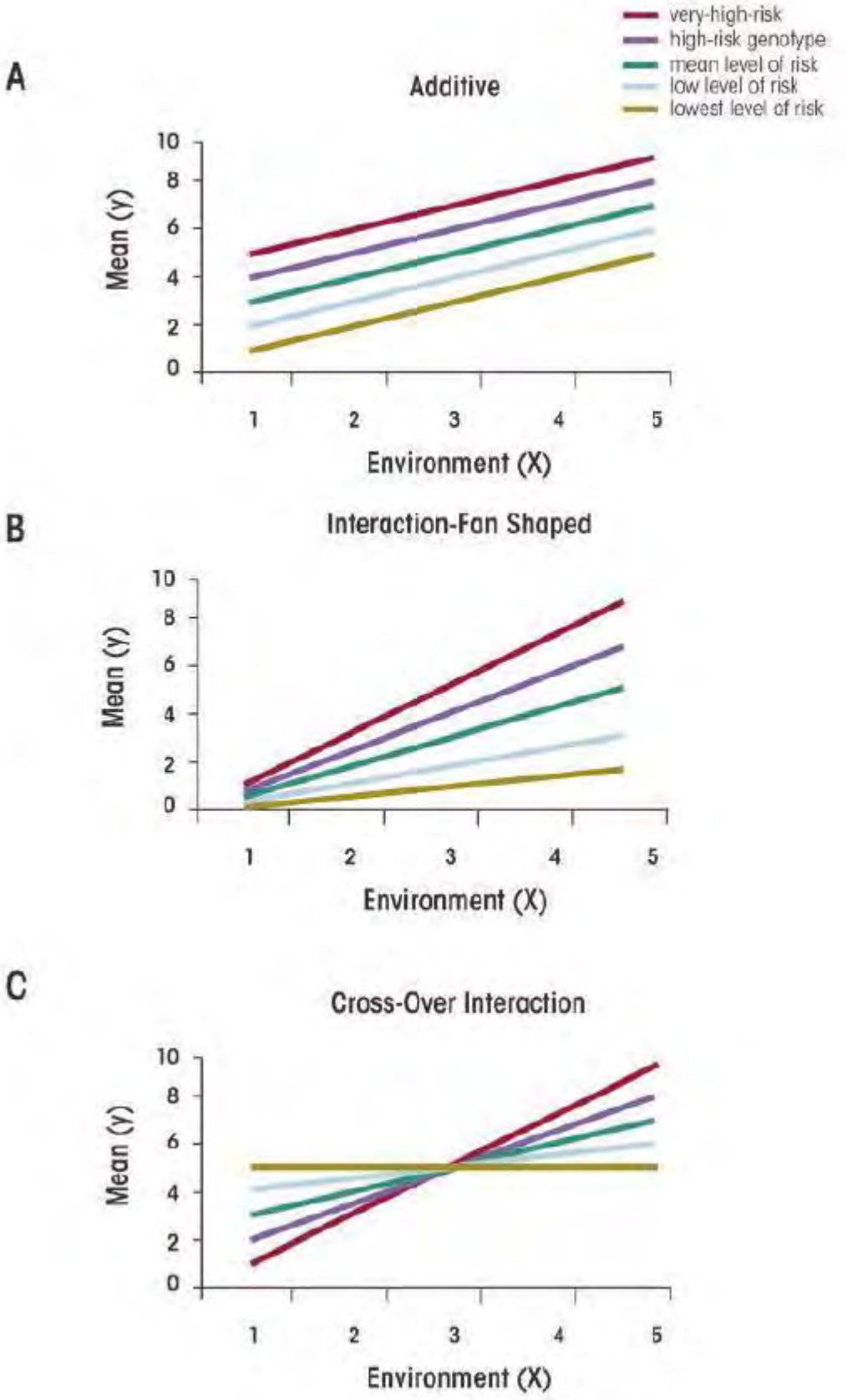

Figure 13: Representation of the three types of gene-environment interactions. The lines (in different colours) represent different genotypic risks to develop trait Y. The X-axis represents the environment, where level 1 is a benign environment and level 5 an adverse environment [Dick and Kendler, 2012].

3.2 Materials and Methods

3.2.1 Research Group

The research cohort investigated in this study was originally recruited as part of a collaborative project entitled *Effects of heavy alcohol abuse on adolescent brain structure and function'* conducted at the University of Stellenbosch, for which ethical approval was obtained (ethics reference number: N06/07/128). In addition, for the purposes of the current study, ethical approval was obtained from the local institutional (UCT) HREC (REF 023/2012). Subjects were recruited from 19 schools within the Cape Flats region of the Cape Town metropole. All participants were either English or Afrikaans speaking and were from moderately low socioeconomic backgrounds. Eligibility was assessed after a detailed medical history was taken, and physical and psychiatric examinations were undertaken. In addition, participants underwent urine analysis and breathalyser testing to ensure they were not intoxicated during the testing period. Written informed assent was obtained from each of the study participants and written informed consent was obtained from their parents or guardians. The consent form contained full details of all study-related activities in the language understandable by the study population [Fein et al., 2013].

Exclusion criteria for study participation included diagnoses of mental retardation, lifetime DSM-IV Axis I other than AUD, lifetime dosages exceeding 30 cannabis joints or 4 methamphetamine doses, current use of sedative or psychotropic medication, signs or history of fetal alcohol syndrome or malnutrition, sensory impairment, history of traumatic brain injury with loss of consciousness exceeding 10 minutes; presence of diseases that may affect the CNS, less than 6 years of formal education, and lack of proficiency in English or Afrikaans. Blood samples (3-4ml) were collected by venepuncture for all of these individuals, with the appropriate informed consent.

The final cohort consisted of 80 adolescents with DSM-IV AUD, and 80 age- and gender-matched light (lifetime dosage not exceeding 80 units of alcohol)/non-drinking controls (HC). All subjects (AUD and HC) were of mixed ancestry ethnicity. The Cape mixed ancestry population are a genetically admixed group, in fact, it is reported to have the highest level of intercontinental genetic admixture compared to other global populations [Tishkoff et al., 2009]. The ancestral composition of this unique population group is 32-43% Khoesan, 20-36% Bantu-speaking Africans, 21-28% European and 9-11% Asian [de Wit et al., 2010].

3.2.2 Clinical Measures

Psychiatric Diagnoses and Alcohol Use

To determine whether any of the participants had current or past psychiatric symptoms, the Schedule for Affective Disorders and Schizophrenia for School Aged Children (6–18 years) Lifetime Version (K-SADS-PL) [Kaufman et al., 1997], a semi-structured, clinician administered, diagnostic interview was used. In addition to the K-SADS-PL, the Timeline Followback (TLFB) procedure, a semi-structured, clinician administered assessment, was used to determine lifetime history of alcohol use and drinking patterns [Sobell and Sobell, 1992].

Childhood Adversity

Measures of childhood trauma were obtained for each of the study participants using the 28-item Childhood Trauma Questionnaire-Short Form (CTQ-SF) [Bernstein et al., 2003] (Appendix 2-Childhood Trauma Questionnaire) which is a reduced version of the initial 70-item Childhood Trauma Questionnaire (CTQ) [Bernstein et al., 1994]. The CTQ-SF is a self-report questionnaire consisting of five subscales, each measuring a specific dimension of childhood maltreatment, including: physical abuse (–bodily assaults on a child by an adult or older person that posed a risk of or resulted in injury"), sexual abuse (–sexual contact or conduct between a child younger than 18 years of age and an adult or older person"), emotional abuse (–verbal assaults on a child's sense of worth or well-being or any humiliating or demeaning behaviour directed toward a child by an adult or older person"), physical neglect (–the failure of caretakers to provide for a child's basic physical needs, including food, shelter, clothing, safety, and health care"), and emotional neglect (–the failure of caretakers to meet children's basic emotional and psychological needs, including love, belonging, nurturance, and support"). Each clinical dimension consists of five items which covers the necessary content from the original questionnaire and has sufficient reliability. Participants answer the questions in the context of –when you were growing up". The scores range from one to five for each question, with one corresponding to –never" and five to –very often", thus the CTQ subscale scores range between five and 25 [Bernstein et al., 2003]. The CTQ total score is a summation of the five subscales. A dichotomised clinical cut-off score which differentiates between significant abuse and neglect was generated for each of the subscales and were defined by the following: sexual abuse (CTQ-SA), physical abuse (CTQ-PA) and physical neglect (CTQ-PN) a score equal to eight or more, emotional abuse (CTQ-

EA) a score equal to ten or more, and emotional neglect (CTQ-EN) a score of 15 or more [Bevilacqua et al., 2012].

3.2.3 Genetic Analysis

DNA from the AUD and HC groups were extracted using the Maxwell® 16 Blood DNA purification kit (AS1010) (Promega) using the Maxwell 16 instrument (Promega) at the CPGR (Cape Town, South Africa). Each of the samples was genotyped using the Illumina Infinium iSelect custom 6000 BeadChip (Illumina Inc., San Diego, California, USA). This assay is based on a dual colour, single-base extension protocol and allows the user to prepare the DNA sample in a single tube without involving PCR or ligation steps. Briefly, this method consists of four main components: i) PCR-free whole genome amplification is performed and the DNA is fragmented, ii) array-based hybridization capture, whereby the unlabelled DNA fragment is hybridised to a 50-mer probe on the array, iii) an enzymatic single base, allele-specific primer extension, and iv) amplified signal detection [Steemers et al., 2006].

The Illumina Infinium iSelect custom 6000 BeadChip comprised approximately 5000 SNPs covering haplotype-tagging and previously reported SNPs in 295 candidate genes (genes involved in neurotransmitter and neuroendocrine systems) for PTSD, and 319 SNPs and CNVs which generally yielded statistically –significant hits" in previous psychiatric GWASs. The bead chip was run on the Illumina BeadStation 500G System at the University of Michigan DNA Sequencing Core (Michigan, USA). AUD and HC samples were analysed together. Standard clustering algorithms were utilised in the GenomeStudio software (Illumina) to make genotype calls.

The genotypes of three variants (*BDNF*-rs6265, *COMT*-rs4680 and *neuregulin 1* (*NRG1*)-rs6994992) were validated using the TaqMan® SNP Genotyping Assay, which were run on the AB7900HT qPCR instrument (Life Technologies) at the CPGR (Cape Town, South Africa). Assay QC measures included a no template control and a positive control. The TaqMan® method, which is based on the 5' exonuclease activity of *Taq* polymerase, consists of two locus-specific PCR primers and two allele-specific probes. The TaqMan® probe contains a fluorescent reporter dye at the 5' end and a non-fluorescent quencher dye at the 3' end. When the probe is unbound, reporter dye emission is inhibited by the quencher dye. However, when extension of the product of interest occurs, the exonuclease activity of the *Taq* polymerase cleaves the 3' end of the probe, which results in increased emission of the

fluorescent reporter dye. With each cycle of PCR, the reporter dye fluoresces only if the probe is annealed to the allele of interest [De la Vega et al., 2005].

3.2.4 Imaging Analysis

Magnetic Resonance Imaging (MRI) is able to provide a high resolution, three-dimensional view of soft tissues. The main advantage of this technique is its relative safety as it is non-invasive and does not involve any ionizing radiation [Leondes, 2005]. The MRI is obtained by the use of a tomograph. The main constituent of this instrument is a large cylindrical coil which functions as a magnet. There are additional coils which form part of an electric circuit, whereby the electrical current within the magnet is able to produce a steady magnetic field. The subject is placed inside the cylindrical coil and exposed to the magnetic field [Hennel et al., 1997].

MRI data was obtained for a subset of the cohort (58 AUD and 58 HC), as part of a previous study, on a 3T Siemans Magnetom Allegra Headscanner using Syngo MR software (Siemens Medical Solutions) [Fein et al., 2013]. The scanner is situated at the Cape Universities Brain Imaging Center (CUBIC) at the Stellenbosch University Health Sciences Campus, South Africa. Analysis of the imaging data was conducted in the Department of Psychiatry and Mental Health at UCT. Briefly, for 50 of the subjects, images were acquired using a transaxial T1-weighted acquisition, and 66 were acquired with the sagittal T1 protocol. MRIs in 28 subjects were acquired using both protocols. Manual reorientation and realignment of the cross-hair on the anterior and posterior commissure plane in all the nifti-converted DICOM T1 images were performed. Using the voxel-based morphometry (VBM) unified segmentation approach [Ashburner and Friston, 2005] in SPM8 [www.fil.ucl.acuk/spm8] initial quality control for signal artifacts, morphological changes were calculated in gray matter by segmenting from white matter and cerebrospinal fluid. Gray matter images, based on probability maps at each voxel, were spatially normalised using a pediatric template from the Cincinnati Children´s Hospital old children template [www.irc.cchmc.org/software/pedbrain.php] and then co-registered using the same segmented template [Brooks et al., 2014]. The following regions of interest (ROI) volumes were extracted using SPM8: Total matter, grey matter, white matter, cerebrospinal fluid (CSF), total intracranial, left caudate, right caudate, left amygdala, right amygdala, left superior temporal gyrus, right superior temporal gyrus, left dorsolateral prefrontal cortex (DLPFC), right DLPFC, left insula, right insula, left hippocampus, right hippocampus, left thalamus, right thalamus, left putamen, right putamen, left globus pallidus, right globus

pallidus, left posterior cingulate, right posterior cingulate, left occipital lobe, right occipital lobe, left precuneus, and right precuneus.

3.2.5 Statistical Analysis

3.2.5.1 Demographic and Clinical measures

Using SPSS [IBM, 2012], the Shapiro-Wilk test was used to determine whether the continuous variables, age, years of education, lifetime alcohol dose units, and CTQ measures, were normally distributed. The non-parametric Mann Whitney *U*-test was performed to determine whether there were differences in these variables between the genders and the groups (HC and AUD).

3.2.5.2 IBS Clustering

For case-control association studies, false positive results may be observed due to the presence of population stratification. Population stratification is when there are differences in the frequencies of ethnic groups in cases and controls and thus a difference in allele frequencies not due to the disease/phenotype of interest. This may also occur in admixed population groups, whereby ~~"~~different fractions of ancestry from each ancestral sub-population are observed". Historically this has been overcome by the careful matching of cases and controls and the use of family-based association studies [Enoch et al., 2006].

In this study, cases and controls were matched but in addition to this, IBS clustering was performed using the whole genome association analysis toolset, Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007], in order to account for possible population stratification within the mixed ancestry group. Using pairwise identity by state (IBS) distance, this process uses a linkage agglomerative clustering method to determine whether two individuals belong to the same or different population groups. For this type of analysis, genome-wide SNP data is required, however, only the genotyped SNPs were available. This test was run without any constraints.

3.2.5.3 Association Analysis

a) Single-SNP

Using the program Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007], it was determined that all samples had a call rate of greater than 0.99. Before genotyping and frequency pruning there were 4656 SNPs. A total of 9 SNPs failed the _missingness' test (i.e. only SNPs with a genotyping rate of 90% or more were included) and

583 SNPs were excluded because of a MAF of less than 5%. A total of 4 SNPs were excluded as these were not in Hardy-Weinberg equilibrium (HWE) (p-value less than 0.00001). Therefore, after genotyping and frequency pruning there were 4065 SNPs used for downstream analysis.

Logistic regression was used to test whether any of the SNPs had an association with AUD. IBS cluster ID was included as a covariate, an additive genetic test was used, and all tests were adjusted for multiple testing using the Bonferroni test [Dunn, 1961]. To visualise the chromosomal location of the significant SNPs from the Plink output, the program Haploview 4.2 [Barrett et al., 2005] was used.

b) Gene-Based

Both SNP-based and gene-based tests have their respective advantages and disadvantages. A recent study found that gene-based tests were more powerful than the SNP-based test in detecting susceptibility loci for the addiction phenotype [Guo et al., 2012]. Gene-based tests are thought to be a useful way of overcoming the limitations of GWAS and candidate gene-based association studies [Fabbri et al., 2013].

A gene-based test was performed in Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007], whereby SNPs from the same gene were grouped together and tested for association to AUD. Intergenic and control SNPs were excluded from the analysis. Gene-based tests were calculated in the following way: 1) determined which SNPs were in LD, above a specified r^2 threshold; 2) single SNP association to phenotype was carried out; 3) ~~in~~dependent" SNPs were selected (as defined by step 1) which were lower than the specified p-value. SNPs were selected in order of decreasing statistical significance, after removing SNPs in LD with previously selected SNPs, 4) a set statistic was calculated as the mean of the single SNP statistics, 5) the dataset was permuted 10 000 times, 6) steps 2 to 4 were repeated for each permutation, 7) the empirical p-value was the number of times the permuted set statistic was higher than the original p-value calculated for that set.

A logistic regression gene-based test was run with cluster assignment as a covariate, SNPs with a MAF of less than 0.05 and a _missingness' rate of greater than 90%, excluded. A total of 10 000 permutations were run, with the default values for r^2 (0.5), p-value (0.05) and the maximum number of SNPs (5).

3.2.5.4 Pathway Analysis

In order to determine which biological processes were over-represented in the uncorrected significant SNPs, a "pathway based analysis" was carried using the web-based program Webgestalt (WEB based GEne SeT AnaLysis Toolkit) [http://bioinfo.vanderbilt.edu/webgestalt/]. The function of this program is to "translate gene lists into biological insights". A list of the significant SNPs (uncorrected p-value < 0.05) from the association analysis was used as input. The following enrichment tools were used: Kegg analysis, Pathway Commons analysis and Disease Association analysis. For each separate analysis, the *homo sapiens* genome was selected as the reference genome, and default values were kept for the other parameters (statistical method: hypergeometric, multiple adjustment method: Benjamini & Hochberg, significance level: top 10, minimum number of genes for a category: 2).

3.2.5.5 Polygenic Profile Scoring

GWASs have not had much success in identifying replicable markers which account for the phenotypic variance observed in psychiatric disorders. It is now believed that the unexplained heritability may be due to the host of genetic markers which do not obtain significant associations in GWASs [Dudbridge, 2013]. The polygenic scoring procedure was employed to determine whether there is overlap in common "risk" variants of small effect (not obtaining the threshold p-values in GWASs), between AUD and other psychiatric disorders. The use of this method has had success in identifying variants for SCZ treatment outcomes [Frank et al., 2014]. This procedure was first proposed by Purcell et al. (2009) and the basis of the method followed, is described below.

1) Firstly, data was obtained from a "discovery" dataset. In this instance, genotyping data for BD, MDD and SCZ were obtained from the PGC website [https://pgc.unc.edu/Sharing.php#SharingOpp] (Date accessed: 19 October 2012).

2) Secondly, SNPs were selected which overlapped between the Illumina bead chip and the three PGC datasets and were below a certain p-value threshold. Overlapping SNPs were determined by the use of the "merge" function in the statistical environment R [http://www.r-project.org/]. The number of overlapping SNPs in each dataset at varying thresholds are listed in Table 11.

3) The following formula was used to calculate the polygenic profile score:

Polygenic Profile Score= $S_1*W_1 + S_2*W_2 + S_3*W_3 + ... n_i$

S_i= no. of reference alleles the individual has at SNP_i

W_i= log odds ratio of SNP_i

This score was calculated using the statistical programme Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007] using the –score" option.

4) Logistic regression was used to test whether the polygenic score for each individual had an association with AUD, using the statistical environment R [http://www.r-project.org/].

Table 11: Number of overlapping SNPs for each threshold between the current genotype data and PGC datasets

p-value threshold	Bipolar disorder	Major depressive disorder	Schizophrenia
	No. SNPs	No. SNPs	No. SNPs
All	3528	2470	2479
< 0.1	576	291	424
< 0.2	940	557	693
< 0.3	1319	796	941
< 0.4	1670	1017	1156
< 0.5	1993	1259	1374

3.3.5.6 Gene x Environment Interaction Analysis

To test whether any of the genotypes interacted with an adverse environment to result in a high lifetime alcohol dose, gene x environment calculations were carried out using Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007]. The (dichotomised) CTQ subscales which were significantly different between the AUD and HC groups were used as the environmental risk factors in this analysis.

3.2.5.7 Gene-Imaging Analysis

To test whether the variance in the ROI brain volumes could be explained by the SNPs on the bead chip, a SNP by brain volume association was conducted using Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007] on a subset of the total cohort (58 AUD and 58 HC). A linear regression test was used and the following were included as covariates in the model: age, gender, education, handedness, intracranial volume

and protocol (transaxial vs sagittal). All analyses were adjusted for multiple testing. As with the association tests (with AUD diagnosis), SNPs with a MAF of less than 0.05, not in HWE (p-value < 0.00001) and a genotype ‗missingness' rate of greater than 10% were excluded.

To verify the initial association findings, the main effects of group (AUD and HC) and of the identified significant SNP genotypes on brain volume data, 2 x 2 ANCOVA using VBM in the SPM8 package [www.fil.ucl.acuk/spm8] was implemented. AUD and HC subjects were matched in terms of age, gender, and protocol (transaxial vs sagittal). Age, total matter volume and total CTQ score was were added as covariates of no interest to control for global differences in head size and emphasise local volume differences. All statistical analyses were corrected for multiple comparisons at the peak voxel level using the family-wise error (FWE).

3.3 Results

3.3.1 Research Group

The research cohort consisted of 80 adolescents with AUD and 80 age- and gender- matched control subjects (Table 12). The total cohort consisted of 66 males and 94 females. In both the AUD and HC groups, there were 47 females and 33 males. The median ages of the AUD and HC groups were 14.94 and 14.92 years, respectively. In the AUD group, the median age of onset was 12 years. The majority of the participants were Afrikaans speaking and the median number of years of education was 8.0 years for both groups (AUD and HC). The same variables, as mentioned above, are reported for the imaging cohort (58 AUD and 58 HC) in Table 12. The values for the reported variables do not differ greatly between the larger (n=160) and smaller (n=116) cohorts.

3.3.2 Clinical Measures

The median number of lifetime alcohol dose units for the AUD and HC groups in the total cohort was 1125.50 and 1.0, respectively, where a unit refers to one beer or wine cooler, one glass of wine, or one 43g shot of liquor (on its own, or in a mixed drink) (Table 12). In the total cohort, the median total CTQ score was 42.5 and 36.50 for the AUD and HC groups, respectively. The minimum CTQ total score was 25 and the maximum 83. The median values for each of the CTQ subscales are also reported in Table 12. Figure 14 represents the frequency of participants in the AUD and HC groups reporting clinically significant abuse and neglect. In the AUD group, the most prevalent CTQ subscale was PN, with 60.3% of the participants reporting significant CTQ-PN.

3.3.3 Statistical Analysis

The quantitative variables (age, years of education, alcohol life dose units, and CTQ measures) were not normally distributed (Shapiro-Wilk Test p-value <0.05), therefore non-parametric tests were used in subsequent analyses. As expected, adolescents with AUDs had significantly higher lifetime doses of alcohol than the HC group (Mann Whitney U-test p-value<0.001; Table 13). The difference in median total CTQ scores was statistically significant (Mann Whitney U-test p-value= 0.023) (Table 13). There were significant differences (p-value < 0.05) in CTQ-SA, EA and PN between the AUD and HC groups, whereby these subscale scores were higher in the AUD compared to the HC group. In the AUD group, males reported higher physical and sexual abuse compared to females and this was statistically significant (p-value < 0.05).

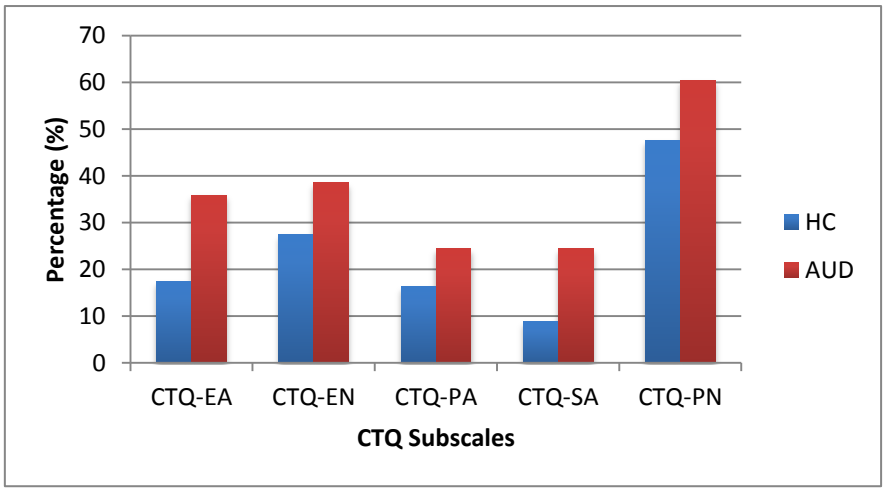

Figure 14: Clinically significant abuse and neglect for HC and AUD groups.

Table 12: Median values and interquartile range for cohort characteristics and clinical measures

Variable	**AUD**			**HC**		
Total cohort *Imaging cohort*[1]	**Females** (*n*= 47) *Females (n= 33)*	**Males** (*n*= 33) *Males (n= 25)*	**Total** (*n*=80) *Total (n=58)*	**Females** (*n*= 47) *Females (n= 33)*	**Males** (*n*= 33) *Males (n= 25)*	**Total** (*n*=80) *Total (n=58)*
Age (years)	14.89 (15.59-14.31) *14.89 (15.64-14.06)*	14.98 (15.51-14.52) *15.17 (15.57-14.52)*	14.94 (15.53-14.47) *14.98(15.60-14.41)*	14.89 (15.32-14.4) *14.71(15.14-14.35)*	14.94 (15.45-14.23) *15.07(15.54-14.32)*	14.92 (15.33-14.36) *14.77 (15.33-14.35)*
Education (years)	8.0 (8.0-7.0) *8.0 (9.0-7.0)*	8.0 (8.0-7.0) *8.0 (8.0-7.0)*	8.0 (8.0-7.0) *8.0 (8.0-7.0)*	8.0 (8.0-7.0) *8.0 (8.0-7.0)*	8.0 (8.0-7.0) *8.0 (8.0-7.0)*	8.0 (8.0-7.0) *8.0 (8.0-7.0)*
% Afrikaans-speaking	63.8 *60.6*	75.8 *72.0*	68.8 *65.5*	63.8 *69.7*	78.8 *84*	70.0 *75.9*
Alcohol life dose units[2]	1152.0 (2008.0-480.0) *892.0 (1980.0-442.0)*	1012.0 (2076.0-396.0) *1012.0 (2076.0-396.0)*	1125.50 (2032.0-441.0) *962.0 (1987.0-429.0)*	1.0 (9.00-0) *1.0 (9.00-0)*	1.0 (4.0-0) *1.0 (3.5-0)*	1.0 (5.75-0.0) *1.0 (4.25-0.0)*
CTQ-Physical Abuse	5.0 (7.0-5.0) *5.0 (7.0-5.0)*	7.0 (11.0-5.0) *7.0 (12.0-5.0)*	5.0 (7.25 -5.0) *6.0 (8.5-5.0)*	5.0 (7.0-5.0) *5.0 (6.5-5.0)*	5.0 (6.0-5.0) *5.0 (6.0-5.0)*	5.0 (7.0-5.0) *5.0 (6.0-5.0)*
CTQ-Sexual abuse	5.0 (6.0-5.0) *5.0 (5.0-5.0)*	6.0 (9.0-5.0) *7.0 (12.0-5.0)*	5.0 (7.25-5.0) *5.0 (7.0-5.0)*	5.0 (5.0-5.0) *5.0 (5.0-5.0)*	5.0 (5.0-5.0) *5.0 (5.0-5.0)*	5.0 (5.0-5.0) *5.0 (5.0-5.0)*
CTQ-Emotional Abuse	8.0 (11.0-6.0) *8.0 (11.0-6.0)*	9.0 (12.0-5.0) *9.0 (12.0-6.0)*	8.5 (11.25-6.0) *8.0 (11.0-6.0)*	6.0 (9.0-5.0) *5.0 (8.5-5.0)*	6.0 (8.0-5.0) *6.0 (8.5-5.0)*	6.0 (8.75-5.0) *6.0 (8.25-5.0)*
CTQ-Physical Neglect	9.0 (11.0-7.0) *8.0 (11.0-6.5)*	9.0 (13.0-6.0) *9.0 (13.0-6.0)*	9.0 (12.0-6.0) *8.5 (12.0-6.25)*	6.0 (9.0-5.0) *5.0 (9.0-5.0)*	8.0 (10.5-5.0) *7.0 (12.0-5.5)*	7.0 (9.0-5.0) *7.0 (9.25-5.0)*

CTQ-Emotional Neglect	12.0 (17.0-7.0) *12.0 (17.5-7.5)*	12.0 (19.0-8.0) *12.0 (18.0-8.0)*	12.0 (18.0-8.0) *12.0 (17.75-8.0)*	10.0 (13.0-6.0) *10.0 (13.0-6.0)*	11.0 (18.5-8.0) *13.0 (21.0-9.0)*	11.0 (15.0-7.0) *11.0 (17.0-7.75)*
CTQ-total score	42.0 (51.0-34.0) *42.0 (48.0-34.5)*	44.0 (61.0-36.0) *44.0 (70.0-37.0)*	42.5 (54.25-34.75) *42.0 (52.0-36.0)*	35.0 (46.0-29.0) *35.0 (46.0-29.0)*	39.0 (47.0-30.5) *39.0 (52.0-30.5)*	36.50 (46.0-29.25) *36.0 (49.0-29.75)*

[1] Imaging cohort values indicated in italics

[2] A unit refers to one beer or wine cooler, one glass of wine, or one 43g shot of liquor

Table 13: Comparison of cohort characteristics for HC and AUD groups- p-values from Mann Whitney U- test

	AUD	**HC**	**HC vs AUD**		
	Females vs Males	Females vs Males	Females	Males	Total
Age (years)	0.581 *0.388*[2]	0.949 *0.162*	0.589 *0.254*	0.401 *0.535*	0.313 *0.159*
Education (years)	0.646 *0.729*	0.132 *0.689*	0.517 *0.706*	0.192 *0.671*	0.751 *0.577*
% Afrikaans-speaking[1]	0.257 *0.366*	0.151	1.000 *0.438*	0.769 *0.306*	0.864 *0.221*
Alcohol life dose units	0.503 *0.881*	0.385 *0.538*	**<0.001** ***<0.001***	**<0.001** ***<0.001***	**<0.001** ***<0.001***
CTQ-Physical Abuse	**0.004** ***0.008***	0.561 *0.807*	0.983 *0.678*	**0.003** ***0.001***	0.055 *0.031*
CTQ-Sexual abuse	**0.002** ***0.036***	0.455 *0.729*	0.398 *0.615*	**<0.001** ***0.001***	**0.001** ***0.030***
CTQ-Emotional Abuse	0.996 *0.950*	0.806 *0.967*	**0.025** ***0.034***	**0.020** ***0.012***	**0.001** ***0.005***
CTQ-Physical Neglect	0.644 *0.658*	0.199 *0.084*	**0.014** ***0.025***	0.322 *0.388*	**0.013** *0.074*
CTQ-Emotional Neglect	0.645 *0.747*	0.109 *0.072*	0.128 *0.226*	0.984 *0.542*	0.263 *0.881*
CTQ-total score	0.200 *0.362*	0.384 *0.203*	**0.048** *0.080*	0.056 *0.090*	**0.009** *0.062*

[1]Pearson chi-squared test (*d.f.* =1).

[2]Imaging cohort values are indicated in italics.

3.3.3.1 IBS Clustering

The cohort was divided into four clusters, indicating population substructure within the mixed ancestry group. The cluster assignment for each individual was used as a covariate in subsequent association testing. The biggest and smallest clusters consisted of 65 and 14 individuals, respectively.

3.3.3.2 Association Analysis

a) Single-SNP

Logistic regression was used to test whether any of the SNPs had an association with AUD. A total of 192 SNPs obtained a p-value of less than 0.05. However, after correction for multiple testing, none of these SNPs remained statistically significant. Figure 15 represents

the 192 SNPs and the respective chromosomal locations of these SNPs. Table 14 lists the top ten SNPs with the lowest p-values for the single SNP association. The most significant SNP was rs7105258 (t-statistic= 3.12, OR= 3.091, uncorrected p-value=0.0018), located in the gene *glutamate receptor, ionotropic kainate 4* (*GRIK4*) (chr11q22.3). These results indicate that the A-allele of this SNP increases the risk of having an AUD. Appendix 2 (Single-SNP Association Results) lists all the 192 SNPs and the corresponding test statistics and p-values.

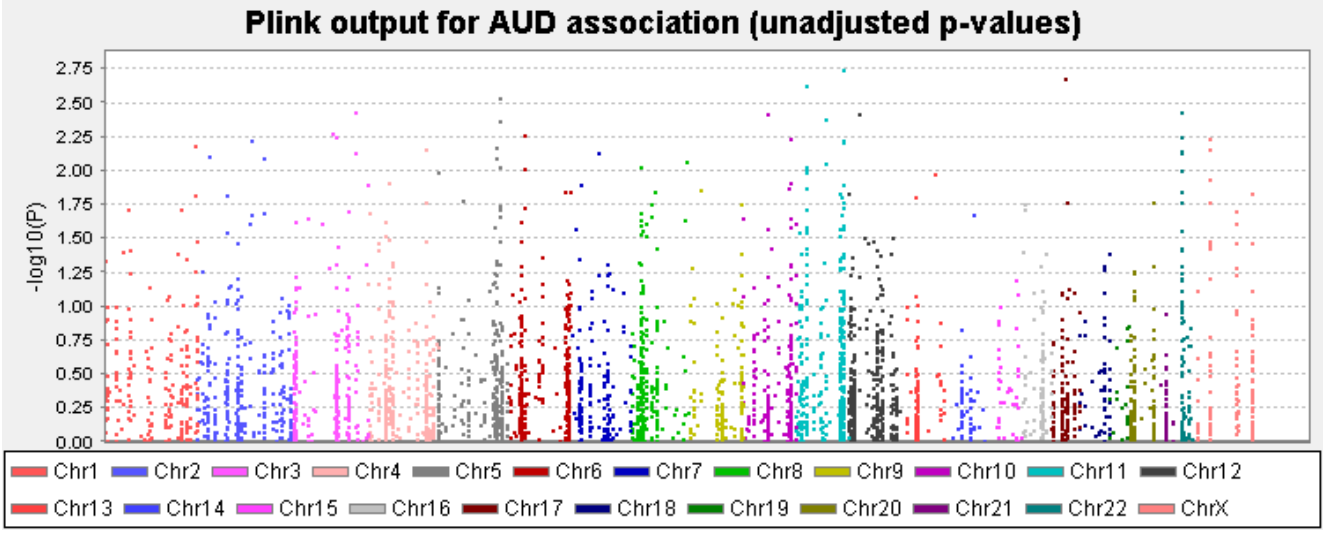

Figure 15: Haploview output. Manhattan Plot with Plink association results for AUD (Y-axis represents the uncorrected -log p-values).

b) Gene-Based

The gene which obtained the most significant association to AUD was *nuclear receptor subfamily 1, group D, member 1* (*NR1D1*) (p-value=0.003), consisting of 5 SNPs in the set, with 1 SNP being significant (rs2071570). The second-most significant SNP (p-value=0.006) is located in the gene, *basic helix-loop-helix family, member e41* (*BHLHE41*, also known as *BHLHB3* and *DEC2*). This set consisted of four SNPs, with rs3809140 being the only significant SNP in the set. Both of the above-mentioned genes form part of the circadian pathway and were in the top ten SNPs for the single SNP association (Table 14). Appendix 2 (Gene-Based Results) lists the results for all the genes and corresponding SNPs analysed.

Table 14: Top ten results from single SNP association to AUD

Chromosome	SNP	Gene	Minor allele	Odds Ratio	t-statistic	Uncorrected p-value
11	rs7105258	*GRIK4*	A	3.091	3.12	0.0018
17	rs2071570	*NR1D1*	A	0.3624	-3.077	0.0021
11	rs11030119	*BDNF*	A	0.4038	-3.034	0.0024
5	rs10515806	*ADRA1B*	A	0.1805	-2.971	0.0030
5	rs10515805	*ADRA1B*	A	0.1805	-2.971	0.0030
22	rs5993882	*COMT*	C	2.225	2.897	0.0038
3	rs11925572	Downstream from *BCHE*	A	3.356	2.896	0.0038
12	rs3809140	*BHLHE41*	G	0.4546	-2.892	0.0038
10	rs10509117	*ANK3*	A	2.771	2.886	0.0039
11	rs528833	*ARRB1*	A	0.3552	-2.857	0.0043

3.3.3.4 Pathway Analysis

Pathway analysis was conducted using the 192 uncorrected significant SNPs (comprising 96 genes). Using Kegg, the *neuroactive ligand-receptor interaction* was the most significant pathway comprising 24 genes and with an adjusted p-value of $5.69e^{-31}$. From the Pathway Commons analysis, the most significantly represented pathway, comprising 19 genes and with an adjusted p-value of $6.91e^{-20}$, was the *g-protein coupled receptor ligand binding* pathway. The mental disorders most significantly represented by the genes were *depression* and *anxiety* with an adjusted p-value of $7.59e^{-43}$ and comprising 28 and 27 genes, respectively.

3.3.3.5 Polygenic Profile Scoring

From the logistic regression analysis, none of the polygenic scores generated for each of the individuals in the cohort, had an association with AUD (p-value < 0.05).

3.3.3.6 Gene x Environment Interaction Analysis

Gene x environment interactions were calculated using a regression analysis. All of the SNPs (n=4065) were tested for interaction with the CTQ subscales SA, EA and PN. The outcome measure was lifetime alcohol dosage. None of the SNPs obtained a significant interaction (corrected p-value < 0.00001) for any of the CTQ measures. For SA, the top SNP was

rs1538974 (z-score = -3.98, p-value = 6.904e-005) located in the gene *disrupted in schizophrenia 1* (*DISC1*). For EA, the top SNP was rs12010790 (z-score = -4.301, p-value = 1.7e-005) upstream of the gene *gamma-aminobutyric acid A receptor, theta* (*GABRQ*) and PN, rs2298703 (z-score = -3.913, p-value = 9.128e-005) located in the gene *neural cell adhesion molecule 1* (NCAM1).

3.3.3.7 Gene-Imaging Analysis

From the association analysis, only one SNP, rs219927, located in an intron of the gene *glutamate receptor, ionotropic, N-methyl D-aspartate 2B* (*GRIN2B*) (chr 12p12) was associated with ROI brain volume in the left posterior cingulate cortex (t-stat=-4.806, beta = -0.03358, corrected p-value< 0.05). However, this SNP had a relatively small effect size. From this, four ~~"f~~unctional" variants (two exonic [rs1806201 and rs7301328] and two located in the 3'UTR [rs890 and rs1805502]) within the *GRIN2B* gene were investigated to determine whether these variants were associated with variation in brain volume that was not detected in the earlier association tests due to the multiple testing burden. It was found that the 3'UTR SNP, rs890, was associated with brain volume in the left and right DLPFC (t-stat=-2.736, beta = -0.0178, corrected p-value< 0.05).

ANCOVA and post-hoc t-tests for brain volume and genotype (rs219927 and rs890 in two separate ANCOVA analyses) were conducted using VBM. There were no FWE-corrected significant findings and no main effects for group (AUD and HC) were observed for any of the brain volumes. In terms of uncorrected findings, a main effect for rs219927 was found in the left orbitofrontal cortex (OFC) (x=-21, y=37, z=-16, uncorrected p-value< 0.05) (Table 15). Post-hoc tests indicated that volume in the left OFC was smaller in individuals with AUD and a GG genotype, although this association was only nominally significant (uncorrected p-value= 0.05). Furthermore, t-tests indicated that AUD individuals with the AG genotype had larger volume in the left-mid posterior cingulate (x=-1, y=-48, z=55, uncorrected p-value< 0.05) (Table 15). For the SNP, rs890, a main effect of genotype was observed in the left parahippocampal gyrus (x=-24, y=-30, z=-21, uncorrected p-value< 0.001) (Table 16) from the ANCOVA analysis. However, this finding was not confirmed with post-hoc t-tests.

Table 15: 2 x 2 ANCOVA with intronic non-functional SNP rs219927 matched for age, gender, group and protocol (with total matter volume age, gender and total CTQ as covariates of no interest).

	MNI Coordinates						
Brain region	***x***	**y**	**z**	**Brodmann Area**	**Cluster Size (Voxels)**	**Z-statistic**	**P-value**
Ancova Analyses							
Main effect of group							
Right posterior cingulate/parietal lobe	19	-57	49	23	379	4.64	0.08
Main effect of genotype							
Left orbitofrontal cortex	-21	37	-16	11	891	5.12	0.01
Post-Hoc t-tests							
Between-group genotype (AA): HC > AUD							
---	---	---	---	---	---	---	---
Between-group genotype (AA): AUD > HC							
Right posterior cingulate/parietal lobe	46	-34	41	40	712	3.96	0.05
Between-group genotype (AG): HC > AUD							
---	---	---	---	---	---	---	---
Between-group genotype (AG): AUD > HC							
Left-mid posterior cingulate/parietal lobe	-1	-48	55	5	2578	4.09	0.01
Between-group genotype (GG): HC >AUD							
Right posterior cingulate/parietal lobe	21	-51	50	7	572	4.37	0.05
Left orbitofrontal cortex	-38	37	-1	47	402	4.20	0.05

Between-group genotype (GG): AUD > HC							
---	---	---	---	---	---	---	---

MNI-Montreal Neurological Institute Coordinates, CTQ-Childhood Trauma Questionnaire

Table 16: 2 x 2 ANCOVA with functional SNP rs890 matched for age, gender, group and protocol (with total matter volume age, gender and total CTQ as covariates of no interest).

	MNI Coordinates						
Brain region	***x***	**y**	**z**	**Brodmann Area**	**Cluster Size (Voxels)**	**Z-statistic**	**P-value**
Ancova Analyses							
Main effect of group							
Right posterior cingulate/parietal lobe	19	-57	49	23/5	379	4.64	0.08
Main effect of genotype							
Left parahippocampal gyrus	-24	-30	-21	36	1073	2.91	<0.001

MNI-Montreal Neurological Institute Coordinates, CTQ-Childhood Trauma Questionnaire

3.4 Discussion

This investigation sought to determine the complex biological interactions underlying adolescent AUD using genetic, brain imaging, and environmental data. From the analysis of the genetic data, no single SNP was found to be associated with adolescent AUDs, after correction for multiple testing. However, the circadian rhythm genes *NR1D1* and *BHLHE41* were associated with AUD in a gene-based test. Childhood trauma was shown to be higher in adolescents with AUD, compared to matched controls. No significant gene x environment interaction was found for lifetime alcohol units. The gene-imaging analysis identified a SNP in the *GRIN2B* gene as being associated with brain volume in the left OFC and posterior cingulate.

No single SNP was found to be statistically associated with AUD. This finding adds further evidence that the aetiology of AUD may be as a result of multiple genes of small effect [Johnson et al., 2006; Heath et al., 2011; Yang et al., 2014] that were not detectable in this relatively small sample size. Although no single SNP reached statistical significance, it is worth noting that the most significant SNP was the rs7105258 intronic SNP, located in the glutamate ionotropic receptor gene, *GRIK4* (11q22.3). Variation within *GRIK4* has previously been associated with BD and SCZ [Pickard et al., 2006; Pickard et al., 2008]. While this is the first time that this particular SNP and gene have been implicated in AUD, previous studies have highlighted the role of the glutamate system in AUDs, with variation in the *glutamate receptor, ionotropic, kainite 1* (*GRIK1*) gene associated with AD [Kranzler et al., 2009] and variants in the *glutamate receptor, ionotropic, N-methyl D-aspartate 2A* (*GRIN2A*) gene shown to have an association with AD, positive family history, an earlier age of onset, risky drinking patterns in adolescents and maximum number of drinks for adults [Schumann et al., 2008]. Further work with larger samples is needed to explore the role of *GRIK4* in particular.

From the gene-based analysis, *NR1D1* and *BHLHE41* were identified as being significantly associated with AUD. Both *NRID1* (also known as *REV-ERBα*) and *BHLHE41* play essential roles in the circadian pathway (the protein encoded by the *BHLHE41* gene is a transcriptional repressor) [Fujimoto et al., 2007]. This is consistent with animal and human evidence that circadian disturbances may be relevant to AUDs [Chen et al., 2004; Hatonen et al., 2008]. *NR1D1* is an orphan nuclear hormone receptor gene which is a ―gatekeeper" for appropriate co-ordination of the circadian response [Duez and Staels, 2009] by regulating the transcription of the clock gene, *aryl hydrocarbon receptor nuclear translocator-like* (*ARNTL*)

[Preitner et al., 2002]. This is the first time this particular gene variant has been associated with AUD but it has been associated with BD [Kripke et al., 2009] and it may well play a role in a range of neuropsychiatric disorders given the importance of circadian rhythms in a variety of psychobiological processes [Merrow et al., 2005]. Similarly, *BHLHE41* which is rhythmically expressed in the suprachiasmatic nucleus, is involved in the repression of the clock circadian regulator, (*CLOCK*)/*ARNTL* heterodimer dependent transcription of the *period circadian clock 1* (*PER1*) gene [Honma et al., 2002]. As with *NR1D1*, this gene and variant has also not previously been implicated in AUD.

Even though AUD has proven to have a considerable genetic heritability [Prescott and Kendler, 1999; Epps and Holt, 2011], it is clear that environment plays a substantial role in whether an individual will abuse illicit substances or not. In this study, childhood trauma was more prevalent in adolescents with AUD compared to control individuals. In particular, sexual and emotional abuse, and physical neglect were higher in the alcohol using group. This has been found in previous studies whereby an adverse childhood environment predicted substance use later in life [Duncan et al., 1996; Felitti et al., 1998; Dube et al., 2006; Khoury et al., 2010; Nelson et al., 2010; Copeland et al., 2011; Sartor et al., 2013]. Childhood maltreatment is also associated with a higher occurrence of comorbid personality disorders in individuals who abuse substances [Bernstein et al., 1998]. These findings clearly illustrate the long-term effects that adverse events during childhood have on psychopathology during adolescence and eventually adulthood.

From the gene-imaging analysis, it was noted that a SNP within the *GRIN2B* gene was associated with differential brain volume. *GRIN2B* (12p12) encodes the 2B subunit of the ionotropic N-methyl-D-aspartate (NMDA) glutamate receptor [Collingridge et al., 2009] and in brain tissue is primarily expressed in the fronto-parieto-temporal cortex and the hippocampal pyramidal cells [Schito et al., 1997]. The NMDA receptor is an ion gated-channel which plays a role in the process of long term potentiation (LTP) and is thought to be involved in learning and memory [Ishii et al., 1993]. In this investigation we found a nominal association between the intronic SNP, rs219927, and smaller volume in the left OFC and larger volume in the left posterior cingulate. The OFC has been implicated in the neural process of decision-making [Bechara et al., 2000] and reward-related behaviour in response to taste, smell and visual cues [Rolls, 2000; Kringelbach, 2005] and the posterior cingulate is involved in memory retrieval [Maddock et al., 2001]. Both the posterior cingulate and orbitofrontal cortices have previously been implicated in the response to alcohol and

addictive behaviour [Volkow and Fowler, 2000; Tapert et al., 2003; Hill et al., 2009; Acheson et al., 2014; Camchong et al., 2014]. Genetic polymorphisms within *GRIN2B* have previously been associated with variation in brain volume. In particular the SNP, rs890 in *GRIN2B* was shown to have an association with reduced fractional anisotropy (FA) in several brain areas, including the bilateral frontal region and left cingulate gyrus, in individuals with BD [Kuswanto et al., 2013]. Similar to our findings, a previous study reported that the rs1805476 *GRIN2B* SNP was associated with smaller OFC volume in individuals with obsessive compulsive disorder [Arnold et al., 2009a]. Another study reported that the rs1019385 *GRIN2B* SNP was associated with smaller anterior cingulate glutamatergic concentration [Arnold et al., 2009b]. Also, the 3'UTR *GRIN2B* SNP, rs1805476, was associated with smaller anterior cingulate cortical volume [Arnold et al., 2009a]. Another study showed increased *glutamate receptor, ionotropic, N-methyl-D-aspartate 3A* (*GRIN3A*) mRNA, an NMDA receptor subunit, levels in the OFC of alcoholics [Jin et al., 2014]. The intronic *GRIN2B* SNP, rs219927, has not previously been associated with any brain volume or psychiatric phenotype. As mentioned, *GRIN2B* is involved in glutamatergic signalling suggesting that this pathway may possibly be associated with differential brain volumes particularly in individuals with AUD. These preliminary findings implicating *GRIN2B* should be investigated further in larger cohorts.

One of the main limitations of this study is the small sample size. With a bigger sample, SNPs which were nominally significant may prove to actually be associated with AUD. A post-hoc power calculation demonstrated that in order to obtain 80% power to detect an association between AUD and the 4065 SNPs, a sample size of 409 would be required. Nevertheless, even a small sample may be useful in providing signals for further investigation, and the value of this sample lies in the early onset of the phenotype, and in the lack of comorbidity. Another limitation is the fact that the MRIs were acquired using two different protocols. However, an attempt to overcome this was made by matching imaging scans based on protocol and have shown previously that images obtained from two different protocols could be combined for analysis [Fein et al., 2013].

The findings from this study imply that the onset of AUD in adolescents may be as a result of dysfunction in the circadian and glutamatergic pathways. Both of these pathways have previously been implicated in BD and suggest that a common pathophysiology between AUD and BD may exist. These pathways should be investigated further in future studies. Also, it

appears that childhood trauma may predict AUD in adolescence. The next chapter of this thesis investigates alcohol use and genetic variation in the HPA-axis in a large birth cohort.

Chapter 4: An Investigation of Alcohol Use in a Group of Adolescents from the ALSPAC Cohort

Abstract

There is evidence that specific genetic variants within components of the HPA-axis and exposure to childhood adversity are associated with AUD. There is also evidence that internalising behaviour may be a risk factor for AUD. The aim of this study was to determine whether variants within genes involved in the HPA-axis play a role in predisposing individuals to AUDs and whether these variants interact with childhood adversity to predict AUDs in adolescence. This study also set out to determine whether the internalising behaviour observed during early childhood has an association with adolescent alcohol use. The experimental group consisted of 8123 individuals from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort. Alcohol use was measured with the Alcohol Use Disorders Identification Test (AUDIT) questionnaire, childhood life events were measured at 42 months and the emotional scores from the Strength and Difficulties Questionnaire (SDQ), part of which measures internalising behaviour, were obtained. Genotype data was acquired for six HPA-axis genes. None of the genetic variants were associated with AUDIT score. However, from the gene-based analysis, *UCN3* for the SNP rs12783734 was associated with AUDIT score. No significant gene x environment interactions were detected and SDQ was not associated with AUDIT score. These results suggest that the HPA-axis genetic variants may not interact with childhood life events to predict adolescent alcohol use. Also, it appears that childhood internalising behaviour is not associated with alcohol consumption in adolescence.

4.1 Introduction

There is evidence from previous studies that both particular genes and particular environments contribute to vulnerability to AUD. However, there is relatively little data on gene x environment interactions in the aetiology of AUDs [Van Der Zwaluw and Engels, 2009]. The results from Chapter 3 showed that childhood trauma may predict AUD in adolescence, possibly indicating that early life stress may have an effect on drinking patterns later in life. However, no statistically significant gene x environment interaction was detected in the relatively small sample size. Genetic variants within the HPA-axis, which mediates the stress response, have previously been associated with alcohol use. In this chapter we examined possible genetic and environmental predictors of AUD and their interactions, in a

large European birth cohort. Further, little is known about the role of internalising behaviour in the development AUD. In this chapter we also investigated whether internalising behaviour has an association with alcohol use in adolescence.

4.1.1 Hypothalamic-Pituitary-Adrenal Axis

Homeostasis is the maintenance of a constant internal environment. "Normal" physiological processes aim to maintain this equilibrium when the body is confronted by internal or external forces, known as stressors. During a stressful situation, brain function is heightened and focussed on the stressor [Tsigos and Chrousos, 2002]. The stress response is mediated by the stress system which forms part of the central and peripheral nervous systems. Stimulation of the stress system gives rise to a host of physical and behavioural alterations in a time-limited manner which ultimately promotes the survival of the individual [Chrousos, 1998]. The HPA axis is the main centre involved in the physiological response to stress through its interactions with stress-sensitive regions in the brain and neuroendocrine systems in the hypothalamic paraventricular nucleus (PVN). This complex process is outlined in a simple diagram in Figure 16. After the stimulation by stress or circadian signals from the suprachiasmatic nucleus (SCN), neurons in the PVN secrete CRH and arginine vasopressin (AVP) into the portal vein which stimulates the secretion of the adrenocorticotrophin hormone (ACTH) (also known as corticotrophin) from the corticotroph cells in the pituitary gland. An increase in circulating levels of ACTH results in the synthesis and secretion of glucocorticoids, such as corticosterone and cortisol, by the adrenal cortex [Herman and Cullinan, 1997]. In humans, cortisol is the primary glucocorticoid which initiates a wide range of physiological responses in the body. For example, cortisol plays a major role in metabolism in order to supply the body with sufficient energy during a stress response. It is also involved in the immune and cardiovascular systems and plays a role in cognitive processes [Kudielka and Kirschbaum, 2005]. Also involved in the stress system is the locus ceruleus and noradrenergic neurons in the medulla and pons [Chrousos, 1998]. The HPA-axis also has an auto-regulatory feedback mechanism whereby cortisol binds to glucocorticoid receptors in the HPA-axis and hippocampus. This auto-regulation is important for the maintenance of homeostasis in the HPA-axis [Watson and Mackin, 2006]. Continued or over-secretion of glucocorticoids from the adrenal glands, as a result of stress, can be physiologically and psychologically detrimental [Herman and Cullinan, 1997].

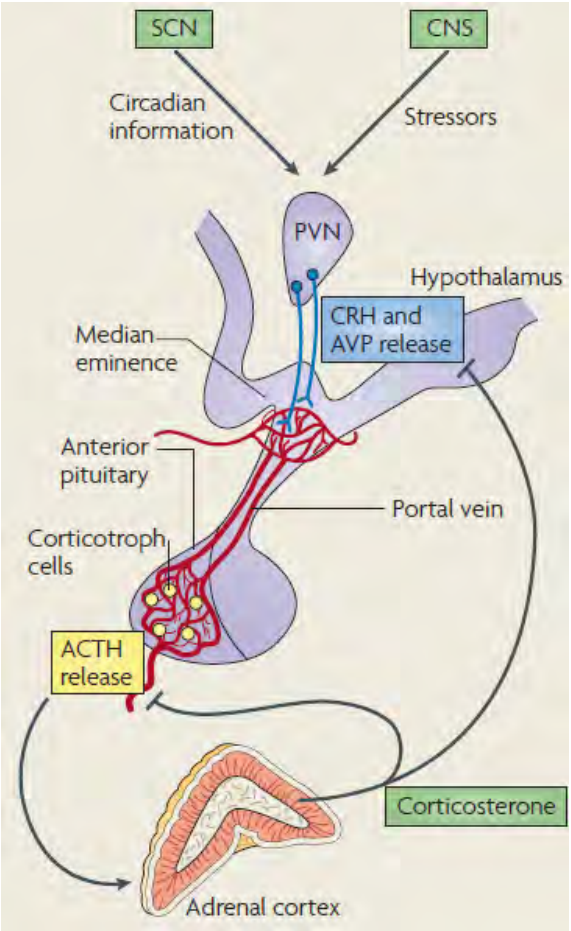

Figure 16: A representation of the HPA axis. The hypothalamus receives input from the central nervous system (CNS) and suprachiasmatic nucleus (SCN) and stimulates the appropriate response, which results in the release of glucocorticoids from the adrenal cortex [Lightman and Conway-Campbell, 2010].

4.1.2 Stress and Alcohol Use

Heavy alcohol use is positively associated with the experience of environmental stressors such as job, health-related, social and legal stress [Dawson et al., 2005; Enoch, 2012]. A study conducted in 1991 showed that peer-reared rhesus macaques had higher levels of alcohol consumption, higher levels of plasma cortisol and corticotrophin compared to those who were maternally reared. In addition, when the maternally-reared monkeys were separated from their mothers, their alcohol consumption increased to a level nearly as high as the peer-reared monkeys. These results suggested that rearing experiences and early stressful life situations can lead to an increase in alcohol consumption [Higley et al., 1991]. Similar results were found in a subsequent study conducted on rhesus macaques which concluded that early rearing experiences and elevated plasma cortisol levels after stressful experiences in early life are good predictors of alcohol consumption [Fahlke et al., 2000]. In a study

involving rats, alcohol consumption increased with exposure to the stress hormone, corticosterone [Besheer et al., 2013]. University students suffering from examination stress, with low social support, increased their alcohol consumption by an average of 18.5% compared to students in the same group with high social support, who on the other hand, decreased their consumption by an average of 17.5% [Steptoe et al., 1996]. Upon alcohol withdrawal, activation of the HPA stress response system is observed [Adinoff et al., 1991]. These studies indicate a close relationship between the experience of stress and alcohol consumption.

4.1.2.1 HPA-Axis Genetics and Alcohol Use

The HPA-axis involves the following genes: *CRH* (also known as *corticotropin releasing factor* (*CRF*)), *CRHR1*, *corticotropin releasing hormone receptor 2* (*CRHR2*), *urocortin* (*UCN*), *urocortin 2* (*UCN2*), *urocortin 3* (*UCN3*), and *proopiomelanocortin* (*POMC*), amongst others. The interactions of these gene products (blue ovals) within the context of the HPA-axis are illustrated in Figure 17 [Whirl-Carrillo et al., 2012]. These genes are relatively unexplored in terms of vulnerability to AD; however, it is thought that the products of these genes interact with each other in specific brain regions to regulate alcohol consumptive behaviours [Ryabinin et al., 2012]. Below are descriptions of each of these HPA-axis genes and their possible involvement with alcohol use:

4.1.2.1.1 *CRH*

The *CRH* gene, located on chromosome 8q13, encodes the 41 amino acid corticotropin-releasing hormone which is released from the PVN. Upon release from the PVN, CRH binds to its receptors located on the anterior pituitary corticotroph cells to stimulate the release of ACTH. The CRH protein, first characterised by [Vale et al., 1981], is hypothesised to be the key regulator in the HPA-axis. This protein is purported to play roles in learning, memory, depression, and anxiety disorders [Raadsheer et al., 1994; Raadsheer et al., 1995; Radulovic et al., 1999; Blank et al., 2002; Roozendaal et al., 2002].

In rats characterised as ~~"e~~thanol-preferring", an increase in basal levels of CRH was observed in the amygdala [Richter et al., 2000] and in a separate study, increased levels of ACTH was detected in the plasma of rats after alcohol consumption. This effect was attenuated when CRH receptors were blocked suggesting that the mechanism of ACTH increase is mediated by CRH [Rivier and Lee, 1996]. *Crh*-knockout (KO) mice consumed less alcohol and had lower blood alcohol concentration than their wild-type (WT) counterparts [Kaur et al., 2012].

A cell culture model has shown that alcohol administration increases the expression of the *CRH* gene [Li et al., 2005].

Variations within the *CRH* gene have previously been reported to be associated with alcohol-use behaviour [Barr et al., 2008; Barr et al., 2009]. A *CRH* SNP (-2232 C>G) was shown to be associated with decreased levels of CRH in CSF, increased levels of ACTH in CSF and increased alcohol consumption in rhesus macaques [Barr et al., 2008]. Another *CRH* polymorphism (-248C>T), located in the promoter of the gene, was shown to be associated with greater alcohol consumption after early life adversity in rhesus macaques [Barr et al., 2009]. Two receptors which bind CRH, CRHR1 and CRHR2, have been identified and are described in greater detail below.

4.1.2.1.2 *CRHR1*

The 415 amino acid receptor protein, CRHR1, was first cloned in 1993. This protein is a G-protein coupled receptor consisting of seven membrane spanning domains [Chen et al., 1993] and is expressed in the CNS, particularly the cortex, cerebellum, and sensory relay structures [Chalmers et al., 1995; Van Pett et al., 2000]. It has been suggested that CRH receptor antagonists may be good treatment options for alcoholism [Lowery and Thiele, 2010; Zorrilla et al., 2013], suggesting a role for this protein in the pathophysiology of AUD. The gene encoding this receptor is located on chromosome 17q12-q22. A few studies have shown the effects of this gene on alcohol use in animal models [Sillaber et al., 2002; Kaur et al., 2012; Molander et al., 2012]. One such study showed that *Crhr1*-KO mice consumed less alcohol than WT mice [Kaur et al., 2012]. In contrast, others have found that mice without a functional *Crhr1* consume more alcohol after being exposed to stress [Sillaber et al., 2002; Molander et al., 2012]. Several studies have looked at *CRHR1* polymorphisms in human subjects and, similar to the findings of the animal studies, results have been conflicting. One such study found that two haplotype-tagging SNPs (rs242938 and rs1876831) within *CRHR1* had been associated with higher alcohol consumption and binge drinking in adult and adolescent groups [Treutlein et al., 2006]. Similarly, [Chen et al., 2010] found that several SNPs in *CRHR1* had an association with AD. In contrast, another study did not find any association between the following *CRHR1* SNPs: hCV449763, rs7209436, rs242924, rs1396862, rs878887, and AD [Dahl et al., 2005].

4.1.2.1.3 *CRHR2*

The CRHR2 receptor consists of 431 amino acids and shares 68% similarity with the CRHR1 protein [Perrin et al., 1995]. This receptor has a distinct expression profile in the brain compared to the Type 1 receptor, and is primarily expressed in the lateral septal nucleus, ventromedial hypothalamic nucleus, hippocampus and the choroid plexus [Chalmers et al., 1995; Van Pett et al., 2000]. CRH preferentially binds CRHR1 with high affinity, suggesting the 2 receptors have different functions [Lovenberg et al., 1995]. Furthermore, there are two splice variants of CRHR2, namely $CRHR2_{\alpha}$ and $CRHR2_{\beta}$ [Lovenberg et al., 1995]. The *CRHR2* gene is located on chromosome 7p14.3. Of the few studies investigating the effects of *CRHR2* in terms of alcohol consumption, one reported that ~~"a~~lcohol-preferring" rats showed decreased *CRHR2* mRNA levels compared to ~~"n~~on-preferring" rats [Yong et al., 2014].

4.1.2.1.4 *UCN*

Urocortins are members of the CRH family of peptides which illicit similar physiological responses to CRH by binding to the CRH receptors. UCN binds with high affinity to CRHR1 and CRHR2 [Suda et al., 2004] and administration of UCN results in ACTH and corticosterone secretion [Vaughan et al., 1995; Asaba et al., 1998]. UCN is primarily expressed in the region known as the Edinger-Westphal nuclei in the brain [Bittencourt et al., 1999]. The gene encoding the 40 amino acid protein, urocortin, is located on chromosome 2p23 [Donaldson et al., 1996]. In terms of alcohol consumption, ~~"e~~thanol-preferring" mice had higher levels of *Ucn1* (*UCN* ortholog) positive cells than ~~"e~~thanol-avoiding mice", after consumption of alcohol, suggesting a possible role of UCN in alcohol-use behaviour [Bachtell et al., 2003; Weitemier and Ryabinin, 2005]. Similar results were also shown in studies on rats [Turek et al., 2005; Fonareva et al., 2009]. No studies have been reported regarding the association of variants within *UCN* and AUD in humans.

4.1.2.1.5 *UCN2*

The mature UCN2 protein (also known as stresscopin-related peptide) consists of 38 amino acids and selectively binds to the receptor CRHR2 [Reyes et al., 2001]. The gene encoding this protein is located on chromosome 3p21.3. To the best of the knowledge of the author, neither the UCN2 protein nor its encoding gene has been investigated in terms of alcohol-related phenotypes.

4.1.2.1.6 *UCN3*

UCN3 (also known as stresscopin) also encodes a 38 amino acid protein, which has a 90% homology to mouse UCN protein, and selectively binds to the type 2 CRH receptor [Lewis et al., 2001]. *UCN3* is also thought to play a role in alcohol-use behaviour as central administration of Ucn3, in a saline solution, to non-dependent mice reduced alcohol intake but not the self-administration of water [Sharpe and Phillips, 2009]. Also, *Ucn3*-KO mice consumed more alcohol compared to WT mice [Smith et al., 2014].

4.1.2.1.7 *POMC*

POMC is a hormone precursor protein which is mainly expressed in the pituitary gland where it undergoes differential post-translational processing to produce hormones such as ACTH and β-LPH. The gene for POMC is located on chromosome 2p23.3 and encodes for a protein of 267 amino acids [Chang et al., 1980; Whitfeld et al., 1982]. Animal studies have shown that alcohol consumption decreases *POMC* transcriptional activity [Gangisetty et al., 2014; Zhou and Lapingo, 2014]. Also, rat pups who consumed alcohol displayed an increase in *POMC* methylation [Gangisetty et al., 2014]. Similarly, in humans, differential methylation patterns in the 5‘ promoter region of the *POMC* gene were observed in individuals with an AD diagnosis compared to controls. The authors of this study suggested that alcohol consumption may have resulted in epigenetic changes associated with alcohol craving [Muschler et al., 2010]. The results from all of these studies suggest that *POMC* may be an important candidate in terms of the aetiology of AD. However, no association was found between seven SNPs, namely rs1042571, rs2071345, rs6713532, rs12473543, rs8192605, rs934778, and rs1009388, located in this gene, and an AD phenotype [Xuei et al., 2007].

4.1.3 HPA-Axis Gene x Environment Interactions

Studies involving animal models have shown that polymorphisms within the *CRHR1*, together with the experience of environmental stress factors, result in greater alcohol consumption [Sillaber et al., 2002; Hansson et al., 2006]. Similarly, in humans, polymorphisms in the *CRHR1* gene (and adjacent chromosomal regions) interact with negative life events to predict heavy alcohol use [Blomeyer et al., 2008; Nelson et al., 2010; Ray et al., 2013] and earlier age of first drink [Schmid et al., 2010]. In fact, it has been shown that *CRHR1* polymorphisms interact with measures of childhood abuse to predict depressive symptoms in adults [Bradley et al., 2008] and have an effect on cortisol response in the dexamethasone/CRH test which assesses alterations in HPA axis activity [Tyrka et al., 2009].

These findings suggest a possible relationship between variants in HPA-axis genes and environmental stressors in AUD.

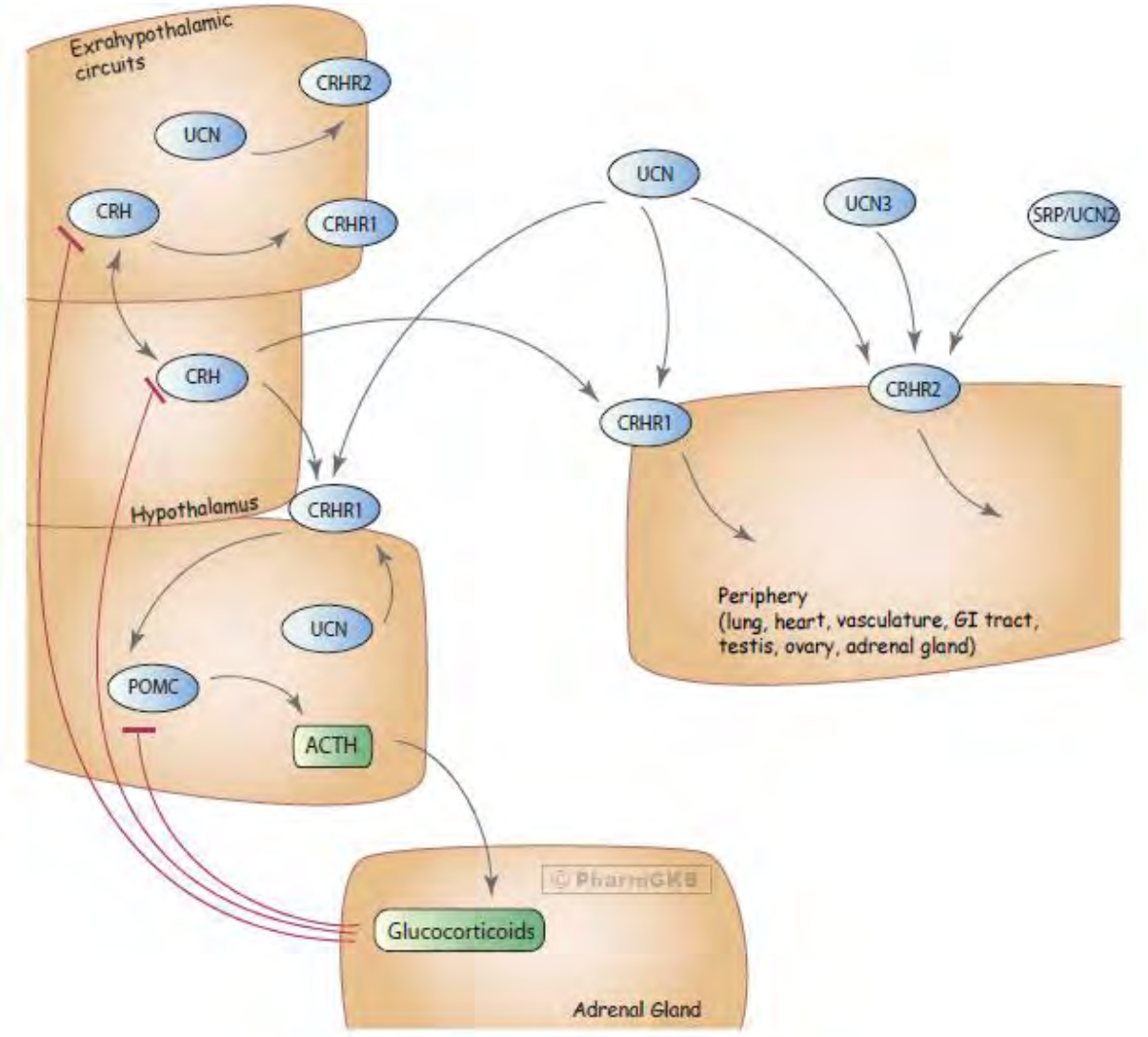

Figure 17: Simplified illustration of glucocorticoid release via the hypothalamic-pituitary-adrenal (HPA) axis [Whirl-Carrillo et al., 2012].

4.1.4 Internalising Behaviour and Alcohol Use

It is often argued that AUD is characterised by externalising behaviour [Kumpulainen, 2000; Englund et al., 2008]. Externalising symptoms or behavioural disinhibition is the ~~–i~~nability to constrain impulses to behave in socially undesirable ways" [Iacono et al., 2008]. It has been shown that there is an increased risk for AUD when an individual presents with behavioural

disinhibition together with an environmental risk factor, such as poor family management [Hill et al., 2010].

Findings regarding the link between behavioural inhibition/internalizing symptoms and AUD are less emphasised in the literature, although a few studies have shown that internalising symptoms, such as anxiety and depression, are associated with alcohol and substance use [Loeber et al., 1999; Sood et al., 2001; Fite et al., 2006; Saraceno et al., 2009; Edwards et al., 2014]. Behavioural disinhibition is further defined as ~~–e~~onsistent tendency of some children to demonstrate fear and withdrawal in novel situations" [Svihra and Katzman, 2004]. A study has shown an association between AUD and anxiety disorders [Merikangas et al., 1998c]. However, it appears that this association is greater in females than in males. This could possibly be due to the low rate of anxiety disorders in males [Merikangas et al., 1998c]. Results from the International Consortium in Psychiatric Epidemiology, which combined previous epidemiological data from Canada, Germany, Mexico, the Netherlands, and the United States, found that 32% of individuals affected with an AUD met the diagnosis for an anxiety disorder [Merikangas et al., 1998b]. A separate study, involving German participants, found that anxiety disorders had the highest rate of comorbidity amongst AD subjects who were undergoing treatment [Schneider et al., 2001].

There are three possible causal mechanisms of comorbidity between AUD and anxiety disorders: i) the anxiety disorder encourages the use of alcohol and thus the onset of AUDs. This has been shown in previous studies where anxiety disorders commonly preceded AUDs [Chambless et al., 1987; Merikangas et al., 1998c; Zimmermann et al., 2003; Schneier et al., 2010], ii) Alcohol use promotes anxiety disorders, and iii) the presence of a common causal factor for both disorders which may be genetic or environmental [Kushner et al., 2000].

4.1.5 HPA-Axis Genes and Internalising Behaviour

The HPA-axis has previously been associated with emotional dysregulation, whereby adolescents with internalising behaviour had lower evening cortisol levels, a measure of HPA-axis activity, than those with externalising behaviour [Marsman et al., 2008]. Another study found that dysregulation of cortisol levels predicted internalising symptoms in young children [Saridjan et al., 2014]. Evidence of a genetic basis to developing internalising symptoms is provided by a meta-analysis which found that generalised anxiety disorders have a heritability of 32% and that it exhibits familial clustering [Hettema et al., 2001]. The anxiety sensitivity index, which is an intermediate phenotype for anxiety disorders, has been

shown to have a heritability of 45% [Stein et al., 1999]. Relatives of an individual with an anxiety disorder are twice more likely to have an anxiety disorder than control individuals [Merikangas et al., 1998c].

Variations within HPA-axis genes have previously been associated with internalising behaviour. Polymorphisms within *CRHR1* have been shown to have a significant association with anxious temperament and to have an influence on the metabolic activity of local brain regions in rhesus macaques [Rogers et al., 2013]. Mice lacking a functional *Crhr1* gene have reduced ACTH and corticosterone secretion in response to stress, and display less anxiety-like behaviour at baseline and during alcohol withdrawal [Timpl et al., 1998]. In contrast, mice with deficient *Crhr2* have an increased sensitivity to stress and exhibit increased anxiety-like behaviour [Bale et al., 2000; Kishimoto et al., 2000]. Mice with deficient *Ucn* have no changes in the stress response but increased anxiety-like behaviour in maze and open-field tests [Vetter et al., 2002]. *Ucn2* KO mice exhibited less depressive-like behaviour compared to WT mice [Chen et al., 2006]. These findings indicate an important role for CRH receptors and their ligands in the regulation of the stress response and anxious behaviour. A study conducted in humans found that an intronic variant within the *CRHR1* gene, rs110402, had an effect on alcohol consumption in adolescents which was mediated by internalising behaviour [Glaser et al., 2014]. Another study found that genetic variants within *CRHR1* and *5-HTTLPR* interact with childhood trauma to predict higher internalising behaviour [Cicchetti et al., 2011]. It has also been suggested, as with the treatment of AUD, that there is a potential role for CRHR1 antagonists in the treatment of anxiety disorders [Holsboer and Ising, 2008].

4.1.6 The ALSPAC Study: "Children of the 90s"

The Avon Longitudinal Study of Parents and Children (ALSPAC) initiative was first initiated at a WHO meeting in Moscow in 1985 and forms part of a series of longitudinal studies in Europe [Boyd et al., 2013]. The main aim of this prospective, trans-generational, observational project was to measure how genotype interacts with environmental factors to affect health and development in children. The study is defined by the following criteria [ALSPAC Study Team, 2001]:

1) Has to include all pregnant mothers from a defined geographical region with an expected delivery date in a predefined time period.

2) The study data is collected from mothers and partners from self-completed questionnaires and is linked to health records.
3) All participant data is anonymised and unable to be linked to a particular individual from statistical data.
4) The study is observational and intervention should be kept to a minimum.
5) The same questions should be asked, in the same way, by all participating study centres except for a few culturally modified questions.
6) As much detail should be added as possible, but core variables should all be obtained.
7) A copy of the data should be cleaned and sent to the Bristol (UK) study co-ordinating office for analyses.

Enrolment to the study first began in September 1990 [ALSPAC Study Team, 2001]. Individuals were considered eligible for the study if they resided in one of the three health districts of the county of Avon (Bristol & Weston, Frenchay and Southmead) in the UK, and gave birth between 1 April 1991 and 31 December 1992. Initially, the study had recruited the children of 14 541 pregnancies. Each participant has been assessed 68 times between birth and 18 years of age. A total of 59 of those assessments were questionnaires (34 child-completed and 25 child-based completed by the mother or main caregiver) and the remaining 9 assessments were clinical visits. The ALSPAC study is unique in the wealth and range of data that has been collected for the participants over the course of the study, at key developmental time points. This includes detailed biological, phenotypic and environmental variables [Boyd et al., 2013].

It is clear that the ALSPAC study is an excellent resource and provides a unique opportunity to try and determine the relationship between genes and environment in the complex AUD phenotype, as this dataset includes information on genetic variants and on adverse environments (e.g. stressful life events). The examination of this cohort could also aid in the determination of whether AUD is as a result of internalising behaviours such as anxiety or depressive disorders experienced during early childhood, as this dataset includes comprehensive information on internalising behaviour experienced during childhood.

4.1.7 Aims and Objectives

The first aim of this study was to determine whether variants within genes involved in the HPA-axis play a role in predisposing individuals to AUDs and whether these variants interact

with adverse childhood events to predict AUDs in adolescence. The second aim was to determine whether internalising behaviour observed during early childhood is a risk factor for adolescent alcohol use behaviour.

4.1.7.1 Objectives

The objectives of this investigation were as follows:

1) To determine whether variants in the seven HPA-axis genes have an association with the Alcohol Use Disorders Identification Test (AUDIT) score at 198 months (16.5 years).
2) To determine whether adverse childhood events occurring before 42 months of age (3.5 years) predicts a high AUDIT score at 198 months of age.
3) To determine whether the HPA-axis variants interact with adverse childhood events occurring before 42 months of age (3.5 years) to predict a high AUDIT score at 198 months of age.
4) To determine if there is an association between HPA-axis variants and the emotional subscale of the Strength and Difficulties Questionnaire (SDQ) at 81 months (6.8 years).
5) To determine if the emotional subscale of the SDQ, which correlates with internalising behaviour, has an association with AUDIT score.

4.2 Materials and Methods

4.2.1 ALSPAC Variables of Interest and Cohort Details

Access was granted from the ALSPAC Executive Committee for the genotype data of the selected HPA-axis genes and the following ALSPAC data variables: AUDIT scores, weighted life events score and the emotional subscale of the SDQ. These variables are described below.

4.2.1.1 Cohort Details

The initial ALSPAC dataset contained information for 15 455 individuals. A total of 406 of these individuals were twins and were excluded from the analyses to ensure that there were no related individuals in the cohort. To avoid population stratification, 595 individuals were excluded as they were not of Caucasian ethnicity. The cohort was further filtered to include only individuals with available genotype data (described below).

4.2.1.2 Genotype Data

All the SNPs from the 7 selected genes (*CRH*, *CRHR1*, *CRHR2*, *UCN*, *UCN2*, *UCN3*, and *POMC*) involved in the HPA-axis were extracted from the NCBI dbSNP (short genetic variations database) website [http://www.ncbi.nlm.nih.gov/SNP]. Of those variants, 82 SNPs (corresponding to six genes) had genotype information available from the ALSPAC cohort and were utilised for downstream analysis. Observed genotypes were obtained using the Illumina HumanHap550 quad genome-wide SNP genotyping platform (Illumina Inc., San Diego, California, USA) by 23 and Me (California, USA), sub-contracted by the Wellcome Trust Sanger Institute, (Cambridge, UK) and the Laboratory Corporation of America, (Burlington, NC, USA). Imputed genotypes were obtained using the MACH 1.0.16 Markov Chain Haplotyping software, using CEPH individuals from phase 2 of the HapMap project as a reference (release 22) [Paternoster et al., 2011]. A total of 19 SNP genotypes were observed in addition to being imputed. The imputed SNPs had an average quality score (average posterior probability for the most likely genotype) of 0.986 and an average Rsq score (estimate of the squared correlation between imputed and observed genotypes) of 0.966 (most imputation programs recommend a cut-off of less than 0.3). A full list of the SNPs and genes investigated in this study are listed in Appendix 3 (Genes and SNPs).

4.2.1.3 AUDIT Scores

The aim of the AUDIT, developed by the WHO, is to assess drinking patterns, identify excessive drinking and AD. It aids in reducing alcohol consumption by identifying risky drinking behaviour. The AUDIT has been validated and shown to be applicable across gender, individuals of all ages and cultural backgrounds [Babor et al., 2001] and has also been shown to have a high specificity and sensitivity [Moussas et al., 2009]. AUDIT scores were calculated by adding the scores of each of the 10 questions which comprise this questionnaire (see Appendix 3-AUDIT Questionnaire). Participants were assigned a score of zero if they answered ~~"n~~o" to the stem question or if they ever had an alcoholic drink. Similarly, if the participant scored zero ("~~n~~ever") for the first question of the AUDIT questionnaire, the rest of the questions were skipped until question 9 and 10. Also, if the participant scored zero for both questions 2 and 3, the rest of the questions were skipped until questions 9 and 10. An AUDIT categorical variable was created by classifying individuals with an AUDIT score of less than 8 as "~~n~~on-hazardous", equal to or greater than 8 and less than or equal to 15 as "~~h~~azardous" and those with a score of equal to or greater than 16 but less than or equal to 42 as "~~h~~armful" [Heron et al., 2012]. A hazardous drinking pattern is

characterised by –alcohol consumption that increases the risk of harmful consequences for the user or others". A harmful pattern is one in which the –alcohol consumption results in consequences to physical and mental health" [Babor et al., 2001]. A binary AUDIT variable was created by combining the –harmful" and –hazardous" groups (score of greater than or equal to 8 but less than 42) and a score of less than 8 was deemed –non-hazardous".

4.2.1.4 Adverse Childhood Events

To determine whether any of the study participants experienced any traumatic events during childhood, the weighted life events score was derived from Section D (–upsetting events") of the parent-rated ALSPAC questionnaire –My Son's/Daughter's Health and Behaviour-42 months" was used. This section of the questionnaire (which can be found in Appendix 3-Adverse Childhood Events) is based on life-events scales developed by [Barnett et al., 1983]. This section of the questionnaire consists of several questions dealing with events occurring in the child's life. Each question consists of five possible responses ranging from whether the event occurred or not, to the effect the event had on the child's life. A score of zero was assigned if a particular life event had not occurred and if it had occurred, a score of one to four was given dependent on the severity of the event (ranging from –not affected at all" to –severely affected") [Dorrington et al., 2014]. The weighted life events score is the summation of the scores for each of the questions. In this study, a binary life events score was derived whereby any individual with a life event was given a score of one and an individual without any life events given a score of zero.

4.2.1.5 SDQ

Internalising behaviour was measured by the parent-rated, emotional subscale of the SDQ [Goodman, 1997], administered at 81 months of age (~7 years). This brief behavioural screening questionnaire, found in Appendix 3 (SDQ), is a widely used tool for assessing mental health in children [Goodman et al., 2010]. It contains 25 questions consisting of five hypothesised subscales measuring emotional symptoms, conduct problems, hyperactivity, peer relationship problems, and prosocial behaviour. Each subscale is scored from zero to ten, with a higher score indicating more emotional problems [Peacock et al., 2011]. The SDQ emotional symptoms subscale has shown to correlate with internalising symptoms [Stone et al., 2010].

The emotional scores were categorised into 3 groups:, namely –normal" for scores less than or equal to three, –borderline" for scores of four, and –abnormal" for scores of greater than or

equal to five and less than or equal to ten [Goodman, 1997]. A binary emotional score variable was created by combining the "borderline" and "abnormal" groups (scores equal to and greater than four and less than ten) and the "normal" group (scores less than or equal to three).

4.2.2 Statistical Analysis

Using the statistical program Stata 11 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP), t-tests (with unequal variances), chi-squared and linear regression were performed to determine if there were associations between the AUDIT, SDQ-emotional subscale and life events variables and whether there were any differences for these variables across gender.

Before genotyping and frequency pruning there were 82 SNPs. Using the program Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007], none of the SNPs failed the missingness test (i.e. only SNPs with a genotyping rate of 90% or more were included) and 5 SNPs were excluded because of a MAF of less than 5%. One SNP was excluded as this SNP was not in HWE (p-value less than 0.0006). Therefore, after genotyping and frequency pruning there were 76 SNPs used for downstream analysis.

4.2.2.1 Association Analysis of Genetic Variants and Alcohol Use Measures

Using Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007], linear and logistic regression was used to test whether any of the SNPs had an association with the continuous variable, total AUDIT score and the binary AUDIT variable, respectively. Gender was included as a covariate, an additive genetic test was used, and all tests were adjusted for multiple testing using the Bonferroni Correction [Dunn, 1961]. A gene-based analysis, using linear regression, was performed to determine whether any of the genes have an association with the total AUDIT score. A total of 10 000 permutations were run, gender was included as a covariate, with the default values for r^2 (0.5), p-value (0.05) and the maximum number of SNPs (5).

4.2.2.2 Gene x Environment Interactions for Total AUDIT Score

A gene x environment interaction, using regression analysis, was performed using Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007]. Total AUDIT score was used as the outcome measure and the environmental measure was the binary life events variable. All 76 variants were analysed and the Bonferroni Correction [Dunn, 1961] was used to adjust for multiple testing.

4.2.2.3 Association Analysis of Genetic Variants and SDQ

Chi-squared tests implemented in Stata 11 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP) were used to determine if there were any significant associations between the genotypes for each of the variants and the categorical SDQ-emotional subscale variable. Logistic regression, using Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007], was performed to test whether any of the SNPs had an association with the binary SDQ-emotional subscale variable. An additive genetic test was used, and all tests were adjusted for multiple testing using the Bonferroni Correction [Dunn, 1961].

4.3 Results

4.3.1 ALSPAC Variables of Interest and Cohort Details

4.3.1.1 Cohort Details

The final cohort consisted of 8123 individuals. Of this, 4159 were male and 3964 females. All of these individuals were of the same age and of Caucasian ethnicity. Not all of the 8123 individuals had data available for all of the variables under investigation. Figure 18 illustrates a breakdown of the number of individuals with available data for each of the variables.

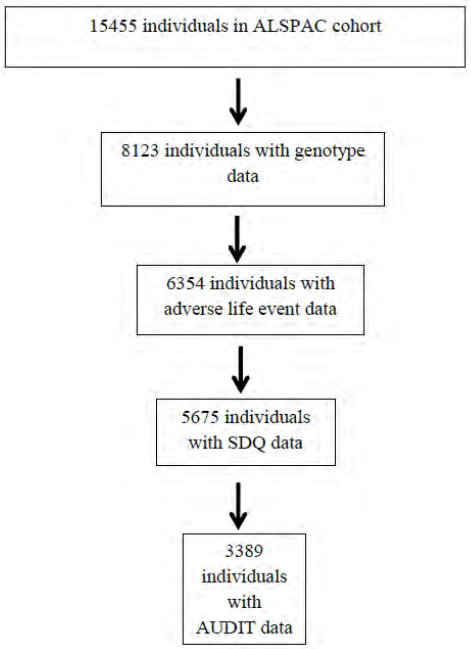

Figure 18: Number of individuals with available data for each of the variables of interest.

4.3.1.2 Genotype Data

There were 8123 individuals with genotype data for each of the 82 SNPs. All SNPs had imputed genotype calls and a total of 19 SNPs had observed calls. For SNPs with both observed and imputed genotype calls, only the observed genotypes were used in downstream analyses. The SNPs rs9839959 (*UCN2*), rs13319651 (*UCN2*), rs3769671 (*POMC*), rs7812133 (*CRHR2*) and rs6982394 (*CRH*) had a MAF of less than 5% and the SNP rs255102 (*CRHR2*) was out of HWE. These six SNPs were excluded from the downstream analyses.

4.3.1.3 AUDIT Scores

In total, there were 3389 participants with an AUDIT score. The mean score was 6.71 (SD= 5.20) and the minimum and maximum scores were 0 and 33, respectively. The distribution of total AUDIT scores is represented in Figure 19. Figure 20 is a box plot of the total AUDIT score for males and females. The numbers of participants belonging to each AUDIT category by gender are listed in Table 17. There were 1258 individuals in the "harmful/hazardous" group and 2131 individuals in the "non-hazardous" group.

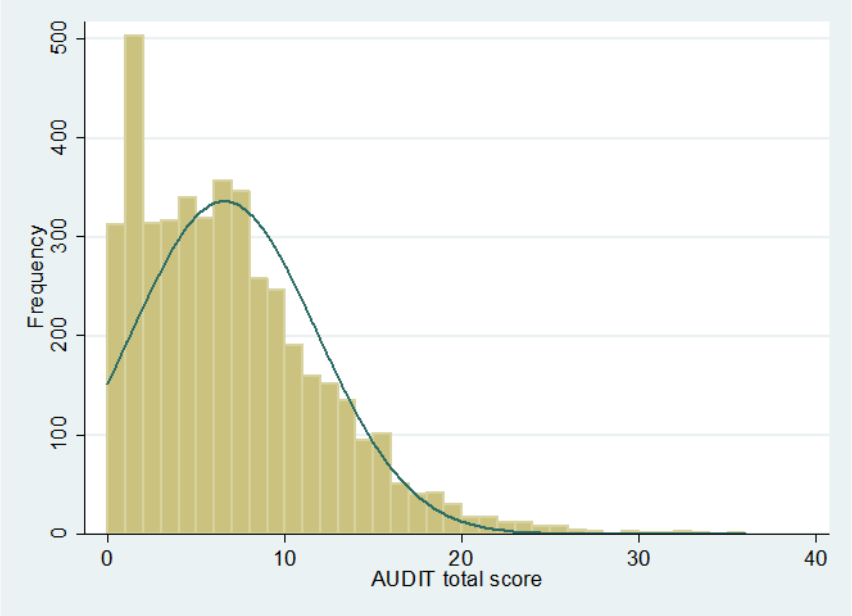

Figure 19: Distribution of total AUDIT scores

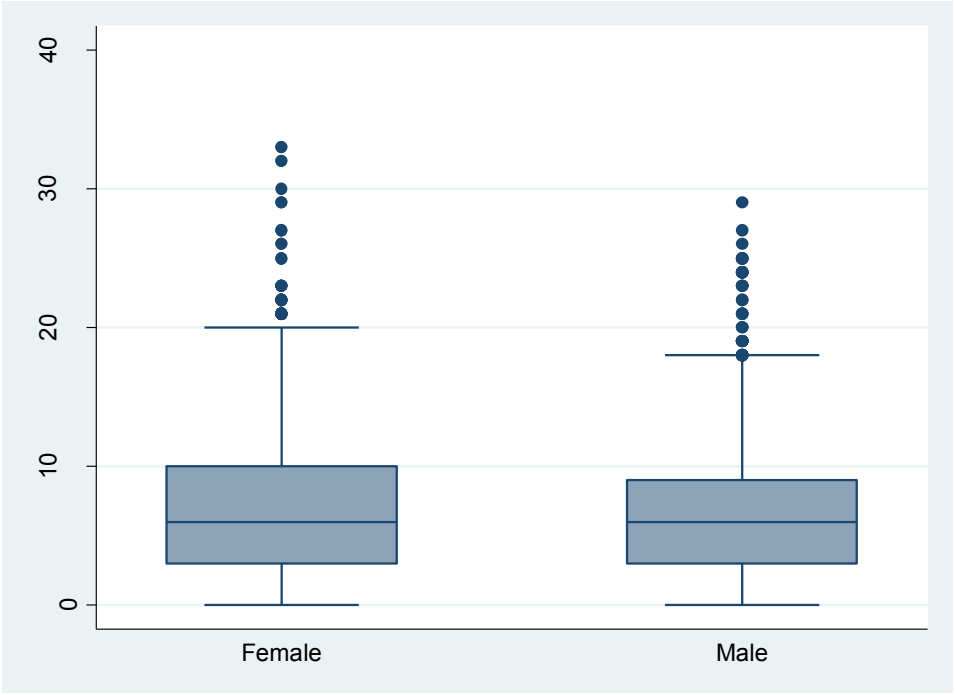

Figure 20: Box plot of total AUDIT scores for females and males.

Table 17: Number of individuals in each AUDIT category by gender

AUDIT category	Score	Number of males	Number of females	Total
Non-hazardous	< 8	918	1213	2131
Hazardous	8 ≥ and ≤ 15	413	642	1055
Harmful	16 ≥ and ≤ 42	69	134	203
Total		**1400**	**1989**	**3389**

4.3.1.4 Adverse Childhood Events

There were 6354 individuals with a weighted life events score. The mean score was 2.74 (SD= 2.57), the minimum and maximum scores were zero and 21, respectively. For the binary life events variable, there were 1118 individuals with no adverse life event and 5236 individuals with at least one adverse life event.

4.3.1.5 SDQ

There were 5675 individuals with an emotional score. The mean score was 1.50 (SD= 1.66) and the minimum and maximum scores were zero and ten, respectively. The number of individuals belonging to each emotional score category by gender are listed in Table 18.

There were 715 individuals in the SDQ binary category –Abnormal/Borderline" and 4960 individuals in the 4960 –Normal" category.

Table 18: Number of individuals in each emotional score category by gender

Emotional Score category	Score	Number of males	Number of females	Total
Normal	≤3	2546	2414	4960
Borderline	4	169	188	357
Abnormal	5 ≥ and ≤ 10	179	179	358
Total		**2894**	**2781**	**5675**

4.3.2 Statistical Analysis

Total AUDIT score was significantly different between males and females (t=3.1, df= 3124.75, two-tailed p-value = 0.001) whereby females had a higher mean total AUDIT score than males. There was no difference in the SDQ-emotional subscale categorical variable between males and females (Pearson chi-squared = 2.2750, df =2, p-value = 0.321). Similarly, for the life events score there was no difference across gender (t=0.4360, df= 6311.91, two-tailed p-value = 0.3314). There was no difference in mean total AUDIT score between the borderline/abnormal and normal SDQ-emotional subscale groups (t= -1.1214, df= 436.038, two-tailed p-value = 0.8686). There was no association between life events and total AUDIT score (t-stat= 0.83, co-efficient= .0319214, p-value= 0.405).

4.3.2.1 Association Analysis of Genetic Variants and Alcohol Use Measures

From the linear and logistic regressions, none of the SNPs obtained a significant association with the total AUDIT score or the binary AUDIT category after correction for multiple testing. However, the top SNPs with uncorrected p-values of less than 0.05 are listed in Table 19. Three of these SNPs are located in the gene *UCN3* and the other two in *POMC*. The *UCN3* variants are all upstream of the gene and the *POMC* SNPs are both intronic.

Only five genes were included in the gene-based analysis as both *UCN2* SNPs had a MAF of less than 5%. From the gene-based analysis only one SNP, rs12783734, located upstream of the gene *UCN3* obtained a p-value less than 0.05 (p-value = 0.0384).

4.3.2.2 Gene x Environment Interactions for Total AUDIT Score

There were no statistically significant interactions between any of the variants and the binary life events variable, after correction for multiple testing. Interactions which obtained uncorrected p-values of less than 0.05 are listed in Table 20. All of these *CRHR2* SNPs are intronic. All the results from this analysis can be found in Appendix 3 (Gene x Environment Interaction Results).

4.3.2.3 Association Analysis of Genetic Variants and SDQ

There were no significant differences in the genotypic distributions of the 76 variants between the three SDQ-emotional subscale categories. From the logistic regression analysis, none of the SNPs obtained a significant association with the binary SDQ-emotional subscale variable, after correction for multiple testing. Three SNPs obtained uncorrected p-values less than 0.05, listed in Table 21. Two of these SNPs are located in *POMC* and the other in *CRH*.

Table 19: Linear and logistic regression results for total AUDIT score and binary AUDIT score

Chromosome	SNP	Gene	Minor allele	Regression co-efficient/ Odds ratio	t-statistic	Uncorrected p-value	Outcome measure
10	rs12783734	*UCN3*	A	0.3581	2.397	0.01657	AUDIT score
10	rs12768198	*UCN3*	A	0.3555	2.382	0.01729	AUDIT score
10	rs11591351	*UCN3*	G	0.3291	2.221	0.02643	AUDIT score
2	rs6545975	*POMC*	C	0.2817	2.201	0.0278	AUDIT score
				1.115	2.133	0.03295	Binary AUDIT
2	rs7565877	*POMC*	G	0.8407	-2.094	0.03622	Binary AUDIT

Table 20: Top results from gene x environment interaction analysis

Chromosome	SNP	Gene	Z score	Uncorrected p-value
7	rs2284217	*CRHR2*	2.253	0.02429
7	rs255100	*CRHR2*	2.159	0.03087
7	rs1076292	*CRHR2*	2.017	0.04365
7	rs2251002	*CRHR2*	2.017	0.04365

Table 21: Logistic regression results for binary SDQ variable

Chromosome	SNP	Gene	Minor allele	Odds ratio	t-statistic	Uncorrected p-value
2	rs6713532	*POMC*	C	1.222	3.065	0.002176
8	rs12721510	*CRH*	T	1.312	2.547	0.01087
2	rs6545975	*POMC*	C	0.8789	-2.206	0.0274

4.4 Discussion

In this chapter, the aim was to determine whether variants within HPA-axis genes play a role in predisposing individuals to AUD and whether these variants interact with childhood adversity to predict AUD in adolescence. The second aim was to determine whether internalising symptoms during childhood, as measured by the emotional subscale of the SDQ, had an association with adolescent alcohol use. No main effects or gene x environment interactions were detected for the for the AUDIT variables. However, from the gene-based analysis, *UCN)* was associated with the total AUDIT score. Specifically, the minor allele of the *UCN3* SNP rs12783734 decreases the risk of having an AUD. Also, internalising behaviour did not have an association with the total AUDIT score.

In this study no significant association was found between any of the genetic variants and the total AUDIT score or the binary AUDIT variable, after correction for multiple testing. This is in agreement with previous studies which did not find an association between HPA-axis genes and alcohol use behaviours [Dahl et al., 2005; Xuei et al., 2007]. Even though there were no statistically significant associations, it is still worth discussing the top SNPs from this analysis. SNPs in the genes *UCN3* (rs12783734, rs12768198, and rs11591351) and *POMC* (rs6545975 and rs7565877) obtained uncorrected p-values of less than 0.05. None of these SNPs have previously been shown to be associated with an alcohol-related phenotype. The gene-based analysis also identified *UCN3* as having an association with the total AUDIT score for the SNP rs12783734. Animal studies have shown that *UCN3* may play a role in

alcohol use behaviour as administration of Ucn3 to mice reduced alcohol intake but not the self-administration of water [Sharpe and Phillips, 2009]. Also, *Ucn3*-KO mice have been shown to consume more alcohol compared to WT mice [Smith et al., 2014]. Animal studies have also shown a role for *POMC* in alcohol use [Chen et al., 2004; Gangisetty et al., 2014; Zhou and Lapingo, 2014]. After alcohol consumption, the circadian rhythm of *POMC* expression in the hypothalamus of rats was dysregulated [Chen et al., 2004]. Studies conducted on humans have shown differential methylation patterns in the 5‘ promoter region of the *POMC* gene in individuals with an AD diagnosis compared to controls [Muschler et al., 2010]. These findings suggest a role for *UCN* and *POMC* in alcohol use behaviour which warrants further exploration.

No association was found between the life events score and the AUDIT score. This suggests that childhood adverse events do not affect alcohol usage later in life. This is in contrast to what was found in Chapter 3, where childhood trauma was found to be significantly associated with adolescent AUD. However, for each of these chapters, different measures of childhood trauma and alcohol use were used. The life events measure used in this chapter included a wide range of childhood traumatic events whereas the CTQ (used in Chapter 3) specifically measures abuse (physical, sexual and emotional) and neglect (physical and emotional). It could be that environmental factors need to be of a certain severity to influence alcohol consumption. Also, the life events assessed in this chapter (by the parent or care-giver) was at an early time-point in the life of the child (before the age of 3.5 years). The CTQ is usually administered to adolescents over the age of 12 years or as adults, and assesses abuse and neglect which could have occurred throughout childhood. Also, the CTQ may be a more reliable measure of childhood maltreatment compared to other methods such as parental observation [Polanczyk et al., 2009]. Another difference between the two chapters was that the alcohol outcome measure (AUDIT score vs DSM-IV AUD) was different for the two analyses. This was because these studies were conducted separately and under completely different conditions.

No significant gene x environment interaction was found in this study. Similarly, another study did not find a significant interaction between the *5-HTTLPR* polymorphism and stressful life events for AD [Dick et al., 2007]. However, a gene x environment interaction should not be ruled out as a possible mechanism for the development of AUD as many previous studies have found significant interactions with different genes and different environmental factors [Caspi et al., 2002; Caspi et al., 2003; Nilsson et al., 2005; Covault et

al., 2007; Kaufman et al., 2007; Ducci et al., 2007; Nilsson et al., 2007; Nelson et al., 2010; Blomeyer et al., 2013; Miranda et al., 2013; Sartor et al., 2014; Kim et al., 2015].

No association was found between the SDQ variable and the AUDIT score. This suggests that childhood internalising behaviour may not have an association with adolescent alcohol use. This is in agreement with previous studies which have also not found strong associations between internalising symptoms and alcohol use [King et al., 2004; Kivimäki et al., 2014]. Some studies report that alcohol use is characterised by externalising behaviour [Kumpulainen, 2000; Englund et al., 2008]. None of the genetic variants were associated with the SDQ-emotional subscale. A previous study did not find an association between variants within *CRHR1* and internalising behaviour [Cicchetti et al., 2011]. Although different measures of internalising behaviour were used across these studies, these findings suggest that internalising behaviour may not be predicted by HPA-axis genes alone.

There were several limitations of this study. Not all of the genes and variants involved in the HPA-axis were investigated in this study, as only the previously genotyped variants were available for analysis. Other variants within the genes of interest, not analysed in this study, could play a role in the development of alcohol use during adolescence. Therefore, the HPA-axis still remains a viable candidate pathway for AUD. Another limitation of this study was that possible population stratification was not controlled for. However, genotype data was only available for a limited number of SNPs, insufficient to do an IBS cluster analysis. Also, a substantial proportion of the data variables were missing. In particular, close to half of the cohort had missing AUDIT scores, the main outcome measure in this study. More statistical power could be achieved with a more complete dataset.

The results from this study indicate that single variants in HPA-axis genes may not have an association with AUD and internalising behaviour. However, the gene *UCN3* showed some preliminary evidence of association to AUD and should be investigated further. Adverse life events do not seem to impact on alcohol usage, however this is in contrast to the results from Chapter 3 regarding the link between childhood trauma and adolescent alcoholism. Also, HPA-axis variants may not interact with adverse childhood events to predict alcohol consumption during adolescence. However, the HPA-axis still represents an important candidate pathway in psychiatric disorders. In Chapter 5, BD-AUD comorbidity is investigated in terms of the glutamate pathway and HPA-axis.

Chapter 5: An Investigation of the Glutamate Pathway and HPA-Axis in BD-AUD Comorbidity

Abstract

Glutamatergic and HPA-axis pathways have been shown to be dysregulated in both BD and AUD. BD is often comorbid with AUD. The effects of the glutamatergic and HPA systems have not been extensively examined in individuals with BD-AUD comorbidity. The main aim of this investigation was to determine whether variants in the glutamatergic and HPA-axis pathways are associated with BD-AUD comorbidity. The cohort consisted of 498 individuals with BDI from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). A subset of the cohort had comorbid current AUD (n=233). A total of 1935 SNPs from both the glutamatergic and HPA-axis pathways were selected from the STEP-BD genome-wide dataset. Using the program Plink, IBS clustering to identify population stratification, single SNP association with logistic regression, and gene-based association testing were performed. No single SNP was associated with BD-AUD comorbidity after correction for multiple testing. However, from the gene-based analysis, the gene *protein kinase C, iota* (*PRKCI*) was significantly associated with current AUD. Although the glutamatergic pathway and HPA axis may not have a major impact on BD-AUD comorbidity, the gene *PRKCI* deserves further investigation.

5.1 Introduction

Most research into the pathophysiology of BD and AUD has primarily focussed on the monoaminergic systems in the brain. Less attention has been focussed on the major excitatory neurotransmitter, glutamate, and its pathways which are now considered to be a therapeutic target for mood disorders. Disruption of the HPA-axis in psychiatric disorders has been researched extensively. In chapter 4 of this thesis, the role of the HPA-axis in AUD was investigated and discussed. In the current chapter, the glutamatergic and the HPA-axis pathways are examined in BD-AUD comorbidity.

5.1.1 The Glutamate Pathway

It is known that excitatory amino acids, acting as neurotransmitters, are responsible for most of the synapses in the CNS [Monaghan et al., 1989]. An estimated 75% of all excitatory neurotransmission in the brain is due to the amino acid, glutamate [Ganong, 1995]. Glutamate is found throughout the brain [Hollmann and Heinemann, 1994] and in order to sustain

normal neuronal functioning; the regulation of this neurotransmitter is tightly controlled [Sanacora et al., 2008]. Glutamate is synthesised in the presynaptic neuronal terminal from glucose and glutamine, via the enzyme, glutaminase. Thereafter it is packaged, at a high concentration into synaptic vesicles, by vesicular glutamate transporters. Glutamatergic neurotransmission is accomplished by the release of glutamate into the synaptic terminals and binding of this substrate to the receptors. Upon binding to the various receptors, excitation of the post-synaptic cell is elicited by the entry of positively charged cations into these cells, which in turn, convey the message to the appropriate target cells [Nakanishi, 1992; Gonzales and Jaworski, 1997; Belsham, 2001] (Figure 21).

There are two types of glutamatergic receptors found on neuronal and glial cells: the ionotropic (gated ion channels) and metabotropic G-protein coupled receptors (mGluRs). The ionotropic receptors (responsible for the passage of calcium, sodium and potassium ions) are further subdivided into the α-amino-3-hydroxy-5-methyl-ioxyzole-4-propionic acid (AMPA), kainate receptors and the voltage-dependent N-methyl-D-aspartate (NMDA) receptors, so named due to the compounds which bind to these receptors [Nakanishi, 1992; Tsai and Coyle, 2002]. The metabotropic receptors consist of eight subtypes which are sub-classified into three groups (Group I, II and III) based on the amino acid sequence of the receptor protein and signal transduction mechanism [Pin and Duvoisin, 1995; Conn and Pin, 1997]. Group I receptors (mGluR1 and mGluR5) stimulate phospholipase C and phosphoinositide (PI) hydrolysis and activate protein kinase C (PKC), Group II receptors (mGluR2 and mGluR3) are responsible for inhibiting the generation of cyclic AMP, and Group III (mGluR4, mGluR6, mGluR7, and mGluR8) inhibit adenyl cyclase (AC) [Pin and Duvoisin, 1995; Conn and Pin, 1997].

Glutamate is reported to have essential roles in neural plasticity, learning and memory [Hollmann and Heinemann, 1994]. In particular, the ionotropic receptors allow the entry of calcium into the cell; thereafter the activation of intracellular signalling pathways and the appropriate gene expression, result in LTP [Bliss and Collingridge, 1993; Ménard and Quirion, 2012]. LTP is a process of long lasting signal transduction whereby there is a high frequency stimulation of excitatory neurotransmission within the hippocampus and other regions in the brain [Bliss and Collingridge, 1993]. LTP is thought to underlie learning and memory formation [Bliss and Collingridge, 1993].

Glutamatergic signalling has multiple downstream physiological effects and has increasingly been implicated in the pathophysiology of mental disorders [Belsham, 2001]. For example, glutamate receptor and transporter expression has been reported to be dysregulated in SCZ and MDD [McCullumsmith and Meador-Woodruff, 2002; Gupta et al., 2005; Beneyto et al., 2007]. Also, it has been suggested that the glutamate pathway is a viable target for psychiatric therapeutics [Krystal et al., 2002; Javitt, 2004]. These studies emphasise the important role that glutamate signalling has in psychiatric disorders, thereby highlighting the need for further investigation of this pathway.

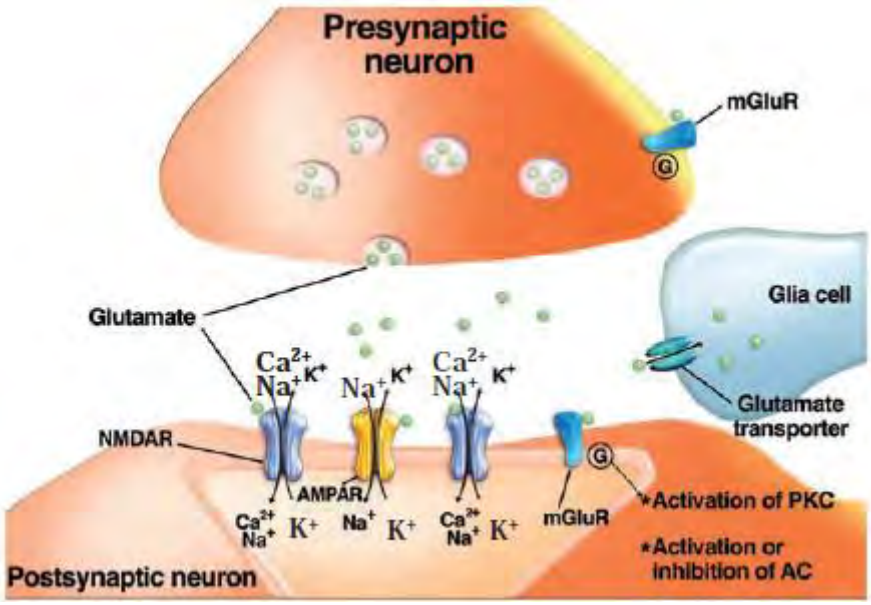

Figure 21: Glutamatergic neurotransmission in the normal state (adapted from [Clapp et al., 2008]). Glutamate is released from the presynaptic neuron where it binds to ionotropic (NMDAR, AMPAR, and Kainate receptors) and metabotropic receptors (mGlur) on the postsynaptic neuron. The appropriate responses are then initiated.

5.1.1.1 The Glutamate Pathway in BD

Several lines of evidence point to glutamatergic neurotransmission being disrupted in patients with BD suggesting that glutamate plays an important role in the aetiology of this disorder [McCullumsmith and Meador-Woodruff, 2002; Hashimoto et al., 2007; McCullumsmith et al., 2007]. Post-mortem studies have found increased glutamate levels in the frontal cortex of individuals with BD [Hashimoto et al., 2007]. Also, the NMDA-signalling complex has been found to be dysregulated in the hippocampus [McCullumsmith et al., 2007] and decreased

expression of glutamate transporters have been shown in the striatum of individuals with BD [McCullumsmith and Meador-Woodruff, 2002].

Numerous studies have investigated the genetic basis of BD with a focus on glutamatergic genes. CNV in the glutamatergic genes *mGluR7*, *calcium channel, voltage-dependent, gamma subunit 2* (*CACNG2*) and *A kinase anchor protein 5* (*AKAP5*) have shown these to be prevalent in post-mortem brains of BD patients, compared to controls [Wilson et al., 2006]. Variation (SNPs and CNVs) within the ionotropic and metabotropic glutamate receptor genes, *N-methyl-D-aspartate subunit 1* (*GRIN1*) [Mundo et al., 2003], *GRIN2A* [Itokawa et al., 2003], *GRIN2B* [Martucci et al., 2006; Dalvie et al., 2010], *glutamate receptor, metabotropic 3* (*GRM3*/*mGluR3*) [Kandaswamy et al., 2013], *glutamate receptor, metabotropic 7* (*GRM7*/*mGluR7*) [Kandaswamy et al., 2014], and *GRIK4* [Pickard et al., 2006; Pickard et al., 2008] have shown an association with BD and BD phenotype severity. A recent GWAS study found that glutamate receptor signalling was one of the most enriched pathways in BD [Xu et al., 2014]. Similarly, a recent BD GWAS meta-analysis identified the glutamatergic pathway as one of the most significantly overrepresented pathways associated with BD [Nurnberger et al., 2014].

It has also been shown that several BD treatments affect glutamate signalling [de Bartolomeis et al., 2014]. A study involving rats showed that treatment with anti-depressants increased the expression of the AMPA receptors whereas anti-manic treatments greatly decreased the expression of these receptors in the hippocampus [Du et al., 2007]. Another study examining the effect of lithium, (which is a mood-stabilising treatment for BD) on glutamate signalling, found that administration of the drug reduced the phosphorylation of the GRIN2B receptor subunit, suggesting that this drug may play a role in the inactivation of NMDA receptors [Hashimoto et al., 2002]. Also, the drug riluzole (sometimes used as an anti-depressant) increases the glutamine/glutamate ratio in the anterior cingulate cortex of individuals with BD, suggesting that this drug increases synaptic glutamate signalling [Brennan et al., 2010]. These studies illustrate that certain BD treatment strategies affect glutamatergic signalling, suggesting a possible role of glutamate in the aetiology of BD.

5.1.1.2 The Glutamate Pathway in AUD

The consumption of alcohol appears to have an effect on the glutamatergic pathway. In this regard, the glutamate-signalling pathway has been implicated in a study investigating an individual‘s level of response to alcohol, an endophenotype of AUD [Joslyn et al., 2010].

After acute alcohol withdrawal, an increase in glutamate neurotransmission and a rise in oxidative stress are observed, which may explain the symptoms of neurotoxicity observed in alcohol-dependent individuals after withdrawal [Tsai et al., 1998].

As previously mentioned (in Chapter 3), alcohol inhibits glutamate signalling, particularly by preventing the flow of ions through the NMDA receptors [Hoffman et al., 1989; Lovinger et al., 1989] (Figure 22). This inhibition has also been shown for the AMPA and kainate receptors although the effects seem to differ amongst the three ionotropic subtypes [Valenzuela et al., 1998; Carta et al., 2003]. After long-term alcohol exposure, as compensation for the inhibition of the NMDA receptors, up-regulation of NMDA receptor subunits is observed in the hippocampus and cerebral cortex of rats [Kalluri et al., 1998]. Likewise, the expression of the glutamate AMPA receptor subtypes GluR2 and GluR3 (products of the genes *glutamate receptor, ionotropic, AMPA 2* (*GRIA2*) and *glutamate receptor, ionotropic, AMPA 3* (*GRIA3*), respectively) were significantly increased in the post-mortem hippocampi of individuals who abused alcohol [Breese et al., 1995]. It has also been shown that disruption of NMDA receptor function alters the subjective response to alcohol which may increase the odds of becoming an alcoholic [Petrakis et al., 2004]. There is not much information regarding the effect of alcohol on the metabotropic glutamate receptors. However, animal studies have shown that microinjection of the mGlur5 antagonist resulted in decreased alcohol self-administration, suggesting that the metabotropic receptors may play a role in the regulation of alcohol intake [Schroeder et al., 2005; Hodge et al., 2006; Besheer et al., 2010].

As with genetic studies conducted on BD, associations between glutamatergic variants and alcohol use have been reported. Variants in the gene *GRIN2A*, which encodes a subunit of an NMDA receptor, has been associated with AD, positive family history, an earlier age of onset, risky drinking patterns in adolescents and maximum number of drinks for adults [Schumann et al., 2008; Domart et al., 2012]. The *GRIN1* and *GRIN2B* genes have also been associated with AD in European and Korean population groups [Wernicke et al., 2003; Kim et al., 2006]. In addition to the ionotropic receptors, variation within the metabotropic glutamate receptor gene, *GRM8*, has shown an association with AD [Chen et al., 2009]. The results of these findings suggest that alterations in glutamatergic neurotransmission may be involved in the development of an AUD diagnosis.

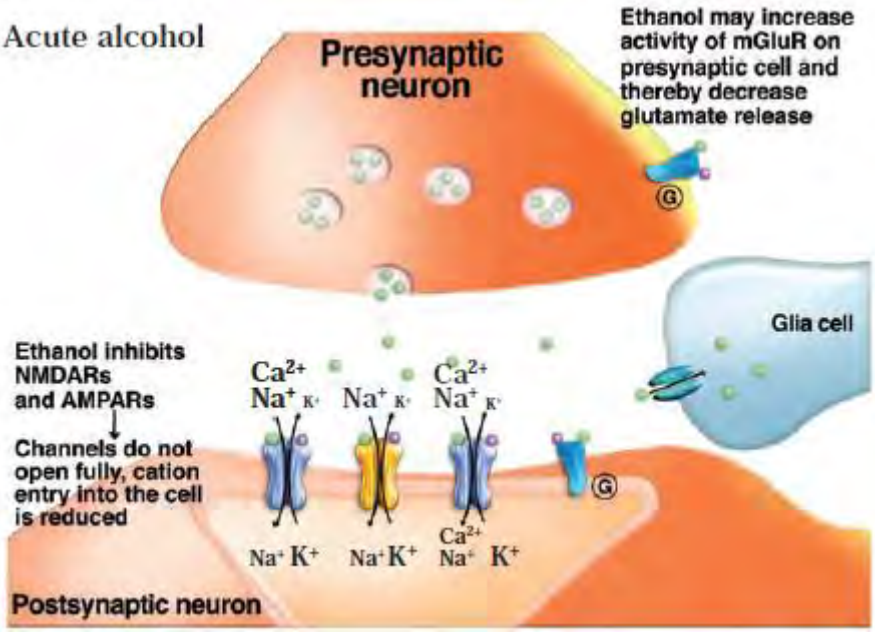

Figure 22: Glutamatergic neurotransmission after alcohol use (adapted from [Clapp et al., 2008]). The inotropic receptors are inhibited after alcohol binds (purple circles). This results in the disruption of cation flow into the post-synaptic cell.

5.1.2 The HPA-Axis in BD

In chapter 4, the role of the HPA-axis in AUD was examined. In the current chapter the HPA-axis is explored in terms of BD-AUD comorbidity.

Glucocorticoids, which are used as treatment for several ailments, produce a range of neuropsychiatric side-effects [Dubovsky et al., 2012]. This suggests a link between the HPA-axis and mental illness. Indeed, dysfunction of the HPA has been shown to be associated with BD, whereby individuals with BD had an elevated cortisol response to the dexamethasone/CRH test [Watson et al., 2004]. As mentioned in Chapter 4, there is a close relationship between the HPA-axis and the circadian pathway. One of the core features of BD is a disrupted circadian rhythm whereby sleep wake-cycles have an effect on the development of a manic or depressive episode [Leibenluft et al., 1996; Perlman et al., 2006].

There are various hypotheses as to how disruption in the HPA-axis may result in mood disorders. One of those hypotheses is that there is hypersensitivity of the HPA-axis as a result of over-secretion of CRH in individuals with mood disorders [Watson and Mackin, 2006]. This was shown in a study where elevated levels of CRH were observed in the CSF of patients with depression [Nemeroff et al., 1984]. Another hypothesis is that the hypersensitivity of the HPA-axis and over-secretion of CRH may occur as a result of impaired glucocorticoid receptor (GR) function [Watson and Mackin, 2006]. A study

involving mice with dysregulated forebrain GR function noted hypersensitivity of the HPA-axis and symptoms similar to that shown in humans with MDD [Boyle et al., 2005]. Other lines of evidence showing a relationship between HPA-axis and BD exist, for example, hair cortisol levels were associated with a more severe BD phenotype characterised by an earlier age of onset and psychiatric comorbidity [Manenschijn et al., 2012]. A study has shown that polymorphisms within the HPA-axis gene, *CRHR1*, have an association with psychotic dimensions in BD [Leszczyńska-Rodziewicz et al., 2013a]. From a BD GWAS meta-analysis, the CRH pathway has been identified to be the most significantly overrepresented pathway in BD [Nurnberger et al., 2014]. The results of these studies strongly suggest a relationship between the HPA-axis and BD. However, the direction of the relationship is still unknown i.e. is disruption of the HPA-axis causative of BD or is the axis disrupted as a result of BD.

5.1.3 Interactions Between Glutamate Pathway and HPA-Axis

The glutamatergic and HPA-axis pathways do not function completely in isolation, but rather, have been shown to have an interacting role with each other via the CRH neurons in the PVN [Herman et al., 2004]. In particular, it has been shown that glutamate regulates the HPA-axis by increasing the secretion of ACTH and corticosterone serum levels [Feldman and Weidenfeld, 1997]. In rats, CRH neurons in the PVN have been shown to express the glutamate ionotropic receptor subunits providing further evidence that glutamate has an effect on the HPA-axis [Aubry et al., 1996]. Furthermore, mGLuR7 KO mice have increased levels of GRs in the brain, reflecting dysregulation of the stress response system [Mitsukawa et al., 2006].

5.1.4 BD-AUD Comorbidity

As stated before, BD and AUD are often found to be comorbid [Hsieh et al., 2012; Schoepf and Heun, 2014], however, the nature of the relationship between these two disorders is not well understood. Approximately 70% of patients with BD abuse alcohol. The presence of alcohol use in BD has a negative effect on the course of the disorder and results in poorer cognitive abilities compared to patients with BD-only and controls [van Gorp et al., 1998]. An MRI study found that BD patients with AUD comorbidity had lower glutamate concentrations in their left DLPFC compared to non-alcoholic BD individuals, suggesting that BD-AUD comorbidity could be as a result of neurochemical imbalance in the glutamatergic pathway [Nery et al., 2010]. Similarly, a study involving MMN (mismatch negativity), a measure of NMDA receptor function, found that the NMDA receptor

dysregulation in BD could be modulated by the effects of alcohol [Chitty et al., 2014]. A specific SNP (rs148754219) within the glutamate receptor gene *GRM3* is thought to increase the risk of developing BD and AUD [Kandaswamy et al., 2013; O'Brien et al., 2014]. There has not been much research in terms of disruption in the HPA-axis and BD-AUD comorbidity. However, as the HPA-axis and the glutamate pathways have been implicated in both AUD and BD, it is plausible that these biological systems may play a role in the comorbid phenotype.

5.1.5 Aims and Objectives

The aim of this investigation was to determine whether genetic variants in the glutamatergic pathway and HPA-axis are associated with BD-AUD comorbidity. Also, to determine whether there is an association between the glutamatergic and HPA-axis variants and BD.

5.1.5.1 Objectives

The objectives of this investigation were as follows:

1) Using publically available genotype data, determine whether there is a significant difference in the genotype distributions for the selected SNPs, in a group of individuals with BD-AUD comorbidity and BD only.
2) Determine whether there is a difference in the genotype distributions for the selected SNPs in the BD-only and control groups.

5.2 Materials and Methods

5.2.1 Overview of STEP-BD

For the current study, the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD) cohort was used to investigate the hypothesis that BD comorbid with an AUD has a distinct genetic aetiology from BD on its own. The STEP-BD cohort is a long-term health study, which recruited 4360 participants, and is the largest government-funded project for BD treatment in the USA. The aim of this project was to determine which medications and combinations thereof resulted in the best treatment for manic and depressive episodes and which of these might have prevented the recurrence of these episodes. This project was different to classical clinical trials in that participants were offered long term care and treatment. All study participants had a diagnosis for BDI, BDII, BDNOS and cyclothymia and were more than 15 years of age. Diagnoses were made by clinical specialists and

psychiatrists using the DSM-IV based MINI-International Neuropsychiatric Interview (MINI) [Lecrubier et al., 1997] and a standardised affective disorder evaluation (ADE), respectively. Subjects had to have a consensus diagnosis from both instruments to be enrolled in the study. Individuals with a comorbid psychiatric diagnosis were also included in the study [Sachs et al., 2003].

Of the 4360 STEP-BD participants, 2089 individuals over the age of 18 years consented for DNA collection. Of this group, 62% had a BDI diagnosis and were of Caucasian ethnicity. A subset of these individuals was genotyped by [Sklar et al., 2008] using the Affymetrix GeneChip Human 500K Mapping Array set at the Center for Genotyping and Analysis at the Broad Institute (Massachusetts Institute of Technology, Boston, USA). Individual samples were excluded based on the following criteria: i) had a genotyping call rate of less than 95%, ii) had increased heterozygosity or homozygosity, iii) after IBS calculations and clustering were found to be sample duplications, close relatives or non-Caucasian. Markers were excluded based on the following criteria: i) a call rate of less than 95%, ii) MAF of less than 1%, iii) not in HWE in control samples ($p < 1x10-6$), iv) difference in missing genotype rates in case and control samples, v) genotyping failure based on haplotype tests, and vi) SNPs on plates with remarkable differences in allele frequencies with SNPs on other plates [Sklar et al., 2008]. The total sample for the [Sklar et al., 2008] study consisted of 955 BDI individuals and 1498 controls, of Caucasian ethnicity, genotyped for 372 193 SNPs. Of this group 1218 were males and 1235 were females [Sklar et al., 2008].

5.2.2 Candidate Gene and Variant Selection

The aim of this study was to examine whether genetic variants within the glutamatergic pathways and HPA-axis pathways have an association with BD-AUD comorbidity. Therefore, 58 glutamatergic genes (and the respective variants) were selected based on those identified in a previous publication [Drago et al., 2011]. Of these glutamate pathway genes, 21 encoded receptors or receptor-subunits, one encoded a receptor-associated protein, one a ribonuclease, 12 were AKAP genes, one encoded a DNA-binding protein, one a glutamate transporter gene, two encoded metabolic enzymes, and 19 encoded protein kinases.

The following 11 HPA-axis genes were selected for analysis: *POMC*, U*CN2*, *UCN3*, *CRHR1*, *CRHR2*, *FK506 binding protein 5* (*FKBP5*), *nuclear receptor subfamily 3, group C, member 1* (*NR3C1*), *nuclear receptor subfamily 3, group C, member 2* (*NR3C2*), *corticotropin releasing hormone binding protein* (*CRHBP*), *melanocortin 2 receptor* (*MC2R*), and *serpin*

peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6 (*SERPINA6*). These genes were chosen based on a search done in PharmGkB [Whirl-Carrillo et al., 2012] for the term "HPA" (date accessed: 13 March 2013) and from the literature [Holliday et al., 2010; Muráni et al., 2010; Schatzberg et al., 2013]. A list of variants (regulatory, intronic and exonic regions) for all of the above-mentioned HPA-axis genes was obtained from NCBI dbSNP [http://www.ncbi.nlm.nih.gov/SNP/] (date accessed: 13 March 2013).

5.2.3 Research Group

Phenotype and clinical information on the STEP-BD 16.01 group (Bipolar Disorder Data Distribution 7.0) was obtained from the National Institute of Mental Health (NIMH) Center for Collaborative Genomic Studies on Mental Disorders [https://www.nimhgenetics.org/]. A total of 498 individuals with BDI with data on current AUD (n= 233) comorbidity was selected. The main outcome variable, current AUD, was derived by combining data from current AD and current AA from the MINI questionnaire. The final list of variants analysed in this study consisted of the glutamatergic and HPA-axis variants which were genotyped in the GWAS study conducted by Sklar et al. (2008) on the STEP-BD 16.01 cohort. Genotype data from the STEP-BD 16.01 dataset was obtained from the above-mentioned NIMH website. All of the variants had a call rate of greater than 95% and a MAF of greater than 1% [Sklar et al., 2008]. For the current study, a total of 1935 SNPs comprising 69 genes were investigated. A list of all the genes and variants analysed are available in Appendix 4 (Genes and SNPs).

Chi-squared and Mann-Whitney *U* tests were used to determine whether there were any significant differences between the two groups (BD and BD-AUD) for each of the clinical (age of onset, number of previous depressive and manic episodes) and demographic (age and gender) variables. This was performed using the statistical program SPSS [IBM, 2012].

5.2.4 Power Calculation

A power calculation was performed using G* Power 3.1.3 software [Faul et al., 2007] for 233 cases and 265 controls. Setting alpha level at 0.00003 (Bonferroni corrected p-value for 1935 SNPs), the present study shows a power of 0.80 to detect genetic effects with an OR of 3.6 (logistic regression model, binomial distribution).

5.2.5 IBS Clustering

To account for possible population stratification, IBS clustering was employed in Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007] using the

entire [Sklar et al., 2008] dataset (955 cases, 1498 controls, and 372193 SNPs). Clustering constraints included a pairwise population concordance of 0.0001 which is a significance test to determine whether two individuals are from the same randomly-mating population group. The cluster output was used as a covariate in the association analysis.

5.2.6 Association Testing: SNP-Based and Gene-Based

To test for association between each of the SNPs and current AUD, logistic regression was used in Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007]. The covariates, age, gender and cluster were included in the analysis. The Bonferroni correction [Dunn, 1961] was used to adjust for multiple testing. As a secondary analysis, the same markers (with covariates) were tested in the larger cohort (955 cases and 1498 controls), to determine whether these 1935 SNPs were associated with BD.

Gene-based tests, as described in Chapter 3, consisting of 54 genes (genes with genotypic data for more than 2 SNPs), were used to test for association with BD-AUD comorbidity. Logistic regression was used, a total of 20 000 permutations were run and age, gender and cluster assignment were included as covariates. This test was also run using Plink (version 1.07) [http://pngu.mgh.harvard.edu/~purcell/plink/] [Purcell et al., 2007]. The gene-based test was also run for the total BD cohort to determine whether any of the genes of interest are associated with BD. This test was run with the same parameters as that for the BD-AUD group.

5.3 Results

5.3.1 Research Group

The final research group consisted of 233 individuals with BD-AUD comorbidity and 265 individuals with BDI only. The number of individuals in each of the groups, together with clinical and demographic data, and results from the chi-squared and Mann-Whitney *U* tests are listed in Table 22. There was no difference for gender and age between the two groups (BD-AUD and BD-only). However, there were significant differences (p-value < 0.01) between the two groups for age at onset, number of previous depressive and manic episodes. The BD-AUD group had an earlier age of onset and more depressive and manic episodes compared to the BD-only group.

5.3.2 IBS Clustering

In total there were 24 clusters. The biggest cluster consisted of 235 individuals and the smallest, nine individuals.

Table 22: Clinical and demographic characteristics of the sample. Distribution and mean±SD were reported as appropriate

Variable	**BD-AUD** (n= 233)	**BD-only** (n=265)	**Pearson chi squared/*U* statistic**	**p-value**
Gender Males:Females	114:119	110:155	2.76	0.097
Age	40.6±11.3	41.1±13.8	30798.5	0.963
Age at onset	15.4±7.1	19.8±9.7	21131.0	< 0.01
Number of previous depressive episodes	5.4±1.8	4.7±2.2	24346.5	< 0.01
Number of previous manic episodes	5.2±2.0	4.3±2.2	23354.5	< 0.01

5.3.3 Association Testing: SNP-Based and Gene-Based

From the single-SNP association analysis, none of the investigated SNPs remained associated with BD-AUD comorbidity after correction for multiple testing. Table 23 lists the SNPs which obtained an unadjusted p-value of less than 0.01. The top SNP, rs2594491 (OR= 0.67, p-value = 0.002), is an intronic variant in the *protein kinase C, epsilon* (*PRKCE)* gene. Individuals with the C-allele of this SNP are at a decreased risk of having BD-AUD comorbidity. The other three variants are all intronic and are located within the glutamate receptors genes, *GRIN2A* and *GRM8*. Individuals with the T and C alleles for the *GRIN2A* rs3104704 and *GRIN2A* rs1875205 SNPs, respectively, are at a decreased risk for having AUD comorbidity with BD. For *GRM8* rs2299556, a G-allele increases the risk for having AUD comorbidity. A total of 61 SNPs obtained an unadjusted p-value of less than 0.05 and are listed in Appendix 4 (Single-SNP Association (BD-AUD)).

In the larger cohort no single-SNP obtained significance after correction for multiple testing. However, the top two SNPs (unadjusted p-value < 0.05) were (i) rs812389 (intronic) (OR= 1.6, t-stat=3.112, p-value = 0.002, allele = G) located in the gene *GRIA1* and, (ii) rs10980210

(intronic) (OR= 2.4, t-stat= 3.077, p-value = 0.002, allele= G) located in *A kinase anchor protein 2* (*AKAP2*). Table 24 lists the SNPs which obtained an unadjusted p-value of less than 0.01. A list of all the SNPs with an unadjusted p-value of less than 0.05 is listed in Appendix 4 (Single-SNP Association (BD)).

From the gene-based analysis, only one gene was significantly associated with BD-AUD. This gene was *protein kinase C, iota* (*PRKCI*) (p-value=0.028), significant for the SNP rs1392366 (a total of 5 *PRKCI* SNPs were included in the analysis). The gene *GRIA2* was approaching significance with a p-value of 0.06 for the SNP rs17035959 (a total of 3 *GRIA2* SNPs were included in the analysis). The results from the gene-based analysis for all the genes investigated are presented in Appendix 4 (Gene-Based Results (BD-AUD)).

In the larger BD cohort three genes obtained a p-value of less than 0.05 for the gene-based analysis. These genes were *AKAP2* (p-value =0.0202) for the intronic SNPs rs10980210 and rs10816924 (40 SNPs were included in the analysis), *POMC* (p-value =0.0117) for the intronic SNP rs7565427 (4 SNPs were included in the analysis) and *NR3C2* (p-value =0.0262) for the intronic SNP rs4027073 (51 SNPs were included in the analysis). All of these genes were in the top results from the single-SNP association (Table 24). The results from the gene-based analysis for all the genes investigated are presented in Appendix 4 (Gene-Based Results (BD)).

Table 23: Top SNPs (p-value < 0.01) from single SNP association for BD-AUD

SNP	Gene	Chromosome	Tested allele	t-statistic	Odds Ratio	Unadjusted p-value	Adjusted p-value
rs2594491	*PRKCE*	2	C	-3.066	0.6677	0.0022	1
rs3104704	*GRIN2A*	16	T	-2.986	0.5604	0.0028	1
rs1875205	*GRIN2A*	16	C	-2.864	0.6155	0.0042	1
rs2299556	*GRM8*	7	G	2.668	1.549	0.0076	1

Table 24: Top SNPs (p-value < 0.01) from single SNP association for BD

SNP	Gene	Chromosome	Tested allele	t-statistic	Odds Ratio	Unadjusted p-value	Adjusted p-value
rs812389	*GRIA1*	5	G	3.112	1.565	0.001858	1
rs10980210	*AKAP2*	9	G	3.077	2.381	0.002093	1
rs16909348	*GRIN2B*	12	A	-3.003	0.3075	0.002672	1
rs4027073	*NR3C2*	4	C	2.867	1.5	0.00414	1
rs10816924	*AKAP2*	9	A	2.843	1.676	0.004462	1
rs7565427	*POMC*	2	A	-2.833	0.4937	0.004615	1
rs10865208	*PRKCE*	2	G	2.742	1.45	0.006098	1
rs2239340	*PRKCB*	16	A	2.738	1.501	0.006186	1
rs16959593	*PRKCA*	17	T	2.716	1.823	0.006607	1
rs9823996	*GRM7*	3	G	2.702	1.463	0.006895	1
rs13142954	*NR3C2*	4	A	2.692	1.494	0.007092	1

5.4 Discussion

The aims of the previous chapters of this thesis were to determine the genetic basis of BD and AUD as separate diagnoses. In this chapter the aim was to determine whether BD comorbid with AUD was genetically distinct to BD without substance-use comorbidity, in terms of the glutamatergic and HPA-axis pathways. When analysing genes, *PRKCI* was found to be associated with BD-AUD comorbidity. After correction for multiple testing, no single SNP was associated with BD-AUD comorbidity or with BD. None of the selected HPA-axis genes were associated with the BD-AUD phenotype.

From the gene-based analysis, *PRKCI*, located on chromosome 3q26.3 was shown to be associated with the BD-AUD phenotype. The SNP within this gene driving the association is rs1392366. *PRKCI* is part of the PKC family of isozymes, which are found abundantly in

neuronal cells [Tanaka and Nishizuka, 1994] and play an important role in cell signalling [Nishizuka, 1989]. PKC proteins regulate many intracellular processes and are activated by diacylglycerol as a result of the hydrolysis of inositol phospholipids. PKC proteins are thought to regulate the efficiency of synapses [Tanaka and Nishizuka, 1994] and to be involved in the pathophysiology of mood disorders [Hahn and Friedman, 1999]. These proteins are targets of lithium treatment [Manji and Lenox, 1999]. The *PRKCI* gene and the SNP rs1392366 have not previously been associated with AUD or BD-AUD comorbidity. However, another intronic SNP within *PRKCI*, rs2140825, has previously been associated with BD [Iwamoto et al., 2011]. Further investigation would be needed to verify whether this gene plays a role in determining whether a BD affected individual will go on to abuse and become dependent on alcohol.

No single SNP was significantly associated with BD-AUD comorbidity. This is consistent with previous studies which have not found an association between BD, AUD and SNPs in the glutamatergic pathway or HPA axis [Feder et al., 1985; Martí et al., 2002; Dahl et al., 2005; Tadic et al., 2005; Leszczyńska-Rodziewicz et al., 2013b]. Although no single SNP obtained a statistically significant association with BD-AUD comorbidity, it is worth discussing the genes which had SNPs with pre-corrected p-values approaching significance as these may have biological significance to the phenotype and could potentially be investigated in future studies. These genes were *PRKCE*, *GRIN2A*, and *GRM8*. *PRKCE* is also part of the PKC family of isozymes. Cytosolic levels of PRKCE have been shown to be reduced in the post-mortem brains of BD subjects [Wang and Friedman, 1996] as well as the brains of rats treated with lithium [Manji and Lenox, 1999]. In terms of AUD, the activity of PRKCE is inhibited by ethanol via an alcohol binding site present on the protein [Das et al., 2009]. The *GRIN2A* gene located on chromosome 16p13.2 encodes a subunit of the glutamate ionotropic NMDA receptor. A variable number tandem repeat in the 5‘ region of this gene has previously been implicated in the aetiology of BD and AUD [Itokawa et al., 2003; Domart et al., 2012]. Two other SNPs in *GRIN2A*, rs2072450 and rs9924016 showed an association with AD, positive family history, an earlier age of onset, risky drinking patterns in adolescents and maximum number of drinks for adults [Schumann et al., 2008]. *GRM8* is located on chromosome 7q31 [Scherer et al., 1996] and encodes a metabotropic glutamate receptor. Variation within this gene has previously been associated with AD [Chen et al., 2009] but not BD.

As a secondary analysis, the larger BD cohort was investigated in terms of the glutamatergic and HPA-axis SNPs to determine whether these SNPs were associated with the BD diagnosis. As with the BD-AUD group, none of the SNPs were significantly associated with BD after correction for multiple testing. However, SNPs in the genes *GRIA1* and *AKAP2* obtained p-values less than 0.01 prior to correction for multiple testing. *GRIA1* located on chromosome 5q31.1 encodes an ionotropic glutamate receptor. This gene has previously been implicated in BD with psychosis, but this was for SNPs other than those reported in this study [Kerner et al., 2009]. Also, the *GRIA1* SNP rs2926835 obtained genome-wide significance in a BD GWAS. *AKAP2* located at chromosome 9q31.3 encodes an A-kinase anchoring protein which, as the name suggests, functions as an ~~"a~~nchor" to protein kinase A (PKA), by binding to its regulatory subunit, thereby directing it to the appropriate location in the cell for PKA to fulfil its function [Colledge and Scott, 1999; Feliciello et al., 2001]. The *AKAP2* gene was also significantly associated with BD from the gene-based analysis. *AKAP2* has not been associated with BD before. Although *GRIA1* and *AKAP2* did not reach statistical significance they are still plausible candidates for BD and should be investigated in future studies.

None of the HPA-axis SNPs obtained even a nominal association with BD-AUD. However, from the gene-based analysis for BD only, two genes from the HPA-axis pathway, *POMC* and *NR3C2* were associated with BD. *POMC*, located on chromosome 2p23.3 encodes a hormone precursor protein, producing hormones such as ACTH [Chang et al., 1980; Whitfeld et al., 1982]. A recent proteomic study found increased levels of the POMC protein in the post-mortem pituitary glands of BD patients [Stelzhammer et al., 2015]. Also, the gene-ontology category of ~~"h~~ormone activity" was found to be over-represented in the significant hits from a BD GWAS meta-analysis, and this category included the gene *POMC* [Holmans et al., 2009]. *NR3C2*, located on chromosome 4q31.1, encodes a mineralocorticoid receptor (MR) which is involved in electrolyte homeostasis [Arriza et al., 1987]. The expression of this gene was significantly decreased in the anterior cingulate cortex and DLPFC of depressed patients [Qi et al., 2013]. SNPs within this gene have been moderately associated with BD [Ceulemans et al., 2011]. However, another study did not find an association between SNPs within *NR3C2* and BD, but found that the GR gene, *NR3C1*, had an association with clinical characteristics of BD [Spijker et al., 2011]. Previous studies have found significant evidence for the role of the HPA-axis in BD and alcohol-use phenotypes separately [Watson et al., 2004; Adinoff et al., 1991]. This suggests that the HPA-axis may

not play a role in the aetiology of BD comorbid with AUD but rather that it may be associated with BD, with no comorbidity.

One of the limitations of this study is the relatively small sample size. An odds ratio of 3.6 is required to detect a statistically significant association. For certain psychiatric disorders, including BD, it is hypothesised that the underlying aetiology is as a result of multiple variants of small effect [Purcell et al., 2009]. The same could be true for psychiatric comorbid phenotypes. Thus it is likely that a larger sample size may detect an association between the variants of interest and BD-AUD. Another limitation of this study was that the individuals under investigation could possibly have had other comorbidities, besides AUD, which they were not assessed for. This would have confounded the analysis and future studies should have a BD-AUD comorbid group free of other comorbid diagnoses. Also, only two pathways were investigated in this study and genotype data was not available for all of the genes present in either pathway and were therefore not included in this analysis. Variants not investigated in this study could play a role in the aetiology of BD-AUD. Therefore, the glutamatergic and HPA-axis pathways could still have a potential role in the aetiology of BD-AUD. Also, there are several other pathways such as the serotonergic and circadian pathways which warrant exploration in terms of BD-AUD comorbidity as these have previously been found to have an association with AUD comorbid with BD and depression [Yasseen et al., 2009; Sjoholm et al., 2010].

The results from this study indicate that BD-AUD is not genetically distinct from BD in terms of the glutamatergic pathways and the HPA-axis. However, *PRKCI* appears to be an interesting candidate gene for follow-up studies for BD-AUD comorbidity. In addition, the genes *GRIA1*, *AKAP2*, *POMC* and *NR3C2* should be further investigated in terms of BD. In the following chapter the overall conclusions of the entire thesis are outlined.

Chapter 6: Conclusions

6.1 Summary of Findings

The aim of this thesis, in its entirety, was to identify the aetiology of the complex psychiatric disorders, BD and AUD, using bioinformatics, high-throughput genomic technologies, brain-imaging and environmental measures. An additional aim was to assess the genetic aetiology of BD-AUD comorbidity. These aims were achieved firstly by performing WGS and whole-genome linkage analysis in an Afrikaner family with BD. Secondly, the aetiology of AUD was investigated by integrating high-throughput genotyping data with brain imaging and environmental measures in two adolescent groups: a South African group and an international birth cohort. Thirdly, the genetic aetiology of BD-AUD comorbidity was assessed by investigating the glutamatergic pathway and HPA-axis using publically-available data on a well-established BD cohort.

In conclusion, this study was unable to identify variants and genes which are highly likely to underlie BD and AUD, based on strong statistical evidence. Using the Bonferroni correction method to adjust for multiple testing, none of the genetic variants under examination were statistically significant. This may reflect, i) that there are no genuine statistical associations between the SNPs and phenotypes of interest, or ii) that there is some association and this disappears upon correction for multiple testing. The Bonferroni correction decreases Type I error (the probability of rejecting the null hypothesis, when it is true). This method is often used as it is relatively simple to calculate and can be applied to most multiple-testing scenarios [Bender and Lange, 2001]. However, the Bonferroni correction has often been criticized as being overly conservative and increases the chances of Type II error (the probability of rejecting the alternate hypothesis, when it is true) [Perneger, 1998]. In light of this, it is worth re-examining the uncorrected findings for each of the chapters of this thesis.

The aims of Chapter 2 were to identify susceptibility variants for BD in a large Afrikaner family using whole-genome technology. Although no chromosomal regions obtained a significant LOD score, using the Axiom™ Genome-wide CEU 1 array plate, the four regions which obtained the highest LOD scores, i.e.: 6p25, 10p14-10p15.1, 11q23-11q25, and 13q21-22 were further investigated. The interrogation of WGS data, while filtering for these four regions, identified variants and pathways which may play a role in the aetiology of BD. The TCR signalling pathway, which is involved in the immune response, was the most enriched pathway in individuals with BD. Disruption of immune function has previously been

identified in psychiatric disorders such as SCZ [Maes et al., 1995; Theodoropoulou et al., 2001; Steiner et al., 2010]. The exact mechanism as to how a disruption of the immune system results in an altered mental state is still unknown, however it is thought to possibly occur via several pathways, including the HPA-axis and various neurotransmitter pathways [Dantzer et al., 2008].

The aims of Chapter 3 were to determine the complex biological interactions underlying adolescent AUD using genetic, brain imaging, and environmental data. From the analysis of the genetic data, no single SNP was found to be associated with adolescent AUD, after correction for multiple testing. However, the SNP with the lowest uncorrected p-value was located in the glutamate-receptor gene, *GRIK4*. Furthermore, the circadian rhythm genes *NR1D1* and *BHLHE41* were associated with AUD in a gene-based test. Childhood trauma was shown to be higher in adolescents with AUD compared to matched controls. No significant gene x environment interactions were found for lifetime alcohol units consumed. Uncorrected findings from the gene-imaging analysis identified a SNP in the glutamate receptor gene, *GRIN2B*, as being associated with brain volume in the left OFC and posterior cingulate. These findings indicate that the circadian and glutamatergic pathways may play a role in AUD and should be further investigated. Both the circadian and glutamatergic pathways have previously been implicated in BD and suggest a common pathophysiology between AUD and BD may exist.

The aim of Chapter 4 was to determine whether HPA-axis variants were associated with alcohol usage in adolescence and whether these variants interact with adverse childhood events. An additional aim was to determine whether childhood internalising behaviour was associated with alcohol use in adolescence. No single SNP was significantly associated with AUDIT scores; however the ~~–to~~p" SNPs from this analysis were located in the genes *UCN3* and *POMC*. Additionally, from the gene-based analysis, *UCN3* was associated with the AUDIT scores. No significant gene x environment interaction was detected and the SDQ-emotional subscale was not associated with AUDIT score. No association was found between life events score and AUDIT scores. None of the HPA-axis genes were associated with the SDQ-emotional subscale. These results suggest that the HPA-axis genetic variants may not interact with childhood life events to predict adolescent alcohol use. Also, it appears that childhood internalising behaviour is not associated with alcohol consumption in adolescence.

In Chapter 5, the aim was to determine whether BD comorbid with AUD was genetically distinct to BD without substance-use comorbidity, in terms of the glutamatergic and HPA-axis pathways. When analysing genes, *PRKCI* was found to be associated with BD-AUD comorbidity. After correction for multiple testing, no single SNP was associated with BD-AUD comorbidity. Nonetheless, it is worth noting that the SNPs with the lowest p-values were located in the genes *PRKCE*, *GRIN2A*, and *GRM8*. None of the selected HPA-axis genes were associated with the BD-AUD phenotype. When examining BD on its own, the genes *AKAP2*, *POMC* and *NR3C2* obtained uncorrected p-values of less than 0.05.

6.2 Emerging Themes

This study was able to detect pathways which may be common to BD and AUD. Firstly; immune dysfunction may be involved in the pathophysiology of BD. The immune system has previously been implicated in BD [Breunis et al., 2003; Ortiz-Domínguez et al., 2007; Brietzke et al., 2009; Drexhage et al., 2011; Wieck et al., 2013; Barbosa et al., 2014]. The excessive consumption of alcohol has a negative effect on the immune system which could result in immunodeficiency and autoimmunity [Cook, 1998; Szabo and Mandrekar, 2009]. Post-mortem brains of individuals with AD showed an increase in expression of a pro-inflammatory cytokine protein and an increase in microglial markers compared to control brains [He and Crews, 2008]. Similarly, another study found differential expression of immune-related genes in the post-mortem brains of individuals with AD [Ökvist et al., 2007]. Besides the effect of alcohol on the immune system, variants in the immune system genes have been shown to play a role in predisposing individuals to AUD. For example, variation within interleukin-related genes have been associated with AD [Pastor et al., 2000; Pastor et al., 2005; Saiz et al., 2009]. These studies suggest a role for the immune system in AUD and further studies are required to investigate the full extent of the association between this system and BD and AUD.

Secondly, circadian dysfunction may underlie the pathophysiology of AUD. This is consistent with animal and human evidence that circadian disturbances may be relevant to AUDs [Chen et al., 2004; Hatonen et al., 2008]. Due to the observed sleep disturbances characterising the BD phenotype, the circadian rhythm has often been investigated in terms of BD [Leibenluft et al., 1996; Perlman et al., 2006; Harvey, 2008]. Several circadian rhythm genes have previously been associated with BD [Benedetti et al., 2003; Mansour et al., 2006; Nievergelt et al., 2006; Shi et al., 2008; Soria et al., 2010; Karthikeyan et al., 2014], BD symptom dimensions [Maciukiewicz et al., 2014] and BD phenotype severity [Benedetti et

al., 2008]. A post-mortem brain study conducted on individuals with BD found dysregulation of expression of circadian rhythm genes in those who abused alcohol [Gonzalez et al., 2014]. Therefore, disruption within the circadian rhythm may be a feature which is common to both BD and AUD.

Thirdly, variation within the HPA-axis does not appear to have an effect on the development of AUD. However variation within the *POMC* gene may be involved in the aetiology of AUD and BD, separately, but not when these two disorders are comorbid. *POMC* has quite a close relationship with the immune system since immune cells (e.g. lymphocytes) have been shown to express *POMC* [Ottaviani et al., 1995]. *POMC* mRNA expression was significantly increased in inflamed epidermis tissue compared to normal tissue [Kono et al., 2001]. Similarly, *POMC* also plays a role in the circadian rhythm [Dickmeis et al., 2007]. Disruption of the circadian rhythm of *POMC* expression in the hypothalamus of rats was observed after alcohol administration [Chen et al., 2004]. Thus *POMC* has an association with the immune system and circadian pathways, both of which may play role in the development of BD and AUD.

It is likely that the aetiology of psychiatric phenotypes is as a result of multiple variants interacting with the environment [Uher, 2009; Uher, 2014]. Although in this study we were unable to detect significant gene x environment interactions for AUD, we were able to identify common pathways, possibly interacting with each other, for BD and AUD. The immune system has a tight bi-directional relationship with the HPA-axis [Silverman et al., 2005; Dunn, 2007]. The HPA-axis has a close relationship with circadian rhythms [Dickmeis et al., 2007; Nader et al., 2010; Kalsbeek et al., 2012]. In turn, the immune system also has an interaction with the circadian pathways [Keller et al., 2009; Scheiermann et al., 2013; Nakao, 2014]. Thus, it is possible that variants of small effect within these three pathways, which were statistically undetectable in this study, interact with each other to produce the complex phenotypes of BD and AUD.

6.3 Future Considerations

Based on the findings from this study, there are several possible considerations to be made for future research in the field of psychiatric genetics. The immune, circadian, and HPA-axis pathways should be investigated further in terms of BD and AUD, particularly in terms of possible epistatic relationships between variants in the genes constituting these pathways. In addition to the kinds of studies reported in this thesis, the expression and epigenetic profiles

of the genes constituting these pathways could be obtained from the post-mortem brain tissue of individuals with BD and AUD. Another approach would be to investigate the expression profiles of microRNAs (miRNAs) which regulate the genes in the immune, circadian, and HPA-axis pathways in individuals with BD and AUD. A recent study has found dysregulated miRNA expression levels in individuals at a high genetic risk for BD [Walker et al., 2015].

Further, in this study only SNPs were investigated. Previous studies have shown increased prevalence of CNVs in several psychiatric disorders including AD and BD [Lachman et al., 2007; Zhang et al., 2008; Lin et al., 2012; Ye et al., 2012]. Future studies could investigate whether there are an increased number of large structural variants in the genes of the three pathways of interest (immune, circadian and HPA-axis) in individuals with BD and AUD. In addition to the genetic analysis of the immune, circadian, and HPA-axis pathways, the proteomic profiles of these pathways could be explored in individuals with BD and AUD.

The use of longitudinal studies may aid in identifying environmental risk factors for the development of psychiatric disorders. In the current study, the longitudinal ALSPAC birth cohort was investigated. No association was found between adverse childhood events collected at a particular time point and adolescent AUD. Also, no significant gene x environment interactions were detected for AUD. Future studies should assess traumatic events throughout childhood and determine if these environmental factors interact with variants in the immune and circadian pathways to predict risk for the development of AUD in adolescence and adulthood.

In this study, statistically significant signals were not detected. Therefore, future studies should have larger sample sizes to have adequate power to detect association between genetic variation and psychiatric phenotypes. Previous studies conducted on large sample numbers (for example 36,989 cases and 113,075 controls) have had success in identifying possible causal variants for SCZ [Ripke et al., 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014].

As described above, disruption in the immune system has also been associated with AUD. The consumption of alcohol affects the immune system [Cook, 1998; Szabo and Mandrekar, 2009] and genetic variants in immune-related genes may predispose individuals to develop AUD [Pastor et al., 2000; Pastor et al., 2005; Saiz et al., 2009], thereby suggesting a bi-directional relationship between alcohol use and the immune system [Robinson et al., 2014]. Another possible direction for future research would be to collect data on substance abuse for

members of Family 30. It would be interesting to determine whether any members of this family had an AUD and whether the immune system would be implicated in this psychiatric phenotype. These family members should also be investigated to determine possible endophenotypes for BD and if present in this family, AUD.

Another possible direction for future studies would be to investigate clinical subgroups of BD in a case-only manner. In this regard, a previous GWAS found different groups of genes associated with two different subtypes of mania: elated and irritable [Greenwood et al., 2013]. Other studies have found significant associations by stratifying affected individuals by symptom dimensions [Labbe et al., 2012; Meier et al., 2012].

In the current investigation and in previous studies, NGS technology has been used to determine the aetiology of BD and other psychiatric disorders such as SCZ. To date, only one WES study has been conducted for an AUD phenotype [Zuo et al., 2013a]. This study found that the most promising candidate genes for AD were *low density lipoprotein receptor-related protein 8, apolipoprotein e receptor* (*APOER2*) and *ubiquitin associated protein 2* (*UBAP2*) [Zuo et al., 2013a]. New genomic technologies, such as WGS and WES may hold the key to determining the complex genetic architecture underlying AUD and should be pursued further.

One of the key challenges of studying psychiatric disorders is the absence of an accurate cell model. To help overcome this problem, researchers have obtained patient-derived induced pluripotent stem cells (iPSCs) to determine *in vitro*, the mechanism underlying psychiatric disorders such as SCZ [Yoon et al., 2014]. A recent study was able to reprogram BD patients‘ fibroblast cells to neuronal cells in order to determine the transcriptional profile of these individuals compared to controls [Chen et al., 2014]. Thus, this approach appears to be an exciting avenue to pursue in future research.

References

1000 Genomes Project Consortium. 2012. An integrated map of genetic variation from 1,092 human genomes. Nature 491:56-65.

Abecasis GR, Burt RA, Hall D, Bochum S, Doheny KF, Lundy SL, Torrington M, Roos JL, Gogos JA, Karayiorgou M. 2004. Genomewide scan in families with schizophrenia from the founder population of afrikaners reveals evidence for linkage and uniparental disomy on chromosome 1. The American Journal of Human Genetics 74:403-417.

Acheson A, Tagamets MA, Rowland LM, Mathias CW, Wright SN, Hong LE, Kochunov P, Dougherty DM. 2014. Increased forebrain activations in youths with family histories of alcohol and other substance use disorders performing a go/NoGo task. Alcoholism: Clinical and Experimental Research 38:2944-2951.

Adinoff B, Risher-Flowers D, De Jong J, Ravitz B, Bone GH, Nutt DJ, Roehrich L, Martin PR, Linnoila M. 1991. Disturbances of hypothalamic-pituitary-adrenal axis functioning during ethanol withdrawal in six men. Am J Psychiatry 148:1023-1025.

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. 2010. A method and server for predicting damaging missense mutations. Nature Methods 7:248-249.

Agartz I, Momenan R, Rawlings RR, Kerich MJ, Hommer DW. 1999. Hippocampal volume in patients with alcohol dependence. Arch Gen Psychiatry 56:356-366.

ALSPAC Study Team. 2001. ALSPAC–the avon longitudinal study of parents and children. Paediatr Perinat Epidemiol 15:74-87.

American Psychiatric Association. Task Force on DSM-IV. 1994. DSM-IV: Diagnostic and statistical manual of mental disorders. In: American Psychiatric Association Washington, DC.

Angst J, Sellaro R. 2000. Historical perspectives and natural history of bipolar disorder. Biol Psychiatry 48:445-457.

Arnold PD, MacMaster FP, Hanna GL, Richter MA, Sicard T, Burroughs E, Mirza Y, Easter PC, Rose M, Kennedy JL. 2009a. Glutamate system genes associated with ventral prefrontal and thalamic volume in pediatric obsessive-compulsive disorder. Brain Imaging and Behavior 3:64-76.

Arnold PD, MacMaster FP, Richter MA, Hanna GL, Sicard T, Burroughs E, Mirza Y, Easter PC, Rose M, Kennedy JL. 2009b. Glutamate receptor gene (GRIN2B) associated with reduced anterior cingulate glutamatergic concentration in pediatric obsessive–compulsive disorder. Psychiatry Research: Neuroimaging 172:136-139.

Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM. 1987. Cloning of human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. Science 237:268-275.

Asaba K, Makino S, Hashimoto K. 1998. Effect of urocortin on ACTH secretion from rat anterior pituitary in vitro and in vivo: Comparison with corticotropin-releasing hormone. Brain Res 806:95-103.

Ashburner J, Friston KJ. 2005. Unified segmentation. Neuroimage 26:839-851.

Aston C, Jiang L, Sokolov B. 2005. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. Mol Psychiatry 10:309-322.

Aubry J, Bartanusz V, Pagliusi S, Schulz P, Kiss JZ. 1996. Expression of ionotropic glutamate receptor subunit mRNAs by paraventricular corticotropin-releasing factor (CRF) neurons. Neurosci Lett 205:95-98.

Baaré WF, Pol HEH, Boomsma DI, Posthuma D, de Geus EJ, Schnack HG, van Haren NE, van Oel CJ, Kahn RS. 2001. Quantitative genetic modeling of variation in human brain morphology. Cerebral Cortex 11:816-824.

Babor TF, Higgins-Biddle JC, Saunders JB, Monteiro MG. 2001. Audit. The Alcohol use Disorders Identification Test (AUDIT): Guidelines for use in Primary Care .

Bachtell RK, Weitemier AZ, Galvan-Rosas A, Tsivkovskaia NO, Risinger FO, Phillips TJ, Grahame NJ, Ryabinin AE. 2003. The edinger-westphal-lateral septum urocortin pathway and its relationship to alcohol consumption. J Neurosci 23:2477-2487.

Badner J, Gershon E. 2002. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol Psychiatry 7:405-411.

Baik I, Cho NH, Kim SH, Han BG, Shin C. 2011. Genome-wide association studies identify genetic loci related to alcohol consumption in korean men. Am J Clin Nutr 93:809-816.

Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, Koob GF, Vale WW, Lee K. 2000. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. Nat Genet 24:410-414.

Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, Nickerson DA, Shendure J. 2011. Exome sequencing as a tool for mendelian disease gene discovery. Nature Reviews Genetics 12:745-755.

Barbosa IG, Rocha NP, Assis F, Vieira EL, Soares JC, Bauer ME, Teixeira AL. 2014. Monocyte and lymphocyte activation in bipolar disorder: A new piece in the puzzle of immune dysfunction in mood disorders. Int J Neuropsychopharmacol 1-7.

Barnett BE, Hanna B, Parker G. 1983. Life event scales for obstetric groups. J Psychosom Res 27:313-320.

Barr CS, Dvoskin RL, Yuan Q, Lipsky RH, Gupte M, Hu X, Zhou Z, Schwandt ML, Lindell SG, McKee M. 2008. CRH haplotype as a factor influencing cerebrospinal fluid levels of corticotropin-releasing hormone, hypothalamic-pituitary-adrenal axis activity, temperament, and alcohol consumption in rhesus macaques. Arch Gen Psychiatry 65:934-944.

Barr CS, Dvoskin RL, Gupte M, Sommer W, Sun H, Schwandt ML, Lindell SG, Kasckow JW, Suomi SJ, Goldman D, Higley JD, Heilig M. 2009. Functional CRH variation increases stress-induced alcohol consumption in primates. Proc Natl Acad Sci U S A 106:14593-14598.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21:263-265.

Baum A, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, Schulze T, Cichon S, Rietschel M, Nöthen M. 2008. A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. Mol Psychiatry 13:197-207.

Baysal BE, Willett-Brozick JE, Badner JA, Corona W, Ferrell RE, Nimgaonkar VL, Detera-Wadleigh SD. 2002. A mannosyltransferase gene at 11q23 is disrupted by a translocation breakpoint that co-segregates with bipolar affective disorder in a small family. Neurogenetics 4:43-53.

Bechara A, Damasio H, Damasio AR. 2000. Emotion, decision making and the orbitofrontal cortex. Cereb Cortex 10:295-307.

Bellis MD, Narasimhan A, Thatcher DL, Keshavan MS, Soloff P, Clark DB. 2005. Prefrontal cortex, thalamus, and cerebellar volumes in adolescents and young adults with Adolescent-Onset alcohol use disorders and comorbid mental disorders. Alcoholism: Clinical and Experimental Research 29:1590-1600.

Belmonte Mahon P, Pirooznia M, Goes FS, Seifuddin F, Steele J, Lee PH, Huang J, Hamshere ML, DePaulo JR, Kelsoe JR. 2011. Genome-wide association analysis of age at onset and psychotic symptoms in bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 156:370-378.

Belsham B. 2001. Glutamate and its role in psychiatric illness. Hum Psychopharmacol Clin Exp 16:139-146.

Bender R, Lange S. 2001. Adjusting for multiple testing—when and how? J Clin Epidemiol 54:343-349.

Benedetti F, Dallaspezia S, Colombo C, Pirovano A, Marino E, Smeraldi E. 2008. A length polymorphism in the circadian clock gene Per3 influences age at onset of bipolar disorder. Neurosci Lett 445:184-187.

Benedetti F, Serretti A, Colombo C, Barbini B, Lorenzi C, Campori E, Smeraldi E. 2003. Influence of CLOCK gene polymorphism on circadian mood fluctuation and illness recurrence in bipolar depression. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 123:23-26.

Beneyto M, Kristiansen LV, Oni-Orisan A, McCullumsmith RE, Meador-Woodruff JH. 2007. Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. Neuropsychopharmacology 32:1888-1902.

Bentley DR. 2006. Whole-genome re-sequencing. Curr Opin Genet Dev 16:545-552.

Bergen S, O'dushlaine C, Ripke S, Lee P, Ruderfer D, Akterin S, Moran J, Chambert K, Handsaker R, Backlund L. 2012. Genome-wide association study in a swedish population yields support for greater CNV and MHC involvement in schizophrenia compared with bipolar disorder. Mol Psychiatry 17:880-886.

Bergen AW, Yang XR, Bai Y, Beerman MB, Goldstein AM, Goldin LR, Framingham Heart Study. 2003. Genomic regions linked to alcohol consumption in the framingham heart study. BMC Genet 4 Suppl 1:S101.

Bernstein DP, Stein JA, Handelsman L. 1998. Predicting personality pathology among adult patients with substance use disorders: Effects of childhood maltreatment. Addict Behav 23:855-868.

Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, Stokes J, Handelsman L, Medrano M, Desmond D. 2003. Development and validation of a brief screening version of the childhood trauma questionnaire. Child Abuse Negl 27:169-190.

Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, Sapareto E, Ruggiero J. 1994. Initial reliability and validity of a new retrospective measure of child abuse and neglect. Am J Psychiatry 151:1132-1136.

Besheer J, Fisher KR, Lindsay TG, Cannady R. 2013. Transient increase in alcohol self-administration following a period of chronic exposure to corticosterone. Neuropharmacology 72:139-147.

Besheer J, Grondin JJ, Cannady R, Sharko AC, Faccidomo S, Hodge CW. 2010. Metabotropic glutamate receptor 5 activity in the nucleus accumbens is required for the maintenance of ethanol self-administration in a rat genetic model of high alcohol intake. Biol Psychiatry 67:812-822.

Bevilacqua L, Carli V, Sarchiapone M, George DK, Goldman D, Roy A, Enoch M. 2012. Interaction between FKBP5 and childhood trauma and risk of aggressive behavior. Arch Gen Psychiatry 69:62-70.

Bierut LJ, Agrawal A, Bucholz KK, Doheny KF, Laurie C, Pugh E, Fisher S, Fox L, Howells W, Bertelsen S, Hinrichs AL, Almasy L, Breslau N, Culverhouse RC, Dick DM, Edenberg HJ, Foroud T, Grucza RA, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Krueger RF, Kuperman S, Lynskey M, Mann K, Neuman RJ, Nothen MM, Nurnberger JI,Jr, Porjesz B, Ridinger M, Saccone NL, Saccone SF, Schuckit MA, Tischfield

JA, Wang JC, Rietschel M, Goate AM, Rice JP, Gene, Environment Association Studies Consortium. 2010. A genome-wide association study of alcohol dependence. Proc Natl Acad Sci U S A 107:5082-5087.

Bittencourt JC, Vaughan J, Arias C, Rissman RA, Vale WW, Sawchenko PE. 1999. Urocortin expression in rat brain: Evidence against a pervasive relationship of Urocortin-Containing projections with targets bearing type 2 CRF receptors. J Comp Neurol 415:285-312.

Black SA, Markides KS. 1993. Acculturation and alcohol consumption in puerto rican, cuban-american, and mexican-american women in the united states. Am J Public Health 83:890-893.

Blank T, Nijholt I, Eckart K, Spiess J. 2002. Priming of long-term potentiation in mouse hippocampus by corticotropin-releasing factor and acute stress: Implications for hippocampus-dependent learning. J Neurosci 22:3788-3794.

Bliss TV, Collingridge GL. 1993. A synaptic model of memory: Long-term potentiation in the hippocampus. Nature 361:31-39.

Blomeyer D, Buchmann AF, Lascorz J, Zimmermann US, Esser G, Desrivieres S, Schmidt MH, Banaschewski T, Schumann G, Laucht M. 2013. Association of PER2 genotype and stressful life events with alcohol drinking in young adults. PloS One 8:e59136.

Blomeyer D, Treutlein J, Esser G, Schmidt MH, Schumann G, Laucht M. 2008. Interaction between CRHR1 gene and stressful life events predicts adolescent heavy alcohol use. Biol Psychiatry 63:146-151.

Bobak M, McKee M, Rose R, Marmot M. 1999. Alcohol consumption in a national sample of the russian population. Addiction 94:857-866.

Böhm D, Hoffmann K, Laccone F, Wilken B, Dechent P, Frahm J, Bartels I, Bohlander S. 2006. Association of jacobsen syndrome and bipolar affective disorder in a patient with a de novo 11q terminal deletion. American Journal of Medical Genetics Part A 140:378-382.

Boileau I, Assaad J, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay RE, Dagher A. 2003. Alcohol promotes dopamine release in the human nucleus accumbens. Synapse 49:226-231.

Boomsma DI, Wijmenga C, Slagboom EP, Swertz MA, Karssen LC, Abdellaoui A, Ye K, Guryev V, Vermaat M, van Dijk F. 2014. The genome of the netherlands: Design, and project goals. European Journal of Human Genetics 22:221-227.

Botha MC, Beighton P. 1983. Inherited disorders in the afrikaner population of southern africa. part I. historical and demographic background, cardiovascular, neurological, metabolic and intestinal conditions. S Afr Med J 64:609-612.

Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. 2013. Cohort profile: The 'children of the 90s'--the index offspring of the avon longitudinal study of parents and children. Int J Epidemiol 42:111-127.

Boyle MP, Brewer JA, Funatsu M, Wozniak DF, Tsien JZ, Izumi Y, Muglia LJ. 2005. Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. Proc Natl Acad Sci U S A 102:473-478.

Bradley RG, Binder EB, Epstein MP, Tang Y, Nair HP, Liu W, Gillespie CF, Berg T, Evces M, Newport DJ. 2008. Influence of child abuse on adult depression: Moderation by the corticotropin-releasing hormone receptor gene. Arch Gen Psychiatry 65:190-200.

Breese CR, Freedman R, Leonard SS. 1995. Glutamate receptor subtype expression in human postmortem brain tissue from schizophrenics and alcohol abusers. Brain Res 674:82-90.

Brennan BP, Hudson JI, Jensen JE, McCarthy J, Roberts JL, Prescot AP, Cohen BM, Pope HG, Renshaw PF, Öngür D. 2010. Rapid enhancement of glutamatergic neurotransmission in bipolar depression following treatment with riluzole. Neuropsychopharmacology 35:834-846.

Breunis MN, Kupka RW, Nolen WA, Suppes T, Denicoff KD, Leverich GS, Post RM, Drexhage HA. 2003. High numbers of circulating activated T cells and raised levels of serum IL-2 receptor in bipolar disorder. Biol Psychiatry 53:157-165.

Brietzke E, Sant‘anna MK, Jackowski A, Grassi-Oliveira R, Bucker J, Zugman A, Mansur RB, Bressan RA. 2012. Impact of childhood stress on psychopathology. Revista Brasileira De Psiquiatria 34:480-488.

Brietzke E, Stertz L, Fernandes BS, Kauer-Sant'Anna M, Mascarenhas M, Vargas AE, Chies JA, Kapczinski F. 2009. Comparison of cytokine levels in depressed, manic and euthymic patients with bipolar disorder. J Affect Disord 116:214-217.

Brooks SJ, Dalvie S, Cuzen NL, Cardenas V, Fein G, Stein DJ. 2014. Childhood adversity is linked to differential brain volumes in adolescents with alcohol use disorder: A voxel-based morphometry study. Metab Brain Dis 29:311-321.

Calabrese J, Hirschfeld R, Reed M, Davies M, Frye M, Keck P, Lewis L, McElroy S, McNulty J, Wagner K. 2003. Impact of bipolar disorder on a US community sample. J Clin Psychiatry 64:425-432.

Camchong J, MacDonald AW, Mueller BA, Nelson B, Specker S, Slaymaker V, Lim KO. 2014. Changes in resting functional connectivity during abstinence in stimulant use disorder: A preliminary comparison of relapsers and abstainers. Drug Alcohol Depend 139:145-151.

Capuron L, Ravaud A. 1999. Prediction of the depressive effects of interferon alfa therapy by the patient's initial affective state. N Engl J Med 340:1370-1370.

Cardoso BM, Sant'Anna MK, Dias VV, Andreazza AC, Ceresér KM, Kapczinski F. 2008. The impact of co-morbid alcohol use disorder in bipolar patients. Alcohol 42:451-457.

Carta M, Ariwodola OJ, Weiner JL, Valenzuela CF. 2003. Alcohol potently inhibits the kainate receptor-dependent excitatory drive of hippocampal interneurons. Proc Natl Acad Sci U S A 100:6813-6818.

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R. 2002. Role of genotype in the cycle of violence in maltreated children. Science 297:851-854.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. 2003. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science 301:386-389.

Ceulemans S, De Zutter S, Heyrman L, Norrback K, Nordin A, Nilsson L, Adolfsson R, Del-Favero J, Claes S. 2011. Evidence for the involvement of the glucocorticoid receptor gene in bipolar disorder in an isolated northern swedish population. Bipolar Disord 13:614-623.

Chalmers DT, Lovenberg TW, De Souza EB. 1995. Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: Comparison with CRF1 receptor mRNA expression. J Neurosci 15:6340-6350.

Chambless DL, Cherney J, Caputo GC, Rheinstein BJ. 1987. Anxiety disorders and alcoholism:: A study with inpatient alcoholics. J Anxiety Disord 1:29-40.

Chang AC, Cochet M, Cohen SN. 1980. Structural organization of human genomic DNA encoding the pro-opiomelanocortin peptide. Proc Natl Acad Sci U S A 77:4890-4894.

Chen AC, Manz N, Tang Y, Rangaswamy M, Almasy L, Kuperman S, Nurnberger J, O'connor SJ, Edenberg HJ, Schuckit MA. 2010. Single-Nucleotide polymorphisms in corticotropin releasing hormone receptor 1 gene (CRHR1) are associated with quantitative trait of Event-Related potential and alcohol dependence. Alcoholism: Clinical and Experimental Research 34:988-996.

Chen AC, Tang Y, Rangaswamy M, Wang JC, Almasy L, Foroud T, Edenberg HJ, Hesselbrock V, Nurnberger J, Kuperman S. 2009. Association of single nucleotide polymorphisms in a glutamate receptor gene (GRM8) with theta power of event-related oscillations and alcohol dependence. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 150:359-368.

Chen CP, Kuhn P, Advis JP, Sarkar DK. 2004. Chronic ethanol consumption impairs the circadian rhythm of pro-opiomelanocortin and period genes mRNA expression in the hypothalamus of the male rat. J Neurochem 88:1547-1554.

Chen D, Jiang X, Akula N, Shugart Y, Wendland J, Steele C, Kassem L, Park J, Chatterjee N, Jamain S. 2013a. Genome-wide association study meta-analysis of european and asian-ancestry samples identifies three novel loci associated with bipolar disorder. Mol Psychiatry 18:195-205.

Chen H, DeLong C, Bame M, Rajapakse I, Herron T, McInnis M, O'Shea K. 2014. Transcripts involved in calcium signaling and telencephalic neuronal fate are altered in induced pluripotent stem cells from bipolar disorder patients. Translational Psychiatry 4:e375.

Chen Y, Prescott CA, Walsh D, Patterson DG, Riley BP, Kendler KS, Kuo P. 2011. Different phenotypic and genotypic presentations in alcohol dependence: Age at onset matters. Journal of Studies on Alcohol and Drugs 72:752-762.

Chen Y, Carter H, Parla J, Kramer M, Goes FS, Pirooznia M, Zandi PP, McCombie WR, Potash JB, Karchin R. 2013b. A hybrid likelihood model for sequence-based disease association studies. PLoS Genetics 9:e1003224.

Chen A, Zorrilla E, Smith S, Rousso D, Levy C, Vaughan J, Donaldson C, Roberts A, Lee KF, Vale W. 2006. Urocortin 2-deficient mice exhibit gender-specific alterations in circadian hypothalamus-pituitary-adrenal axis and depressive-like behavior. J Neurosci 26:5500-5510.

Chen R, Lewis KA, Perrin MH, Vale WW. 1993. Expression cloning of a human corticotropin-releasing-factor receptor. Proc Natl Acad Sci U S A 90:8967-8971.

Cheng R, Juo S, Loth J, Nee J, Iossifov I, Blumenthal R, Sharpe L, Kanyas K, Lerer B, Lilliston B. 2006. Genome-wide linkage scan in a large bipolar disorder sample from the national institute of mental health genetics initiative suggests putative loci for bipolar disorder, psychosis, suicide, and panic disorder. Mol Psychiatry 11:252-260.

Chetcuti A, Adams LJ, Mitchell PB, Schofield PR. 2006. Altered gene expression in mice treated with the mood stabilizer sodium valproate. The International Journal of Neuropsychopharmacology 9:267-276.

Chitty KM, Kaur M, Lagopoulos J, Hickie IB, Hermens DF. 2014. Risky alcohol use predicts temporal mismatch negativity impairments in young people with bipolar disorder. Biol Psychol 99:60-68.

Chrousos GP. 1998. Stressors, stress, and neuroendocrine integration of the adaptive response: The 1997 hans selye memorial lecture. Ann N Y Acad Sci 851:311-335.

Cicchetti D, Rogosch FA, Oshri A. 2011. Interactive effects of corticotropin releasing hormone receptor 1, serotonin transporter linked polymorphic region, and child maltreatment on diurnal cortisol regulation and internalizing symptomatology. Dev Psychopathol 23:1125-1138.

Cichon S, Mühleisen TW, Degenhardt FA, Mattheisen M, Miró X, Strohmaier J, Steffens M, Meesters C. 2011. Genome-wide association study identifies genetic variation in neurocan as a susceptibility factor for bipolar disorder. The American Journal of Human Genetics 88:372-388.

Cichon S, Schumacher J, Muller DJ, Hurter M, Windemuth C, Strauch K, Hemmer S, Schulze TG, Schmidt-Wolf G, Albus M, Borrmann-Hassenbach M, Franzek E, Lanczik M, Fritze J, Kreiner R, Reuner U, Weigelt B, Minges J, Lichtermann D, Lerer B, Kanyas K, Baur MP, Wienker TF, Maier W, Rietschel M, Propping P, Nothen MM. 2001. A genome screen for genes predisposing to bipolar affective disorder detects a new susceptibility locus on 8q. Hum Mol Genet 10:2933-2944.

Clapp P, Bhave SV, Hoffman PL. 2008. How adaptation of the brain to alcohol leads to dependence: A pharmacological perspective. Alcohol Res Health 31:310-339.

Colledge M, Scott JD. 1999. AKAPs: From structure to function. Trends Cell Biol 9:216-221.

Collingridge GL, Olsen RW, Peters J, Spedding M. 2009. A nomenclature for ligand-gated ion channels. Neuropharmacology 56:2-5.

Conn PJ, Pin J. 1997. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37:205-237.

Constant A, Castera L, Dantzer R, Couzigou P, de Ledinghen V, Demotes-Mainard J, Henry C. 2005. Mood alterations during interferon-alfa therapy in patients with chronic hepatitis C: Evidence for an overlap between manic/hypomanic and depressive symptoms. J Clin Psychiatry 66:1050-1057.

Cook RT. 1998. Alcohol abuse, alcoholism, and damage to the immune system—a review. Alcoholism: Clinical and Experimental Research 22:1927-1942.

Cooper ML, Frone MR, Russell M, Mudar P. 1995. Drinking to regulate positive and negative emotions: A motivational model of alcohol use. J Pers Soc Psychol 69:990-1005.

Copeland WE, Magnusson Å, Göransson M, Heilig MA. 2011. Genetic moderators and psychiatric mediators of the link between sexual abuse and alcohol dependence. Drug Alcohol Depend 115:183-189.

Costin B, Miles M. 2014. Molecular and neurologic responses to chronic alcohol use. Alcohol and the Nervous System: Handbook of Clinical Neurology 125:157-171.

Covault J, Tennen H, Armeli S, Conner TS, Herman AI, Cillessen AH, Kranzler HR. 2007. Interactive effects of the serotonin transporter 5-HTTLPR polymorphism and stressful life events on college student drinking and drug use. Biol Psychiatry 61:609-616.

Cross-Disorder Group of the Psychiatric Genomics Consortium. 2013a. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet 45:984-994.

Cross-Disorder Group of the Psychiatric Genomics Consortium. 2013b. Identification of risk loci with shared effects on five major psychiatric disorders: A genome-wide analysis. The Lancet 381:1371-1379.

Cruceanu C, Ambalavanan A, Spiegelman D, Gauthier J, Lafrenière RG, Dion PA, Alda M, Turecki G, Rouleau GA, Scherer S. 2013. Family-based exome-sequencing approach identifies rare susceptibility variants for lithium-responsive bipolar disorder 1. Genome 56:634-640.

Curtis D, Vine AE, McQuillin A, Bass NJ, Pereira A, Kandaswamy R, Lawrence J, Anjorin A, Choudhury K, Datta SR, Puri V, Krasucki R, Pimm J, Thirumalai S, Quested D, Gurling HM. 2011. Case-case genome-wide association analysis shows markers differentially associated with schizophrenia and bipolar disorder and implicates calcium channel genes. Psychiatr Genet 21:1-4.

Daff E, Thomas SD. 2014. Bipolar disorder and criminal offending: A data linkage study. Soc Psychiatry Psychiatr Epidemiol 49:1985-1991.

Dahl JP, Doyle GA, Oslin DW, Buono RJ, Ferraro TN, Lohoff FW, Berrettini WH. 2005. Lack of association between single nucleotide polymorphisms in the corticotropin releasing hormone receptor 1 (CRHR1) gene and alcohol dependence. J Psychiatr Res 39:475-479.

Dalvie S, Horn N, Nossek C, van der Merwe L, Stein D, Ramesar R. 2010. Psychosis and relapse in bipolar disorder are related to GRM3, DAOA, and GRIN2B genotype. African Journal of Psychiatry 13:297-301.

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. 2008. From inflammation to sickness and depression: When the immune system subjugates the brain. Nature Reviews Neuroscience 9:46-56.

Das J, Pany S, Rahman GM, Slater SJ. 2009. PKC epsilon has an alcohol-binding site in its second cysteine-rich regulatory domain. Biochem J 421:405-413.

Davidson D, Palfai T, Bird C, Swift R. 1999. Effects of naltrexone on alcohol Self-Administration in heavy drinkers. Alcoholism: Clinical and Experimental Research 23:195-203.

Dawson DA, Grant BF, Ruan WJ. 2005. The association between stress and drinking: Modifying effects of gender and vulnerability. Alcohol Alcohol 40:453-460.

de Bartolomeis A, Buonaguro EF, Iasevoli F, Tomasetti C. 2014. The emerging role of dopamine-glutamate interaction and of the postsynaptic density in bipolar disorder pathophysiology: Implications for treatment. J Psychopharmacol 28:505-526.

de Baumont A, Maschietto M, Lima L, Carraro DM, Olivieri EH, Fiorini A, Barreta LAN, Palha JA, Belmonte-de-Abreu P, Moreira Filho CA. 2014. Innate immune response is differentially dysregulated between bipolar disease and schizophrenia. Schizophr Res 161:215-221.

De Bellis MD, Clark DB, Beers SR, Soloff PH, Boring AM, Hall J, Kersh A, Keshavan MS. 2000. Hippocampal volume in adolescent-onset alcohol use disorders. Am J Psychiatry 157:737-744.

De la Vega FM, Lazaruk KD, Rhodes MD, Wenz MH. 2005. Assessment of two flexible and compatible SNP genotyping platforms: TaqMan SNP genotyping assays and the SNPlex genotyping system. Mutat Res 573:111-135.

de Wit E, Delport W, Rugamika CE, Meintjes A, Möller M, van Helden PD, Seoighe C, Hoal EG. 2010. Genome-wide analysis of the structure of the south african coloured population in the western cape. Hum Genet 128:145-153.

Dean OM, Data-Franco J, Giorlando F, Berk M. 2012. Minocycline. CNS Drugs 26:391-401.

Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G, Rollins DY, Moses T, Sanders AR, Karkera JD, Esterling LE, Zeng J, Ferraro TN, Guroff JJ, Kazuba D, Maxwell ME, Nurnberger JI,Jr, Gershon ES. 1999. A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. Proc Natl Acad Sci U S A 96:5604-5609.

Dick DM, Plunkett J, Hamlin D, Nurnberger Jr J, Kuperman S, Schuckit M, Hesselbrock V, Edenberg H, Bierut L. 2007. Association analyses of the serotonin transporter gene with lifetime depression and alcohol dependence in the collaborative study on the genetics of alcoholism (COGA) sample. Psychiatr Genet 17:35-38.

Dick DM, Foroud T, Flury L, Bowman ES, Miller MJ, Rau NL, Moe PR, Samavedy N, El-Mallakh R, Manji H. 2003. Genomewide linkage analyses of bipolar disorder: A new sample of 250 pedigrees from the national institute of mental health genetics initiative. The American Journal of Human Genetics 73:107-114.

Dick DM, Nurnberger J, Edenberg HJ, Goate A, Crowe R, Rice J, Bucholz KK, Kramer J, Schuckit MA, Smith TL. 2002. Suggestive linkage on chromosome 1 for a quantitative Alcohol-Related phenotype. Alcoholism: Clinical and Experimental Research 26:1453-1460.

Dick DM, Kendler KS. 2012. The impact of gene-environment interaction on alcohol use disorders. Alcohol Res 34:318-324.

Dickmeis T, Lahiri K, Nica G, Vallone D, Santoriello C, Neumann CJ, Hammerschmidt M, Foulkes NS. 2007. Glucocorticoids play a key role in circadian cell cycle rhythms. PLoS Biology 5:e78.

Dielman T, Campanelli PC, Shope JT, Butchart AT. 1987. Susceptibility to peer pressure, self-esteem, and health locus of control as correlates of adolescent substance abuse. Health Education & Behavior 14:207-221.

Dieset I, Djurovic S, Tesli M, Hope S, Mattingsdal M, Michelsen A, Joa I, Larsen TK, Agartz I, Melle I. 2014. Up-regulation of NOTCH4 gene expression in bipolar disorder.Am J Psychiatry 169:1292-1300.

Djurovic S, Gustafsson O, Mattingsdal M, Athanasiu L, Bjella T, Tesli M, Agartz I, Lorentzen S, Melle I, Morken G. 2010. A genome-wide association study of bipolar disorder in norwegian individuals, followed by replication in icelandic sample. J Affect Disord 126:312-316.

Domart M, Benyamina A, Lemoine A, Bourgain C, Blecha L, Debuire B, Reynaud M, Saffroy R. 2012. Association between a polymorphism in the promoter of a glutamate receptor subunit gene (GRIN2A) and alcoholism. Addict Biol 17:783-785.

Donaldson CJ, Sutton SW, Perrin MH, Corrigan AZ, Lewis KA, Rivier JE, Vaughan JM, Vale WW. 1996. Cloning and characterization of human urocortin. Endocrinology 137:2167-2170.

Dorrington S, Zammit S, Asher L, Evans J, Heron J, Lewis G. 2014. Perinatal maternal life events and psychotic experiences in children at twelve years in a birth cohort study. Schizophr Res 152:158-163.

Drago A, Crisafulli C, Sidoti A, Serretti A. 2011. The molecular interaction between the glutamatergic, noradrenergic, dopaminergic and serotoninergic systems informs a detailed genetic perspective on depressive phenotypes. Prog Neurobiol 94:418-460.

Drexhage RC, Hoogenboezem TH, Versnel MA, Berghout A, Nolen WA, Drexhage HA. 2011. The activation of monocyte and T cell networks in patients with bipolar disorder. Brain Behav Immun 25:1206-1213.

Du J, Suzuki K, Wei Y, Wang Y, Blumenthal R, Chen Z, Falke C, Zarate CA, Manji HK. 2007. The anticonvulsants lamotrigine, riluzole, and valproate differentially regulate AMPA receptor membrane localization: Relationship to clinical effects in mood disorders. Neuropsychopharmacology 32:793-802.

Dube SR, Miller JW, Brown DW, Giles WH, Felitti VJ, Dong M, Anda RF. 2006. Adverse childhood experiences and the association with ever using alcohol and initiating alcohol use during adolescence. Journal of Adolescent Health 38:444. e1-444. e10.

Dubovsky AN, Arvikar S, Stern TA, Axelrod L. 2012. The neuropsychiatric complications of glucocorticoid use: Steroid psychosis revisited. Psychosomatics 53:103-115.

Ducci F, Enoch M, Hodgkinson C, Xu K, Catena M, Robin R, Goldman D. 2007. Interaction between a functional MAOA locus and childhood sexual abuse predicts alcoholism and antisocial personality disorder in adult women. Mol Psychiatry 13:334-347.

Dudbridge F. 2013. Power and predictive accuracy of polygenic risk scores. PLoS Genetics 9:e1003348.

Duez H, Staels B. 2009. Rev-erb-alpha: An integrator of circadian rhythms and metabolism. J Appl Physiol (1985) 107:1972-1980.

Duncan LE, Keller MC. 2011. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. Perspectives 168:1041-1049.

Duncan LE, Pollastri AR, Smoller JW. 2014. Mind the gap: Why many geneticists and psychological scientists have discrepant views about gene–environment interaction (G× E) research. Am Psychol 69:249-268.

Duncan RD, Saunders BE, Kilpatrick DG, Hanson RF, Resnick HS. 1996. Childhood physical assault as a risk factor for PTSD, depression, and substance abuse: Findings from a national survey. Am J Orthopsychiatry 66:437-448.

Dunn AJ. 2007. The HPA axis and the immune system: A perspective. NeuroImmune Biology 7:3-15.

Dunn OJ. 1961. Multiple comparisons among means. Journal of the American Statistical Association 56:52-64.

Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO, Crowe RR, Goate A, Hesselbrock V, Jones K. 2004. Variations in GABRA2, encoding the α2 subunit of the GABAA receptor, are associated with alcohol dependence and with brain oscillations. The American Journal of Human Genetics 74:705-714.

Edenberg HJ, Koller DL, Xuei X, Wetherill L, McClintick JN, Almasy L, Bierut LJ, Bucholz KK, Goate A, Aliev F. 2010. Genome-Wide association study of alcohol dependence implicates a region on chromosome 11. Alcoholism: Clinical and Experimental Research 34:840-852.

Edenberg HJ, Xuei X, Chen HJ, Tian H, Wetherill LF, Dick DM, Almasy L, Bierut L, Bucholz KK, Goate A. 2006. Association of alcohol dehydrogenase genes with alcohol dependence: A comprehensive analysis. Hum Mol Genet 15:1539-1549.

Edenberg HJ, Bierut LJ, Boyce P, Cao M, Cawley S, Chiles R, Doheny KF, Hansen M, Hinrichs T, Jones K, Kelleher M, Kennedy GC, Liu G, Marcus G, McBride C, Murray SS, Oliphant A, Pettengill J, Porjesz B, Pugh EW, Rice JP, Rubano T, Shannon S, Steeke R, Tischfield JA, Tsai YY, Zhang C, Begleiter H. 2005. Description of the data from the collaborative study on the genetics of alcoholism (COGA) and single-nucleotide polymorphism genotyping for genetic analysis workshop 14. BMC Genet 6 Suppl 1:S2.

Edwards AC, Latendresse SJ, Heron J, Cho SB, Hickman M, Lewis G, Dick DM, Kendler KS. 2014. Childhood internalizing symptoms are negatively associated with early adolescent alcohol use. Alcoholism: Clinical and Experimental Research 38:1680-1688.

Ekholm JM, Kieseppa T, Hiekkalinna T, Partonen T, Paunio T, Perola M, Ekelund J, Lonnqvist J, Pekkarinen-Ijas P, Peltonen L. 2003. Evidence of susceptibility loci on 4q32 and 16p12 for bipolar disorder. Hum Mol Genet 12:1907-1915.

Englund MM, Egeland B, Oliva EM, Collins WA. 2008. Childhood and adolescent predictors of heavy drinking and alcohol use disorders in early adulthood: A longitudinal developmental analysis. Addiction 103:23-35.

Enoch M. 2006. Genetic and environmental influences on the development of alcoholism. Ann N Y Acad Sci 1094:193-201.

Enoch M. 2012. The influence of gene–environment interactions on the development of alcoholism and drug dependence. Curr Psychiatry Rep 14:150-158.

Enoch MA, Shen PH, Xu K, Hodgkinson C, Goldman D. 2006. Using ancestry-informative markers to define populations and detect population stratification. J Psychopharmacol 20:19-26.

Epps C, Holt L. 2011. The genetic basis of addiction and relevant cellular mechanisms. Int Anesthesiol Clin 49:3-14.

Etain B, Mathieu F, Rietschel M, Maier W, Albus M, Mckeon P, Roche S, Kealey C, Blackwood D, Muir W. 2006. Genome-wide scan for genes involved in bipolar affective disorder in 70 european families ascertained through a bipolar type I early-onset proband: Supportive evidence for linkage at 3p14. Mol Psychiatry 11:685-694.

Ewald H, Flint T, Kruse T, Mors O. 2002. A genome-wide scan shows significant linkage between bipolar disorder and chromosome 12q24. 3 and suggestive linkage to chromosomes 1p22-21, 4p16, 6q14-22, 10q26 and 16p13. 3. Mol Psychiatry 7:734-744.

Fabbri C, Drago A, Serretti A. 2013. Early antidepressant efficacy modulation by glutamatergic gene variants in the STAR< sup>∗ D. European Neuropsychopharmacology 23:612-621.

Fahlke C, Lorenz JG, Long J, Champoux M, Suomi SJ, Higley JD. 2000. Rearing experiences and Stress-Induced plasma cortisol as early risk factors for excessive alcohol consumption in nonhuman primates. Alcoholism: Clinical and Experimental Research 24:644-650.

Faul F, Erdfelder E, Lang A, Buchner A. 2007. G* power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 39:175-191.

Feder J, Gurling HM, Darby J, Cavalli-Sforza LL. 1985. DNA restriction fragment analysis of the proopiomelanocortin gene in schizophrenia and bipolar disorders. Am J Hum Genet 37:286-294.

Fein G, Greenstein D, Cardenas VA, Cuzen NL, Fouche J, Ferrett H, Thomas K, Stein DJ. 2013. Cortical and subcortical volumes in adolescents with alcohol dependence but without substance or psychiatric comorbidities. Psychiatry Research: Neuroimaging 214:1-8.

Feldman S, Weidenfeld J. 1997. Hypothalamic mechanisms mediating glutamate effects on the hypothalamo-pituitary-adrenocortical axis. J Neural Transm 104:633-642.

Feliciello A, Gottesman ME, Avvedimento EV. 2001. The biological functions of A-kinase anchor proteins. J Mol Biol 308:99-114.

Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, Koss MP, Marks JS. 1998. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The adverse childhood experiences (ACE) study. Am J Prev Med 14:245-258.

Ferreira MAR, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L, Fan J, Kirov G, Perlis RH, Green EK. 2008. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet 40:1056-1058.

Fiorentino A, O'Brien NL, Locke DP, McQuillin A, Jarram A, Anjorin A, Kandaswamy R, Curtis D, Blizard RA, Gurling HMD. 2014. Analysis of ANK3 and CACNA1C variants identified in bipolar disorder whole genome sequence data. Bipolar Disord 16:583-591.

First M, Spitzer R, Gibbon M, Williams J. 1996. Structured clinical interview for DSM-IV axis I disorders - patient edition (SCID-I/P, version 2.0). Structured Clinical Interview for DSM-IV Axis I Disorders .

Fite PJ, Colder CR, O'Connor RM. 2006. Childhood behavior problems and peer selection and socialization: Risk for adolescent alcohol use. Addict Behav 31:1454-1459.

Fonareva I, Spangler E, Cannella N, Sabino V, Cottone P, Ciccocioppo R, Zorrilla EP, Ryabinin AE. 2009. Increased perioculomotor urocortin 1 immunoreactivity in genetically selected alcohol preferring rats. Alcoholism: Clinical and Experimental Research 33:1956-1965.

Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL, Bierut LJ, Conneally PM, Nurnberger JI, Bucholz KK. 2000. Alcoholism susceptibility loci: Confirmation studies in a replicate sample and further mapping. Alcoholism: Clinical and Experimental Research 24:933-945.

Frank J, Lang M, Witt S, Strohmaier J, Rujescu D, Cichon S, Degenhardt F, Nöthen M, Collier D, Ripke S. 2014. Identification of increased genetic risk scores for schizophrenia in treatment-resistant patients. Mol Psychiatry 1-2.

Frank J, Cichon S, Treutlein J, Ridinger M, Mattheisen M, Hoffmann P, Herms S, Wodarz N, Soyka M, Zill P. 2012. Genome-wide significant association between alcohol dependence and a variant in the ADH gene cluster. Addict Biol 17:171-180.

Franke B, Neale BM, Faraone SV. 2009. Genome-wide association studies in ADHD. Hum Genet 126:13-50.

Frye MA, Altshuler LL, McElroy SL, Suppes T, Keck PE, Denicoff K, Nolen WA, Kupka R, Leverich GS, Pollio C. 2003. Gender differences in prevalence, risk, and clinical correlates of alcoholism comorbidity in bipolar disorder. Am J Psychiatry 160:883-889.

Fujimoto K, Hamaguchi H, Hashiba T, Nakamura T, Kawamoto T, Sato F, Noshiro M, Bhawal UK, Suardita K, Kato Y. 2007. Transcriptional repression by the basic helix-loop-helix protein Dec2: Multiple mechanisms through E-box elements. Int J Mol Med 19:925-932.

Fullerton JM, Donald JA, Mitchell PB, Schofield PR. 2010. Two-dimensional genome scan identifies multiple genetic interactions in bipolar affective disorder. Biol Psychiatry 67:478-486.

Gangisetty O, Bekdash R, Maglakelidze G, Sarkar DK. 2014. Fetal alcohol exposure alters proopiomelanocortin gene expression and hypothalamic-pituitary-adrenal axis function via increasing MeCP2 expression in the hypothalamus. PloS One 9:e113228.

Ganong WF. 1995. Review of medical physiology. McGraw-Hill/Appleton & Lange.

Garcia-Alcalde F, Okonechnikov K, Carbonell J, Cruz LM, Gotz S, Tarazona S, Dopazo J, Meyer TF, Conesa A. 2012. Qualimap: Evaluating next-generation sequencing alignment data. Bioinformatics 28:2678-2679.

Gazdzinski S, Durazzo TC, Studholme C, Song E, Banys P, Meyerhoff DJ. 2005. Quantitative brain MRI in alcohol dependence: Preliminary evidence for effects of concurrent chronic cigarette smoking on regional brain volumes. Alcoholism: Clinical and Experimental Research 29:1484-1495.

Gelernter J, Kranzler H, Sherva R, Almasy L, Koesterer R, Smith A, Anton R, Preuss U, Ridinger M, Rujescu D. 2014. Genome-wide association study of alcohol dependence: Significant findings in african-and european-americans including novel risk loci. Mol Psychiatry 19:41-49.

Gelernter J, Kranzler HR, Panhuysen C, Weiss RD, Brady K, Poling J, Farrer L. 2009. Dense genomewide linkage scan for alcohol dependence in african americans: Significant linkage on chromosome 10. Biol Psychiatry 65:111-115.

Genome of the Netherlands Consortium. 2014. Whole-genome sequence variation, population structure and demographic history of the dutch population. Nat Genet 46:818-825.

Georgi B, Craig D, Kember RL, Liu W, Lindquist I, Nasser S, Brown C, Egeland JA, Paul SM, Bućan M. 2014. Genomic view of bipolar disorder revealed by whole genome sequencing in a genetic isolate. PLoS Genetics 10:e1004229.

Gibbs RA, Belmont JW, Hardenbol P, Willis TD, Yu F, Yang H, Ch'ang L, Huang W, Liu B, Shen Y. 2003. The international HapMap project. Nature 426:789-796.

Gil OD, Zanazzi G, Struyk AF, Salzer JL. 1998. Neurotrimin mediates bifunctional effects on neurite outgrowth via homophilic and heterophilic interactions. J Neurosci 18:9312-9325.

Gilpin NW, Koob GF. 2008. Neurobiology of alcohol dependence: Focus on motivational mechanisms. Alcohol Research & Health: The Journal of the National Institute on Alcohol Abuse and Alcoholism 31:185-195.

Glaser D. 2000. Child abuse and neglect and the brain—a review. Journal of Child Psychology and Psychiatry 41:97-116.

Glaser YG, Zubieta JK, Hsu DT, Villafuerte S, Mickey BJ, Trucco EM, Burmeister M, Zucker RA, Heitzeg MM. 2014. Indirect effect of corticotropin-releasing hormone receptor 1 gene variation on negative emotionality and alcohol use via right ventrolateral prefrontal cortex. J Neurosci 34:4099-4107.

Goes FS, Zandi PP, Miao K, McMahon FJ, Steele J, Willour VL, MacKinnon DF, Mondimore FM, Schweizer B, Nurnberger Jr JI. 2007. Mood-incongruent psychotic features in bipolar disorder: Familial aggregation and suggestive linkage to 2p11-q14 and 13q21-33. Am J Psychiatry 164:236-247.

Goes F, Hamshere M, Seifuddin F, Pirooznia M, Belmonte-Mahon P, Breuer R, Schulze T, Nöthen M, Cichon S, Rietschel M. 2012. Genome-wide association of mood-incongruent psychotic bipolar disorder. Translational Psychiatry 2:e180.

Gonzales RA, Jaworski JN. 1997. Alcohol and glutamate. Alcohol Health Res World 21:120-126.

Gonzalez R, Bernardo C, Cruz D, Walss-Bass C, Thompson PM. 2014. The relationships between clinical characteristics, alcohol and psychotropic exposure, and circadian gene expression in human postmortem samples of affective disorder and control subjects. Psychiatry Res 218:359-362.

Goodman A, Lamping DL, Ploubidis GB. 2010. When to use broader internalising and externalising subscales instead of the hypothesised five subscales on the strengths and difficulties questionnaire (SDQ): Data from british parents, teachers and children. J Abnorm Child Psychol 38:1179-1191.

Goodman R. 1997. The strengths and difficulties questionnaire: A research note. Journal of Child Psychology and Psychiatry 38:581-586.

Gottesman II, Gould TD. 2003. The endophenotype concept in psychiatry: Etymology and strategic intentions. Am J Psychiatry 160:636-645.

Grant BF, Dawson DA. 1997. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: Results from the national longitudinal alcohol epidemiologic survey. J Subst Abuse 9:103-110.

Grant JD, Scherrer JF, Lynskey MT, Lyons MJ, Eisen SA, Tsuang MT, True WR, Bucholz KK. 2006. Adolescent alcohol use is a risk factor for adult alcohol and drug dependence: Evidence from a twin design. Psychol Med 36:109-118.

Gratacòs M, González JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X. 2007. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: Meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. Biol Psychiatry 61:911-922.

Greeff JM. 2007. Deconstructing jaco: Genetic heritage of an afrikaner. Ann Hum Genet 71:674-688.

Greenberg DA, Berger B. 1994. Using lod-score differences to determine mode of inheritance: A simple, robust method even in the presence of heterogeneity and reduced penetrance. Am J Hum Genet 55:834-840.

Greenwood TA, Akiskal HS, Akiskal KK, Study BG, Kelsoe JR. 2012. Genome-wide association study of temperament in bipolar disorder reveals significant associations with three novel loci. Biol Psychiatry 72:303-310.

Greenwood TA, Kelsoe JR, Bipolar Genome Study (BiGS) Consortium. 2013. Genome-wide association study of irritable vs. elated mania suggests genetic differences between clinical subtypes of bipolar disorder. PloS One 8:e53804.

Gudmundsson J, Sulem P, Gudbjartsson DF, Masson G, Agnarsson BA, Benediktsdottir KR, Sigurdsson A, Magnusson OT, Gudjonsson SA, Magnusdottir DN. 2012. A study based on whole-genome sequencing yields a rare variant at 8q24 associated with prostate cancer. Nat Genet 44:1326-1329.

Guo X, Liu Z, Wang X, Zhang H. 2012. Large scale association analysis for drug addiction: Results from SNP to gene. ScientificWorldJournal 2012:939584.

Gupta DS, McCullumsmith RE, Beneyto M, Haroutunian V, Davis KL, Meador-Woodruff JH. 2005. Metabotropic glutamate receptor protein expression in the prefrontal cortex and striatum in schizophrenia. Synapse 57:123-131.

Hahn C, Friedman E. 1999. Abnormalities in protein kinase C signaling and the pathophysiology of bipolar disorder. Bipolar Disord 1:81-86.

Hanisch U, Kettenmann H. 2007. Microglia: Active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10:1387-1394.

Hansson AC, Cippitelli A, Sommer WH, Fedeli A, Bjork K, Soverchia L, Terasmaa A, Massi M, Heilig M, Ciccocioppo R. 2006. Variation at the rat Crhr1 locus and sensitivity to relapse into alcohol seeking induced by environmental stress. Proc Natl Acad Sci U S A 103:15236-15241.

Harvey AG. 2008. Sleep and circadian rhythms in bipolar disorder: Seeking synchrony, harmony, and regulation. Am J Psychiatry 165:820-829.

Hashimoto K, Sawa A, Iyo M. 2007. Increased levels of glutamate in brains from patients with mood disorders. Biol Psychiatry 62:1310-1316.

Hashimoto R, Hough C, Nakazawa T, Yamamoto T, Chuang D. 2002. Lithium protection against glutamate excitotoxicity in rat cerebral cortical neurons: Involvement of NMDA receptor inhibition possibly by decreasing NR2B tyrosine phosphorylation. J Neurochem 80:589-597.

Hasin DS, Stinson FS, Ogburn E, Grant BF. 2007. Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the united states: Results from the national epidemiologic survey on alcohol and related conditions. Arch Gen Psychiatry 64:830-842.

Hatonen T, Forsblom S, Kieseppa T, Lonnqvist J, Partonen T. 2008. Circadian phenotype in patients with the co-morbid alcohol use and bipolar disorders. Alcohol Alcohol 43:564-568.

Hattori E, Toyota T, Ishitsuka Y, Iwayama Y, Yamada K, Ujike H, Morita Y, Kodama M, Nakata K, Minabe Y. 2009. Preliminary genome-wide association study of bipolar disorder in the japanese population. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 150:1110-1117.

He J, Crews FT. 2008. Increased MCP-1 and microglia in various regions of the human alcoholic brain. Exp Neurol 210:349-358.

Heath AC, Whitfield JB, Martin NG, Pergadia ML, Goate AM, Lind PA, McEvoy BP, Schrage AJ, Grant JD, Chou Y. 2011. A quantitative-trait genome-wide association study of alcoholism risk in the community: Findings and implications. Biol Psychiatry 70:513-518.

Hennel JW, Kryst-Widźgowska T, Klinowski J. 1997. A primer of magnetic resonance imaging. Imperial College Pr.

Herman AA, Stein DJ, Seedat S, Heeringa SG, Moomal H, Williams DR. 2009. The south african stress and health (SASH) study: 12-month and lifetime prevalence of common mental disorders. SAMJ: South African Medical Journal 99:339-344.

Herman JP, Cullinan WE. 1997. Neurocircuitry of stress: Central control of the hypothalamo–pituitary–adrenocortical axis. Trends Neurosci 20:78-84.

Herman JP, Mueller NK, Figueiredo H. 2004. Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. Ann N Y Acad Sci 1018:35-45.

Heron J, Macleod J, Munafo MR, Melotti R, Lewis G, Tilling K, Hickman M. 2012. Patterns of alcohol use in early adolescence predict problem use at age 16. Alcohol Alcohol 47:169-177.

Hettema JM, Neale MC, Kendler KS. 2001. A review and meta-analysis of the genetic epidemiology of anxiety disorders. Am J Psychiatry 158:1568-1578.

Higley JD, Hasert MF, Suomi SJ, Linnoila M. 1991. Nonhuman primate model of alcohol abuse: Effects of early experience, personality, and stress on alcohol consumption. Proc Natl Acad Sci U S A 88:7261-7265.

Hill KG, Hawkins JD, Bailey JA, Catalano RF, Abbott RD, Shapiro VB. 2010. Person–environment interaction in the prediction of alcohol abuse and alcohol dependence in adulthood. Drug Alcohol Depend 110:62-69.

Hill SY, Lichenstein S, Wang S, Carter H, McDermott M. 2013. Caudate volume in offspring at ultra high risk for alcohol dependence: COMT Val158Met, DRD2, externalizing disorders, and working memory. Advances in Molecular Imaging 3:43-54.

Hill SY, Wang S, Carter H, Tessner K, Holmes B, McDermott M, Zezza N, Stiffler S. 2011. Cerebellum volume in high-risk offspring from multiplex alcohol dependence families: Association with allelic variation in GABRA2 and BDNF. Psychiatry Research: Neuroimaging 194:304-313.

Hill SY, Wang S, Kostelnik B, Carter H, Holmes B, McDermott M, Zezza N, Stiffler S, Keshavan MS. 2009. Disruption of orbitofrontal cortex laterality in offspring from multiplex alcohol dependence families. Biol Psychiatry 65:129-136.

Hirschfeld R, Vornik LA. 2005. Bipolar disorder—costs and comorbidity. Am J Manag Care 11:S85-90.

Hirschhorn JN, Daly MJ. 2005. Genome-wide association studies for common diseases and complex traits. Nature Reviews Genetics 6:95-108.

Hodge CW, Miles MF, Sharko AC, Stevenson RA, Hillmann JR, Lepoutre V, Besheer J, Schroeder JP. 2006. The mGluR5 antagonist MPEP selectively inhibits the onset and maintenance of ethanol self-administration in C57BL/6J mice. Psychopharmacology (Berl) 183:429-438.

Hoffman PL, Rabe CS, Moses F, Tabakoff B. 1989. N-Methyl-D-Aspartate receptors and ethanol: Inhibition of calcium flux and cyclic GMP production. J Neurochem 52:1937-1940.

Holliday KL, Nicholl BI, Macfarlane GJ, Thomson W, Davies KA, McBeth J. 2010. Genetic variation in the hypothalamic-pituitary-adrenal stress axis influences susceptibility to musculoskeletal pain: Results from the EPIFUND study. Ann Rheum Dis 69:556-560.

Hollmann M, Heinemann S. 1994. Cloned glutamate receptors. Annu Rev Neurosci 17:31-108.

Holmans P, Green EK, Pahwa JS, Ferreira MA, Purcell SM, Sklar P, Owen MJ, O'Donovan MC, Craddock N, Wellcome Trust Case-Control Consortium. 2009. Gene ontology analysis of GWA study data sets provides insights into the biology of bipolar disorder. The American Journal of Human Genetics 85:13-24.

Holsboer F, Ising M. 2008. Central CRH system in depression and anxiety—Evidence from clinical studies with CRH 1 receptor antagonists. Eur J Pharmacol 583:350-357.

Homer JP, Flodman PL, Spence MA. 1997. Bipolar disorder: Dominant or recessive on chromosome 5? Genet Epidemiol 14:647-651.

Honma S, Kawamoto T, Takagi Y, Fujimoto K, Sato F, Noshiro M, Kato Y, Honma K. 2002. Dec1 and Dec2 are regulators of the mammalian molecular clock. Nature 419:841-844.

Hsieh MH, Tang C, Hung S, Lee IH, Lin Y, Yang YK. 2012. Comorbid prevalence of alcohol dependence, substance abuse, and external cause of injury in patients with bipolar disorder in a nationwide representative sample in taiwan. Bipolar Disord 14:677-679.

Hu M, Lee S, Wang T, Chen S, Chang Y, Chen S, Chu C, Wang C, Lee IH, Yeh TL. 2013. Association study of DRD2 and MAOA genes with subtyped alcoholism comorbid with bipolar disorder in han chinese. Prog Neuro-Psychopharmacol Biol Psychiatry 40:144-148.

Huang J, Perlis RH, Lee PH, Rush AJ, Fava M, Sachs GS, Lieberman J, Hamilton SP, Sullivan P, Sklar P. 2014. Cross-disorder genomewide analysis of schizophrenia, bipolar disorder, and depression Am J Psychiatry.

Hui P. 2014. Next generation sequencing: Chemistry, technology and applications. In: Chemical diagnostics. Top Curr Chem 336:1-18.

Iacono WG, Malone SM, McGue M. 2008. Behavioral disinhibition and the development of early-onset addiction: Common and specific influences. Annu.Rev.Clin.Psychol. 4:325-348.

IBM C. 2012. IBM SPSS statistics for windows.

Insel TR. 2008. Assessing the economic costs of serious mental illness. Am J Psychiatry 165:663-665.

Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, Akazawa C, Shigemoto R, Mizuno N, Masu M. 1993. Molecular characterization of the family of the N-methyl-D-aspartate receptor subunits. J Biol Chem 268:2836-2843.

Itokawa M, Yamada K, Iwayama-Shigeno Y, Ishitsuka Y, Detera-Wadleigh S, Yoshikawa T. 2003. Genetic analysis of a functional GRIN2A promoter (GT) n repeat in bipolar disorder pedigrees in humans. Neurosci Lett 345:53-56.

Iwamoto K, Ueda J, Bundo M, Kojima T, Kato T. 2011. Survey of the effect of genetic variations on gene expression in human prefrontal cortex and its application to genetics of psychiatric disorders. Neurosci Res 70:238-242.

Jablensky A, Angelicheva D, Donohoe G, Cruickshank M, Azmanov D, Morris D, McRae A, Weickert C, Carter K, Chandler D. 2012. Promoter polymorphisms in two overlapping 6p25 genes implicate mitochondrial proteins in cognitive deficit in schizophrenia. Mol Psychiatry 17:1328-1339.

Javitt D. 2004. Glutamate as a therapeutic target in psychiatric disorders. Mol Psychiatry 9:984-997.

Jiang Y, Zhang H. 2011. Propensity score-based nonparametric test revealing genetic variants underlying bipolar disorder. Genet Epidemiol 35:125-132.

Jin Z, Bhandage AK, Bazov I, Kononenko O, Bakalkin G, Korpi ER, Birnir B. 2014. Selective increases of AMPA, NMDA, and kainate receptor subunit mRNAs in the hippocampus and orbitofrontal cortex but not in prefrontal cortex of human alcoholics. Frontiers in Cellular Neuroscience 29:8-11.

Johnson C, Drgon T, Liu Q, Walther D, Edenberg H, Rice J, Foroud T, Uhl GR. 2006. Pooled association genome scanning for alcohol dependence using 104,268 SNPs: Validation and use to identify alcoholism vulnerability loci in unrelated individuals from the collaborative study on the genetics of alcoholism. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 141:844-853.

Joo E, Greenwood TA, Schork N, McKinney RA, Sadovnick AD, Remick RA, Keck PE, McElroy SL, Kelsoe JR. 2010. Suggestive evidence for linkage of ADHD features in bipolar disorder to chromosome 10p14. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 153:260-268.

Joslyn G, Ravindranathan A, Brush G, Schuckit M, White RL. 2010. Human variation in alcohol response is influenced by variation in neuronal signaling genes. Alcoholism: Clinical and Experimental Research 34:800-812.

Kalluri HS, Mehta AK, Ticku MK. 1998. Up-regulation of NMDA receptor subunits in rat brain following chronic ethanol treatment. Mol Brain Res 58:221-224.

Kalsbeek A, Van der Spek R, Lei J, Endert E, Buijs R, Fliers E. 2012. Circadian rhythms in the hypothalamo–pituitary–adrenal (HPA) axis. Mol Cell Endocrinol 349:20-29.

Kamburov A, Wierling C, Lehrach H, Herwig R. 2009. ConsensusPathDB--a database for integrating human functional interaction networks. Nucleic Acids Res 37:D623-8.

Kandaswamy R, McQuillin A, Curtis D, Gurling H. 2014. Allelic association, DNA resequencing and copy number variation at the metabotropic glutamate receptor GRM7 gene locus in bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 165:365-372.

Kandaswamy R, McQuillin A, Sharp SI, Fiorentino A, Anjorin A, Blizard RA, Curtis D, Gurling HM. 2013. Genetic association, mutation screening, and functional analysis of a kozak sequence variant in the metabotropic glutamate receptor 3 gene in bipolar disorder. JAMA Psychiatry 70:591-598.

Kapoor M, Wang J, Wetherill L, Le N, Bertelsen S, Hinrichs AL, Budde J, Agrawal A, Bucholz K, Dick D. 2013. A meta-analysis of two genome-wide association studies to identify novel loci for maximum number of alcoholic drinks. Hum Genet 132:1141-1151.

Karayiorgou M, Torrington M, Abecasis GR, Pretorius H, Robertson B, Kaliski S, Lay S, Sobin C, Möller N, Lundy SL. 2004. Phenotypic characterization and genealogical tracing in an afrikaner schizophrenia database. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 124:20-28.

Karthikeyan R, Marimuthu G, Chellamuthu Ramasubramanian GA, BaHammam AS, Spence DW, Cardinali DP, Brown GM, Pandi-Perumal SR. 2014. Association of Per3 length polymorphism with bipolar I disorder and schizophrenia. Neuropsychiatric Disease and Treatment 10:2325-2330.

Kaufman J, Yang BZ, Douglas-Palumberi H, Crouse-Artus M, Lipschitz D, Krystal JH, Gelernter J. 2007. Genetic and environmental predictors of early alcohol use. Biol Psychiatry 61:1228-1234.

Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, Williamson D, Ryan N. 1997. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): Initial reliability and validity data. Journal of the American Academy of Child & Adolescent Psychiatry 36:980-988.

Kaur S, Li J, Stenzel-Poore MP, Ryabinin AE. 2012. Corticotropin-Releasing factor acting on Corticotropin-Releasing factor receptor type 1 is critical for binge alcohol drinking in mice. Alcoholism: Clinical and Experimental Research 36:369-376.

Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk HD, Kramer A, Maier B. 2009. A circadian clock in macrophages controls inflammatory immune responses. Proc Natl Acad Sci U S A 106:21407-21412.

Kelsoe JR, Spence MA, Loetscher E, Foguet M, Sadovnick AD, Remick RA, Flodman P, Khristich J, Mroczkowski-Parker Z, Brown JL. 2001. A genome survey indicates a possible susceptibility locus for bipolar disorder on chromosome 22. Proceedings of the National Academy of Sciences 98:585-590.

Kendler KS. 2013. What psychiatric genetics has taught us about the nature of psychiatric illness and what is left to learn. Mol Psychiatry 18:1058-1066.

Kendler KS, Gardner CO, Prescott CA. 1997. Religion, psychopathology, and substance use and abuse: A multimeasure, genetic-epidemiologic study. Am J Psychiatry 154:322-329.

Kendler KS, Kalsi G, Holmans PA, Sanders AR, Aggen SH, Dick DM, Aliev F, Shi J, Levinson DF, Gejman PV. 2011a. Genomewide association analysis of symptoms of alcohol dependence in the molecular genetics of schizophrenia (MGS2) control sample. Alcoholism: Clinical and Experimental Research 35:963-975.

Kendler K, Jaffee S, Romer D. 2011b. The dynamic genome and mental health. New York: Oxford.

Kerner B, Jasinska AJ, DeYoung J, Almonte M, Choi O, Freimer NB. 2009. Polymorphisms in the GRIA1 gene region in psychotic bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 150:24-32.

Kerner B, Lambert CG, Muthen BO. 2011. Genome-wide association study in bipolar patients stratified by co-morbidity. PloS One 6:e28477.

Kerner B, Rao AR, Christensen B, Dandekar S, Yourshaw M, Nelson SF. 2013. Rare genomic variants link bipolar disorder with anxiety disorders to CREB-regulated intracellular signaling pathways. Frontiers in Psychiatry 4:1-11.

Kessler RC, Aguilar-Gaxiola S, Alonso J, Chatterji S, Lee S, Ormel J, Üstün TB, Wang PS. 2009. The global burden of mental disorders: An update from the WHO world mental health (WMH) surveys. Epidemiol Psichiatr Soc 18:23-33.

Kessler RC, Chiu WT, Demler O, Walters EE. 2005. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the national comorbidity survey replication. Arch Gen Psychiatry 62:617-627.

Kessler RC, Angermeyer M, Anthony JC, DE Graaf R, Demyttenaere K, Gasquet I, DE Girolamo G, Gluzman S, Gureje O, Haro JM, Kawakami N, Karam A, Levinson D, Medina Mora ME, Oakley Browne MA, Posada-Villa J, Stein DJ, Adley Tsang CH, Aguilar-Gaxiola S, Alonso J, Lee S, Heeringa S, Pennell BE, Berglund P, Gruber MJ, Petukhova M, Chatterji S, Ustun TB. 2007. Lifetime prevalence and age-of-onset distributions of

mental disorders in the world health organization's world mental health survey initiative. World Psychiatry 6:168-176.

Khoury L, Tang YL, Bradley B, Cubells JF, Ressler KJ. 2010. Substance use, childhood traumatic experience, and posttraumatic stress disorder in an urban civilian population. Depress Anxiety 27:1077-1086.

Kim JH, Park M, Yang SY, Jeong BS, Yoo HJ, Kim J, Chung J, Kim S. 2006. Association study of polymorphisms in N-methyl-d-aspartate receptor 2B subunits (GRIN2B) gene with korean alcoholism. Neurosci Res 56:220-223.

Kim J, Park A, Glatt SJ, Eckert TL, Vanable PA, Scott-Sheldon LA, Carey KB, Ewart CK, Carey MP. 2015. Interaction effects between the 5-hydroxy tryptamine transporter-linked polymorphic region (5-HTTLPR) genotype and family conflict on adolescent alcohol use and misuse. Addiction 110:289-299.

King SM, Iacono WG, McGue M. 2004. Childhood externalizing and internalizing psychopathology in the prediction of early substance use. Addiction 99:1548-1559.

Kishimoto T, Radulovic J, Radulovic M, Lin CR, Schrick C, Hooshmand F, Hermanson O, Rosenfeld MG, Spiess J. 2000. Deletion of crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. Nat Genet 24:415-419.

Kivimäki P, Kekkonen V, Valtonen H, Tolmunen T, Honkalampi K, Tacke U, Hintikka J, Lehto SM, Laukkanen E. 2014. Alcohol use among adolescents, aggressive behaviour, and internalizing problems. J Adolesc 37:945-951.

Konishi T, Calvillo M, Leng A, Lin K, Wan YY. 2004. Polymorphisms of the dopamine D2 receptor, serotonin transporter, and GABA A receptor β 3 subunit genes and alcoholism in mexican-americans. Alcohol 32:45-52.

Kono Y, Yoneda H, Sakai T, Nonomura Y, Inayama Y, Koh J, Sakai J, Inada Y, Imamichi H, Asaba H. 1997. Association between early-onset alcoholism and the dopamine D2 receptor gene. Am J Med Genet 74:179-182.

Kono M, Nagata H, Umemura S, Kawana S, Osamura RY. 2001. In situ expression of corticotropin-releasing hormone (CRH) and proopiomelanocortin (POMC) genes in human skin. FASEB J 15:2297-2299.

Kranzler HR, Gelernter J, Anton RF, Arias AJ, Herman A, Zhao H, Burian L, Covault J. 2009. Association of markers in the 3′ region of the GluR5 kainate receptor subunit gene to alcohol dependence. Alcoholism: Clinical and Experimental Research 33:925-930.

Kringelbach ML. 2005. The human orbitofrontal cortex: Linking reward to hedonic experience. Nature Reviews Neuroscience 6:691-702.

Kripke DF, Nievergelt CM, Joo E, Shekhtman T, Kelsoe JR. 2009. Circadian polymorphisms associated with affective disorders. J Circadian Rhythms 7:1-10.

Krystal J, Sanacora G, Blumberg H, Anand A, Charney D, Marek G, Epperson C, Goddard A, Mason G. 2002. Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. Mol Psychiatry 7:S71-S80.

Ku C, Naidoo N, Pawitan Y. 2011. Revisiting mendelian disorders through exome sequencing. Hum Genet 129:351-370.

Kudielka BM, Kirschbaum C. 2005. Sex differences in HPA axis responses to stress: A review. Biol Psychol 69:113-132.

Kumpulainen K. 2000. Psychiatric symptoms and deviance in early adolescence predict heavy alcohol use 3 years later. Addiction 95:1847-1857.

Kuo P, Chuang L, Liu J, Liu C, Huang M, Lin S, Sun HS, Hsieh M, Hung H, Lu R. 2014. Identification of novel loci for bipolar I disorder in a multi-stage genome-wide association study. Prog Neuro-Psychopharmacol Biol Psychiatry 51:58-64.

Kushner MG, Abrams K, Borchardt C. 2000. The relationship between anxiety disorders and alcohol use disorders: A review of major perspectives and findings. Clin Psychol Rev 20:149-171.

Kuswanto CN, Sum MY, Thng CRZ, Zhang YB, Yang GL, Nowinski WL, Sitoh YY, Low CM, Sim K. 2013. GRIN2B gene and associated brain cortical white matter changes in bipolar disorder: A preliminary combined platform investigation. BioMed Research International 2013: 635131.

Labbe A, Bureau A, Moreau I, Roy M, Chagnon Y, Maziade M, Merette C. 2012. Symptom dimensions as alternative phenotypes to address genetic heterogeneity in schizophrenia and bipolar disorder. European Journal of Human Genetics 20:1182-1188.

Lachman HM, Pedrosa E, Petruolo OA, Cockerham M, Papolos A, Novak T, Papolos DF, Stopkova P. 2007. Increase in GSK3β gene copy number variation in bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 144:259-265.

Lambert D, Middle F, Hamshere ML, Segurado R, Raybould R, Corvin A, Green E, O'Mahony E, Nikolov I, Mulcahy T. 2005. Stage 2 of the wellcome trust UK–Irish bipolar affective disorder sibling-pair genome screen: Evidence for linkage on chromosomes 6q16–q21, 4q12–q21, 9p21, 10p14–p12 and 18q22. Mol Psychiatry 10:831-841.

Lander E, Kruglyak L. 1995. Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. Nat Genet 11:241-247.

Latvala A, Dick DM, Tuulio-Henriksson A, Suvisaari J, Viken RJ, Rose RJ, Kaprio J. 2011. Genetic correlation and gene-environment interaction between alcohol problems and educational level in young adulthood. J Stud Alcohol Drugs 72:210-220.

Laucht M, Blomeyer D, Buchmann AF, Treutlein J, Schmidt MH, Esser G, Jennen-Steinmetz C, Rietschel M, Zimmermann US, Banaschewski T. 2012. Catechol-O-methyltransferase Val158Met genotype, parenting practices and adolescent alcohol use: Testing the differential susceptibility hypothesis. Journal of Child Psychology and Psychiatry 53:351-359.

Lecrubier Y, Sheehan D, Weiller E, Amorim P, Bonora I, Sheehan KH, Janavs J, Dunbar G. 1997. The mini international neuropsychiatric interview (MINI). A short diagnostic structured interview: Reliability and validity according to the CIDI. European Psychiatry 12:224-231.

Lee H, Woo HG, Greenwood TA, Kripke DF, Kelsoe JR. 2013. A genome-wide association study of seasonal pattern mania identifies NF1A as a possible susceptibility gene for bipolar disorder. J Affect Disord 145:200-207.

Lee M, Chen C, Lee C, Chen C, Chong M, Ouyang W, Chiu N, Chuo L, Chen C, Tan H. 2011. Genome-wide association study of bipolar I disorder in the han chinese population. Mol Psychiatry 16:548-556.

Lee S, Rivier C. 1997. Alcohol increases the expression of type 1, but not type 2α corticotropin-releasing factor (CRF) receptor messenger ribonucleic acid in the rat hypothalamus. Mol Brain Res 52:78-89.

Leibenluft E, Albert PS, Rosenthal NE, Wehr TA. 1996. Relationship between sleep and mood in patients with rapid-cycling bipolar disorder. Psychiatry Res 63:161-168.

Leondes CT. 2005. Medical imaging systems technology:Modalities. World Scientific Pub Co Inc.

Lesch KP. 2005. Alcohol dependence and gene x environment interaction in emotion regulation: Is serotonin the link? Eur J Pharmacol 526:113-124.

Leszczyńska-Rodziewicz A, Maciukiewicz M, Szczepankiewicz A, Pogłodziński A, Hauser J. 2013a. Association between OPCRIT dimensions and polymorphisms of HPA axis genes in bipolar disorder. J Affect Disord 151:744-747.

Leszczyńska-Rodziewicz A, Szczepankiewicz A, Dmitrzak-Węglarz M, Rajewska-Rager A, Skibińska M, Hauser J. 2013b. No association between polymorphisms and haplotypes of the AVPR1b, CRHR1 and NR3C1 genes and depression with melancholic features in the course of bipolar disorder. Psychiatry Res 207:140-142.

Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, Vaughan J, Reyes TM, Gulyas J, Fischer W, Bilezikjian L, Rivier J, Sawchenko PE, Vale WW. 2001. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc Natl Acad Sci U S A 98:7570-7575.

Li D, Zhao H, Gelernter J. 2011. Strong association of the alcohol dehydrogenase 1B gene (ADH1B) with alcohol dependence and alcohol-induced medical diseases. Biol Psychiatry 70:504-512.

Li Z, Kang SS, Lee S, Rivier C. 2005. Effect of ethanol on the regulation of corticotropin-releasing factor (CRF) gene expression. Molecular and Cellular Neuroscience 29:345-354.

Li H, Durbin R. 2009. Fast and accurate short read alignment with burrows-wheeler transform. Bioinformatics 25:1754-1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-2079.

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM. 2009. Common genetic determinants of schizophrenia and bipolar disorder in swedish families: A population-based study. The Lancet 373:234-239.

Lichtermann D, Hranilovic D, Trixler M, Franke P, Jernej B, Delmo CD, Knapp M, Schwab SG, Maier W, Wildenauer DB. 2000. Support for allelic association of a polymorphic site in the promoter region of the serotonin transporter gene with risk for alcohol dependence. Am J Psychiatry 157:2045-2047.

Lightman SL, Conway-Campbell BL. 2010. The crucial role of pulsatile activity of the HPA axis for continuous dynamic equilibration. Nature Reviews Neuroscience 11:710-718.

Lin P, Hartz SM, Wang J, Agrawal A, Zhang T, McKenna N, Bucholz K, Brooks AI, Tischfield JA, Edenberg HJ. 2012. Copy number variations in 6q14. 1 and 5q13. 2 are associated with alcohol dependence. Alcoholism: Clinical and Experimental Research 36:1512-1518.

Lind PA, Macgregor S, Vink JM, Pergadia ML, Hansell NK, de Moor MH, Smit AB, Hottenga JJ, Richter MM, Heath AC, Martin NG, Willemsen G, de Geus EJ, Vogelzangs N, Penninx BW, Whitfield JB, Montgomery GW, Boomsma DI, Madden PA. 2010. A genomewide association study of nicotine and alcohol dependence in australian and dutch populations. Twin Res Hum Genet 13:10-29.

Liu J, Juo S, Dewan A, Grunn A, Tong X, Brito M, Park N, Loth J, Kanyas K, Lerer B. 2003. Evidence for a putative bipolar disorder locus on 2p13–16 and other potential loci on 4q31, 7q34, 8q13, 9q31, 10q21–24, 13q32, 14q21 and 17q11–12. Mol Psychiatry 8:333-342.

Liu J, Zhou Z, Hodgkinson CA, Yuan Q, Shen P, Mulligan CJ, Wang A, Gray RR, Roy A, Virkkunen M. 2011a. Haplotype-Based study of the association of Alcohol-Metabolizing genes with alcohol dependence in four independent populations. Alcoholism: Clinical and Experimental Research 35:304-316.

Liu L, Li Y, Li S, Hu N, He Y, Pong R, Lin D, Lu L, Law M. 2012. Comparison of next-generation sequencing systems. BioMed Research International 2012: 251364.

Liu Y, Blackwood DH, Caesar S, de Geus EJ, Farmer A, Ferreira MA, Ferrier IN, Fraser C, Gordon-Smith K, Green EK. 2011b. Meta-analysis of genome-wide association data of bipolar disorder and major depressive disorder. Mol Psychiatry 16:2-4.

Loeber R, Stouthamer-Loeber M, Raskin White H. 1999. Developmental aspects of delinquency and internalizing problems and their association with persistent juvenile substance use between ages 7 and 18. J Clin Child Psychol 28:322-332.

Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E, Bennett PH, Goldman D. 1998. Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an american indian population. Am J Med Genet 81:216-221.

Loveland-Cherry CJ, Leech S, Laetz VB, Dielman TE. 1996. Correlates of alcohol use and misuse in fourth-grade children: Psychosocial, peer, parental, and family factors. Health Education & Behavior 23:497-511.

Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. 1995. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci U S A 92:836-840.

Lovinger DM, White G, Weight FF. 1989. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 243:1721-1724.

Lowery EG, Thiele TE. 2010. Pre-clinical evidence that corticotropin-releasing factor (CRF) receptor antagonists are promising targets for pharmacological treatment of alcoholism. CNS Neurol Disord Drug Targets 9:77-86.

Lund C, Myer L, Stein DJ, Williams DR, Flisher AJ. 2013. Mental illness and lost income among adult south africans. Soc Psychiatry Psychiatr Epidemiol 48:845-851.

Lydall GJ, Bass NJ, McQuillin A, Lawrence J, Anjorin A, Kandaswamy R, Pereira A, Guerrini I, Curtis D, Vine AE, Sklar P, Purcell SM, Gurling HM. 2011. Confirmation of prior evidence of genetic susceptibility to alcoholism in a genome-wide association study of comorbid alcoholism and bipolar disorder. Psychiatr Genet 21:294-306.

Macgregor S, Lind PA, Bucholz KK, Hansell NK, Madden PAF, Richter MM, Montgomery GW, Martin NG, Heath AC, Whitfield JB. 2009. Associations of ADH and ALDH2 gene variation with self report alcohol reactions, consumption and dependence: An integrated analysis. Hum Mol Genet 18:580-593.

Maciukiewicz M, Dmitrzak-Weglarz M, Pawlak J, Leszczynska-Rodziewicz A, Zaremba D, Skibinska M, Hauser J. 2014. Analysis of genetic association and epistasis interactions between circadian clock genes and symptom dimensions of bipolar affective disorder. Chronobiol Int 31:770-778.

Maclean PD. 1954. The limbic system and its hippocampal formation; studies in animals and their possible application to man. J Neurosurg 11:29-44.

Maddock R, Garrett A, Buonocore M. 2001. Remembering familiar people: The posterior cingulate cortex and autobiographical memory retrieval. Neuroscience 104:667-676.

Maes M, Bosmans E, Calabrese J, Smith R, Meltzer HY. 1995. Interleukin-2 and interleukin-6 in schizophrenia and mania: Effects of neuroleptics and mood stabilizers. J Psychiatr Res 29:141-152.

Makris N, Oscar-Berman M, Jaffin SK, Hodge SM, Kennedy DN, Caviness VS. 2008. Decreased volume of the brain reward system in alcoholism. Biol Psychiatry 64:192-202.

Mancuso SG, Morgan VA, Mitchell PB, Berk M, Young A, Castle DJ. 2015. A comparison of schizophrenia, schizoaffective disorder, and bipolar disorder: Results from the second australian national psychosis survey. J Affect Disord 172:30-37.

Manenschijn L, Spijker AT, Koper JW, Jetten AM, Giltay EJ, Haffmans J, Hoencamp E, van Rossum EF. 2012. Long-term cortisol in bipolar disorder: Associations with age of onset and psychiatric co-morbidity. Psychoneuroendocrinology 37:1960-1968.

Manji HK, Lenox RH. 1999. Protein kinase C signaling in the brain: Molecular transduction of mood stabilization in the treatment of manic-depressive illness. Biol Psychiatry 46:1328-1351.

Mansour H, Wood J, Logue T, Chowdari K, Dayal M, Kupfer D, Monk T, Devlin B, Nimgaonkar V. 2006. Association study of eight circadian genes with bipolar I disorder, schizoaffective disorder and schizophrenia. Genes, Brain and Behavior 5:150-157.

Mantere O, Suominen K, Valtonen HM, Arvilommi P, Leppamaki S, Paunio T, Isometsa ET. 2012. Concomitants of family histories of mood disorders and alcoholism in a clinical cohort of patients with bipolar I and II disorder. J Nerv Ment Dis 200:388-394.

Mardis ER. 2008. Next-generation DNA sequencing methods. Annu.Rev.Genomics Hum.Genet. 9:387-402.

Marsman R, Swinkels SH, Rosmalen JG, Oldehinkel AJ, Ormel J, Buitelaar JK. 2008. HPA-axis activity and externalizing behavior problems in early adolescents from the general population: The role of comorbidity and gender: The TRAILS study. Psychoneuroendocrinology 33:789-798.

Martí SB, Cichon S, Propping P, Nöthen M. 2002. Metabotropic glutamate receptor 3 (GRM3) gene variation is not associated with schizophrenia or bipolar affective disorder in the german population. Am J Med Genet 114:46-50.

Martínez C, Galván S, Garcia-Martin E, Ramos MI, Gutiérrez-Martín Y, Agúndez JA. 2010. Variability in ethanol biodisposition in whites is modulated by polymorphisms in the ADH1B and ADH1C genes. Hepatology 51:491-500.

Martucci L, Wong AHC, De Luca V, Likhodi O, Wong GWH, King N, Kennedy JL. 2006. N-methyl-d-aspartate receptor NR2B subunit gene GRIN2B in schizophrenia and bipolar disorder: Polymorphisms and mRNA levels. Schizophr Res 84:214-221.

Mathers CD, Loncar D. 2006. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Medicine 3:e442.

Matsuo K, Hiraki A, Hirose K, Ito H, Suzuki T, Wakai K, Tajima K. 2007. Impact of the Alcohol-Dehydrogenase (ADH) 1C and ADH1B polymorphisms on drinking behavior in nonalcoholic japanese. Hum Mutat 28:506-510.

May PA, Brooke L, Gossage JP, Croxford J, Adnams C, Jones KL, Robinson L, Viljoen D. 2000. Epidemiology of fetal alcohol syndrome in a south african community in the western cape province. Am J Public Health 90:1905-1912.

McCullumsmith RE, Kristiansen LV, Beneyto M, Scarr E, Dean B, Meador-Woodruff JH. 2007. Decreased NR1, NR2A, and SAP102 transcript expression in the hippocampus in bipolar disorder. Brain Res 1127:108-118.

McCullumsmith RE, Meador-Woodruff JH. 2002. Striatal excitatory amino acid transporter transcript expression in schizophrenia, bipolar disorder, and major depressive disorder.Neuropsychopharm 26:368-375.

McElroy SL, Altshuler LL, Suppes T, Keck Jr PE, Frye MA, Denicoff KD, Nolen WA, Kupka RW, Leverich GS, Rochussen JR. 2001. Axis I psychiatric comorbidity and its relationship to historical illness variables in 288 patients with bipolar disorder. Am J Psychiatry 158:420-426.

McGrath LM, Cornelis MC, Lee PH, Robinson EB, Duncan LE, Barnett JH, Huang J, Gerber G, Sklar P, Sullivan P. 2013. Genetic predictors of risk and resilience in psychiatric disorders: A cross-disorder genome-wide association study of functional impairment in major depressive disorder, bipolar disorder, and schizophrenia. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 162:779-788.

McGue M, Zhang Y, Miller MB, Basu S, Vrieze S, Hicks B, Malone S, Oetting WS, Iacono WG. 2013. A genome-wide association study of behavioral disinhibition. Behav Genet 43:363-373.

McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. 2003. The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. Arch Gen Psychiatry 60:497-502.

McInnis MG, Dick DM, Willour VL, Avramopoulos D, MacKinnon DF, Simpson SG, Potash JB, Edenberg HJ, Bowman ES, McMahon FJ. 2003. Genome-wide scan and conditional analysis in bipolar disorder: Evidence for genomic interaction in the national institute of mental health genetics initiative bipolar pedigrees. Biol Psychiatry 54:1265-1273.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297-1303.

McQueen MB, Devlin B, Faraone SV, Nimgaonkar VL, Sklar P, Smoller JW, Jamra RA, Albus M, Bacanu S, Baron M. 2005. Combined analysis from eleven linkage studies of bipolar disorder provides strong evidence of susceptibility loci on chromosomes 6q and 8q. The American Journal of Human Genetics 77:582-595.

Medina KL, McQueeny T, Nagel BJ, Hanson KL, Schweinsburg AD, Tapert SF. 2008. Prefrontal cortex volumes in adolescents with alcohol use disorders: Unique gender effects. Alcoholism: Clinical and Experimental Research 32:386-394.

Meier S, Mattheisen M, Vassos E, Strohmaier J, Treutlein J, Josef F, Breuer R, Degenhardt F, Mühleisen TW, Müller-Myhsok B. 2012. Genome-wide significant association between a ‚negative mood delusions' dimension in bipolar disorder and genetic variation on chromosome 3q26. 1. Translational Psychiatry 2:e165.

Ménard C, Quirion R. 2012. Group 1 metabotropic glutamate receptor function and its regulation of learning and memory in the aging brain. Frontiers in Pharmacology 3:182.

Merikangas KR, Akiskal HS, Angst J, Greenberg PE, Hirschfeld RMA, Petukhova M, Kessler RC. 2007. Lifetime and 12-month prevalence of bipolar spectrum disorder in the national comorbidity survey replication. Arch Gen Psychiatry 64:543-552.

Merikangas KR, Mehta RL, Molnar BE, Walters EE, Swendsen JD, Aguilar-Gaziola S, Bijl R, Borges G, Caraveo-Anduaga JJ, Dewit DJ. 1998a. Comorbidity of substance use disorders with mood and anxiety disorders: Results of the international consortium in psychiatric epidemiology. Addict Behav 23:893-907.

Merikangas KR, Mehta RL, Molnar BE, Walters EE, Swendsen JD, Aguilar-Gaziola S, Bijl R, Borges G, Caraveo-Anduaga JJ, Dewit DJ. 1998b. Comorbidity of substance use disorders with mood and anxiety disorders: Results of the international consortium in psychiatric epidemiology. Addict Behav 23:893-907.

Merikangas KR, Stevens D, Fenton B, Stolar M, O'malley S, Woods S, Risch N. 1998c. Co-morbidity and familial aggregation of alcoholism and anxiety disorders. Psychol Med 28:773-788.

Merikangas KR, Jin R, He JP, Kessler RC, Lee S, Sampson NA, Viana MC, Andrade LH, Hu C, Karam EG, Ladea M, Medina-Mora ME, Ono Y, Posada-Villa J, Sagar R, Wells JE, Zarkov Z. 2011. Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. Arch Gen Psychiatry 68:241-251.

Merrow M, Spoelstra K, Roenneberg T. 2005. The circadian cycle: Daily rhythms from behaviour to genes. EMBO Rep 6:930-935.

Metzker ML. 2010. Sequencing technologies—the next generation. Nature Reviews Genetics 11:31-46.

Middleton F, Pato M, Gentile K, Morley C, Zhao X, Eisener A, Brown A, Petryshen T, Kirby A, Medeiros H. 2004. Genomewide linkage analysis of bipolar disorder by use of a high-density single-nucleotide–polymorphism (SNP) genotyping assay: A comparison with microsatellite marker assays and finding of significant linkage to chromosome 6q22. The American Journal of Human Genetics 74:886-897.

Miller K. 2006. Bipolar disorder: Etiology, diagnosis, and management. J Am Acad Nurse Pract 18:368-373.

Miranda R, Reynolds E, Ray L, Justus A, Knopik VS, McGeary J, Meyerson LA. 2013. Preliminary evidence for a gene–environment interaction in predicting alcohol use disorders in adolescents. Alcoholism: Clinical and Experimental Research 37:325-331.

Mitsukawa K, Mombereau C, Lötscher E, Uzunov DP, Van Der Putten H, Flor PJ, Cryan JF. 2006. Metabotropic glutamate receptor subtype 7 ablation causes dysregulation of the HPA axis and increases hippocampal BDNF protein levels: Implications for stress-related psychiatric disorders. Neuropsychopharmacology 31:1112-1122.

Molander A, Vengeliene V, Heilig M, Wurst W, Deussing JM, Spanagel R. 2012. Brain-specific inactivation of the Crhr1 gene inhibits post-dependent and stress-induced alcohol intake, but does not affect relapse-like drinking. Neuropsychopharmacology 37:1047-1056.

Monaghan DT, Bridges RJ, Cotman CW. 1989. The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 29:365-402.

Moore AA, Gould R, Reuben DB, Greendale GA, Carter MK, Zhou K, Karlamangla A. 2005. Longitudinal patterns and predictors of alcohol consumption in the united states. Am J Public Health 95:458-465.

Morris PL, Hopwood M, Whelan G, Gardiner J, Drummond E. 2001. Naltrexone for alcohol dependence: A randomized controlled trial. Addiction 96:1565-1573.

Most D, Ferguson L, Harris RA. 2014. Molecular basis of alcoholism. Handb Clin Neurol 125:89-111.

Moussas G, Dadouti G, Douzenis A, Poulis E, Tzelembis A, Bratis D, Christodoulou C, Lykouras L. 2009. The alcohol use disorders identification test (AUDIT): Reliability and validity of the greek version. Ann Gen Psychiatry 8:1-5.

Mühleisen TW, Leber M, Schulze TG, Strohmaier J, Degenhardt F, Treutlein J, Mattheisen M, Forstner AJ, Schumacher J, Breuer R. 2014. Genome-wide association study reveals two new risk loci for bipolar disorder. Nature Communications 5:3339.

Müller-Oerlinghausen B, Berghöfer A, Bauer M. 2002. Bipolar disorder. The Lancet 359:241-247.

Mullins N, Perroud N, Uher R, Butler AW, Cohen-Woods S, Rivera M, Malki K, Euesden J, Power RA, Tansey KE. 2014. Genetic relationships between suicide attempts, suicidal ideation and major psychiatric disorders: A genome-wide association and polygenic scoring study. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 165:428-437.

Mundo E, Tharmalingham S, Neves-Pereira M, Dalton E, Macciardi F, Parikh S, Bolonna A, Kerwin R, Arranz M, Makoff A. 2003. Evidence that the N-methyl-D-aspartate subunit 1 receptor gene (GRIN1) confers susceptibility to bipolar disorder. Mol Psychiatry 8:241-245.

Muráni E, Ponsuksili S, D'Eath RB, Turner SP, Kurt E, Evans G, Thölking L, Klont R, Foury A, Mormède P. 2010. Association of HPA axis-related genetic variation with stress reactivity and aggressive behaviour in pigs. BMC Genetics 11:74.

Muschler MAN, Hillemacher T, Kraus C, Kornhuber J, Bleich S, Frieling H. 2010. DNA methylation of the POMC gene promoter is associated with craving in alcohol dependence. J Neural Transm 117:513-519.

Nader N, Chrousos GP, Kino T. 2010. Interactions of the circadian CLOCK system and the HPA axis. Trends in Endocrinology & Metabolism 21:277-286.

Nagel BJ, Schweinsburg AD, Phan V, Tapert SF. 2005. Reduced hippocampal volume among adolescents with alcohol use disorders without psychiatric comorbidity. Psychiatry Research: Neuroimaging 139:181-190.

Nakanishi S. 1992. Molecular diversity of glutamate receptors and implications for brain function. Science 258:597-603.

Nakao A. 2014. Temporal regulation of cytokines by the circadian clock. Journal of Immunology Research 2014: 614529.

Nedic G, Nikolac M, Sviglin KN, Muck-Seler D, Borovecki F, Pivac N. 2011. Association study of a functional catechol-O-methyltransferase (COMT) Val108/158Met polymorphism and suicide attempts in patients with alcohol dependence. International Journal of Neuropsychopharmacology 14:377-388.

Nelson EC, Agrawal A, Pergadia ML, Wang JC, Whitfield JB, Saccone FS, Kern J, Grant JD, Schrage AJ, Rice JP. 2010. GENETIC STUDY: H2 haplotype at chromosome 17q21. 31 protects against childhood sexual abuse-associated risk for alcohol consumption and dependence. Addict Biol 15:1-11.

Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts CD, Loosen PT, Vale W. 1984. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science 226:1342-1344.

Nery FG, Stanley JA, Chen H, Hatch JP, Nicoletti MA, Serap Monkul E, Lafer B, Soares JC. 2010. Bipolar disorder comorbid with alcoholism: A 1H magnetic resonance spectroscopy study. J Psychiatr Res 44:278-285.

Nestler EJ. 2005. Is there a common molecular pathway for addiction? Nat Neurosci 8:1445-1449.

Newbury AJ, Rosen GD. 2012. Genetic, morphometric, and behavioral factors linked to the midsagittal area of the corpus callosum. Front Genet 3:91.

Ng PC, Henikoff S. 2001. Predicting deleterious amino acid substitutions. Genome Res 11:863-874.

Nievergelt CM, Kripke DF, Barrett TB, Burg E, Remick RA, Sadovnick AD, McElroy SL, Keck Jr PE, Schork NJ, Kelsoe JR. 2006. Suggestive evidence for association of the circadian genes PERIOD3 and ARNTL with bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 141:234-241.

Nilsson KW, Sjoberg RL, Wargelius HL, Leppert J, Lindstrom L, Oreland L. 2007. The monoamine oxidase A (MAO-A) gene, family function and maltreatment as predictors of destructive behaviour during male adolescent alcohol consumption. Addiction 102:389-398.

Nilsson KW, Sjöberg RL, Damberg M, Alm PO, Öhrvik J, Leppert J, Lindström L, Oreland L. 2005. Role of the serotonin transporter gene and family function in adolescent alcohol consumption. Alcoholism: Clinical and Experimental Research 29:564-570.

Nishizuka Y. 1989. The family of protein kinase C for signal transduction. JAMA 262:1826-1833.

Nurnberger Jr JI, Foroud T, Flury L, Su J, Meyer ET, Hu K, Crowe R, Edenberg H, Goate A, Bierut L. 2001. Evidence for a locus on chromosome 1 that influences vulnerability to alcoholism and affective disorder. Am J Psychiatry 158:718-724.

Nurnberger JI, Koller DL, Jung J, Edenberg HJ, Foroud T, Guella I, Vawter MP, Kelsoe JR. 2014. Identification of pathways for bipolar disorder: A meta-analysis. JAMA Psychiatry 71:657-664.

Nyholt DR. 2000. All LODs are not created equal. The American Journal of Human Genetics 67:282-288.

O'Brien NL, Way MJ, Kandaswamy R, Fiorentino A, Sharp SI, Quadri G, Alex J, Anjorin A, Ball D, Cherian R, Dar K, Gormez A, Guerrini I, Heydtmann M, Hillman A, Lankappa S, Lydall G, O'Kane A, Patel S, Quested D, Smith I, Thomson AD, Bass NJ, Morgan MY, Curtis D, McQuillin A. 2014. The functional GRM3 kozak

sequence variant rs148754219 affects the risk of schizophrenia and alcohol dependence as well as bipolar disorder. Psychiatr Genet 24:277-278.

Ökvist A, Johansson S, Kuzmin A, Bazov I, Merino-Martinez R, Ponomarev I, Mayfield RD, Harris RA, Sheedy D, Garrick T. 2007. Neuroadaptations in human chronic alcoholics: Dysregulation of the NF-κB system. PLoS One 2:e930.

Olfson E, Bierut LJ. 2012. Convergence of Genome-Wide association and candidate gene studies for alcoholism. Alcoholism: Clinical and Experimental Research 36:2086-2094.

Olive MF, Koenig HN, Nannini MA, Hodge CW. 2001. Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine, and amphetamine. J Neurosci 21:RC184.

Oquendo MA, Currier D, Liu SM, Hasin DS, Grant BF, Blanco C. 2010. Increased risk for suicidal behavior in comorbid bipolar disorder and alcohol use disorders: Results from the national epidemiologic survey on alcohol and related conditions (NESARC). J Clin Psychiatry 71:902-909.

Ortiz-Domínguez A, Hernández ME, Berlanga C, Gutiérrez-Mora D, Moreno J, Heinze G, Pavón L. 2007. Immune variations in bipolar disorder: Phasic differences. Bipolar Disord 9:596-602.

Oscar-Berman M, Marinković K. 2007. Alcohol: Effects on neurobehavioral functions and the brain. Neuropsychol Rev 17:239-257.

Ottaviani E, Capriglione T, Franceschi C. 1995. Invertebrate and vertebrate immune cells express pro-opiomelanocortin (POMC) mRNA. Brain Behavior and Immunity 9:1-8.

Padmos RC, Hillegers MH, Knijff EM, Vonk R, Bouvy A, Staal FJ, de Ridder D, Kupka RW, Nolen WA, Drexhage HA. 2008. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. Arch Gen Psychiatry 65:395-407.

Palmer R, McGeary JE, Francazio S, Raphael B, Lander A, Heath AC, Knopik V. 2012. The genetics of alcohol dependence: Advancing towards systems-based approaches. Drug Alcohol Depend 125:179-191.

Pan Y, Luo X, Liu X, Wu L, Zhang Q, Wang L, Wang W, Zuo L, Wang K. 2013. Genome-wide association studies of maximum number of drinks. J Psychiatr Res 47:1717-1724.

Pan Y, Wang K, Aragam N. 2011. NTM and NR3C2 polymorphisms influencing intelligence: Family-based association studies. Prog Neuro-Psychopharmacol Biol Psychiatry 35:154-160.

Papiol S, Rosa A, Gutierrez B, Martin B, Salgado P, Catalan R, Arias B, Fananas L. 2004. Interleukin-1 cluster is associated with genetic risk for schizophrenia and bipolar disorder. J Med Genet 41:219-223.

Park BL, Kim JW, Cheong HS, Kim LH, Lee BC, Seo CH, Kang T, Nam Y, Kim G, Shin HD. 2013. Extended genetic effects of ADH cluster genes on the risk of alcohol dependence: From GWAS to replication. Hum Genet 132:657-668.

Parkin J, Cohen B. 2001. An overview of the immune system. The Lancet 357:1777-1789.

Parry CD. 2005. South africa: Alcohol today. Addiction 100:426-429.

Parry CD, Myers B, Morojele NK, Flisher AJ, Bhana A, Donson H, Plüddemann A. 2004. Trends in adolescent alcohol and other drug use: Findings from three sentinel sites in south africa (1997–2001). J Adolesc 27:429-440.

Pastor IJ, Laso FJ, Àvila JJ, Rodriguez RE, González-Sarrniento R. 2000. Polymorphism in the Interleukin-1 receptor antagonist gene is associated with alcoholism in spanish men. Alcoholism: Clinical and Experimental Research 24:1479-1482.

Pastor IJ, Laso FJ, Romero A, Gonzalez-Sarmiento R. 2005. Interleukin-1 gene cluster polymorphisms and alcoholism in spanish men. Alcohol Alcohol 40:181-186.

Paternoster L, Howe LD, Tilling K, Weedon MN, Freathy RM, Frayling TM, Kemp JP, Smith GD, Timpson NJ, Ring SM, Evans DM, Lawlor DA. 2011. Adult height variants affect birth length and growth rate in children. Hum Mol Genet 20:4069-4075.

Pato CN, Pato M, Kirby A, Petryshen T, Medeiros H, Carvalho C, Macedo A, Dourado A, Coelho I, Valente J. 2004. Genome-wide scan in portuguese island families implicates multiple loci in bipolar disorder: Fine

mapping adds support on chromosomes 6 and 11. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 127:30-34.

Paus T, Keshavan M, Giedd JN. 2008. Why do many psychiatric disorders emerge during adolescence? Nature Reviews Neuroscience 9:947-957.

Peacock PJ, Lewis G, Northstone K, Wiles NJ. 2011. Childhood diet and behavioural problems: Results from the ALSPAC cohort. Eur J Clin Nutr 65:720-726.

Perlis RH, Smoller JW, Ferreira MA, McQuillin A, Bass N, Lawrence J, Psych M, Sachs GS, Nimgaonkar V, Scolnick EM. 2009. A genomewide association study of response to lithium for prevention of recurrence in bipolar disorder. Am J Psychiatry 166:718-725.

Perlman CA, Johnson SL, Mellman TA. 2006. The prospective impact of sleep duration on depression and mania. Bipolar Disord 8:271-274.

Perneger TV. 1998. What's wrong with bonferroni adjustments. Br Med J 316:1236.

Perrin M, Donaldson C, Chen R, Blount A, Berggren T, Bilezikjian L, Sawchenko P, Vale W. 1995. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. Proc Natl Acad Sci U S A 92:2969-2973.

Petrakis IL, Limoncelli D, Gueorguieva R, Jatlow P, Boutros NN, Trevisan L, Gelernter J, Krystal JH. 2004. Altered NMDA glutamate receptor antagonist response in individuals with a family vulnerability to alcoholism. Am J Psychiatry 161:1776-1782.

Phillips J. 2008. Alcohol use among black female adolescents in a south african community: A mixed methods investigation. JCHS 3:22-30.

Pickard B, Malloy M, Christoforou A, Thomson P, Evans K, Morris S, Hampson M, Porteous D, Blackwood D, Muir W. 2006. Cytogenetic and genetic evidence supports a role for the kainate-type glutamate receptor gene, GRIK4, in schizophrenia and bipolar disorder. Mol Psychiatry 11:847-857.

Pickard BS, Knight HM, Hamilton RS, Soares DC, Walker R, Boyd JK, Machell J, Maclean A, McGhee KA, Condie A, Porteous DJ, St Clair D, Davis I, Blackwood DH, Muir WJ. 2008. A common variant in the 3'UTR of the GRIK4 glutamate receptor gene affects transcript abundance and protects against bipolar disorder. Proc Natl Acad Sci U S A 105:14940-14945.

Pin J, Duvoisin R. 1995. The metabotropic glutamate receptors: Structure and functions. Neuropharmacology 34:1-26.

Plüddemann A, Dada S, Parry C, Bhana A, Perreira T, Carelsen A, Kitleli N, Gerber W, Rosslee C, Fourie D. 2008. Monitoring Alcohol & drug Abuse trends in south africa (july 1996 – june 2008).

Polanczyk G, Caspi A, Williams B, Price TS, Danese A, Sugden K, Uher R, Poulton R, Moffitt TE. 2009. Protective effect of CRHR1 gene variants on the development of adult depression following childhood maltreatment: Replication and extension. Arch Gen Psychiatry 66:978-985.

Posthuma D, De Geus E, Neale M, Pol HH, Baare W, Kahn R, Boomsma D. 2000. Multivariate genetic analysis of brain structure in an extended twin design. Behav Genet 30:311-319.

Potash JB, Zandi PP, Willour VL, Lan T, Huo Y, Avramopoulos D, Shugart YY, MacKinnon DF, Simpson SG, McMahon FJ. 2003. Suggestive linkage to chromosomal regions 13q31 and 22q12 in families with psychotic bipolar disorder. Am J Psychiatry 160:680-686.

Preitner N, Damiola F, Zakany J, Duboule D, Albrecht U, Schibler U. 2002. The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell 110:251-260.

Prescott C, Sullivan P, Kuo P, Webb B, Vittum J, Patterson D, Thiselton D, Myers J, Devitt M, Halberstadt L. 2006. Genomewide linkage study in the irish affected sib pair study of alcohol dependence: Evidence for a susceptibility region for symptoms of alcohol dependence on chromosome 4. Mol Psychiatry 11:603-611.

Prescott CA, Kendler KS. 1999. Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. Am J Psychiatry 156:34-40.

Pruitt KD, Tatusova T, Maglott DR. 2005. NCBI reference sequence (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res 33:D501-4.

Psychiatric GWAS Consortium Bipolar Disorder Working Group. 2011. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet 43:977-983.

Pulst SM. 1999. Genetic linkage analysis. Arch Neurol 56:667-672.

Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P, Ruderfer DM, McQuillin A, Morris DW. 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460:748-752.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, De Bakker PI, Daly MJ. 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81:559-575.

Qi X, Kamphuis W, Wang S, Wang Q, Lucassen PJ, Zhou J, Swaab DF. 2013. Aberrant stress hormone receptor balance in the human prefrontal cortex and hypothalamic paraventricular nucleus of depressed patients. Psychoneuroendocrinology 38:863-870.

Quillen EE, Chen X, Almasy L, Yang F, He H, Li X, Wang X, Liu T, Hao W, Deng H. 2014. ALDH2 is associated to alcohol dependence and is the major genetic determinant of –daily maximum drinks" in a GWAS study of an isolated rural chinese sample. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 165:103-110.

Raadsheer F, Hoogendijk WJ, Stam F, Tilders F, Swaab DF. 1994. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. Neuroendocrinology 60:436-444.

Raadsheer FC, van Heerikhuize JJ, Lucassen PJ, Hoogendijk WJ, Tilders FJ, Swaab DF. 1995. Increased corticotropin-releasing hormone (CRH) mRNA in the paraventricular nucleus of patients with alzheimer's disease or depression. Am J Psychiatry 152:1372-1376.

Radhakrishna U, Senol S, Herken H, Gucuyener K, Gehrig C, Blouin JL, Akarsu NA, Antonarakis SE. 2001. An apparently dominant bipolar affective disorder (BPAD) locus on chromosome 20p11.2-q11.2 in a large turkish pedigree. Eur J Hum Genet 9:39-44.

Radulovic J, Ruhmann A, Liepold T, Spiess J. 1999. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: Differential roles of CRF receptors 1 and 2. J Neurosci 19:5016-5025.

Ramchandani V, Umhau J, Pavon F, Ruiz-Velasco V, Margas W, Sun H, Damadzic R, Eskay R, Schoor M, Thorsell A. 2010. A genetic determinant of the striatal dopamine response to alcohol in men. Mol Psychiatry 1-9.

Ray LA, Sehl M, Bujarski S, Hutchison K, Blaine S, Enoch M. 2013. The CRHR1 gene, trauma exposure, and alcoholism risk: A test of G× E effects. Genes, Brain and Behavior 12:361-369.

Reardon W, Dunlop A, Holden ST, Blennerhassett R. 2007. Bipolar affective disorder associated with 11q24. 2 disruption—A second report. American Journal of Medical Genetics Part A 143:1263-1267.

Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, Goodwin FK. 1990. Comorbidity of mental disorders with alcohol and other drug abuse: Results from the epidemiologic catchment area (ECA) study. JAMA 264:2511-2518.

Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, Foroud T, Hesselbrock V, Schuckit MA, Bucholz K, Porjesz B, Li TK, Conneally PM, Nurnberger JI,Jr, Tischfield JA, Crowe RR, Cloninger CR, Wu W, Shears S, Carr K, Crose C, Willig C, Begleiter H. 1998. Genome-wide search for genes affecting the risk for alcohol dependence. Am J Med Genet 81:207-215.

Reyes TM, Lewis K, Perrin MH, Kunitake KS, Vaughan J, Arias CA, Hogenesch JB, Gulyas J, Rivier J, Vale WW, Sawchenko PE. 2001. Urocortin II: A member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc Natl Acad Sci U S A 98:2843-2848.

Richter RM, Zorrilla EP, Basso AM, Koob GF, Weiss F. 2000. Altered amygdalar CRF release and increased Anxiety-Like behavior in sardinian Alcohol-Preferring rats: A microdialysis and behavioral study. Alcoholism: Clinical and Experimental Research 24:1765-1772.

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M. 2013. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150-1159.

Risch NJ. 2000. Searching for genetic determinants in the new millennium. Nature 405:847-856.

Rivier C, Lee S. 1996. Acute alcohol administration stimulates the activity of hypothalamic neurons that express corticotropin-releasing factor and vasopressin. Brain Res 726:1-10.

Robb JC, Young LT, Cooke RG, Joffe RT. 1998. Gender differences in patients with bipolar disorder influence outcome in the medical outcomes survey (SF-20) subscale scores. J Affect Disord 49:189-193.

Robinson G, Most D, Ferguson LB, Mayfield J, Harris RA, Blednov YA. 2014. Neuroimmune pathways in alcohol consumption: Evidence from behavioral and genetic studies in rodents and humans. Int Rev Neurobiol 118:13-39.

Rogers J, Raveendran M, Fawcett G, Fox A, Shelton S, Oler J, Cheverud J, Muzny D, Gibbs R, Davidson R. 2013. CRHR1 genotypes, neural circuits and the diathesis for anxiety and depression. Mol Psychiatry 18:700-707.

Rohde P, Lewinsohn PM, Kahler CW, Seeley JR, Brown RA. 2001. Natural course of alcohol use disorders from adolescence to young adulthood. Journal of the American Academy of Child & Adolescent Psychiatry 40:83-90.

Rolls ET. 2000. The orbitofrontal cortex and reward. Cereb Cortex 10:284-294.

Roozendaal B, Brunson KL, Holloway BL, McGaugh JL, Baram TZ. 2002. Involvement of stress-released corticotropin-releasing hormone in the basolateral amygdala in regulating memory consolidation. Proc Natl Acad Sci U S A 99:13908-13913.

Ruderfer D, Fanous A, Ripke S, McQuillin A, Amdur R, Gejman P, O'Donovan M, Andreassen O, Djurovic S, Hultman C. 2014. Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. Mol Psychiatry 19:1017-1024.

Ryabinin AE, Tsoory MM, Kozicz T, Thiele TE, Neufeld-Cohen A, Chen A, Lowery-Gionta EG, Giardino WJ, Kaur S. 2012. Urocortins: CRF's siblings and their potential role in anxiety, depression and alcohol drinking behavior. Alcohol 46:349-357.

Sachs GS, Thase ME, Otto MW, Bauer M, Miklowitz D, Wisniewski SR, Lavori P, Lebowitz B, Rudorfer M, Frank E. 2003. Rationale, design, and methods of the systematic treatment enhancement program for bipolar disorder (STEP-BD). Biol Psychiatry 53:1028-1042.

Saiz PA, Garcia-Portilla MP, Florez G, Corcoran P, Arango C, Morales B, Leza JC, Alvarez S, Díaz EM, Alvarez V. 2009. Polymorphisms of the IL-1 gene complex are associated with alcohol dependence in spanish caucasians: Data from an association study. Alcoholism: Clinical and Experimental Research 33:2147-2153.

Salloum IM, Thase ME. 2000. Impact of substance abuse on the course and treatment of bipolar disorder. Bipolar Disord 2:269-280.

San José E, Sahuquillo AG, Bragado R, Alarcón B. 1998. Assembly of the TCR/CD3 complex: CD3ϵ/δ and CD3ϵ/γ dimers associate indistinctly with both TCRα and TCRβ chains. evidence for a double TCR heterodimer model. Eur J Immunol 28:12-21.

Sanacora G, Zarate CA, Krystal JH, Manji HK. 2008. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. Nature Reviews Drug Discovery 7:426-437.

Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL. 2012. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485:237-241.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74:5463-5467.

Saraceno L, Munafó M, Heron J, Craddock N, Van Den Bree M. 2009. Genetic and non-genetic influences on the development of co-occurring alcohol problem use and internalizing symptomatology in adolescence: A review. Addiction 104:1100-1121.

Saridjan NS, Velders FP, Jaddoe VW, Hofman A, Verhulst FC, Tiemeier H. 2014. The longitudinal association of the diurnal cortisol rhythm with internalizing and externalizing problems in pre-schoolers. the generation R study. Psychoneuroendocrinology 50:118-129.

Sartor CE, Waldron M, Duncan AE, Grant JD, McCutcheon VV, Nelson EC, Madden PA, Bucholz KK, Heath AC. 2013. Childhood sexual abuse and early substance use in adolescent girls: The role of familial influences. Addiction 108:993-1000.

Sartor CE, Wang Z, Xu K, Kranzler HR, Gelernter J. 2014. The joint effects of ADH1B variants and childhood adversity on alcohol related phenotypes in African-American and European-American women and men. Alcoholism: Clinical and Experimental Research 38:2907-2914.

Saunders EH, Scott LJ, McInnis MG, Burmeister M. 2008. Familiality and diagnostic patterns of subphenotypes in the national institutes of mental health bipolar sample. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 147:18-26.

Saunders CJ, Miller NA, Soden SE, Dinwiddie DL, Noll A, Alnadi NA, Andraws N, Patterson ML, Krivohlavek LA, Fellis J, Humphray S, Saffrey P, Kingsbury Z, Weir JC, Betley J, Grocock RJ, Margulies EH, Farrow EG, Artman M, Safina NP, Petrikin JE, Hall KP, Kingsmore SF. 2012. Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. Sci Transl Med 4:154ra135.

Schatzberg A, Keller J, Tennakoon L, Lembke A, Williams G, Kraemer F, Sarginson J, Lazzeroni L, Murphy G. 2013. HPA axis genetic variation, cortisol and psychosis in major depression. Mol Psychiatry 19:220-227.

Scheiermann C, Kunisaki Y, Frenette PS. 2013. Circadian control of the immune system. Nature Reviews Immunology 13:190-198.

Scherer S, Heng H, Tsui L. 1996. Localization of two metabotropic glutamate receptor genes, GRM3 and GRM8, to human chromosome 7q. Genomics 31:230-233.

Schito A, Pizzuti A, Di Maria E, Schenone A, Ratti A, Defferrari R, Bellone E, Mancardi G, Ajmar F, Mandich P. 1997. mRNA distribution in adult human brain of GRIN2B, a N-methyl-D-aspartate (NMDA) receptor subunit. Neurosci Lett 239:49-53.

Schizophrenia Working Group of the Psychiatric Genomics Consortium. 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421-427.

Schmid B, Blomeyer D, Treutlein J, Zimmermann US, Buchmann AF, Schmidt MH, Esser G, Rietschel M, Banaschewski T, Schumann G. 2010. Interacting effects of CRHR1 gene and stressful life events on drinking initiation and progression among 19-year-olds. International Journal of Neuropsychopharmacology 13:703-714.

Schmidt CW. 2007. Environmental connections: A deeper look into mental illness. Environ Health Perspect 115:A404, A406-10.

Schneider M, Norman R, Parry C, Bradshaw D, Pluddemann A, Collaboration, South African Comparitive Risk Assessment. 2007. Estimating the burden of disease attributable to alcohol use in south africa in 2000. South African Medical Journal 97:664-672.

Schneider U, Altmann A, Baumann M, Bernzen J, Bertz B, Bimber U, Broese T, Broocks A, Burtscheidt W, Cimander KF, Degkwitz P, Driessen M, Ehrenreich H, Fischbach E, Folkerts H, Frank H, Gurth D, Havemann-Reinecke U, Heber W, Heuer J, Hingsammer A, Jacobs S, Krampe H, Lange W, Lay T, Leimbach M, Lemke MR, Leweke M, Mangholz A, Massing W, Meyenberg R, Porzig J, Quattert T, Redner C, Ritzel G, Rollnik JD, Sauvageoll R, Schlafke D, Schmid G, Schroder H, Schwichtenberg U, Schwoon D, Seifert J, Sickelmann I, Sieveking CF, Spiess C, Stiegemann HH, Stracke R, Straetgen HD, Subkowski P, Thomasius R, Tretzel H, Verner LJ, Vitens J, Wagner T, Weirich S, Weiss I, Wendorff T, Wetterling T, Wiese B, Wittfoot J. 2001. Comorbid anxiety and affective disorder in alcohol-dependent patients seeking treatment: The first multicentre study in germany. Alcohol Alcohol 36:219-223.

Schneier FR, Foose TE, Hasin DS, Heimberg RG, Liu S, Grant BF, Blanco C. 2010. Social anxiety disorder and alcohol use disorder co-morbidity in the national epidemiologic survey on alcohol and related conditions. Psychol Med 40:977-988.

Schoepf D, Heun R. 2014. Bipolar disorder and comorbidity: Increased prevalence and increased relevance of comorbidity for hospital-based mortality during a 12.5-year observation period in general hospital admissions. J Affect Disord 169:170-178.

Schroeder JP, Overstreet DH, Hodge CW. 2005. The mGluR5 antagonist MPEP decreases operant ethanol self-administration during maintenance and after repeated alcohol deprivations in alcohol-preferring (P) rats. Psychopharmacology (Berl) 179:262-270.

Schumacher J, Kaneva R, Jamra RA, Diaz GO, Ohlraun S, Milanova V, Lee Y, Rivas F, Mayoral F, Fuerst R. 2005. Genomewide scan and fine-mapping linkage studies in four european samples with bipolar affective disorder suggest a new susceptibility locus on chromosome 1p35-p36 and provides further evidence of loci on chromosome 4q31 and 6q24. The American Journal of Human Genetics 77:1102-1111.

Schumann G, Johann M, Frank J, Preuss U, Dahmen N, Laucht M, Rietschel M, Rujescu D, Lourdusamy A, Clarke T. 2008. Systematic analysis of glutamatergic neurotransmission genes in alcohol dependence and adolescent risky drinking behavior. Arch Gen Psychiatry 65:826-838.

Schumann G, Coin LJ, Lourdusamy A, Charoen P, Berger KH, Stacey D, Desrivieres S, Aliev FA, Khan AA, Amin N, Aulchenko YS, Bakalkin G, Bakker SJ, Balkau B, Beulens JW, Bilbao A, de Boer RA, Beury D, Bots ML, Breetvelt EJ, Cauchi S, Cavalcanti-Proenca C, Chambers JC, Clarke TK, Dahmen N, de Geus EJ, Dick D, Ducci F, Easton A, Edenberg HJ, Esko T, Fernandez-Medarde A, Foroud T, Freimer NB, Girault JA, Grobbee DE, Guarrera S, Gudbjartsson DF, Hartikainen AL, Heath AC, Hesselbrock V, Hofman A, Hottenga JJ, Isohanni MK, Kaprio J, Khaw KT, Kuehnel B, Laitinen J, Lobbens S, Luan J, Mangino M, Maroteaux M, Matullo G, McCarthy MI, Mueller C, Navis G, Numans ME, Nunez A, Nyholt DR, Onland-Moret CN, Oostra BA, O'Reilly PF, Palkovits M, Penninx BW, Polidoro S, Pouta A, Prokopenko I, Ricceri F, Santos E, Smit JH, Soranzo N, Song K, Sovio U, Stumvoll M, Surakk I, Thorgeirsson TE, Thorsteinsdottir U, Troakes C, Tyrfingsson T, Tonjes A, Uiterwaal CS, Uitterlinden AG, van der Harst P, van der Schouw YT, Staehlin O, Vogelzangs N, Vollenweider P, Waeber G, Wareham NJ, Waterworth DM, Whitfield JB, Wichmann EH, Willemsen G, Witteman JC, Yuan X, Zhai G, Zhao JH, Zhang W, Martin NG, Metspalu A, Doering A, Scott J, Spector TD, Loos RJ, Boomsma DI, Mooser V, Peltonen L, Stefansson K, van Duijn CM, Vineis P, Sommer WH, Kooner JS, Spanagel R, Heberlein UA, Jarvelin MR, Elliott P. 2011. Genome-wide association and genetic functional studies identify autism susceptibility candidate 2 gene (AUTS2) in the regulation of alcohol consumption. Proc Natl Acad Sci U S A 108:7119-7124.

Scott LJ, Muglia P, Kong XQ, Guan W, Flickinger M, Upmanyu R, Tozzi F, Li JZ, Burmeister M, Absher D. 2009. Genome-wide association and meta-analysis of bipolar disorder in individuals of european ancestry. Proceedings of the National Academy of Sciences 106:7501-7506.

Sedler MJ. 1983. Falret's discovery: The origin of the concept of bipolar affective illness. translated by M. J. sedler and eric C. dessain. Am J Psychiatry 140:1127-1133.

Segurado R, Detera-Wadleigh SD, Levinson DF, Lewis CM, Gill M, Nurnberger JI, Craddock N, DePaulo JR, Baron M, Gershon ES. 2003. Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: Bipolar disorder. The American Journal of Human Genetics 73:49-62.

Sharpe AL, Phillips TJ. 2009. Central urocortin 3 administration decreases limited-access ethanol intake in nondependent mice. Behav Pharmacol 20:346-351.

Shendure J, Ji H. 2008. Next-generation DNA sequencing. Nat Biotechnol 26:1135-1145.

Sher KJ, Dick DM, Crabbe JC, Hutchison KE, O'Malley SS, Heath AC. 2010. Review: Consilient research approaches in studying gene× environment interactions in alcohol research. Addict Biol 15:200-216.

Shi J, Wittke-Thompson JK, Badner JA, Hattori E, Potash JB, Willour VL, McMahon FJ, Gershon ES, Liu C. 2008. Clock genes may influence bipolar disorder susceptibility and dysfunctional circadian rhythm. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 147:1047-1055.

Shin S, Stewart R, Ferri CP, Kim J, Shin I, Kim S, Yang S, Yoon J. 2010. An investigation of associations between alcohol use disorder and polymorphisms on ALDH2, BDNF, 5-HTTLPR, and MTHFR genes in older korean men. Int J Geriatr Psychiatry 25:441-448.

Silberstein M, Weissbrod O, Otten L, Tzemach A, Anisenia A, Shtark O, Tuberg D, Galfrin E, Gannon I, Shalata A, Borochowitz ZU, Dechter R, Thompson E, Geiger D. 2013. A system for exact and approximate genetic linkage analysis of SNP data in large pedigrees. Bioinformatics 29:197-205.

Sillaber I, Rammes G, Zimmermann S, Mahal B, Zieglgansberger W, Wurst W, Holsboer F, Spanagel R. 2002. Enhanced and delayed stress-induced alcohol drinking in mice lacking functional CRH1 receptors. Science 296:931-933.

Silverman MN, Pearce BD, Biron CA, Miller AH. 2005. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. Viral Immunol 18:41-78.

Sjoholm LK, Kovanen L, Saarikoski ST, Schalling M, Lavebratt C, Partonen T. 2010. CLOCK is suggested to associate with comorbid alcohol use and depressive disorders. J Circadian Rhythms 8:1-3391-8-1.

Sklar P, Smoller J, Fan J, Ferreira M, Perlis R, Chambert K, Nimgaonkar V, McQueen M, Faraone S, Kirby A. 2008. Whole-genome association study of bipolar disorder. Mol Psychiatry 13:558-569.

Sleiman P, Wang D, Glessner J, Hadley D, Gur RE, Cohen N, Li Q, Hakonarson H, Janssen-CHOP Neuropsychiatric Genomics Working Group. 2013. GWAS meta analysis identifies TSNARE1 as a novel schizophrenia/bipolar susceptibility locus. Scientific Reports 3:3075-3080.

Smith E, Bloss C, Badner J, Barrett T, Belmonte P, Berrettini W, Byerley W, Coryell W, Craig D, Edenberg H. 2009. Genome-wide association study of bipolar disorder in european american and african american individuals. Mol Psychiatry 14:755-763.

Smith EN, Koller DL, Panganiban C, Szelinger S, Zhang P, Badner JA, Barrett TB, Berrettini WH, Bloss CS, Byerley W. 2011a. Genome-wide association of bipolar disorder suggests an enrichment of replicable associations in regions near genes. PLoS Genetics 7:e1002134.

Smith KR, Bromhead CJ, Hildebrand MS, Shearer AE, Lockhart PJ, Najmabadi H, Leventer RJ, McGillivray G, Amor DJ, Smith RJ. 2011b. Reducing the exome search space for mendelian diseases using genetic linkage analysis of exome genotypes. Genome Biol 12:R85.

Smith ML, Li J, Ryabinin AE. 2014. Increased alcohol consumption in urocortin 3 knockout mice is unaffected by chronic inflammatory pain. Alcohol Alcohol 50:132-139.

Smoller JW, Finn CT. 2003. Family, twin, and adoption studies of bipolar disorder. Am J Med Genet 123C:48-58.

Sobell LC, Sobell MB. 1992. Timeline follow-back. In: Measuring alcohol consumption. The Human Press Inc. p 41-72.

Sood B, Delaney-Black V, Covington C, Nordstrom-Klee B, Ager J, Templin T, Janisse J, Martier S, Sokol RJ. 2001. Prenatal alcohol exposure and childhood behavior at age 6 to 7 years: I. dose-response effect. Pediatrics 108:E34.

Soria V, Martínez-Amorós È, Escaramís G, Valero J, Pérez-Egea R, García C, Gutiérrez-Zotes A, Puigdemont D, Bayés M, Crespo JM. 2010. Differential association of circadian genes with mood disorders: CRY1 and NPAS2 are associated with unipolar major depression and CLOCK and VIP with bipolar disorder. Neuropsychopharmacology 35:1279-1289.

Soyka M, Preuss U, Hesselbrock V, Zill P, Koller G, Bondy B. 2008. GABA-A2 receptor subunit gene (GABRA2) polymorphisms and risk for alcohol dependence. J Psychiatr Res 42:184-191.

Spear LP. 2000. The adolescent brain and age-related behavioral manifestations. Neuroscience & Biobehavioral Reviews 24:417-463.

Spijker A, Giltay E, van Rossum E, Manenschijn L, DeRijk R, Haffmans J, Zitman F, Hoencamp E. 2011. Glucocorticoid and mineralocorticoid receptor polymorphisms and clinical characteristics in bipolar disorder patients. Psychoneuroendocrinology 36:1460-1469.

Squeglia LM, Jacobus J, Tapert SF. 2009. The influence of substance use on adolescent brain development. Clinical EEG and Neuroscience 40:31-38.

Srivastava V, Buzas B, Momenan R, Oroszi G, Pulay AJ, Enoch MA, Hommer DW, Goldman D. 2009. Association of SOD2, a mitochondrial antioxidant enzyme, with gray matter volume shrinkage in alcoholics. Neuropsychopharmacology 35:1120-1128.

StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP.

Steemers FJ, Chang W, Lee G, Barker DL, Shen R, Gunderson KL. 2006. Whole-genome genotyping with the single-base extension assay. Nature Methods 3:31-33.

Stein MB, Jang KL, Livesley WJ. 1999. Heritability of anxiety sensitivity: A twin study. Am J Psychiatry 156:246-251.

Stein DJ, Seedat S, Herman A, Moomal H, Heeringa SG, Kessler RC, Williams DR. 2008. Lifetime prevalence of psychiatric disorders in south africa. Br J Psychiatry 192:112-117.

Steiner J, Jacobs R, Panteli B, Brauner M, Schiltz K, Bahn S, Herberth M, Westphal S, Gos T, Walter M. 2010. Acute schizophrenia is accompanied by reduced T cell and increased B cell immunity. Eur Arch Psychiatry Clin Neurosci 260:509-518.

Stelzhammer V, Alsaif M, Chan MK, Rahmoune H, Steeb H, Guest PC, Bahn S. 2015. Distinct proteomic profiles in post-mortem pituitary glands from bipolar disorder and major depressive disorder patients. J Psychiatr Res 60:40-48.

Steptoe A, Wardle J, Pollard TM, Canaan L, Davies GJ. 1996. Stress, social support and health-related behavior: A study of smoking, alcohol consumption and physical exercise. J Psychosom Res 41:171-180.

Stine OC, McMahon FJ, Chen L, Xu J, Meyers DA, MacKinnon DF, Simpson S, McInnis MG, Rice JP, Goate A. 1997. Initial genome screen for bipolar disorder in the NIMH genetics initiative pedigrees: Chromosomes 2. American Journal of Medical Genetics (Neuropsychiatric Genetics) 74:263-269.

Stine OC, Xu J, Koskela R, McMahon FJ, Gschwend M, Friddle C, Clark CD, McInnis MG, Simpson SG, Breschel TS, Vishio E, Riskin K, Feilotter H, Chen E, Shen S, Folstein S, Meyers DA, Botstein D, Marr TG, DePaulo JR. 1995. Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect. Am J Hum Genet 57:1384-1394.

Stone LL, Otten R, Engels RC, Vermulst AA, Janssens JM. 2010. Psychometric properties of the parent and teacher versions of the strengths and difficulties questionnaire for 4-to 12-year-olds: A review. Clin Child Fam Psychol Rev 13:254-274.

Stratton M. 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447:661-678.

Strous RD, Shoenfeld Y. 2006. Schizophrenia, autoimmunity and immune system dysregulation: A comprehensive model updated and revisited. J Autoimmun 27:71-80.

Suda T, Kageyama K, Sakihara S, Nigawara T. 2004. Physiological roles of urocortins, human homologues of fish urotensin I, and their receptors. Peptides 25:1689-1701.

Sullivan PF, Daly MJ, O'Donovan M. 2012. Genetic architectures of psychiatric disorders: The emerging picture and its implications. Nature Reviews Genetics 13:537-551.

Svihra M, Katzman MA. 2004. Behavioural inhibition: A predictor of anxiety. Paediatr Child Health 9:547-550.

Szabo G, Mandrekar P. 2009. A recent perspective on alcohol, immunity, and host defense. Alcoholism: Clinical and Experimental Research 33:220-232.

Tadic A, Dahmen N, Szegedi A, Rujescu D, Giegling I, Koller G, Anghelescu I, Fehr C, Klawe C, Preuss UW. 2005. Polymorphisms in the NMDA subunit 2B are not associated with alcohol dependence and alcohol withdrawal–induced seizures and delirium tremens. Eur Arch Psychiatry Clin Neurosci 255:129-135.

Tanaka C, Nishizuka Y. 1994. The protein kinase C family for neuronal signaling. Annu Rev Neurosci 17:551-567.

Tapert SF, Cheung EH, Brown GG, Frank LR, Paulus MP, Schweinsburg AD, Meloy M, Brown SA. 2003. Neural response to alcohol stimuli in adolescents with alcohol use disorder. Arch Gen Psychiatry 60:727-735.

Theodoropoulou S, Spanakos G, Baxevanis CN, Economou M, Gritzapis AD, Papamichail MP, Stefanis CN. 2001. Cytokine serum levels, autologous mixed lymphocyte reaction and surface marker analysis in never medicated and chronically medicated schizophrenic patients. Schizophr Res 47:13-25.

Thompson PM, Cannon TD, Narr KL, Van Erp T, Poutanen V, Huttunen M, Lönnqvist J, Standertskjöld-Nordenstam C, Kaprio J, Khaledy M. 2001. Genetic influences on brain structure. Nat Neurosci 4:1253-1258.

Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JM, Stalla GK, Blanquet V, Steckler T, Holsboer F, Wurst W. 1998. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nat Genet 19:162-166.

Tishkoff SA, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, Froment A, Hirbo JB, Awomoyi AA, Bodo JM, Doumbo O, Ibrahim M, Juma AT, Kotze MJ, Lema G, Moore JH, Mortensen H, Nyambo TB, Omar SA, Powell

K, Pretorius GS, Smith MW, Thera MA, Wambebe C, Weber JL, Williams SM. 2009. The genetic structure and history of africans and african americans. Science 324:1035-1044.

Tran VT, Snyder SH, Major LF, Hawley RJ. 1981. GABA receptors are increased in brains of alcoholics. Ann Neurol 9:289-292.

Treutlein J, Cichon S, Ridinger M, Wodarz N, Soyka M, Zill P, Maier W, Moessner R, Gaebel W, Dahmen N. 2009. Genome-wide association study of alcohol dependence. Arch Gen Psychiatry 66:773.

Treutlein J, Kissling C, Frank J, Wiemann S, Dong L, Depner M, Saam C, Lascorz J, Soyka M, Preuss U. 2006. Genetic association of the human corticotropin releasing hormone receptor 1 (CRHR1) with binge drinking and alcohol intake patterns in two independent samples. Mol Psychiatry 11:594-602.

Tsai G, Coyle JT. 2002. Glutamatergic mechanisms in schizophrenia. Annu Rev Pharmacol Toxicol 42:165-179.

Tsai GE, Ragan P, Chang R, Chen S, Linnoila VM, Coyle JT. 1998. Increased glutamatergic neurotransmission and oxidative stress after alcohol withdrawal. Am J Psychiatry 155:726-732.

Tsiatis AC, Norris-Kirby A, Rich RG, Hafez MJ, Gocke CD, Eshleman JR, Murphy KM. 2010. Comparison of sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: Diagnostic and clinical implications. The Journal of Molecular Diagnostics 12:425-432.

Tsigos C, Chrousos GP. 2002. Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. J Psychosom Res 53:865-871.

Turecki G, Rouleau GA, Alda M. 1999. Family density of alcoholism and linkage information in the analysis of the COGA data. Genet Epidemiol 17:S361-S366.

Turek V, Tsivkovskaia N, Hyytia P, Harding S, Le A, Ryabinin A. 2005. Urocortin 1 expression in five pairs of rat lines selectively bred for differences in alcohol drinking. Psychopharmacology (Berl) 181:511-517.

Tyrka AR, Price LH, Gelernter J, Schepker C, Anderson GM, Carpenter LL. 2009. Interaction of childhood maltreatment with the corticotropin-releasing hormone receptor gene: Effects on hypothalamic-pituitary-adrenal axis reactivity. Biol Psychiatry 66:681-685.

Uher R. 2009. The role of genetic variation in the causation of mental illness: An evolution-informed framework. Mol Psychiatry 14:1072-1082.

Uher R. 2014. Gene–environment interactions in severe mental illness. Frontiers in Psychiatry 5:48.

Vale W, Spiess J, Rivier C, Rivier J. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394-1397.

Valenzuela CF, Bhave S, Hoffman P, Harris RA. 1998. Acute effects of ethanol on pharmacologically isolated kainate receptors in cerebellar granule neurons: Comparison with NMDA and AMPA receptors. J Neurochem 71:1777-1780.

Van Der Zwaluw, Engels RC. 2009. Gene–environment interactions and alcohol use and dependence: Current status and future challenges. Addiction 104:907-914.

van der Zwaluw, Engels RC, Vermulst AA, Rose RJ, Verkes RJ, Buitelaar J, Franke B, Scholte RH. 2010. A serotonin transporter polymorphism (5-HTTLPR) predicts the development of adolescent alcohol use. Drug Alcohol Depend 112:134-139.

van Gorp WG, Altshuler L, Theberge DC, Wilkins J, Dixon W. 1998. Cognitive impairment in euthymic bipolar patients with and without prior alcohol dependence: A preliminary study. Arch Gen Psychiatry 55:41-46.

Van Pett K, Viau V, Bittencourt JC, Chan RK, Li H, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE. 2000. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. J Comp Neurol 428:191-212.

Vandeleur C, Rothen S, Gholam-Rezaee M, Castelao E, Vidal S, Favre S, Ferrero F, Halfon O, Fumeaux P, Merikangas KR. 2012. Mental disorders in offspring of parents with bipolar and major depressive disorders. Bipolar Disord 14:641-653.

Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C. 1995. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor.Nature 378: 287-292.

Vaughn MG, Beaver KM, DeLisi M, Howard MO, Perron BE. 2009. Dopamine D4 receptor gene exon III polymorphism associated with binge drinking attitudinal phenotype. Alcohol 43:179-184.

Vetter DE, Li C, Zhao L, Contarino A, Liberman MC, Smith GW, Marchuk Y, Koob GF, Heinemann SF, Vale W. 2002. Urocortin-deficient mice show hearing impairment and increased anxiety-like behavior. Nat Genet 31:363-369.

Villafuerte S, Heitzeg MM, Foley S, Yau WW, Majczenko K, Zubieta J, Zucker RA, Burmeister M. 2012. Impulsiveness and insula activation during reward anticipation are associated with genetic variants in GABRA2 in a family sample enriched for alcoholism. Mol Psychiatry 17:511-519.

Volkow N, Fowler J, Wang G, Baler R, Telang F. 2009. Imaging dopamine's role in drug abuse and addiction. Neuropharmacology 56:3-8.

Volkow ND, Fowler JS. 2000. Addiction, a disease of compulsion and drive: Involvement of the orbitofrontal cortex. Cereb Cortex 10:318-325.

von Bubnoff A. 2008. Next-generation sequencing: The race is on. Cell 132:721-723.

Walker RM, Rybka J, Anderson SM, Torrance HS, Boxall R, Sussmann JE, Porteous DJ, McIntosh AM, Evans KL. 2015. Preliminary investigation of miRNA expression in individuals at high familial risk of bipolar disorder. J Psychiatr Res 62:48-55.

Wall TL, Shea SH, Luczak SE, Cook TA, Carr LG. 2005. Genetic associations of alcohol dehydrogenase with alcohol use disorders and endophenotypes in white college students. J Abnorm Psychol 114:456-465.

Wang H, Friedman E. 1996. Enhanced protein kinase C activity and translocation in bipolar affective disorder brains. Biol Psychiatry 40:568-575.

Wang J, Foroud T, Hinrichs AL, Le NX, Bertelsen S, Budde JP, Harari O, Koller DL, Wetherill L, Agrawal A. 2013. A genome-wide association study of alcohol-dependence symptom counts in extended pedigrees identifies C15orf53. Mol Psychiatry 18:1218-1224.

Wang K, Liu X, Aragam N. 2010a. A genome-wide meta-analysis identifies novel loci associated with schizophrenia and bipolar disorder. Schizophr Res 124:192-199.

Wang K, Liu X, Zhang Q, Pan Y, Aragam N, Zeng M. 2011. A meta-analysis of two genome-wide association studies identifies 3 new loci for alcohol dependence. J Psychiatr Res 45:1419-1425.

Wang K, Liu X, Zhang Q, Wu L, Zeng M. 2012. Genome-wide association study identifies 5q21 and 9p24. 1 (KDM4C) loci associated with alcohol withdrawal symptoms. J Neural Transm 119:425-433.

Wang JC, Hinrichs AL, Stock H, Budde J, Allen R, Bertelsen S, Kwon JM, Wu W, Dick DM, Rice J, Jones K, Nurnberger JI,Jr, Tischfield J, Porjesz B, Edenberg HJ, Hesselbrock V, Crowe R, Schuckit M, Begleiter H, Reich T, Goate AM, Bierut LJ. 2004. Evidence of common and specific genetic effects: Association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. Hum Mol Genet 13:1903-1911.

Wang K, Li M, Hakonarson H. 2010b. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:e164.

Watson S, Mackin P. 2006. HPA axis function in mood disorders. Psychiatry 5:166-170.

Watson S, Gallagher P, Ritchie JC, Ferrier IN, Young AH. 2004. Hypothalamic-pituitary-adrenal axis function in patients with bipolar disorder. Br J Psychiatry 184:496-502.

Weiss F, Parsons LH, Schulteis G, Hyytia P, Lorang MT, Bloom FE, Koob GF. 1996. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. J Neurosci 16:3474-3485.

Weitemier AZ, Ryabinin AE. 2005. Brain Region–Specific regulation of urocortin 1 innervation and Corticotropin-Releasing factor receptor type 2 binding by ethanol exposure. Alcoholism: Clinical and Experimental Research 29:1610-1620.

Wernicke C, Samochowiec J, Schmidt LG, Winterer G, Smolka M, Kucharska-Mazur J, Horodnicki J, Gallinat J, Rommelspacher H. 2003. Polymorphisms in the N-methyl-D-aspartate receptor 1 and 2B subunits are associated with alcoholism-related traits. Biol Psychiatry 54:922-928.

Wetherill L, Kapoor M, Agrawal A, Bucholz K, Koller D, Bertelsen SE, Le N, Wang J, Almasy L, Hesselbrock V. 2014. Family-Based association analysis of alcohol dependence criteria and severity. Alcoholism: Clinical and Experimental Research 38:354-366.

Whirl-Carrillo M, McDonagh E, Hebert J, Gong L, Sangkuhl K, Thorn C, Altman R, Klein TE. 2012. Pharmacogenomics knowledge for personalized medicine. Clinical Pharmacology & Therapeutics 92:414-417.

Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N. 2013. Global burden of disease attributable to mental and substance use disorders: Findings from the global burden of disease study 2010. The Lancet 382:1575-1586.

Whitfeld P, Seeburg PH, Shine J. 1982. The human pro-opiomelanocortin gene: Organization, sequence, and interspersion with repetitive DNA. DNA 1:133-143.

Wieck A, Grassi-Oliveira R, do Prado CH, Rizzo LB, de Oliveira AS, Kommers-Molina J, Viola TW, Teixeira AL, Bauer ME. 2013. Differential neuroendocrine and immune responses to acute psychosocial stress in women with type 1 bipolar disorder. Brain Behav Immun 34:47-55.

Wildenauer DB, Schwab SG, Blaveri A, Culverhouse R, Curtis D, Edwards J, Foroud T, Gershon E, Kelsoe J, Lichtermann D. 1999. Chromosomes 8 and 10 workshop. Am J Med Genet 88:239-243.

Williams D, Herman A, Stein D, Heeringa S, Jackson P, Moomal H, Kessler R. 2008. Twelve-month mental disorders in south africa: Prevalence, service use and demographic correlates in the population-based south african stress and health study. Psychol Med 38:211-220.

Willour VL, Seifuddin F, Mahon PB, Jancic D, Pirooznia M, Steele J, Schweizer B, Goes FS, Mondimore FM, MacKinnon DF. 2012. A genome-wide association study of attempted suicide. Mol Psychiatry 17:433-444.

Wilson GM, Flibotte S, Chopra V, Melnyk BL, Honer WG, Holt RA. 2006. DNA copy-number analysis in bipolar disorder and schizophrenia reveals aberrations in genes involved in glutamate signaling. Hum Mol Genet 15:743-749.

Winham S, Cuellar-Barboza A, Oliveros A, McElroy S, Crow S, Colby C, Choi D, Chauhan M, Frye M, Biernacka J. 2014. Genome-wide association study of bipolar disorder accounting for effect of body mass index identifies a new risk allele in TCF7L2. Mol Psychiatry 19:1010-1016.

Wise RA. 1996. Addictive drugs and brain stimulation reward. Annu Rev Neurosci 19:319-340.

Wrona D. 2006. Neural–immune interactions: An integrative view of the bidirectional relationship between the brain and immune systems. J Neuroimmunol 172:38-58.

Xu B, Roos JL, Dexheimer P, Boone B, Plummer B, Levy S, Gogos JA, Karayiorgou M. 2011. Exome sequencing supports a de novo mutational paradigm for schizophrenia. Nat Genet 43:864-868.

Xu W, Cohen-Woods S, Chen Q, Noor A, Knight J, Hosang G, Parikh SV, De Luca V, Tozzi F, Muglia P. 2014. Genome-wide association study of bipolar disorder in canadian and UK populations corroborates disease loci including SYNE1 and CSMD1. BMC Medical Genetics 15:2.

Xuei X, Flury-Wetherill L, Bierut L, Dick D, Nurnberger J, Foroud T, Edenberg HJ. 2007. The opioid system in alcohol and drug dependence: Family-based association study. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 144:877-884.

Yang C, Li C, Kranzler HR, Farrer LA, Zhao H, Gelernter J. 2014. Exploring the genetic architecture of alcohol dependence in african-americans via analysis of a genomewide set of common variants. Hum Genet 133:617-624.

Yasseen B, Kennedy JL, Zawertailo LA, Busto UE. 2009. Comorbidity between bipolar disorder and alcohol use disorder: Association of dopamine and serotonin gene polymorphisms. Psychiatry Res 176:30-33.

Ye T, Lipska BK, Tao R, Hyde TM, Wang L, Li C, Choi KH, Straub RE, Kleinman JE, Weinberger DR. 2012. Analysis of copy number variations in brain DNA from patients with schizophrenia and other psychiatric disorders. Biol Psychiatry 72:651-654.

Yong W, Spence JP, Eskay R, Fitz SD, Damadzic R, Lai D, Foroud T, Carr LG, Shekhar A, Chester JA. 2014. Alcohol-Preferring rats show decreased Corticotropin-Releasing Hormone-2 receptor expression and differences in HPA activation compared to Alcohol-Nonpreferring rats. Alcoholism: Clinical and Experimental Research 38:1275-1283.

Yoon H, Kim Y. 2012. The T allele of the interferon-gamma 874A/T polymorphism is associated with bipolar disorder. Nordic Journal of Psychiatry 66:14-18.

Yoon K, Nguyen HN, Ursini G, Zhang F, Kim N, Wen Z, Makri G, Nauen D, Shin JH, Park Y. 2014. Modeling a genetic risk for schizophrenia in iPSCs and mice reveals neural stem cell deficits associated with adherens junctions and polarity. Cell Stem Cell 15:79-91.

Yoon G, Westermeyer J, Kuskowski MA, Nesheim L. 2013. Impact of the number of parents with alcohol use disorder on alcohol use disorder in offspring: A population-based study. J Clin Psychiatry 74:795-801.

Yosifova A, Mushiroda T, Kubo M, Takahashi A, Kamatani Y, Kamatani N, Stoianov D, Vazharova R, Karachanak S, Zaharieva I. 2011. Genome-wide association study on bipolar disorder in the bulgarian population. Genes, Brain and Behavior 10:789-797.

Young AH, Rigney U, Shaw S, Emmas C, Thompson JM. 2011. Annual cost of managing bipolar disorder to the UK healthcare system. J Affect Disord 133:450-456.

Yu TW, Chahrour MH, Coulter ME, Jiralerspong S, Okamura-Ikeda K, Ataman B, Schmitz-Abe K, Harmin DA, Adli M, Malik AN. 2013. Using whole-exome sequencing to identify inherited causes of autism. Neuron 77:259-273.

Zhang D, Cheng L, Qian Y, Alliey-Rodriguez N, Kelsoe JR, Greenwood T, Nievergelt C, Barrett TB, McKinney R, Schork N. 2008. Singleton deletions throughout the genome increase risk of bipolar disorder. Mol Psychiatry 14:376-380.

Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS. 2012. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics 28:3326-3328.

Zhou Y, Lapingo C. 2014. Modulation of proopiomelanocortin gene expression by ethanol in mouse anterior pituitary corticotrope tumor cell AtT20. Regul Pept 192:6-14.

Zimmermann P, Wittchen H, Höfler M, Pfister H, Kessler RC, Lieb R. 2003. Primary anxiety disorders and the development of subsequent alcohol use disorders: A 4-year community study of adolescents and young adults. Psychol Med 33:1211-1222.

Zimmermann US, Blomeyer D, Laucht M, Mann KF. 2007. How gene–stress–behavior interactions can promote adolescent alcohol use: The roles of predrinking allostatic load and childhood behavior disorders. Pharmacology Biochemistry and Behavior 86:246-262.

Zorrilla EP, Heilig M, de Wit H, Shaham Y. 2013. Behavioral, biological, and chemical perspectives on targeting CRF 1 receptor antagonists to treat alcoholism. Drug Alcohol Depend 128:175-186.

Zuo L, Gelernter J, Zhang CK, Zhao H, Lu L, Kranzler HR, Malison RT, Li CR, Wang F, Zhang X. 2012a. Genome-wide association study of alcohol dependence implicates KIAA0040 on chromosome 1q. Neuropsychopharmacology 37:557-566.

Zuo L, Saba L, Wang K, Zhang X, Krystal JH, Tabakoff B, Luo X. 2013a. Exome-wide association study of replicable nonsynonymous variants conferring risk for alcohol dependence. Journal of Studies on Alcohol and Drugs 74:622-625.

Zuo L, Wang K, Zhang X, Krystal JH, Li CR, Zhang F, Zhang H, Luo X. 2013b. NKAIN1–SERINC2 is a functional, replicable and genome-wide significant risk gene region specific for alcohol dependence in subjects of european descent. Drug Alcohol Depend 129:254-264.

Zuo L, Zhang F, Zhang H, Zhang X, Wang F, Li CR, Lu L, Hong J, Lu L, Krystal J. 2012b. Genome-wide search for replicable risk gene regions in alcohol and nicotine co-dependence. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 159:437-444.

Zuo L, Zhang X, Wang F, Li CR, Lu L, Ye L, Zhang H, Krystal JH, Deng H, Luo X. 2013c. Genome-Wide significant association signals in IPO11-HTR1A region specific for alcohol and nicotine codependence. Alcoholism: Clinical and Experimental Research 37:730-739.

Websites Cited

1) http://www.unodc.org/unodc/en/data-and-analysis/WDR-2010.html
2) http://www.who.int/substance_abuse/publications/global_alcohol_report/en/
3) http://www.who.int/substance_abuse/en/
4) http://www.who.int/mediacentre/factsheets/fs349/en/
5) http://www.genome.gov/gwastudies/
6) https://pgc.unc.edu/index.php
7) https://molgenis26.target.rug.nl/downloads/gonl_public/variants/release5/
8) http://covarisinc.com/technology/how-it-works/
9) http://www.ncbi.nlm.nih.gov/SNP
10) http://www.affymetrix.com/support/technical/sample_data/axiom_db/axiomdb_data.affx
11) http://hpc.uct.ac.za
12) http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc
13) http://broadinstitute.github.io/picard/
14) http://www.broadinstitute.org/gatk/guide/
15) http://www.ncbi.nlm.nih.gov/dbvar/browse/
16) ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502
17) https://www.cog-genomics.org/plink2/
18) http://www.r-project.org/
19) http://www.bioinf.wits.ac.za/software/genesis/downloads.html
20) www.fil.ucl.acuk/spm8
21) www.irc.cchmc.org/software/pedbrain.php
22) http://pngu.mgh.harvard.edu/~purcell/plink/
23) http://bioinfo.vanderbilt.edu/webgestalt/
24) https://pgc.unc.edu/Sharing.php#SharingOpp
25) https://www.nimhgenetics.org/

Appendices

Appendix 1

A1.1 Family Tree (A subset of Family 30). Individuals with numerical identifiers were genotyped and/or sequenced

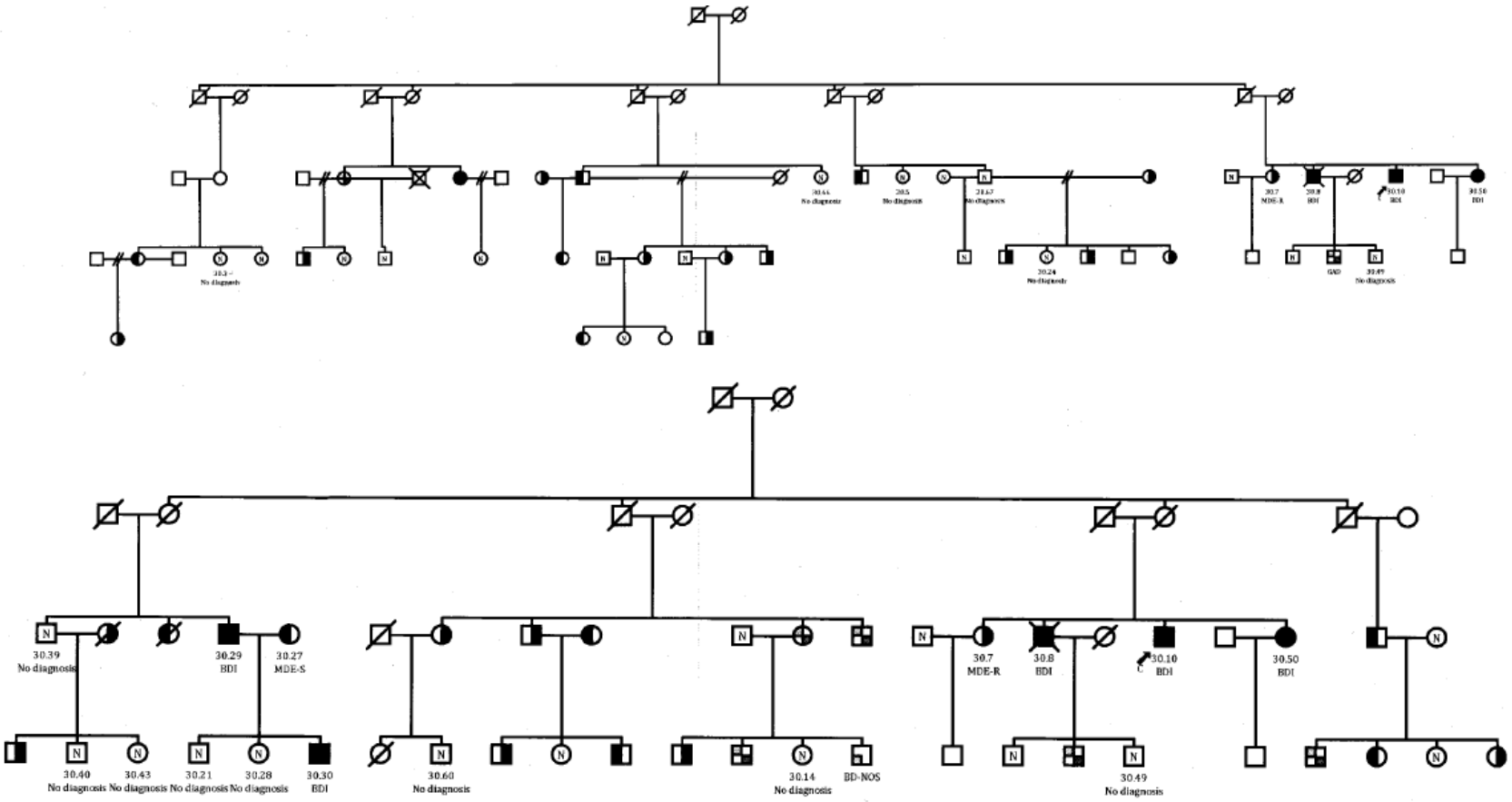

Figure A1.1: Two branches of family 30. Individuals with numerical identifiers were genotyped and/or sequenced. BDI: bipolar disorder type I, BDNOS: bipolar disorder not otherwise specified, GAD: general anxiety disorder, MDE-R: major depressive episode-recurrent, MDE-S: Major depressive episode-single.

A1.2 Example of Patient Consent Form

REQUEST FOR MOLECULAR STUDIES (DNA)

Molecular Laboratory

Division of Human Genetics
IIDMM, LEVEL 3
UCT Medical School, Observatory 7925

Tel: (021) 406 6425 Fax: (021) 406 6826

Blood should be drawn in 2 plastic EDTA Tubes (Purple top) +/- 10ml each using a yellow barrel. Each tube should be inverted to mix and should be clearly labelled with the patient's name and DOB
Keep blood in fridge at 4°C until able to send to laboratory

Please DO NOT send specimens on ice or frozen.

Please fill in all the information requested:

Surname:_____First Name(s):_____

New Family: Yes ☐ No ☐ (If no, please fill in family name) Family name: _____

Medical Aid: _____Medical Aid No: _____

Sex: M ☐ F ☐ Date of Birth: Year:_____ Month:_____ Day: _____

Number of children: _____

Ethnic Origin : (please indicate ancestry of both your mother and father)_____

Contact Address: _____ Town: _____ Fax: Tel: _____

Referring Doctor/Sister: _____ Town: _____ Fax: Tel: _____

Hospital or Address: _____ Town: _____ Fax: Tel: _____

Reason for Referral (Clinical diagnosis):

Affected ☐ At Risk ☐ Carrier ☐ Spouse ☐ Query ☐ Unaffected ☐

Becker Muscular Dys.	☐	Duchenne Muscular Dys	☐	Colonic Carcinoma	☐
Fragile-X Syndrome	☐	Bipolar Disorder	☐	Huntington Disease	☐
Retinitis Pigmentosa	☐	Spinocerebellar Ataxia	☐	Waardenberg Syndrome	☐

Additional disorders (apparent or previously treated): _____

Additional family history _____

Clinical Details:

Physical disability ☐ Mental retardation ☐ Deafness ☐ Impaired vision ☐ Night blindness ☐

Other: _____

Have samples from this patient been sent to a DNA lab before? (DELETE WHERE NOT APPLICABLE) YES / NO / Don't Know

If Yes, where: _____

For Laboratory use only:

DNA number: _____ *Vol.Blood:* _____*(ml)* *Other:* _____

Date Received: Year: _____ *Month:* _____ *Day:* _____ *Computer Index No:*_____

CONSENT FOR DNA ANALYSIS AND STORAGE

1. I, _____ , request that an attempt be made using genetic material to assess the probability that: I / my child / my unborn child (DELETE WHERE NOT APPLICABLE) might have inherited a disease-causing mutation in the gene for:_____ _____

2. I understand that the genetic material for analysis is to be obtained from: blood cells/skin sample/other (specify) (DELETE WHERE NOT APPLICABLE) :

3. I request that **no** portion of the sample be stored for later use. ☐ **(**MARK IF APPLICABLE)
   Or
   I request that a portion of the sample be stored indefinitely for (DELETE WHERE NOT APPLICABLE):
   (a) possible re-analysis
   (b) analysis for the benefit of members of my immediate family
   (c) research purposes, subject to the approval of the University of Cape Town Research Ethics Committee, provided that any information from such research will remain confidential.

4. The results of the analysis carried out on this sample of stored biological material will be made known to me,
   via my doctor, in accordance with the relevant protocol, if and when available.
   In addition, I authorise that they may be made known to: (DELETE WHERE NOT APPLICABLE) :
   other doctors involved in my care
   the following family members:
   _____
   other:
   _____
   _

5. I authorise / do not authorise my doctor(s) (DELETE WHERE NOT APPLICABLE) to provide relevant clinical details to the Division of Human Genetics, UCT.

6. I have been informed that:
   (a) there are risks and benefits associated with genetic analysis and storage of biological material and these have been explained to me.
   (b) the analysis procedure is specific to the genetic condition mentioned above and cannot determine the complete genetic makeup of an individual.
   (c) the genetics laboratory is under an obligation to respect medical confidentiality .
   (d) genetic analysis may not be informative for some families or family members.
   (e) even under the best conditions, current technology of this type is not perfect and could lead to incorrect results.
   (f) where biological material is used for research purposes, there may be no direct benefit to me.

7. I understand that I may withdraw my consent for any aspect of the above at any time without this affecting my future medical care.

8. **ALL OF THE ABOVE HAS BEEN EXPLAINED TO ME IN A LANGUAGE THAT I UNDERSTAND AND MY QUESTIONS ANSWERED BY:**

_____ DATE:

_____

Patient signature _____ **Witnessed consent** _____

NOTE - PLEASE INSERT A FAMILY PEDIGREE DRAWING ON THE REVERSE OF THIS FORM

A1.3 DNA Isolation Protocol

- Added 900 microlitres (µl) red blood cell lysis solution to microcentrifuge tube
- Added 500µl whole blood and mixed by inverting
- Incubated for one minute at room temperature
- Centrifuged for 20 seconds at 13 000rpm to pellet white blood cells. Discarded supernatant
- Added 300µl cell lysis solution and vortexed
- Incubated at 37°C for ten minutes
- Added 100µl protein precipitation solution and inverted
- Centrifuged for one minute at 13 000rpm
- Added supernatant to clean microcentrifuge tube with 300µl isopropanol
- Mixed by inversion until DNA was visible as threads or clumps
- Centrifuged for one minute at 13 000rpm
- Discarded supernatant
- Added 300µl 70% ethanol and inverted several times
- Centrifuged for one minute 13 000rpm
- Discarded supernatant
- Rehydrated with 50µl DNA hydration solution

A1.4 DNA Integrity Check

For agarose gel electrophoresis, a volume containing 200ng of DNA together with loading buffer (Fermentas Life Sciences, Hanover, USA) was loaded onto a 1% agarose gel. To estimate integrity of DNA fragments, the first lane of the gel was loaded with a 100bp molecular weight marker (MWM) (Fermentas Life Sciences, Hanover, USA). To enable DNA visualization, 30ng of ethidium bromide (EtBr) (Sigma, England), a DNA intercalating agent, was added to the agarose prior to solidification. Gel electrophoresis was carried out in a gel tank with 1x Tris Borate EDTA (TBE) electrophoretic buffer, at 160V for approximately 30 minutes. Thereafter, it was placed in the Uvipro Gold transilluminator (UVITec, Cambridge, UK) for the illumination and image capture of the DNA fragments.

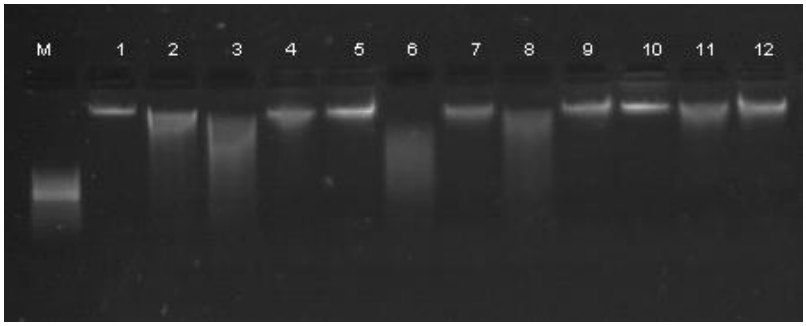

Figure A1.4.1: Integrity Gel with 200ng DNA. M: Molecular weight marker, 1-12 DNA samples.

A1.5 WGS Protocol

WGS consists of three steps: library creation, clustering and sequencing, each of which are described below.

1) **Library Creation**

DNA shearing

- Random DNA shearing on Covaris instrument
- Switch on machine 1hr before use. Add distilled water to chamber (between 10 and 15 left-hand side measure)
- Require temperature to be between 3-4°C
- Setting at P1
- De-gas
- Shearing: duration 40 seconds- should give fragments of 300bp

Clean-up of fragmented DNA

- Use Qiagen kit

End repair (ER)

- This step gets rid of 3‘ and 5‘ overhangs
- Consists of: ER control, ER mix, resuspension buffer
- Use 96-well plate
- Add 10µl ER control added to each sample
- Add all DNA sample (~50µl fragmented DNA)
- Add 40µl ER mix
- Place seal on plate
- Place plate in thermal cycler at 30°C for 30 minutes

Clean-up with AmPure XP beads

- Shake beads
- Add 160µl beads to sample
- Mix well
- Incubate at room temp for 15 mins
- Incubate at room temp for 15 mins on magnetic plate (pulls beads to the bottom of well)
- Discard supernatant without disturbing pellet (brown clump of beads)
- Add 200µl 80% ethanol and incubate for 30 seconds at room temperature
- Discard supernatant and add 200µl 80% ethanol
- Incubate at room temp for 15 minutes to dry out pellet (may see a crack in the pellet)

- Add 17.5µl resuspension buffer, mix with pellet (mix well), make sure pellet is evenly dispersed and no pellet remains on the walls of the well
- Incubate at room temperature for 2 minutes
- Incubate at room temperature for 5 minutes on magnetic plate (or until liquid is clear)
- Transfer 15µl supernatant to clean well

Adenylate 3' ends

- Add 2.5µl A-tailing control
- Add 12.5µl A-tailing mix
- Incubate at 37°C on thermal cycler for 30 minutes

Ligate Adapters

- Add 2.5µl ligase control to each sample
- Add 2.5µl ligase mix
- Add 2.5µl adapter index (different adapter index for each sample)
- Mix thoroughly
- Incubate at 30°C for 10 minutes

Clean-up with AmPure XP beads

- Shake beads
- Add 45µl beads to sample
- Follow protocol as above
- Transfer 20µl of supernatant to clean well

Purify products on gel (Invitrogen e-gel)

- Load 20µl DNA product into each well (x2 for each sample)
- Load 10µl 50bp MWM
- Load water into empty wells
- Add 20µl water into second lane of wells after 400bp fragment reaches second lane
- Collect (pipette from second lane wells) before 500bp fragment reaches second lane

Clean-up with Qiagen kit

PCR of products

- Add 5µl PCR primer cocktail to wells in PCR plate
- Add 25µl PCR master mix
- Add all of the cleaned up product
- Pop air bubbles and seal plate

Clean-up PCR products with AmPure XP beads

- Add 50µl AmPure beads
- Step 12- take out entire volume. Do not remove any beads

Check concentration of product: Nanodrop (concentration) **and Agilent Analyser (**size of fragments and molar conc.)

Agilent analyser

- Use DNA 1000 chip

- add 9µl of gel dye mix (from 4°C fridge) in the well marked ~~–G~~"

- Place chip on priming station

- Push down syringe until chip is locked into position (~~–p~~ressurising" spreads gel into capillaries)

- Add 9µl gel-dye mix into other G-wells

- load 5µl marker (DNA 1000) into every other well not marked ~~–G~~"

- load 1µl ladder into ladder well

- load 1µl sample into other wells (not G-wells)

- load 1µl water into empty wells

- ensure that there are no air bubbles

- vortex for 1 min

- Computer setting- DNA 1000 series assay

2) Clustering

- Thaw reagent plate and hybridisation buffer at room temp

DNA dilution

- Need to have a DNA conc. of 10nm
- Therefore, dilute with elution buffer (EB) and Tween
- End concentration of DNA= 10nM

Denaturation

- Starting [DNA]= 10nM
- Add 17µl EB

- Add 2µl DNA template
- Add 1µl 2N NaOH (always add last)
- Vortex and centrifuge
- Incubate at room temperature for 5 minutes
- Place on ice
- Final [DNA]= 1pmol/µl

Serial Dilution

- Add 4µl denatured sample to clean tube
- Add 496µl hybridisation buffer
- Vortex and centrifuge
- Final [DNA]= 8 pmol
- Add 125µl diluted sample to clean tube
- Add 208µl hybridisation buffer
- Vortex and centrifuge
- Final [DNA]= 3pmol
- Add 148.5µl diluted sample to 8 strip tubes
- Add 1.5µl 8pmol phiX control (20µl 20pmol phiX and 30µl hybridisation buffer)
- Put on strip cap, vortex and centrifuge

At cluster station (cBot Illumina)
- Wash reservoir (blot water/wash buffer)
- Load reagents (remove red strip)
- Scan barcode
- Load flowcell (wash with water beforehand)- rainbow side-up
- Put on manifold
- Load template (without strip cap, NB note direction)
- Do pre-check
- Start clustering (runs for ~4 hours)
-

3) Sequencing (Illumina HiSeq 2000)

- Thaw sequencing reagents at 4°C overnight (except dNTPs and polymerase)

Wash instrument

- Place water bottles in panel (A/B)
- Change PE reagents to water (optional)
- Wash ~1hr

Sequencing

- Place cleavage mix in water at room temperature
- Add reagent bottles from TruSeq kit to corresponding colours of black rack
- Split incorporation mix into two bottles (other half for paired end reaction)

- Add both tubes of long read FFN mix to incorporation mix
- Add 1.1ml of polymerase to incorporation mix
- Shake
- Place cleavage mix in rack
- Put on caps with holes
- Add rack to sequencer
- Prime X2 (only for version 3)
- Once primed, remove old gaskets and flowcell
- Wipe flowcell station with alcohol swab
- Put in flowcell (wash with water, then alcohol swab, then dry with wipe) of interest and new gaskets
- Engage vacuum
- Wait for first base read (approx. 30 minutes- 1hr)

Paired-end (PE) cluster generation ("turn-around chemistry")

- Use PE cluster kit
- Thaw reagents
- Prepare 0.1N NaOH (HP3)
- Add 2.85ml of PW1 to 15ml conical tube
- Add 150µl HP3
- Use PE module rack (black, thin rack)
- Add reagents to module- uncap
- Scan lot no. of PE kit
- Add module to PE rack
- Pull lever down

A1.6 FastQC Results

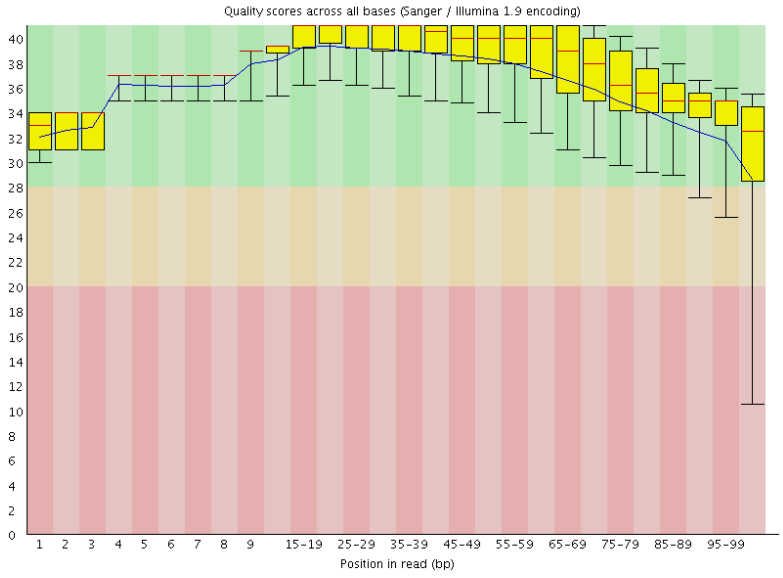

Figure A1.6.1: Per base sequence quality

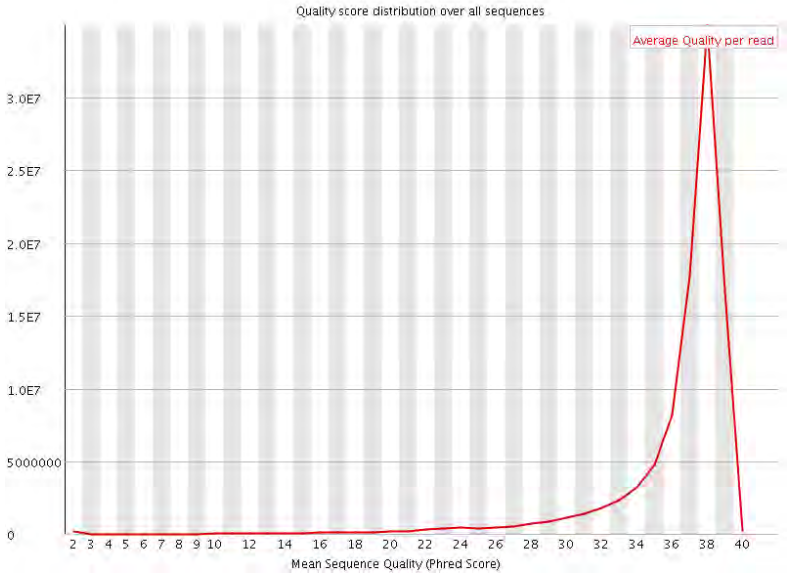

Figure A1.6.2: Per sequence quality scores

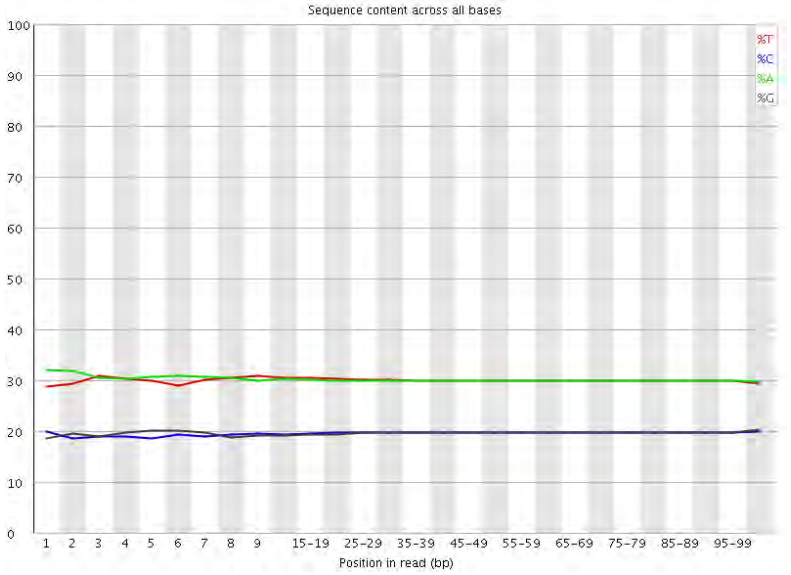

Figure A1.6.3: Per base sequence content

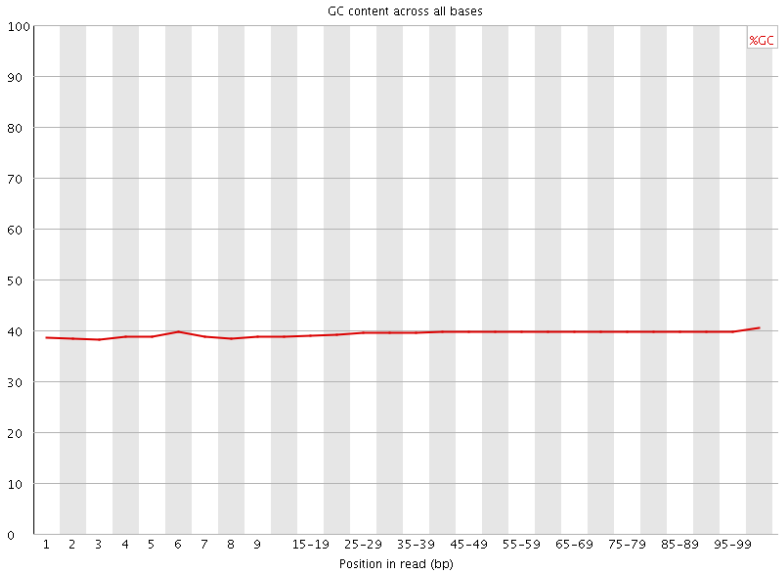

Figure A1.6.4: Per base GC content

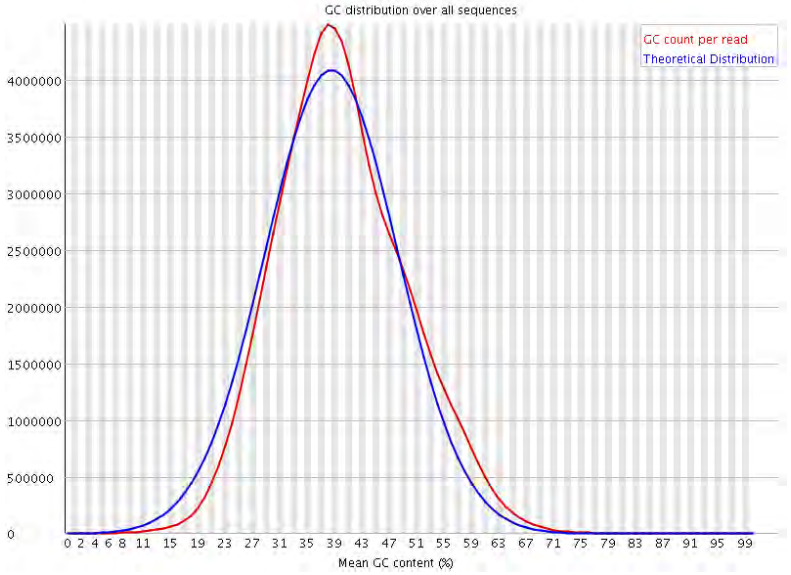

Figure A1.6.5: Per sequence GC content

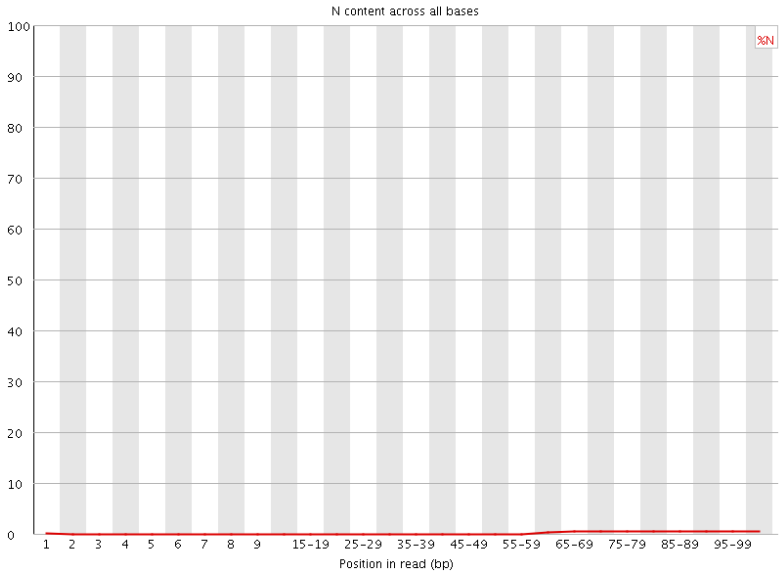

Figure A1.6.6: Per base N content

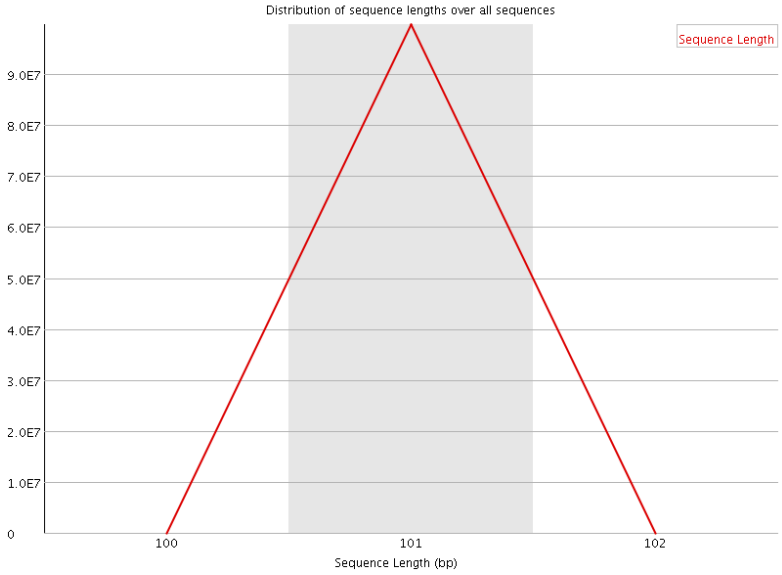

Figure A1.6.7: Sequence Length distribution

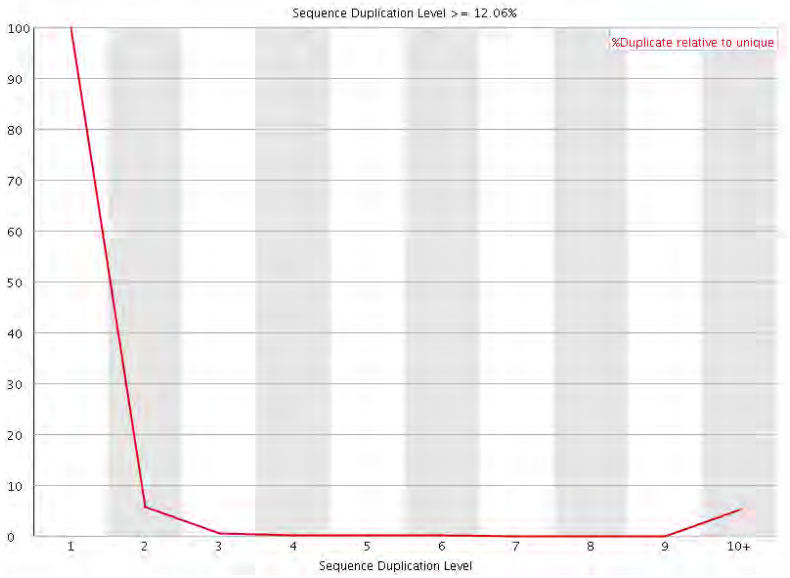

Figure A1.6.8: Sequence duplication levels

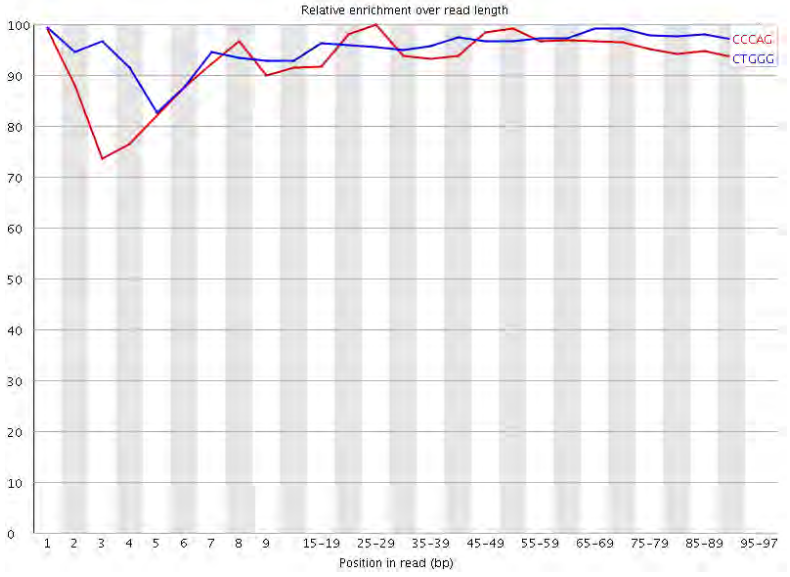

Figure A1.6.9: Kmer content

A1.7 Enrichment Analysis Results

Table A.1.7.1: ConsensusPathDB Results: Pathway Analysis

Pathway name	set size	candidates contained	p-value	q-value	pathway source
TCR Signaling Pathway	92	6 (6.7%)	0.000448	0.148	Wikipathways
Nectin/Necl trans heterodimerization	7	2 (28.6%)	0.00241	0.254	Reactome
TCR signaling in naïve CD8+ T cells	58	4 (7.5%)	0.00265	0.254	PID
Olfactory transduction - Homo sapiens (human)	408	11 (2.7%)	0.00463	0.254	KEGG
IL12 signaling mediated by STAT4	33	3 (9.4%)	0.00504	0.254	PID
Pre-NOTCH Expression and Processing	33	3 (9.4%)	0.00504	0.254	Reactome
TCR signaling in naïve CD4+ T cells	72	4 (6.1%)	0.00585	0.254	PID
Olfactory Signaling Pathway	426	11 (2.6%)	0.0065	0.254	Reactome
Trehalose Degradation	12	2 (16.7%)	0.00731	0.254	SMPDB
lck and fyn tyrosine kinases in initiation of tcr activation	14	2 (15.4%)	0.00858	0.254	BioCarta

Table A1.7.2: ConsensusPathDB Results: GO Analysis

gene ontology term	category,level	set size	candidates contained	p-value	q-value
GO:0032623 interleukin-2 production	BP 4	54	5 (9.4%)	0.000228	0.0356
GO:0032616 interleukin-13 production	BP 4	13	3 (23.1%)	0.000299	0.0356
GO:0007155 cell adhesion	BP 2	1375	28 (2.1%)	0.00037	0.0299
GO:0030424 axon	CC 4	320	11 (3.5%)	0.000477	0.0229
GO:0036477 somatodendritic compartment	CC 3	562	15 (2.7%)	0.000676	0.0369
GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity	MF 3	512	14 (2.8%)	0.000789	0.0355
GO:0097458 neuron part	CC 2	980	21 (2.2%)	0.000963	0.0211
GO:0031225 anchored component of membrane	CC 2	150	7 (4.7%)	0.000995	0.0211
GO:0042105 alpha-beta T cell receptor complex	CC 4	5	2 (40.0%)	0.00107	0.0257
GO:0098742 cell-cell adhesion via plasma-membrane adhesion molecules	BP 4	200	8 (4.0%)	0.00117	0.104

GO:0098609 cell-cell adhesion	BP 3	201	8 (4.0%)	0.00121	0.243
GO:0043005 neuron projection	CC 3	796	18 (2.3%)	0.00125	0.0369
GO:0005886 plasma membrane	CC 2	4776	67 (1.5%)	0.00155	0.0211
GO:0071944 cell periphery	CC 2	4870	68 (1.4%)	0.00162	0.0211
GO:0042608 T cell receptor binding	MF 4	7	2 (28.6%)	0.00221	0.126
GO:0048731 system development	BP 3	4093	59 (1.5%)	0.00224	0.243
GO:0098602 single organism cell adhesion	BP 2	721	16 (2.3%)	0.00298	0.0967
GO:0043025 neuronal cell body	CC 3	350	10 (2.9%)	0.00326	0.064
GO:0006099 tricarboxylic acid cycle	BP 3	29	3 (10.3%)	0.00338	0.243
GO:0007275 multicellular organismal development	BP 3	4621	64 (1.4%)	0.00379	0.243
GO:0016337 single organismal cell-cell adhesion	BP 4	679	15 (2.3%)	0.00411	0.253
GO:0044707 single-multicellular organism process	BP 2	6462	84 (1.3%)	0.00415	0.0967
GO:0004984 olfactory receptor activity	MF 4	425	11 (2.6%)	0.00485	0.138
GO:0005911 cell-cell junction	CC 2	370	10 (2.8%)	0.00485	0.0505
GO:0008038 neuron recognition	BP 4	34	3 (9.1%)	0.0049	0.253
GO:0042107 cytokine metabolic process	BP 4	110	5 (4.8%)	0.00496	0.253
GO:0031224 intrinsic component of membrane	CC 2	5571	73 (1.4%)	0.00615	0.0533
GO:0045058 T cell selection	BP 2	40	3 (8.1%)	0.00677	0.0967
GO:0051606 detection of stimulus	BP 2	712	15 (2.1%)	0.00689	0.0967
GO:0008037 cell recognition	BP 3	116	5 (4.4%)	0.007	0.358
GO:0048856 anatomical structure development	BP 2	4828	65 (1.4%)	0.00716	0.0967
GO:0044297 cell body	CC 2	399	10 (2.6%)	0.00806	0.0599
GO:0060055 angiogenesis involved in wound healing	BP 4	14	2 (14.3%)	0.00914	0.308
GO:0042101 T cell receptor complex	CC 4	14	2 (14.3%)	0.00914	0.146
GO:0034109 homotypic cell-cell adhesion	BP 4	476	11 (2.4%)	0.00919	0.308
GO:0042110 T cell activation	BP 4	412	10 (2.5%)	0.00922	0.308
GO:0050907 detection of chemical stimulus involved in sensory perception	BP 4	470	11 (2.4%)	0.00977	0.308

Table A1.7.3: ConsensusPathDB Results: Protein complex Analysis

complex name	set size	candidates contained	p-value	q-value	complex source
CD3 epsilon: CD3 gamma with phosphorylated ITAM	2	2 (100.0%)	0.000169	0.00339	Reactome
TCR/CD3/MHC II/CD4/LCK/CBL/Ubiquitin	12	3 (27.3%)	0.000332	0.00339	PID
TCR/CD3/MHC I/CD8/LCK/ZAP-70/CBL/ubiquitin	14	3 (23.1%)	0.000564	0.00339	PID
TCR/CD3/MHC II/CD4/LCK/ZAP-70/CBL/SLAP-2/Ubiquitin	14	3 (23.1%)	0.000564	0.00339	PID
HTR3:5HT	5	2 (40.0%)	0.00165	0.00792	Reactome
Monovalent TCR/CD3	6	2 (33.3%)	0.00245	0.00982	PID
MHC II/TCR-CD3	8	2 (25.0%)	0.0045	0.0122	BioCarta
TCR-CD3/MHC II/CD4	9	2 (22.2%)	0.00574	0.0122	BioCarta
TCR/CD3/MHC II/CD4	10	2 (22.2%)	0.00574	0.0122	PID
CD3Z antigen zeta polypeptide/MHC class II alpha/CD4/LCK/CD3Z antigen zeta polypeptide/TCR-alpha/TCR-beta/CD3G antigen gamma polypeptide/CD3E antigen epsilon polypeptide/CD3E antigen epsilon polypeptide/CD3D antigen delta polypeptide/MHC IIb	10	2 (20.0%)	0.00712	0.0122	BioCarta
TCR/CD3/MHC I/CD8	11	2 (20.0%)	0.00712	0.0122	PID
TCR/CD3/MHC II/CD4/LCK	11	2 (20.0%)	0.00712	0.0122	PID
TCR/CD3/MHC II/CD4/Fyn	12	2 (20.0%)	0.00712	0.0122	PID
TCR-CD3/MHC II/CD4/LCK/ZAP70	11	2 (18.2%)	0.00863	0.0122	BioCarta
TCR/CD3/MHC I/CD8/LCK	12	2 (18.2%)	0.00863	0.0122	PID
TCR/CD3/MHC II/CD4/LCK/ZAP-70	12	2 (18.2%)	0.00863	0.0122	PID
TCR/CD3/MHC I/CD8/Fyn	13	2 (18.2%)	0.00863	0.0122	PID

A1.8 Novel Variants

Chromosome	position	Allele 1	Allele 2	Gene
10	10136103	G	A	N/A
10	10179737	A	AG	N/A
10	10200514	C	CA	N/A
10	10296609	GT	G	N/A
10	10456021	G	C	N/A
10	10548263	G	GTATC	N/A
10	10621683	C	T	N/A
10	10646657	T	TG	N/A
10	10650108	C	CA	N/A
10	10960713	G	T	N/A
10	11061555	CA	C	*CELF2*
10	11237125	A	AAT	*CELF2*
10	11264087	C	CTG	*CELF2*
10	11278801	C	T	*CELF2*
10	11296849	A	ATCCCATCTCTCCCCTTCCTG	*CELF2*
10	11424854	A	G	N/A
10	11428568	T	C	N/A
10	11620617	A	AT	*USP6NL*
10	11801381	A	AC	N/A
10	12126317	TA	T	N/A
10	12161027	T	C	*DHTKD1*
10	12186107	T	C	*SEC61A2*
10	6745418	A	C	N/A
10	6763972	TAC	T	N/A
10	6765464	A	G	N/A
10	6856462	C	CAAATT	*LINC00707*
10	6877709	CAT	C	*LINC00707*
10	6878028	AATTTATATTTACATAAC	A	*LINC00707*
10	6896099	GA	G	N/A
10	6953742	C	CTT	*RP11-799O21.2*
10	6963347	T	TTTTGTTTG	*RP11-799O21.2*
10	7011013	C	CAA	N/A
10	7057102	A	ATTG	N/A
10	7136671	GTGCCA	G	N/A
10	7143901	CTTG	C	N/A
10	7159479	G	GATTT	N/A
10	7425807	C	T	*SFMBT2*
10	7468055	C	CAG	N/A
10	7500300	T	TC	*RP5-1031D4.2*

10	7533074	A	G	N/A
10	7561766	T	A	N/A
10	7625453	A	G	*ITIH5*
10	7631534	A	G	*ITIH5*
10	7686459	AT	A	*ITIH5*
10	7686493	GAT	G	*ITIH5*
10	7686511	GAT	G	*ITIH5*
10	7686876	CAT	C	*ITIH5*
10	8154863	G	GAC	N/A
10	8165301	C	CT	N/A
10	8193066	TTA	T	N/A
10	8229754	CT	C	N/A
10	8423492	T	A	N/A
10	8496602	A	G	*RP11-543F8.2*
10	8716466	GA	G	N/A
10	8721113	T	TTA	N/A
10	8746686	G	GTTA	N/A
10	8812576	G	GA	N/A
10	8931548	G	GA	N/A
10	8932280	TTA	T	N/A
10	8939670	T	TA	*RP11-428L9.1*
10	8948220	A	AGATAT	*RP11-428L9.1*
10	8971599	CA	C	N/A
10	9002088	G	GAA	N/A
10	9020005	T	C	N/A
10	9027420	T	TA	N/A
10	9109503	CTTCT	C	N/A
10	9219127	TG	T	N/A
10	9347135	GT	G	N/A
10	9349704	CA	C	N/A
10	9398543	AAAAAG	A	N/A
10	9407739	G	GTTC	N/A
10	9501148	C	CTAAG	N/A
10	9518661	C	CT	N/A
10	9641099	T	TA	N/A
10	9642117	A	AAATT	N/A
10	9658016	T	C	N/A
10	9727305	TA	T	N/A
10	9783562	CA	C	N/A
10	9864575	T	TTTTTC	N/A
10	9925608	A	C	N/A
11	110920370	C	CT	N/A
11	111106536	C	CT	N/A

11	112125748	GC	G	*C11orf34*
11	114420611	ATAAAAT	A	*NXPE1*
11	114420618	AT	A	*NXPE1*
11	114420624	AATC	A	*NXPE1*
11	114422786	GT	G	*NXPE1*
11	114506823	C	CTT	N/A
11	114677167	GA	G	N/A
11	115239741	A	ATCT	*CADM1*
11	115322872	TGGTGGGGTGGGGG	T	*CADM1*
11	116817836	TA	T	*SIK3*
11	118292682	A	AAG	*ATP5L*
11	119440683	TA	T	N/A
11	120555425	CT	C	*GRIK4*
11	121583989	CAAACAAACAAACAAACA	C	N/A
11	122638014	T	TTTTTGTTTTG	*UBASH3B*
11	125345171	T	TACAC	*FEZ1*
11	125389250	CGCTTGCATCCCCCAGTGCTCAGCA	C	N/A
11	125602506	TG	T	N/A
11	125682372	C	CT	N/A
11	125690149	C	CA	N/A
11	125763566	TC	T	*HYLS1*
11	125875199	AAAAAAAAAAAAAAAACAAACAAACAAAC	A	*CDON*
11	126511290	T	TA	*KIRREL3*
11	126524686	A	AT	*RP11-115C10.1*
11	126576836	C	CT	N/A
11	130151678	ACGGGGTTTCGC	A	*ZBTB44*
11	130168727	GAC	G	*ZBTB44*
11	130180792	T	C	*ZBTB44*
11	130499624	C	T	N/A
11	130613172	C	T	N/A
11	130746519	GT	G	*SNX19*
11	131134998	T	TATATATCTC	*AP002856.5*
11	131527043	C	CAA	*NTM*
11	131657248	G	GATT	*NTM*
11	132505486	C	CA	*OPCML*
11	132635994	TA	T	*OPCML*
11	132714860	AT	A	*OPCML*
11	132935913	C	T	*OPCML*
11	133297113	A	G	*OPCML*
11	133496502	A	G	N/A
11	133517583	A	T	N/A
13	55029955	AC	A	N/A

13	55820910	GT	G	N/A
13	56043847	A	AAT	N/A
13	56134064	A	AC	N/A
13	56469135	AAC	A	N/A
13	57675873	T	TA	N/A
13	57682228	G	GA	N/A
13	57859067	CT	C	N/A
13	57898337	CAT	C	N/A
13	57929785	C	CT	N/A
13	58557942	C	CA	N/A
13	60084333	AT	A	N/A
13	60157779	CAT	C	N/A
13	61437085	G	GT	N/A
13	61567690	A	ACTTCTATCCAAACACCTTCT	N/A
13	61608564	AT	A	N/A
13	61816277	TA	T	N/A
13	62212434	G	GT	N/A
13	62455649	CT	C	N/A
13	62458899	TA	T	N/A
13	62465242	A	ATTAT	N/A
13	62491661	T	TA	N/A
13	62506143	G	GA	N/A
13	63262572	AAT	A	*LINC00448*
13	63462506	TAA	T	N/A
13	63602845	AG	A	N/A
13	63603386	G	GAAAACCACCATTTCACAATTCCACTTGTA	N/A
13	63617372	GTCTGA	G	N/A
13	63621303	CT	C	N/A
13	63630283	T	TTTG	N/A
13	63631658	AT	A	N/A
13	63632180	TTA	T	N/A
13	63633294	TTA	T	N/A
13	63636191	T	TA	N/A
13	63637865	A	AT	N/A
13	63640749	CAAT	C	N/A
13	63643708	TAC	T	N/A
13	63643733	G	GAC	N/A
13	63645763	CA	C	N/A
13	63647632	AAAAT	A	N/A
13	63647877	CT	C	N/A
13	63949360	G	GA	N/A
13	64855636	T	TACAAGGAACTTAA	N/A
13	65368410	G	GTAAGTATATACTTACAGATATATGCA	N/A

13	65406800	CTTTAAA	C	N/A
13	66023171	GTA	G	N/A
13	66038243	ACTGT	A	N/A
13	66190671	ATG	A	N/A
13	66770329	G	GAT	N/A
13	68028753	A	AC	N/A
13	68181900	GAT	G	N/A
13	68182036	A	AAT	N/A
13	70534839	TTGAG	T	*KLHL1*
13	70542462	GATATATTTGC	G	*KLHL1*
13	70734955	ATATATT	A	N/A
13	71748804	AAAAT	A	N/A
13	72072799	A	AAG	*DACH1*
13	72309226	CAT	C	*DACH1*
13	72767168	C	CTG	N/A
13	73912075	CATATGTGTGTGTGTAT	C	N/A
13	74383497	C	CG	*KLF12*
13	75384338	A	G	N/A
13	76011735	C	CT	*TBC1D4*
13	76138056	C	CTGTGTG	*UCHL3*
13	76512262	CAT	C	N/A
13	77645372	G	GTGTA	*MYCBP2*
13	78116534	CAACA	C	*SCEL*
6	1512884	A	AAAAG	*RP11-157J24.1*
6	1531653	C	CT	N/A
6	1653790	G	T	*GMDS*
6	1793139	A	G	*GMDS*
6	1940990	T	C	*GMDS*
6	2016301	TA	T	*GMDS*
6	258665	AT	A	N/A
6	2781978	G	A	*WRNIP1*
6	2878456	C	G	*RP11-420G6.4*
6	3321342	G	GTCCAGGC	*SLC22A23*
6	3440941	AG	A	*SLC22A23*
6	3627192	A	T	N/A
6	3684277	C	CTTGAA	N/A
6	380640	A	G	N/A
6	3836105	T	A	*RP11-420L9.4*
6	438817	T	A	N/A
6	5318630	AAAAC	A	*FARS2*
6	5337300	CT	C	*FARS2*
6	5354173	CT	C	*FARS2*

6	5432761	T	TA	*FARS2*
6	5853542	A	AAC	*RP3-380B8.4*
6	6742716	A	AG	N/A
6	780554	TA	T	*RP11-532F6.5*
6	904746	TTATC	T	*RP5-1077H22.2*
6	974400	T	TGTGTGTGTATATATATATATACCATG	N/A
6	982448	GT	G	N/A

Appendix 2

A2.1 Childhood Trauma Questionnaire

Instructions: These questions ask about some of your experiences growing up as a child and a teenager. For each question, circle the number that best describes how you feel. 1 = Never True 2 = Rarely True 3 = Sometimes True 4 = Often True 5 = Very Often True. Although some of these questions are of a personal nature, please try to answer as honestly as you can. Your answers will be kept confidential.

1. When I was growing up, I didn't have enough to eat.	1	2	3	4	5
2. When I was growing up, I knew that there was someone to take care of me and protect me.	1	2	3	4	5
3. When I was growing up, people in my family called me things like "stupid", "lazy" or "ugly".	1	2	3	4	5
4. When I was growing up, my parents were too drunk or high to take care of the family.	1	2	3	4	5
5. When I was growing up, there was some one in my family who helped me feel that I was important or special	1	2	3	4	5
6. When I was growing up, I had to wear dirty clothes.	1	2	3	4	5
7. When I was growing up, I felt that I was loved.	1	2	3	4	5
8. When I was growing up, I thought my parents wished I had never been born.	1	2	3	4	5
9. When I was growing up, I got hit so hard by someone in my family that I had to see a doctor or go to the hospital.	1	2	3	4	5
10. When I was growing up, people in my family hit me so hard that it left bruises or marks.	1	2	3	4	5
11. When I was growing up, I was punished with a belt, a board, or a cord (or some other	1	2	3	4	5

hard object).					
12. When I was growing up, there was nothing I wanted to change about my family.	1	2	3	4	5
13. When I was growing up, people in my family looked out for each other.	1	2	3	4	5
14. When I was growing up, people in my family said hurtful or insulting things to me.	1	2	3	4	5
15. When I was growing up, I believe that I was physically abused.	1	2	3	4	5
16. When I was growing up, I got hit or beaten so badly that it was noticed by someone like a teacher, neighbour or doctor.	1	2	3	4	5
17. When I was growing up, I felt that someone in my family hated me.	1	2	3	4	5
18. When I was growing up, people in my family felt close to each other.	1	2	3	4	5
19. When I was growing up, someone tried to touch me in a sexual way, or tried to make me touch them.	1	2	3	4	5
20. When I was growing up, someone threatened to hurt me or tell lies about me unless I did something sexual with them.	1	2	3	4	5
21. When I was growing up, I had the perfect childhood.	1	2	3	4	5
22. When I was growing up, someone tried to make me do sexual things or watch sexual things.	1	2	3	4	5
23. When I was growing up, someone molested me.	1	2	3	4	5
24. When I was growing up, I believe that I was emotionally abused.	1	2	3	4	5
25. When I was growing up, there was someone to take me to the doctor if I needed it.	1	2	3	4	5
26. When I was growing up, I had the best family in the world.	1	2	3	4	5
27. When I was growing up, I believe that I was sexually abused.	1	2	3	4	5
28. When I was growing up, my family was a source of strength and support.	1	2	3	4	5

A2.2 Single SNP Association Results (uncorrected p-value less than 0.05)

Chromosome	SNP	Minor allele	Odds Ratio	t-statistic	p-value
11	rs7105258	A	3.091	3.12	0.001807
17	rs2071570	A	0.3624	-3.077	0.002093
11	rs11030119	A	0.4038	-3.034	0.00241
5	rs10515806	A	0.1805	-2.971	0.002964
5	rs10515805	A	0.1805	-2.971	0.002964
22	rs5993882	C	2.225	2.897	0.003767
3	rs11925572	A	3.356	2.896	0.00378
12	rs3809140	G	0.4546	-2.892	0.003828
10	rs10509117	A	2.771	2.886	0.003906
11	rs528833	A	0.3552	-2.857	0.004274
5	rs752266	G	0.3441	-2.848	0.004398
3	rs1503079	A	2.144	2.781	0.005422
6	rs3025035	A	0.2992	-2.776	0.005498
3	rs16823934	A	0.4083	-2.769	0.005624
22	rs5993883	A	0.4762	-2.765	0.005689
23	rs5906729	C	0.3862	-2.759	0.005802
10	rs2420941	C	0.4699	-2.752	0.005921
11	rs7105286	A	2.17	2.747	0.00601
2	rs309180	A	2.316	2.742	0.006111
11	rs4936556	A	2.535	2.739	0.006171
1	rs2487453	G	0.4915	-2.717	0.006594
5	rs1042713	A	2.185	2.701	0.006919
23	rs4570308	A	0.4007	-2.7	0.006935
23	rs1465108	G	0.4007	-2.7	0.006935
4	rs10026568	A	0.4498	-2.696	0.007013
22	rs1063311	A	2.091	2.683	0.007307
3	rs2668196	T	2.162	2.679	0.007377
22	rs174675	A	2.01	2.679	0.007381
7	rs3793243	G	2.086	2.672	0.00754
2	rs590557	A	2.29	2.652	0.008011
2	rs12185692	A	2.63	2.647	0.008129
5	rs12654778	A	2.313	2.641	0.008255
8	rs4907376	T	2.746	2.625	0.008657
11	rs2276310	A	2	2.616	0.008886
11	rs11030108	A	0.4549	-2.59	0.009591
8	rs17053612	A	1.892	2.589	0.009626
5	rs6888306	A	0.5173	-2.589	0.009635
6	rs6458307	G	0.508	-2.582	0.009814

11	rs11030121	A	0.5019	-2.576	0.01001
22	rs933271	G	1.941	2.575	0.01003
5	rs393795	A	0.4628	-2.565	0.01031
11	rs2030324	G	1.983	2.565	0.01032
11	rs10835215	A	1.983	2.565	0.01032
13	rs1536375	G	0.3261	-2.55	0.01078
23	rs7883073	G	2.4	2.518	0.0118
4	rs10000512	A	2.435	2.505	0.01226
10	rs1649200	G	0.4926	-2.497	0.01252
11	rs2276319	A	2.046	2.492	0.01271
7	rs10226318	A	1.969	2.491	0.01274
3	rs6771393	A	0.4404	-2.484	0.01298
10	rs1801253	C	0.442	-2.47	0.01352
11	rs10767665	A	1.935	2.466	0.01367
9	rs10511887	G	0.359	-2.459	0.01392
22	rs740603	A	0.5466	-2.45	0.01428
6	rs9497515	T	0.5218	-2.45	0.01429
6	rs827677	C	0.5255	-2.445	0.0145
8	rs3912537	C	2.041	2.442	0.01459
11	rs1800497	A	0.4746	-2.438	0.01476
23	rs5925079	A	0.3049	-2.438	0.01477
12	rs555044	A	1.926	2.437	0.01482
2	rs3771827	G	1.982	2.426	0.01526
1	rs1538974	A	2.17	2.424	0.01535
13	rs2148656	G	2.031	2.416	0.0157
11	rs11214775	A	2.115	2.413	0.01583
5	rs1364043	C	0.5236	-2.395	0.01662
20	rs2427430	C	0.5014	-2.385	0.01706
23	rs7890174	A	2.182	2.385	0.0171
22	rs2283793	G	1.948	2.383	0.01718
17	rs12103667	G	4.172	2.383	0.0172
11	rs7128320	A	1.982	2.378	0.01738
4	rs17483979	C	3.393	2.377	0.01747
9	rs2073837	A	0.5427	-2.37	0.01778
8	rs7016275	G	2.464	2.367	0.01792
16	rs1070487	A	0.5252	-2.365	0.01805
5	rs11953285	C	3.364	2.358	0.01836
11	rs3133846	G	0.5148	-2.352	0.01869
6	rs6900017	A	0.4031	-2.351	0.01872
11	rs12225735	A	0.4352	-2.345	0.01905
1	rs1342809	A	6.376	2.338	0.0194

1	rs6587924	A	0.5501	-2.335	0.01955
11	rs7103873	G	1.864	2.334	0.0196
11	rs2049046	A	1.864	2.334	0.0196
5	rs13171967	A	0.533	-2.333	0.01966
16	rs837692	A	1.924	2.332	0.01969
23	rs6579495	A	0.5192	-2.327	0.01999
3	rs6763202	G	0.547	-2.324	0.02011
4	rs3898050	A	0.4177	-2.319	0.0204
8	rs1563945	C	0.457	-2.312	0.02076
2	rs3828275	A	2.007	2.31	0.02087
11	rs17614942	A	0.3192	-2.304	0.02123
14	rs4645856	G	0.4064	-2.302	0.02134
2	rs4988235	A	2.481	2.3	0.02147
2	rs182549	A	2.481	2.3	0.02147
8	rs3802281	G	1.783	2.294	0.02182
5	rs6892282	A	0.5561	-2.288	0.02214
3	rs2029127	A	0.4346	-2.282	0.02251
10	rs9420317	G	2.158	2.279	0.02266
10	rs17156146	A	0.4764	-2.277	0.02278
8	rs979148	G	2.193	2.269	0.02327
8	rs351784	G	0.56	-2.262	0.02368
4	rs4695226	C	0.5223	-2.256	0.02407
6	rs1343796	G	1.872	2.255	0.02415
3	rs4481143	T	0.5393	-2.254	0.02422
10	rs6413419	A	0.3666	-2.249	0.0245
23	rs518147	G	1.849	2.245	0.02474
2	rs755503	G	1.795	2.244	0.02484
8	rs13270574	G	0.5535	-2.243	0.0249
3	rs1563382	G	0.5323	-2.243	0.02492
11	rs6589846	C	2.098	2.235	0.02539
8	rs472151	A	1.74	2.231	0.0257
5	rs12656106	G	1.904	2.226	0.02599
10	rs10788154	C	1.889	2.223	0.0262
11	rs2155258	G	1.833	2.222	0.02631
11	rs962369	G	0.5069	-2.218	0.02658
11	rs2282586	A	1.942	2.213	0.02688
10	rs6479700	A	1.788	2.211	0.02706
11	rs674246	G	0.5648	-2.209	0.02717
7	rs4721415	A	2.454	2.206	0.02735
22	rs4485648	G	1.818	2.204	0.02754
8	rs3757930	A	1.868	2.202	0.02766

11	rs7127507	G	0.5582	-2.2	0.0278
2	rs3771811	A	0.5084	-2.187	0.02877
11	rs3793993	C	0.458	-2.185	0.02886
11	rs1464896	G	0.3774	-2.181	0.02916
8	rs488323	A	0.5815	-2.176	0.02955
11	rs2212449	C	0.5776	-2.168	0.03014
4	rs6447528	A	1.785	2.159	0.03085
8	rs1050275	G	2.269	2.157	0.03097
12	rs3741775	C	1.999	2.153	0.03134
12	rs2730891	G	0.5813	-2.149	0.03161
4	rs6837710	G	0.5586	-2.139	0.03242
6	rs7770710	A	0.4644	-2.132	0.03301
4	rs6857487	A	0.5769	-2.13	0.03315
1	rs6688165	A	0.5274	-2.129	0.03325
12	rs3021530	C	0.3919	-2.123	0.03373
5	rs7737796	G	1.756	2.123	0.03374
23	rs3027458	A	1.981	2.122	0.03385
2	rs356643	A	1.705	2.121	0.03396
4	rs1800855	A	0.6014	-2.12	0.03401
12	rs10875749	A	0.452	-2.117	0.03429
23	rs2269416	C	1.881	2.116	0.03436
23	rs498207	G	1.77	2.114	0.03449
23	rs12843268	G	0.4857	-2.111	0.03476
23	rs3027405	T	2.038	2.1	0.03569
23	rs12394221	G	1.944	2.097	0.03596
3	rs1698042	C	0.5855	-2.091	0.03653
11	rs12273363	G	0.3371	-2.082	0.0373
10	rs4148913	G	1.737	2.078	0.03771
22	rs174690	A	0.5173	-2.077	0.03776
8	rs7006334	G	0.4406	-2.075	0.03801
23	rs3810709	A	0.4796	-2.074	0.03805
23	rs5905859	A	0.4796	-2.074	0.03805
23	rs2072744	G	0.4796	-2.074	0.03805
22	rs3810608	A	2.738	2.068	0.0386
12	rs10444584	A	1.796	2.065	0.03896
1	rs2780816	C	0.5951	-2.063	0.03914
4	rs2000978	G	2.425	2.062	0.03922
16	rs10500336	G	1.842	2.059	0.03949
16	rs2939960	A	2.57	2.052	0.04013
1	rs2295633	A	1.722	2.047	0.04063
9	rs3025369	A	0.3779	-2.047	0.04066

18	rs1944545	G	1.991	2.042	0.04113
11	rs12800734	A	1.74	2.04	0.0413
12	rs10746075	A	0.5705	-2.04	0.04136
11	rs4430518	C	1.799	2.04	0.04137
11	rs4910306	A	1.733	2.04	0.04139
1	rs6424922	A	1.703	2.037	0.04167
16	rs17498325	A	0.4063	-2.036	0.04176
11	rs1491851	G	0.5671	-2.036	0.04178
12	rs711159	A	1.589	2.029	0.04251
11	rs10892643	A	1.767	2.024	0.04296
12	rs2269355	G	0.5932	-2.02	0.04342
11	rs3758987	G	0.5555	-2.018	0.04361
11	rs3132780	G	0.3817	-2.016	0.0438
6	rs806379	T	0.6032	-2.01	0.04443
7	rs2066992	A	2.181	2.008	0.04466
11	rs1076562	A	1.704	2.007	0.04476
1	rs3024498	G	2.498	1.999	0.04562
1	rs16824627	G	1.741	1.994	0.04616
5	rs874083	A	2.462	1.994	0.04618
11	rs4274224	A	1.645	1.989	0.04673
4	rs10021525	G	1.673	1.984	0.04727
8	rs2927385	C	0.5946	-1.982	0.04746
11	rs2027760	A	1.655	1.978	0.04797
3	rs1837205	A	1.923	1.971	0.04871
3	rs4687002	G	1.65	1.97	0.04883
7	rs3917550	A	2.163	1.97	0.04886
5	rs11167557	G	0.6331	-1.969	0.04896
5	rs6884105	A	0.6176	-1.966	0.04932
10	rs10886849	A	0.5122	-1.963	0.04965
11	rs2883187	A	1.754	1.962	0.04974
8	rs526302	A	0.5261	-1.96	0.04998

A2.3 Gene-Based Results

SET	NSNP	NSIG	ISIG	EMP1	SNPS
Per3	13	0	0	1	NA
MTHFR	1	0	0	1	NA
OPRD1	18	0	0	1	NA
JUN	2	0	0	1	NA
NFIA	1	0	0	1	NA
PTGER3	2	0	0	1	NA
NRAS	1	0	0	1	NA
NGFB	12	0	0	1	NA
S100A10	5	0	0	1	NA
CHRNB2	5	0	0	1	NA
CRP	1	0	0	1	NA
RGS4	8	0	0	1	NA
F5	11	0	0	1	NA
AVPR1B	7	0	0	1	NA
PLXNA2	6	0	0	1	NA
IL1B	16	0	0	1	NA
NTSR2	5	0	0	1	NA
POMC	11	0	0	1	NA
FOSL2	8	0	0	1	NA
PRKCE	19	0	0	1	NA
REG3A	1	0	0	1	NA
ADRA2B	3	0	0	1	NA
IL1R2	13	0	0	1	NA
IL1A	7	0	0	1	NA
IL1RN	2	0	0	1	NA
DBI	6	0	0	1	NA
NR4A2	4	0	0	1	NA
STAT1	10	0	0	1	NA
FZD7	4	0	0	1	NA
CREB	8	0	0	1	NA
FEV	4	0	0	1	NA
DNAJB2	1	0	0	1	NA
HTR2B	5	0	0	1	NA
HES6	3	0	0	1	NA
Per2	22	0	0	1	NA
BHLHB2	7	0	0	1	NA
GRM7	2	0	0	1	NA
OXTR	21	0	0	1	NA
SLC6A1	16	0	0	1	NA
RAF1	3	0	0	1	NA
RBMS3	0	0	0	1	NA
FHIT	2	0	0	1	NA

DRD3	24	0	0	1	NA
GSK3B	11	0	0	1	NA
LRRC31	1	0	0	1	NA
FGFR3	1	0	0	1	NA
ADRA2C	5	0	0	1	NA
DRD5	2	0	0	1	NA
GABRA2	20	0	0	1	NA
REST	8	0	0	1	NA
AMTN	0	0	0	1	NA
IL8	1	0	0	1	NA
ADH1A	0	0	0	1	NA
NFKB1	9	0	0	1	NA
IL2	2	0	0	1	NA
FGF2	11	0	0	1	NA
NPY2R	3	0	0	1	NA
GLRB	8	0	0	1	NA
NPY1R	12	0	0	1	NA
NPY5R	4	0	0	1	NA
PRLR	2	0	0	1	NA
CART	0	0	0	1	NA
CRHBP	13	0	0	1	NA
IL5	1	0	0	1	NA
IL4	1	0	0	1	NA
FGF1	21	0	0	1	NA
CSNK1A1	7	0	0	1	NA
PPARGC1B	1	0	0	1	NA
SLC6A7	7	0	0	1	NA
GABRB2	30	0	0	1	NA
GABRA6	12	0	0	1	NA
GABRA1	8	0	0	1	NA
GABRG2	2	0	0	1	NA
DRD1	12	0	0	1	NA
TFAP2A	7	0	0	1	NA
DTNBP1	2	0	0	1	NA
PRL	3	0	0	1	NA
GABBR1	9	0	0	1	NA
LTA	2	0	0	1	NA
TNFA	6	0	0	1	NA
GRM4	13	0	0	1	NA
TULP1	1	0	0	1	NA
VEGF	5	0	0	1	NA
SLC29A1	5	0	0	1	NA
IL17	2	0	0	1	NA
HCRTR1	0	0	0	1	NA

HTR1B	13	0	0	1	NA
HTR1E	1	0	0	1	NA
SYNE1	47	0	0	1	NA
OPRM1	65	0	0	1	NA
ACHE	5	0	0	1	NA
NPY	15	0	0	1	NA
CREB5	2	0	0	1	NA
CRHR2	18	0	0	1	NA
DDC	21	0	0	1	NA
EGFR	5	0	0	1	NA
GRM3	14	0	0	1	NA
TAC1	5	0	0	1	NA
NRCAM	6	0	0	1	NA
LEP	2	0	0	1	NA
CSMD1	4	0	0	1	NA
CLDN23	4	0	0	1	NA
SLC18A1	15	0	0	1	NA
ADRB3	5	0	0	1	NA
FGFR1	2	0	0	1	NA
ADAM18	3	0	0	1	NA
PENK	5	0	0	1	NA
CALB1	2	0	0	1	NA
SLC1A1	3	0	0	1	NA
JAK2	5	0	0	1	NA
MPDZ	11	0	0	1	NA
ALDH1A1	13	0	0	1	NA
RORB	2	0	0	1	NA
NTRK2	36	0	0	1	NA
NFIL3	3	0	0	1	NA
GRIN1	6	0	0	1	NA
GAD2	20	0	0	1	NA
ADRA2A	10	0	0	1	NA
SLC18A2	30	0	0	1	NA
DRD1IP	4	0	0	1	NA
DRD4	4	0	0	1	NA
TH	9	0	0	1	NA
ARNTL	30	0	0	1	NA
TPH1	11	0	0	1	NA
CAT	0	0	0	1	NA
CRY2	7	0	0	1	NA
DRD4	15	0	0	1	NA
FOSL1	3	0	0	1	NA
ADRBK1	3	0	0	1	NA
GAL	1	0	0	1	NA

IL18	4	0	0	1	NA
HTR3A	14	0	0	1	NA
CACNA1C	44	0	0	1	NA
TNFRSF1A	3	0	0	1	NA
SCNN1A	1	0	0	1	NA
GRIN2B	76	0	0	1	NA
PDE6H	0	0	0	1	NA
timeless	4	0	0	1	NA
TPH2	79	0	0	1	NA
KCNC2	2	0	0	1	NA
SYT1	20	0	0	1	NA
SLC6A15	30	0	0	1	NA
ALDH2	6	0	0	1	NA
NOS1	2	0	0	1	NA
CIT	2	0	0	1	NA
P2RX7	15	0	0	1	NA
FGF9	8	0	0	1	NA
HTR2A	54	0	0	1	NA
DAOA	9	0	0	1	NA
MAMDC1	26	0	0	1	NA
GPHN	16	0	0	1	NA
GABRB3	0	0	0	1	NA
SLC12A6	12	0	0	1	NA
AP3B2	8	0	0	1	NA
MAPK3	2	0	0	1	NA
ADCY7	14	0	0	1	NA
NET1	40	0	0	1	NA
ARRB2	7	0	0	1	NA
PER1	13	0	0	1	NA
VAMP2	3	0	0	1	NA
SLC6A4	42	0	0	1	NA
CDK5R1	3	0	0	1	NA
PPP1R1B	4	0	0	1	NA
PNMT	0	0	0	1	NA
HCRT	1	0	0	1	NA
STAT5	0	0	0	1	NA
ACE1	14	0	0	1	NA
GRIN2C	3	0	0	1	NA
GALR2	2	0	0	1	NA
CSNK1D	0	0	0	1	NA
MC2R	4	0	0	1	NA
MYO5B	3	0	0	1	NA
TCF4	3	0	0	1	NA
BCL2	4	0	0	1	NA

SerpinB2	0	0	0	1	NA
SerpinB10	4	0	0	1	NA
SerpinB8	11	0	0	1	NA
CDH7	6	0	0	1	NA
EPOR	1	0	0	1	NA
APOE	8	0	0	1	NA
PDYN	10	0	0	1	NA
OXT	16	0	0	1	NA
SNAP25	45	0	0	1	NA
SLC32A1	2	0	0	1	NA
CHRNA4	15	0	0	1	NA
OPRL1	6	0	0	1	NA
GRIK1	46	0	0	1	NA
DIP2	3	0	0	1	NA
ADRBK2	14	0	0	1	NA
XBP1	2	0	0	1	NA
SYN3	2	0	0	1	NA
GALR3	0	0	0	1	NA
CSNK1E	14	0	0	1	NA
GLRA2	12	0	0	1	NA
GPR50	9	0	0	1	NA
GABRA3	20	0	0	1	NA
GABRQ	6	0	0	1	NA
NR1D1	5	1	1	0.0032	rs2071570
BHLHB3	4	1	1	0.005799	rs3809140
BCHE	11	2	2	0.0113	rs11925572|rs2668196
STX1A	5	1	1	0.0188	rs3793243
ARRB1	10	2	2	0.0225	rs528833|rs2276310
ADRB2	11	2	1	0.0236	rs1042713
HES1	4	1	1	0.0283	rs6771393
ADRB1	4	1	1	0.0352	rs1801253
DISC1	5	2	2	0.0372	rs2487453|rs1538974
ADRA1B	15	7	5	0.0457	rs10515805|rs752266|rs6888306|rs11953285|rs6892282
VEGFA	11	2	2	0.0468	rs3025035|rs6900017
GRIK4	67	6	5	0.05139	rs7105258|rs7105286|rs4936556|rs2276319|rs3133846
MAOB	18	4	1	0.05469	rs7883073
HTR1A	7	1	1	0.05569	rs1364043
FOS	3	1	1	0.05879	rs4645856
WFS1	5	1	1	0.06719	rs3898050
TSPAN8	2	1	1	0.06979	rs10444584
SLC6A3	45	1	1	0.07379	rs393795
MAOA	21	8	2	0.07459	rs5906729|rs3027405
MAPK1	15	3	2	0.07669	rs1063311|rs3810608
CCK	6	1	1	0.08149	rs2029127

GAD1	19	2	2	0.08529	rs12185692|rs3828275
AP3M2	4	2	2	0.08529	rs13270574|rs1050275
ANK3	49	2	2	0.09779	rs10509117|rs6479700
SLC6A13	16	1	1	0.1036	rs555044
GRM1	18	1	1	0.1044	rs9497515
CYP2E1	6	1	1	0.1047	rs6413419
OPRK1	12	2	2	0.1123	rs7016275|rs3802281
COMT	36	7	5	0.1143	rs5993882|rs5993883|rs740603|rs4485648|rs174690
PNOC	9	2	2	0.1155	rs1563945|rs351784
RGS2	17	1	1	0.1169	rs1342809
CLOCK	32	2	2	0.1185	rs10000512|rs6837710
GRIN2A	9	2	2	0.1207	rs1070487|rs837692
NTSR1	14	1	1	0.1212	rs2427430
GABRE	8	2	2	0.1271	rs5925079|rs2269416
GABRD	3	1	1	0.1272	rs16824627
GALNTL4	5	1	1	0.1308	rs4910306
DGKH	26	1	1	0.1324	rs2148656
CRHR1	31	1	1	0.1445	rs12103667
BDNF	41	13	5	0.1489	rs11030119|rs2030324|rs1464896|rs12273363|rs1491851
CCKBR	9	1	1	0.1528	rs3793993
HTR2C	37	3	2	0.1652	rs6579495|rs518147
MAPK14	8	1	1	0.1721	rs7770710
DAO	9	1	1	0.1794	rs3741775
CRY1	6	1	1	0.1954	rs10746075
IL10	15	1	1	0.1997	rs3024498
GAP43	31	2	2	0.2037	rs16823934|rs1837205
GLRA1	8	1	1	0.2158	rs11167557
GNB3	7	1	1	0.2164	rs2269355
CCKAR	10	2	2	0.2178	rs1800855|rs2000978
NR3C2	51	3	3	0.2185	rs10026568|rs17483979|rs6857487
HTR3B	21	3	3	0.2266	rs11214775|rs17614942|rs3758987
JAK1	14	1	1	0.2355	rs2780816
AVPR1A	17	1	1	0.2447	rs3021530
SLC6A11	21	1	1	0.2618	rs4481143
TACR1	29	2	2	0.2689	rs3771827|rs3771811
GALR1	9	1	1	0.2713	rs1944545
NR3C1	36	1	1	0.285	rs12656106
FAAH	11	1	1	0.3018	rs2295633
CNR1	11	1	1	0.3141	rs806379
SLC9A9	43	1	1	0.3308	rs6763202
IL6	15	1	1	0.337	rs2066992
FGFR2	64	5	5	0.3398	rs2420941|rs1649200|rs9420317|rs10788154|rs10886849
DBH	31	2	2	0.3834	rs2073837|rs3025369
NCAM1	28	2	2	0.4025	rs674246|rs2212449

GABRB1	36	2	2	0.4365	rs4695226|rs6447528
CRH	33	1	1	0.4427	rs7006334
FKBP5	98	1	1	0.4617	rs1343796
PON1	21	1	1	0.499	rs3917550
NRG1	45	1	1	0.514	rs3757930
NPAS2	34	1	1	0.5301	rs356643
CAMK2A	24	1	1	0.557	rs874083
DRD2	36	2	2	0.5597	rs1076562|rs4274224
ADRA1A	56	3	2	0.6272	rs472151|rs526302
LPHN3	31	1	1	0.6833	rs10021525

Appendix 3

A3.1 Genes and SNPs

Gene	SNP	Gene	SNP
CRHR2	rs1003929	*CRHR1*	rs241027
CRHR2	rs10271509	*CRHR1*	rs241030
UCN3	rs10400011	*CRHR1*	rs241031
CRHR2	rs107540	*CRHR1*	rs241033
CRHR2	rs1076292	*CRHR1*	rs241035
UCN3	rs10795268	*CRHR1*	rs241036
UCN3	rs10904479	*CRHR1*	rs241039
UCN3	rs10904481	*CRHR2*	rs255100
CRHR1	rs110402	*CRHR2*	rs255102
UCN3	rs11253130	*CRHR2*	rs255105
UCN3	rs11591351	*CRHR2*	rs255106
CRHR2	rs12701020	*CRHR2*	rs255108
CRH	rs12721510	*CRHR2*	rs255110
UCN3	rs12768198	*CRHR2*	rs255112
UCN3	rs12783734	*CRHR2*	rs255113
UCN2	rs13319651	*CRHR2*	rs255115
CRHR1	rs171440	*CRHR2*	rs255121
CRHR1	rs171441	*CRHR2*	rs255122
CRHR2	rs17159371	*CRH*	rs3176921
CRHR2	rs17159372	*CRHR1*	rs3418
CRHR1	rs173365	*POMC*	rs3769671
CRHR2	rs2014663	*CRHR2*	rs3779250
CRHR2	rs2190242	*CRHR2*	rs4722999
CRHR2	rs2240403	*CRHR2*	rs4723000
CRHR2	rs2240404	*CRHR2*	rs4723002
CRHR2	rs2251002	*CRHR2*	rs4723003
CRHR2	rs2267710	*CRHR2*	rs6462219
CRHR2	rs2267712	*CRH*	rs6472257

CRHR2	rs2267715	*POMC*	rs6545975
CRHR2	rs2267716	*UCN3*	rs6601952
CRHR2	rs2267717	*POMC*	rs6713532
CRHR2	rs2284216	*CRH*	rs6982394
CRHR2	rs2284217	*CRH*	rs6999780
CRHR2	rs2284218	*CRHR2*	rs733453
CRHR2	rs2284219	*POMC*	rs7565427
CRHR2	rs2284220	*POMC*	rs7565877
CRHR1	rs241022	*CRHR2*	rs7812133
CRHR1	rs241023	*CRHR1*	rs81189
CRHR1	rs241026	*CRHR2*	rs929377
POMC	rs934778	*CRHR2*	rs975537
CRHR2	rs973002	*UCN3*	rs9839959

A3.2 AUDIT Questionnaire

Box 4

The Alcohol Use Disorders Identification Test: Interview Version

Read questions as written. Record answers carefully. Begin the AUDIT by saying "Now I am going to ask you some questions about your use of alcoholic beverages during this past year." Explain what is meant by "alcoholic beverages" by using local examples of beer, wine, vodka, etc. Code answers in terms of "standard drinks". Place the correct answer number in the box at the right.

1. How often do you have a drink containing alcohol?
   (0) Never [Skip to Qs 9-10]
   (1) Monthly or less
   (2) 2 to 4 times a month
   (3) 2 to 3 times a week
   (4) 4 or more times a week

2. How many drinks containing alcohol do you have on a typical day when you are drinking?
   (0) 1 or 2
   (1) 3 or 4
   (2) 5 or 6
   (3) 7, 8, or 9
   (4) 10 or more

3. How often do you have six or more drinks on one occasion?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily
   Skip to Questions 9 and 10 if Total Score for Questions 2 and 3 = 0

4. How often during the last year have you found that you were not able to stop drinking once you had started?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

5. How often during the last year have you failed to do what was normally expected from you because of drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

7. How often during the last year have you had a feeling of guilt or remorse after drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
   (0) Never
   (1) Less than monthly
   (2) Monthly
   (3) Weekly
   (4) Daily or almost daily

9. Have you or someone else been injured as a result of your drinking?
   (0) No
   (2) Yes, but not in the last year
   (4) Yes, during the last year

10. Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down?
    (0) No
    (2) Yes, but not in the last year
    (4) Yes, during the last year

Record total of specific items here

If total is greater than recommended cut-off, consult User's Manual.

A3.3 Adverse Childhood Events

SECTION D: UPSETTING EVENTS

Below are listed some events that might upset some children. Please state whether any of these happened in the past 12 months?

		Yes and he was very upset	Yes and he was quite upset	Yes and he was a bit upset	Yes but he wasn't upset	No did not happen
D1.	He was taken * into care	1	2	3	4	5
D2.	A pet died	1	2	3	4	5
D3.	He moved home	1	2	3	4	5
D4.	He had a shock * or fright	1	2	3	4	5
D5.	He was physically hurt by someone	1	2	3	4	5
D6.	He was sexually * abused*	1	2	3	4	5
D7.	He was separated * from his mother for at least a week	1	2	3	4	5

		Yes and he was very upset	Yes and he was quite upset	Yes and he was a bit upset	Yes but he wasn't upset	No did not happen
D8.	He was separated * from his father for at least a week	1	2	3	4	5
D9.	He acquired a new * parent	1	2	3	4	5
D10.	He had a new brother or sister	1	2	3	4	5
D11.	He was admitted to hospital	1	2	3	4	5
D12.	He changed carer/ care giver	1	2	3	4	5
D13.	He was separated * from someone else	1	2	3	4	5
D14.	He started a new creche or nursery	1	2	3	4	5
D15.	Something else *	1	2	3	4	5

If yes, to any marked *, please give details below:

.....

.....

A3.4 SDQ

Strengths and Difficulties Questionnaire

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of the child's behaviour over the last six months or this school year.

Child's Name ..... Male/Female

Date of Birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children (treats, toys, pencils etc.)	☐	☐	☐
Often has temper tantrums or hot tempers	☐	☐	☐
Rather solitary, tends to play alone	☐	☐	☐
Generally obedient, usually does what adults request	☐	☐	☐
Many worries, often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, down-hearted or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Thinks things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets on better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Sees tasks through to the end, good attention span	☐	☐	☐

Signature ..... Date .....

Parent/Teacher/Other (please specify:)

Thank you very much for your help

A3.5 Gene x Environment Interaction Results

Chromsome	SNP	regression co-efficient in first group	standard error regression co-efficient in first group	regression co-efficient in second group	standard error regression co-efficient in second group	Z score	P-value_GXE
7	rs2284217	0.8502	0.4279	-0.1952	0.1798	2.253	0.02429
7	rs255100	0.7212	0.3825	-0.1732	0.1592	2.159	0.03087
7	rs1076292	0.5794	0.3683	-0.226	0.1541	2.017	0.04365
7	rs2251002	0.5794	0.3683	-0.226	0.1541	2.017	0.04365
7	rs17159371	1.127	0.7061	-0.2369	0.2837	1.792	0.07307
7	rs17159372	1.271	0.7648	-0.1628	0.3085	1.739	0.08206
7	rs107540	0.5768	0.3644	-0.08987	0.1515	1.689	0.09116
7	rs255122	0.5168	0.3552	-0.1098	0.1489	1.627	0.1037
7	rs2284219	0.4105	0.3622	-0.2274	0.1516	1.625	0.1043
7	rs733453	0.4105	0.3622	-0.2223	0.1516	1.612	0.107
7	rs2267715	0.4105	0.3622	-0.2223	0.1516	1.612	0.107
7	rs2284218	0.386	0.3626	-0.2321	0.1515	1.573	0.1157
7	rs255115	0.4937	0.354	-0.09809	0.1487	1.541	0.1233
7	rs255113	0.471	0.3546	-0.1132	0.1502	1.517	0.1293
7	rs255112	0.4604	0.3565	-0.1084	0.1501	1.471	0.1414
7	rs975537	0.6072	0.4257	-0.04953	0.1778	1.423	0.1546
2	rs6713532	0.6058	0.4074	-0.01121	0.1712	1.396	0.1627
7	rs3779250	0.3997	0.3806	-0.1257	0.153	1.281	0.2002
7	rs2267712	0.5778	0.5018	-0.1031	0.2132	1.249	0.2117
7	rs6462219	0.5778	0.5018	-0.1031	0.2132	1.249	0.2117
7	rs2014663	0.5778	0.5018	-0.1031	0.2132	1.249	0.2117
8	rs12721510	0.8856	0.6439	0.02541	0.3004	1.21	0.2261
7	rs255108	0.4179	0.3508	-0.04093	0.1451	1.209	0.2268
7	rs2284220	0.5185	0.5009	-0.1139	0.2131	1.162	0.2453
7	rs1003929	0.5185	0.5009	-0.1139	0.2131	1.162	0.2453
7	rs255106	0.3929	0.3513	-0.04395	0.1453	1.149	0.2504
7	rs4722999	0.4106	0.3948	-0.07582	0.1579	1.144	0.2526
7	rs2267710	0.4106	0.3948	-0.07195	0.1579	1.135	0.2564
17	rs110402	-0.2436	0.3484	0.1491	0.1458	-1.04	0.2985

7	rs10271509	0.4079	0.3821	0.004651	0.1594	0.9741	0.33
17	rs173365	-0.2721	0.3436	0.07394	0.1441	-0.9286	0.3531
7	rs2267716	0.1488	0.4227	-0.2276	0.1758	0.8222	0.411
7	rs2190242	0.1615	0.4305	-0.2092	0.1799	0.7945	0.4269
7	rs255121	0.289	0.592	-0.2215	0.2542	0.7924	0.4281
7	rs4723003	-0.6989	0.6339	-0.1613	0.2576	-0.7856	0.4321
2	rs7565427	0.01557	0.5046	0.44	0.2201	-0.7708	0.4408
2	rs7565877	0.0905	0.5462	-0.3629	0.2331	0.7634	0.4452
8	rs3176921	0.5693	0.6586	0.06115	0.2445	0.7233	0.4695
8	rs6999780	0.5693	0.6586	0.06115	0.2445	0.7233	0.4695
8	rs6472257	0.5693	0.6586	0.06115	0.2445	0.7233	0.4695
7	rs929377	0.1476	0.3961	-0.1608	0.1627	0.7202	0.4714
10	rs10904479	-0.3871	0.3942	-0.08743	0.1624	-0.7029	0.4821
7	rs973002	0.3336	0.4676	0.01813	0.1973	0.6216	0.5342
7	rs4723002	-0.5708	0.6467	-0.1613	0.2576	-0.5883	0.5564
2	rs934778	0.3021	0.3579	0.07771	0.1571	0.574	0.566
7	rs255105	0.1665	0.3634	-0.05159	0.1504	0.5546	0.5792
17	rs81189	-0.09253	0.3472	0.1085	0.144	-0.5349	0.5927
17	rs171440	-0.09253	0.3472	0.08929	0.1443	-0.4836	0.6287
7	rs2284216	0.07005	0.6977	-0.212	0.2701	0.377	0.7061
10	rs10904481	-0.212	0.3881	-0.06231	0.1593	-0.3568	0.7212
10	rs11253130	-0.1904	0.3983	-0.04523	0.1629	-0.3373	0.7359
10	rs6601952	-0.2071	0.3884	-0.07365	0.159	-0.3179	0.7506
7	rs2240404	-0.1315	0.544	-0.3065	0.2301	0.2962	0.7671
10	rs10795268	-0.1393	0.4002	-0.01401	0.1645	-0.2895	0.7722
2	rs6545975	0.2213	0.3286	0.3147	0.1477	-0.2591	0.7955
10	rs11591351	0.2584	0.3853	0.3639	0.1694	-0.2507	0.802
10	rs10400011	-0.1365	0.4003	-0.03172	0.1645	-0.2422	0.8087
7	rs255110	0.06193	0.3759	-0.0329	0.1603	0.232	0.8165
7	rs2240403	-0.4275	0.689	-0.2634	0.2679	-0.222	0.8243
7	rs2267717	0.2292	0.5712	0.106	0.231	0.2	0.8415
10	rs12783734	0.3111	0.3875	0.3948	0.1706	-0.1976	0.8434
10	rs127681	0.3111	0.3875	0.3913	0.1704	-0.1894	0.8498

	98						
7	rs4723000	-0.226	0.5399	-0.3205	0.2311	0.1609	0.8722
7	rs12701020	-0.2376	0.4605	-0.1578	0.1988	-0.159	0.8737
17	rs241030	-0.1779	0.3937	-0.1455	0.1623	-0.07608	0.9394
17	rs241039	-0.1405	0.3979	-0.1229	0.1646	-0.04099	0.9673
17	rs3418	-0.1405	0.3979	-0.1229	0.1646	-0.04099	0.9673
17	rs241036	-0.1405	0.3979	-0.133	0.1642	-0.01755	0.986
17	rs241035	-0.1405	0.3979	-0.133	0.1642	-0.01755	0.986
17	rs241033	-0.1405	0.3979	-0.133	0.1642	-0.01755	0.986
17	rs241031	-0.1405	0.3979	-0.133	0.1642	-0.01755	0.986
17	rs241027	-0.1405	0.3979	-0.133	0.1642	-0.01755	0.986
17	rs241026	-0.1405	0.3979	-0.133	0.1642	-0.01755	0.986
17	rs241023	-0.1405	0.3979	-0.133	0.1642	-0.01755	0.986
17	rs241022	-0.1405	0.3979	-0.133	0.1642	-0.01755	0.986
17	rs171441	0.2618	0.6437	0.262	0.2869	-0.0003641	0.9997

Appendix 4

A4.1 Genes and SNPs

Gene	SNP	Gene	SNP	Gene	SNP	Gene	SNP
AKAP1	rs15944	*GRIK4*	rs10502240	*GRM8*	rs1008260	*GRIK3*	rs544932
AKAP1	rs2058578	*GRIK4*	rs10790394	*GRM8*	rs1008906	*GRIK3*	rs547390
AKAP1	rs2160115	*GRIK4*	rs10790395	*GRM8*	rs1008907	*GRIK3*	rs553719
AKAP1	rs2230772	*GRIK4*	rs10790400	*GRM8*	rs10232001	*GRIK3*	rs569858
AKAP1	rs2301702	*GRIK4*	rs10790402	*GRM8*	rs1024380	*GRIK3*	rs571524
AKAP1	rs3095505	*GRIK4*	rs10892599	*GRM8*	rs10254233	*GRIK3*	rs6687320
AKAP1	rs3785484	*GRIK4*	rs10892612	*GRM8*	rs10256873	*GRIK3*	rs7517274
AKAP1	rs3826296	*GRIK4*	rs10892629	*GRM8*	rs10274867	*GRIK3*	rs7531813
AKAP1	rs4583	*GRIK4*	rs10892631	*GRM8*	rs10487463	*GRIK3*	rs7555221
AKAP1	rs7209653	*GRIK4*	rs10892640	*GRM8*	rs10487466	*PRKCH*	rs4312236
AKAP1	rs721427	*GRIK4*	rs10892643	*GRM8*	rs1155597	*PRKCH*	rs4899050
AKAP1	rs998113	*GRIK4*	rs10892648	*GRM8*	rs1155657	*PRKCH*	rs4902046
AKAP10	rs119672	*GRIK4*	rs11217956	*GRM8*	rs11563302	*PRKCH*	rs4902069
AKAP10	rs17604987	*GRIK4*	rs11217973	*GRM8*	rs11563486	*PRKCH*	rs6573385
AKAP10	rs203457	*GRIK4*	rs11217974	*GRM8*	rs11563496	*PRKCH*	rs7155214
AKAP10	rs203466	*GRIK4*	rs11218005	*GRM8*	rs11563701	*PRKCH*	rs7158720
AKAP10	rs2108978	*GRIK4*	rs11218016	*GRM8*	rs11563720	*PRKCH*	rs767755
AKAP10	rs850622	*GRIK4*	rs11218029	*GRM8*	rs11563773	*PRKCH*	rs8008798
AKAP11	rs9525607	*GRIK4*	rs11218032	*GRM8*	rs11563801	*PRKCH*	rs8013398
AKAP11	rs9594724	*GRIK4*	rs11218067	*GRM8*	rs11763603	*PRKCH*	rs8019776
AKAP12	rs10457871	*GRIK4*	rs11218069	*GRM8*	rs11978873	*PRKCH*	rs912617

AKAP12	rs12195960	*GRIK4*	rs11218071	*GRM8*	rs1204515	*PRKCH*	rs991110
AKAP12	rs12202922	*GRIK4*	rs11218072	*GRM8*	rs1204516	*PRKCI*	rs1392366
AKAP12	rs17080959	*GRIK4*	rs11218078	*GRM8*	rs1204556	*PRKCI*	rs1684885
AKAP12	rs17081113	*GRIK4*	rs11603586	*GRM8*	rs1204578	*PRKCI*	rs2291930
AKAP12	rs2294796	*GRIK4*	rs11607732	*GRM8*	rs1204585	*PRKCI*	rs2292386
AKAP12	rs2786748	*GRIK4*	rs12226536	*GRM8*	rs1204590	*PRKCI*	rs7620870
AKAP12	rs4870006	*GRIK4*	rs12284730	*GRM8*	rs1211384	*PRKCQ*	rs2453
AKAP12	rs4870007	*GRIK4*	rs2004676	*GRM8*	rs12154960	*PRKCQ*	rs4750439
AKAP12	rs6557123	*GRIK4*	rs2155258	*GRM8*	rs12706735	*PRKCQ*	rs582052
AKAP12	rs6907228	*GRIK4*	rs2298723	*GRM8*	rs12706739	*PRKCZ*	rs262641
AKAP12	rs7742224	*GRIK4*	rs2850808	*GRM8*	rs12706740	*PRKCZ*	rs3107146
AKAP12	rs7764018	*GRIK4*	rs2852212	*GRM8*	rs13222700	*PRKCZ*	rs3107157
AKAP12	rs926557	*GRIK4*	rs2852241	*GRM8*	rs13240418	*PRKCZ*	rs3128309
AKAP12	rs9322311	*GRIK4*	rs3824978	*GRM8*	rs13240504	*PRKCZ*	rs3753242
AKAP12	rs9383875	*GRIK4*	rs3901285	*GRM8*	rs1361981	*PRKCZ*	rs385039
AKAP12	rs9383876	*GRIK4*	rs4414232	*GRM8*	rs1361990	*PRKCZ*	rs626479
AKAP12	rs9383877	*GRIK4*	rs4451741	*GRM8*	rs1419442	*PRKCZ*	rs7513222
AKAP12	rs9383882	*GRIK4*	rs4526780	*GRM8*	rs1419484	*SERPIN A6*	rs10141408
AKAP13	rs10520589	*GRIK4*	rs4582985	*GRM8*	rs1419496	*SERPIN A6*	rs11160168
AKAP13	rs10520594	*GRIK4*	rs4635090	*GRM8*	rs1419508	*SERPIN A6*	rs11160169
AKAP13	rs10520595	*GRIK4*	rs4936566	*GRM8*	rs1419521	*SERPIN A6*	rs12437224
AKAP13	rs10520596	*GRIK4*	rs499804	*GRM8*	rs17150350	*SERPIN A6*	rs1956177
AKAP13	rs10520599	*GRIK4*	rs645156	*GRM8*	rs17612819	*SERPIN A6*	rs1998056
AKAP13	rs11073403	*GRIK4*	rs646543	*GRM8*	rs17627206	*SERPIN A6*	rs2144834
AKAP13	rs11074223	*GRIK4*	rs658696	*GRM8*	rs17683462	*SERPIN A6*	rs7161521
AKAP13	rs1111107	*GRIK4*	rs6589833	*GRM8*	rs17691394	*SERPIN A6*	rs8005533
AKAP13	rs11631660	*GRIK4*	rs6589846	*GRM8*	rs1981696	*SERPIN A6*	rs941599
AKAP13	rs11633893	*GRIK4*	rs669397	*GRM8*	rs1989850	*SLC1A1*	rs10491731
AKAP13	rs11634177	*GRIK4*	rs7104543	*GRM8*	rs2023735	*SLC1A1*	rs10758629
AKAP13	rs11634651	*GRIK4*	rs7116106	*GRM8*	rs2066902	*SLC1A1*	rs10814993
AKAP13	rs11635711	*GRIK4*	rs7128009	*GRM8*	rs2106190	*SLC1A1*	rs10814997
AKAP13	rs11637726	*GRIK4*	rs7128320	*GRM8*	rs2106193	*SLC1A1*	rs10974616
AKAP13	rs11638762	*GRIK4*	rs751368	*GRM8*	rs2106200	*SLC1A1*	rs10974623
AKAP13	rs12101598	*GRIK4*	rs751369	*GRM8*	rs2106353	*SLC1A1*	rs10974624
AKAP13	rs12440599	*GRIK4*	rs751370	*GRM8*	rs2157752	*SLC1A1*	rs12342908
AKAP13	rs12442591	*GRIK4*	rs7925270	*GRM8*	rs2188187	*SLC1A1*	rs12551465
AKAP13	rs12708553	*GRIK5*	rs3785906	*GRM8*	rs2237776	*SLC1A1*	rs17812372
AKAP13	rs12906580	*GRIN2A*	rs10500370	*GRM8*	rs2237784	*SLC1A1*	rs2026828

AKAP13	rs12909648	*GRIN2A*	rs10500373	*GRM8*	rs2237786	*SLC1A1*	rs2072657
AKAP13	rs13379755	*GRIN2A*	rs10518151	*GRM8*	rs2237790	*SLC1A1*	rs3780414
AKAP13	rs16940997	*GRIN2A*	rs1070476	*GRM8*	rs2237794	*SLC1A1*	rs3824259
AKAP13	rs16941653	*GRIN2A*	rs1070484	*GRM8*	rs2283061	*SLC1A1*	rs4742003
AKAP13	rs16942698	*GRIN2A*	rs1070548	*GRM8*	rs2283062	*SLC1A1*	rs4742008
AKAP13	rs16943236	*GRIN2A*	rs1102972	*GRM8*	rs2283064	*SLC1A1*	rs4742009
AKAP13	rs16943741	*GRIN2A*	rs11074502	*GRM8*	rs2283079	*SLC1A1*	rs6476878
AKAP13	rs16977800	*GRIN2A*	rs11074546	*GRM8*	rs2283092	*SLC1A1*	rs7022369
AKAP13	rs16977810	*GRIN2A*	rs11074547	*GRM8*	rs2299476	*SLC1A1*	rs7022772
AKAP13	rs17552700	*GRIN2A*	rs11074593	*GRM8*	rs2299492	*SLC1A1*	rs7031998
AKAP13	rs17552971	*GRIN2A*	rs11074605	*GRM8*	rs2299500	*SLC1A1*	rs7849913
AKAP13	rs17553734	*GRIN2A*	rs11074613	*GRM8*	rs2299503	*SLC1A1*	rs7871243
AKAP13	rs17553790	*GRIN2A*	rs11644367	*GRM8*	rs2299536	*SLC1A1*	rs928209
AKAP13	rs17575933	*GRIN2A*	rs11644461	*GRM8*	rs2299537	*SLC1A1*	rs9886720
AKAP13	rs17618909	*GRIN2A*	rs11645379	*GRM8*	rs2299546	*SLC1A1*	rs9918970
AKAP13	rs17623915	*GRIN2A*	rs11647369	*GRM8*	rs2299555	*SYNGAP1*	rs411136
AKAP13	rs17632140	*GRIN2A*	rs11647877	*GRM8*	rs2299556	*UCN2*	rs171440
AKAP13	rs17635450	*GRIN2A*	rs11648615	*GRM8*	rs2402820	*UCN2*	rs171441
AKAP13	rs17638001	*GRIN2A*	rs11859693	*GRM8*	rs3808122	*UCN3*	rs11253130
AKAP13	rs17638180	*GRIN2A*	rs11862776	*GRM8*	rs3808142		
AKAP13	rs1861856	*GRIN2A*	rs11866328	*GRM8*	rs4141410		
AKAP13	rs2062234	*GRIN2A*	rs11866385	*GRM8*	rs4731330		
AKAP13	rs2169876	*GRIN2A*	rs12596342	*GRM8*	rs6467092		
AKAP13	rs2241267	*GRIN2A*	rs12920919	*GRM8*	rs6947960		
AKAP13	rs2291048	*GRIN2A*	rs12924396	*GRM8*	rs6950264		
AKAP13	rs2430832	*GRIN2A*	rs12932835	*GRM8*	rs6951546		
AKAP13	rs2448026	*GRIN2A*	rs12934281	*GRM8*	rs6951643		
AKAP13	rs338507	*GRIN2A*	rs12935101	*GRM8*	rs6958658		
AKAP13	rs338515	*GRIN2A*	rs13331514	*GRM8*	rs6961760		
AKAP13	rs338518	*GRIN2A*	rs13336632	*GRM8*	rs6975541		
AKAP13	rs338520	*GRIN2A*	rs1345424	*GRM8*	rs6975798		
AKAP13	rs338540	*GRIN2A*	rs1420040	*GRM8*	rs728600		
AKAP13	rs338554	*GRIN2A*	rs1448262	*GRM8*	rs766239		
AKAP13	rs3743321	*GRIN2A*	rs1550961	*GRM8*	rs7784231		
AKAP13	rs3765096	*GRIN2A*	rs1650420	*GRM8*	rs7792592		
AKAP13	rs414374	*GRIN2A*	rs16956781	*GRM8*	rs7794734		
AKAP13	rs4240775	*GRIN2A*	rs16966731	*GRM8*	rs7796391		
AKAP13	rs4281668	*GRIN2A*	rs17570793	*GRM8*	rs7805944		
AKAP13	rs4360875	*GRIN2A*	rs17671033	*GRM8*	rs7806403		
AKAP13	rs4407021	*GRIN2A*	rs17682922	*GRM8*	rs886176		
AKAP13	rs4536419	*GRIN2A*	rs17682940	*GRM8*	rs929174		
AKAP13	rs4638530	*GRIN2A*	rs17750208	*GRM8*	rs952912		
AKAP13	rs4842888	*GRIN2A*	rs17793917	*GRM8*	rs9942681		
AKAP13	rs4843062	*GRIN2A*	rs1868291	*GRM8*	rs994783		

AKAP13	rs4843094	*GRIN2A*	rs1875205	*IDO1*	rs3739319	
AKAP13	rs6496055	*GRIN2A*	rs1875207	*MAPK1*	rs17759598	
AKAP13	rs6497117	*GRIN2A*	rs1875208	*MAPK1*	rs2276005	
AKAP13	rs7165772	*GRIN2A*	rs1900718	*MAPK1*	rs2276006	
AKAP13	rs7168345	*GRIN2A*	rs194355	*MAPK1*	rs2283792	
AKAP13	rs7169429	*GRIN2A*	rs2034369	*MAPK1*	rs2298432	
AKAP13	rs7170370	*GRIN2A*	rs2267772	*MAPK1*	rs2298434	
AKAP13	rs7171364	*GRIN2A*	rs2267786	*MAPK1*	rs363437	
AKAP13	rs7182210	*GRIN2A*	rs2267787	*MAPK1*	rs5755694	
AKAP13	rs725686	*GRIN2A*	rs2267792	*MAPK1*	rs5999515	
AKAP13	rs730372	*GRIN2A*	rs2352740	*MAPK1*	rs5999521	
AKAP13	rs730439	*GRIN2A*	rs2353434	*MC2R*	rs1940907	
AKAP13	rs8025492	*GRIN2A*	rs2442098	*MC2R*	rs4308014	
AKAP13	rs8035127	*GRIN2A*	rs2650434	*MC2R*	rs4519393	
AKAP13	rs8036310	*GRIN2A*	rs2937030	*MC2R*	rs4519394	
AKAP13	rs8041467	*GRIN2A*	rs3104704	*MC2R*	rs4797824	
AKAP13	rs870689	*GRIN2A*	rs3743833	*MC2R*	rs4797825	
AKAP13	rs900573	*GRIN2A*	rs3848332	*NR3C1*	rs10051292	
AKAP14	rs2428236	*GRIN2A*	rs3852745	*NR3C1*	rs10063711	
AKAP3	rs10774250	*GRIN2A*	rs3852746	*NR3C1*	rs10428525	
AKAP3	rs1860342	*GRIN2A*	rs3905837	*NR3C1*	rs10482642	
AKAP3	rs6416316	*GRIN2A*	rs4780717	*NR3C1*	rs10515522	
AKAP3	rs7316317	*GRIN2A*	rs4780746	*NR3C1*	rs12187799	
AKAP6	rs10134827	*GRIN2A*	rs4780751	*NR3C1*	rs12189303	
AKAP6	rs10135053	*GRIN2A*	rs4780817	*NR3C1*	rs12656580	
AKAP6	rs10146478	*GRIN2A*	rs4782108	*NR3C1*	rs13158686	
AKAP6	rs10151252	*GRIN2A*	rs4782258	*NR3C1*	rs13354365	
AKAP6	rs1028537	*GRIN2A*	rs4782263	*NR3C1*	rs1363668	
AKAP6	rs10431651	*GRIN2A*	rs4782265	*NR3C1*	rs1366082	
AKAP6	rs10431652	*GRIN2A*	rs4782266	*NR3C1*	rs1373999	
AKAP6	rs10483416	*GRIN2A*	rs4782271	*NR3C1*	rs1422874	
AKAP6	rs10872865	*GRIN2A*	rs4782275	*NR3C1*	rs1445873	
AKAP6	rs11156742	*GRIN2A*	rs4998386	*NR3C1*	rs153516	
AKAP6	rs11627566	*GRIN2A*	rs6416623	*NR3C1*	rs153518	
AKAP6	rs12435388	*GRIN2A*	rs6497523	*NR3C1*	rs1644431	
AKAP6	rs12818068	*GRIN2A*	rs6497732	*NR3C1*	rs17100309	
AKAP6	rs12878600	*GRIN2A*	rs6497738	*NR3C1*	rs17100323	
AKAP6	rs12882489	*GRIN2A*	rs7173923	*NR3C1*	rs17100330	
AKAP6	rs12887176	*GRIN2A*	rs7188291	*NR3C1*	rs17100416	
AKAP6	rs12889349	*GRIN2A*	rs7190716	*NR3C1*	rs17100498	
AKAP6	rs12897283	*GRIN2A*	rs7191698	*NR3C1*	rs17100500	
AKAP6	rs17091663	*GRIN2A*	rs7195891	*NR3C1*	rs17100506	
AKAP6	rs17098884	*GRIN2A*	rs7196708	*NR3C1*	rs17100739	
AKAP6	rs17099235	*GRIN2A*	rs7203315	*NR3C1*	rs171569	

AKAP6	rs17099285	*GRIN2A*	rs7204654	*NR3C1*	rs17287758	
AKAP6	rs17099538	*GRIN2A*	rs7205594	*NR3C1*	rs17339831	
AKAP6	rs17414154	*GRIN2A*	rs7499321	*NR3C1*	rs17342167	
AKAP6	rs17484233	*GRIN2A*	rs8044064	*NR3C1*	rs17346995	
AKAP6	rs1805261	*GRIN2A*	rs8045712	*NR3C1*	rs17348257	
AKAP6	rs1885721	*GRIN2A*	rs8055598	*NR3C1*	rs17348488	
AKAP6	rs1950702	*GRIN2A*	rs8060093	*NR3C1*	rs17401682	
AKAP6	rs1956210	*GRIN2A*	rs8060992	*NR3C1*	rs181786	
AKAP6	rs1957015	*GRIN2A*	rs837692	*NR3C1*	rs184458	
AKAP6	rs1957030	*GRIN2A*	rs837694	*NR3C1*	rs2028833	
AKAP6	rs2300831	*GRIN2A*	rs837696	*NR3C1*	rs2398660	
AKAP6	rs2300832	*GRIN2A*	rs837698	*NR3C1*	rs247623	
AKAP6	rs2300833	*GRIN2A*	rs837703	*NR3C1*	rs2918417	
AKAP6	rs2383302	*GRIN2A*	rs844395	*NR3C1*	rs2963154	
AKAP6	rs2383340	*GRIN2A*	rs884918	*NR3C1*	rs325258	
AKAP6	rs4981990	*GRIN2A*	rs971035	*NR3C1*	rs325262	
AKAP6	rs4981991	*GRIN2A*	rs971036	*NR3C1*	rs431647	
AKAP6	rs6571548	*GRIN2A*	rs9922383	*NR3C1*	rs4912927	
AKAP6	rs7146377	*GRIN2A*	rs9922871	*NR3C1*	rs4912933	
AKAP6	rs7146681	*GRIN2A*	rs9924226	*NR3C1*	rs6580287	
AKAP6	rs7146994	*GRIN2A*	rs9932138	*NR3C1*	rs6859024	
AKAP6	rs7149335	*GRIN2A*	rs9933832	*NR3C1*	rs6869469	
AKAP6	rs7151141	*GRIN2B*	rs10492132	*NR3C1*	rs6871843	
AKAP6	rs7151681	*GRIN2B*	rs10492134	*NR3C1*	rs6879079	
AKAP6	rs7159519	*GRIN2B*	rs10492136	*NR3C1*	rs6898504	
AKAP6	rs7160156	*GRIN2B*	rs10492141	*NR3C1*	rs7714402	
AKAP6	rs7160301	*GRIN2B*	rs10492142	*NR3C1*	rs7719351	
AKAP6	rs8004777	*GRIN2B*	rs10772694	*NR3C1*	rs7727865	
AKAP6	rs8016916	*GRIN2B*	rs10845809	*NR3C1*	rs7728928	
AKAP6	rs8021057	*GRIN2B*	rs10845814	*NR3C1*	rs867924	
AKAP6	rs926771	*GRIN2B*	rs10845826	*NR3C1*	rs9285657	
AKAP6	rs9322889	*GRIN2B*	rs10845831	*NR3C1*	rs953723	
AKAP6	rs9322896	*GRIN2B*	rs10845838	*NR3C1*	rs17403174	
AKAP6	rs9322899	*GRIN2B*	rs10845852	*NR3C1*	rs17403503	
AKAP6	rs941746	*GRIN2B*	rs10845856	*NR3C1*	rs17413134	
AKAP6	rs9635189	*GRIN2B*	rs10845858	*NR3C1*	rs17414396	
AKAP7	rs1190788	*GRIN2B*	rs10845861	*NR3C2*	rs10010766	
AKAP7	rs1190791	*GRIN2B*	rs10845863	*NR3C2*	rs10031194	
AKAP7	rs1190806	*GRIN2B*	rs11055556	*NR3C2*	rs10032020	
AKAP7	rs11965549	*GRIN2B*	rs11055557	*NR3C2*	rs1040288	
AKAP7	rs1534624	*GRIN2B*	rs11055593	*NR3C2*	rs10434100	
AKAP7	rs17182683	*GRIN2B*	rs11055594	*NR3C2*	rs10519951	
AKAP7	rs1729549	*GRIN2B*	rs11055597	*NR3C2*	rs10519952	
AKAP7	rs1746469	*GRIN2B*	rs11055621	*NR3C2*	rs10519958	

AKAP7	rs17573187	*GRIN2B*	rs11055626	*NR3C2*	rs10519963	
AKAP7	rs17787339	*GRIN2B*	rs11055628	*NR3C2*	rs11099678	
AKAP7	rs1881573	*GRIN2B*	rs11055664	*NR3C2*	rs11099681	
AKAP7	rs2204732	*GRIN2B*	rs11055670	*NR3C2*	rs11099694	
AKAP7	rs3777478	*GRIN2B*	rs11055681	*NR3C2*	rs11725509	
AKAP7	rs3777483	*GRIN2B*	rs11055699	*NR3C2*	rs11737660	
AKAP7	rs3777486	*GRIN2B*	rs11611183	*NR3C2*	rs11936376	
AKAP7	rs3777489	*GRIN2B*	rs12305339	*NR3C2*	rs13116332	
AKAP7	rs3903759	*GRIN2B*	rs12313659	*NR3C2*	rs13116347	
AKAP7	rs6917207	*GRIN2B*	rs12317299	*NR3C2*	rs13133240	
AKAP7	rs6937607	*GRIN2B*	rs12371702	*NR3C2*	rs13137823	
AKAP7	rs6942184	*GRIN2B*	rs12422385	*NR3C2*	rs13142954	
AKAP7	rs9321275	*GRIN2B*	rs12809496	*NR3C2*	rs1403142	
AKAP8	rs12611023	*GRIN2B*	rs1362816	*NR3C2*	rs1403143	
AKAP9	rs10234071	*GRIN2B*	rs16909348	*NR3C2*	rs1512335	
AKAP9	rs10281556	*GRIN2B*	rs16909619	*NR3C2*	rs1512342	
AKAP9	rs10488509	*GRIN2B*	rs17220663	*NR3C2*	rs1512343	
AKAP9	rs12530646	*GRIN2B*	rs172677	*NR3C2*	rs16998733	
AKAP9	rs12531366	*GRIN2B*	rs17760842	*NR3C2*	rs17024387	
AKAP9	rs12536529	*GRIN2B*	rs17760889	*NR3C2*	rs17024443	
AKAP9	rs12537846	*GRIN2B*	rs17833759	*NR3C2*	rs17024482	
AKAP9	rs12540055	*GRIN2B*	rs17833849	*NR3C2*	rs1858416	
AKAP9	rs13231578	*GRIN2B*	rs17833908	*NR3C2*	rs1879827	
AKAP9	rs2282971	*GRIN2B*	rs1805199	*NR3C2*	rs1994624	
AKAP9	rs2299231	*GRIN2B*	rs1805476	*NR3C2*	rs2070949	
AKAP9	rs2299233	*GRIN2B*	rs1805490	*NR3C2*	rs2070950	
AKAP9	rs3753107	*GRIN2B*	rs1805525	*NR3C2*	rs2070951	
AKAP9	rs6942649	*GRIN2B*	rs1805543	*NR3C2*	rs2293162	
AKAP9	rs7785095	*GRIN2B*	rs1805546	*NR3C2*	rs2883930	
CAMK2A	rs1432833	*GRIN2B*	rs1805553	*NR3C2*	rs3846307	
CAMK2A	rs17111079	*GRIN2B*	rs1806191	*NR3C2*	rs3846329	
CAMK2A	rs3822606	*GRIN2B*	rs2160402	*NR3C2*	rs3887856	
CAMK2A	rs4958467	*GRIN2B*	rs2193150	*NR3C2*	rs3910044	
CAMK2A	rs4958468	*GRIN2B*	rs2193151	*NR3C2*	rs3910046	
CAMK2A	rs4958469	*GRIN2B*	rs219872	*NR3C2*	rs3910054	
CAMK2A	rs874083	*GRIN2B*	rs219886	*NR3C2*	rs4027073	
CAMK2B	rs1890135	*GRIN2B*	rs219904	*NR3C2*	rs4260521	
CAMK2B	rs3934888	*GRIN2B*	rs219909	*NR3C2*	rs4835128	
CAMK2	rs10000775	*GRIN2B*	rs219920	*NR3C2*	rs4835488	

D						
CAMK2 D	rs10009286	*GRIN2B*	rs219921	*NR3C2*	rs4835493	
CAMK2 D	rs10023113	*GRIN2B*	rs220578	*NR3C2*	rs4835514	
CAMK2 D	rs10461194	*GRIN2B*	rs2268129	*NR3C2*	rs5522	
CAMK2 D	rs1047187	*GRIN2B*	rs2268132	*NR3C2*	rs5525	
CAMK2 D	rs10488891	*GRIN2B*	rs2268133	*NR3C2*	rs6535577	
CAMK2 D	rs10488894	*GRIN2B*	rs2268135	*NR3C2*	rs6535584	
CAMK2 D	rs11098193	*GRIN2B*	rs2284410	*NR3C2*	rs6535594	
CAMK2 D	rs11946664	*GRIN2B*	rs2284415	*NR3C2*	rs6810951	
CAMK2 D	rs12508566	*GRIN2B*	rs2284428	*NR3C2*	rs6811001	
CAMK2 D	rs12644009	*GRIN2B*	rs2300241	*NR3C2*	rs6828665	
CAMK2 D	rs13144613	*GRIN2B*	rs2300242	*NR3C2*	rs6849903	
CAMK2 D	rs17046126	*GRIN2B*	rs2300245	*NR3C2*	rs7687754	
CAMK2 D	rs17446453	*GRIN2B*	rs2300266	*NR3C2*	rs7693077	
CAMK2 D	rs17592426	*GRIN2B*	rs2300268	*NR3C2*	rs9307847	
CAMK2 D	rs17593296	*GRIN2B*	rs4140764	*NR3C2*	rs17483833	
CAMK2 D	rs17629820	*GRIN2B*	rs4763358	*NR3C2*	rs17484259	
CAMK2 D	rs17630012	*GRIN2B*	rs7295850	*NR3C2*	rs17484678	
CAMK2 D	rs17630328	*GRIN2B*	rs7296762	*NR3C2*	rs17484873	
CAMK2 D	rs17630766	*GRIN2B*	rs7311519	*NR3C2*	rs17581311	
CAMK2 D	rs1859148	*GRIN2B*	rs7313149	*NR3C2*	rs17581884	
CAMK2 D	rs1859150	*GRIN2B*	rs7314871	*NR3C2*	rs17620330	
CAMK2 D	rs1859152	*GRIN2B*	rs7952915	*NR3C2*	rs17620822	
CAMK2 D	rs2107172	*GRIN2B*	rs7970144	*PALM2-AKAP2*	rs1010788	
CAMK2 D	rs2158197	*GRIN2B*	rs7970177	*PALM2-AKAP2*	rs10114649	
CAMK2 D	rs2214392	*GRIN2B*	rs7970407	*PALM2-AKAP2*	rs10512288	
CAMK2 D	rs3733619	*GRIN2B*	rs7977831	*PALM2-AKAP2*	rs10816924	

CAMK2D	rs3815072	*GRIN2B*	rs890	*PALM2-AKAP2*	rs10980164	
CAMK2D	rs4240286	*GRIN2B*	rs918168	*PALM2-AKAP2*	rs10980170	
CAMK2D	rs4834340	*GRIN2B*	rs9971835	*PALM2-AKAP2*	rs10980179	
CAMK2D	rs4834347	*GRIN2C*	rs690046	*PALM2-AKAP2*	rs10980190	
CAMK2D	rs6533700	*GRIN2C*	rs690578	*PALM2-AKAP2*	rs10980194	
CAMK2D	rs6821520	*GRIN2D*	rs1654670	*PALM2-AKAP2*	rs10980195	
CAMK2D	rs6836139	*GRIN2D*	rs2380121	*PALM2-AKAP2*	rs10980207	
CAMK2D	rs7656183	*GRIN2D*	rs276711	*PALM2-AKAP2*	rs10980210	
CAMK2D	rs7675804	*GRIN2D*	rs892200	*PALM2-AKAP2*	rs10980231	
CAMK2D	rs7676842	*GRIN3A*	rs10115450	*PALM2-AKAP2*	rs11790833	
CAMK2D	rs7684265	*GRIN3A*	rs10512287	*PALM2-AKAP2*	rs16914725	
CAMK2D	rs993506	*GRIN3A*	rs10760802	*PALM2-AKAP2*	rs16914837	
CAMK2G	rs11258747	*GRIN3A*	rs10819954	*PALM2-AKAP2*	rs17806457	
CAMK2G	rs12572101	*GRIN3A*	rs10819959	*PALM2-AKAP2*	rs2017392	
CAMK2G	rs17631000	*GRIN3A*	rs12353519	*PALM2-AKAP2*	rs2017393	
CAMK2G	rs2664280	*GRIN3A*	rs12685902	*PALM2-AKAP2*	rs260214	
CAMK2G	rs7080350	*GRIN3A*	rs12708308	*PALM2-AKAP2*	rs260221	
CREB1	rs2551920	*GRIN3A*	rs1323412	*PALM2-AKAP2*	rs491356	
CREB1	rs2709393	*GRIN3A*	rs1323420	*PALM2-AKAP2*	rs491730	
CREB1	rs6785	*GRIN3A*	rs1323421	*PALM2-AKAP2*	rs496709	
CRHBP	rs7721799	*GRIN3A*	rs1323430	*PALM2-AKAP2*	rs4978870	
CRHR1	rs17689471	*GRIN3A*	rs1407877	*PALM2-AKAP2*	rs4978875	
CRHR1	rs17689824	*GRIN3A*	rs1535633	*PALM2-AKAP2*	rs525681	
CRHR1	rs17689882	*GRIN3A*	rs16920504	*PALM2-AKAP2*	rs545955	
CRHR1	rs17762769	*GRIN3A*	rs17189275	*PALM2-AKAP2*	rs6477737	
CRHR1	rs17763104	*GRIN3A*	rs17189604	*PALM2-AKAP2*	rs6477740	
CRHR1	rs241031	*GRIN3A*	rs17197320	*PALM2-AKAP2*	rs7026192	

CRHR1	rs241033	*GRIN3A*	rs17774987	*PALM2-AKAP2*	rs7028076	
CRHR1	rs242939	*GRIN3A*	rs1853447	*PALM2-AKAP2*	rs7038399	
CRHR1	rs413778	*GRIN3A*	rs2146801	*PALM2-AKAP2*	rs7847333	
CRHR1	rs422112	*GRIN3A*	rs2417286	*PALM2-AKAP2*	rs7847354	
CRHR1	rs4277389	*GRIN3A*	rs2485523	*PALM2-AKAP2*	rs7861341	
CRHR1	rs453997	*GRIN3A*	rs2506351	*PALM2-AKAP2*	rs7863709	
CRHR1	rs4640231	*GRIN3A*	rs2506362	*PALM2-AKAP2*	rs7865972	
CRHR1	rs8072451	*GRIN3A*	rs2506363	*PALM2-AKAP2*	rs914356	
CRHR2	rs1003929	*GRIN3A*	rs301434	*PALM2-AKAP2*	rs914358	
CRHR2	rs2190242	*GRIN3A*	rs4742823	*POMC*	rs6545975	
CRHR2	rs2240404	*GRIN3A*	rs7023041	*POMC*	rs7565427	
CRHR2	rs2267710	*GRIN3A*	rs7849782	*POMC*	rs7565877	
CRHR2	rs255121	*GRM1*	rs1014878	*POMC*	rs934778	
CRHR2	rs4723003	*GRM1*	rs10484860	*PRKACB*	rs1016379	
CRHR2	rs929377	*GRM1*	rs10872583	*PRKACB*	rs10493750	
CRHR2	rs973002	*GRM1*	rs11155451	*PRKACB*	rs11163905	
CRHR2	rs975537	*GRM1*	rs12202357	*PRKACB*	rs12405120	
DICER1	rs10498642	*GRM1*	rs1331639	*PRKACB*	rs12723299	
DICER1	rs6575499	*GRM1*	rs1331645	*PRKACB*	rs2134646	
DLG4	rs2239340	*GRM1*	rs1543349	*PRKACB*	rs2134648	
FKBP5	rs1475774	*GRM1*	rs17075834	*PRKACB*	rs2139931	
FKBP5	rs16878806	*GRM1*	rs2268663	*PRKACB*	rs2642186	
FKBP5	rs16879378	*GRM1*	rs2268666	*PRKACB*	rs589373	
FKBP5	rs2766533	*GRM1*	rs2300620	*PRKACB*	rs603939	
FKBP5	rs2817032	*GRM1*	rs2300631	*PRKACB*	rs6661411	
FKBP5	rs4713899	*GRM1*	rs2814863	*PRKACB*	rs6701486	
FKBP5	rs755658	*GRM1*	rs362818	*PRKAR1A*	rs16972996	
FKBP5	rs9368881	*GRM1*	rs362840	*PRKAR1A*	rs2952275	
FKBP5	rs9462104	*GRM1*	rs362842	*PRKAR1A*	rs4265886	
GLS	rs3771316	*GRM1*	rs362844	*PRKAR1A*	rs8076465	
GLS	rs6434429	*GRM1*	rs362845	*PRKAR2A*	rs9859473	
GLS	rs867637	*GRM1*	rs362847	*PRKAR2B*	rs10272371	
GRIA1	rs10035262	*GRM1*	rs362848	*PRKAR2B*	rs12154324	
GRIA1	rs10035289	*GRM1*	rs362851	*PRKAR2B*	rs13224682	
GRIA1	rs10060095	*GRM1*	rs362852	*PRKAR2B*	rs17153823	
GRIA1	rs10070447	*GRM1*	rs362855	*PRKAR2B*	rs17340577	
GRIA1	rs10073988	*GRM1*	rs362894	*PRKAR2B*	rs17425839	
GRIA1	rs10477084	*GRM1*	rs362949	*PRKAR2B*	rs2049900	
GRIA1	rs10515691	*GRM1*	rs3804295	*PRKAR2B*	rs2251838	

GRIA1	rs10515692	*GRM1*	rs4896861	*PRKAR2B*	rs6960708	
GRIA1	rs11167640	*GRM1*	rs56566	*PRKAR2B*	rs9656135	
GRIA1	rs11738883	*GRM1*	rs6570746	*PRKCA*	rs10451280	
GRIA1	rs11739206	*GRM1*	rs6570747	*PRKCA*	rs10491200	
GRIA1	rs11742223	*GRM1*	rs6570757	*PRKCA*	rs12450569	
GRIA1	rs11747180	*GRM1*	rs6914813	*PRKCA*	rs12600582	
GRIA1	rs11955628	*GRM1*	rs7741271	*PRKCA*	rs12601850	
GRIA1	rs12152942	*GRM1*	rs7743911	*PRKCA*	rs1355984	
GRIA1	rs12153489	*GRM1*	rs7750018	*PRKCA*	rs16959216	
GRIA1	rs12153765	*GRM1*	rs7772919	*PRKCA*	rs16959229	
GRIA1	rs12515520	*GRM1*	rs863820	*PRKCA*	rs16959236	
GRIA1	rs12515561	*GRM1*	rs9322052	*PRKCA*	rs16959238	
GRIA1	rs12515563	*GRM1*	rs9390374	*PRKCA*	rs16959447	
GRIA1	rs13354399	*GRM1*	rs9497485	*PRKCA*	rs16959450	
GRIA1	rs1363677	*GRM1*	rs9497521	*PRKCA*	rs16959508	
GRIA1	rs1461232	*GRM1*	rs979775	*PRKCA*	rs16959510	
GRIA1	rs1463747	*GRM3*	rs10238436	*PRKCA*	rs16959514	
GRIA1	rs1493389	*GRM3*	rs10952891	*PRKCA*	rs16959593	
GRIA1	rs1493395	*GRM3*	rs12704286	*PRKCA*	rs16959604	
GRIA1	rs1493403	*GRM3*	rs12704289	*PRKCA*	rs16959714	
GRIA1	rs1540383	*GRM3*	rs16888204	*PRKCA*	rs16959880	
GRIA1	rs1552837	*GRM3*	rs16888210	*PRKCA*	rs16960077	
GRIA1	rs17096210	*GRM3*	rs17160978	*PRKCA*	rs16960114	
GRIA1	rs17114692	*GRM3*	rs17161024	*PRKCA*	rs17643302	
GRIA1	rs17114703	*GRM3*	rs17697445	*PRKCA*	rs17686174	
GRIA1	rs17114836	*GRM3*	rs17697609	*PRKCA*	rs17686720	
GRIA1	rs17114975	*GRM3*	rs2158786	*PRKCA*	rs17688050	
GRIA1	rs17115017	*GRM3*	rs2189812	*PRKCA*	rs17710992	
GRIA1	rs17115020	*GRM3*	rs2237553	*PRKCA*	rs17771145	
GRIA1	rs17115039	*GRM3*	rs2237554	*PRKCA*	rs227915	
GRIA1	rs17356099	*GRM3*	rs2237559	*PRKCA*	rs228884	
GRIA1	rs17515380	*GRM3*	rs2237563	*PRKCA*	rs3803821	
GRIA1	rs17518831	*GRM3*	rs2299221	*PRKCA*	rs3803822	
GRIA1	rs17519054	*GRM3*	rs2299224	*PRKCA*	rs3888658	
GRIA1	rs17519558	*GRM3*	rs274621	*PRKCA*	rs4381631	
GRIA1	rs17519656	*GRM3*	rs2888551	*PRKCA*	rs4536508	
GRIA1	rs17523221	*GRM3*	rs4294091	*PRKCA*	rs4622543	
GRIA1	rs17525192	*GRM3*	rs6955452	*PRKCA*	rs4630584	
GRIA1	rs17591636	*GRM3*	rs6955917	*PRKCA*	rs4630585	
GRIA1	rs1873906	*GRM3*	rs723631	*PRKCA*	rs4644888	
GRIA1	rs1973372	*GRM3*	rs7788115	*PRKCA*	rs4791051	
GRIA1	rs2216649	*GRM3*	rs7804907	*PRKCA*	rs6504424	
GRIA1	rs2452801	*GRM3*	rs802437	*PRKCA*	rs6504425	
GRIA1	rs2910258	*GRM3*	rs802439	*PRKCA*	rs6504441	

GRIA1	rs2963957	*GRM3*	rs802441	*PRKCA*	rs6967	
GRIA1	rs2964018	*GRM3*	rs802459	*PRKCA*	rs7207097	
GRIA1	rs4273649	*GRM3*	rs802467	*PRKCA*	rs7207345	
GRIA1	rs4336409	*GRM4*	rs1109654	*PRKCA*	rs7211524	
GRIA1	rs4424038	*GRM4*	rs13196449	*PRKCA*	rs7217618	
GRIA1	rs4958351	*GRM4*	rs2499690	*PRKCA*	rs7219499	
GRIA1	rs4958676	*GRM4*	rs2499714	*PRKCA*	rs7221805	
GRIA1	rs524905	*GRM4*	rs7751882	*PRKCA*	rs7225164	
GRIA1	rs541518	*GRM4*	rs9368793	*PRKCA*	rs7405806	
GRIA1	rs545098	*GRM4*	rs937039	*PRKCA*	rs7502206	
GRIA1	rs574071	*GRM4*	rs9380406	*PRKCA*	rs8066055	
GRIA1	rs6580024	*GRM4*	rs9394187	*PRKCA*	rs8068129	
GRIA1	rs6580032	*GRM4*	rs9469693	*PRKCA*	rs8069142	
GRIA1	rs6580039	*GRM5*	rs1000061	*PRKCA*	rs8069859	
GRIA1	rs6870761	*GRM5*	rs10501694	*PRKCA*	rs8075066	
GRIA1	rs6889794	*GRM5*	rs10501696	*PRKCA*	rs8080727	
GRIA1	rs6889909	*GRM5*	rs10741500	*PRKCA*	rs956952	
GRIA1	rs6891142	*GRM5*	rs10765186	*PRKCA*	rs980346	
GRIA1	rs7708391	*GRM5*	rs10831251	*PRKCA*	rs9891403	
GRIA1	rs7709884	*GRM5*	rs11018448	*PRKCA*	rs9893560	
GRIA1	rs7711124	*GRM5*	rs11018463	*PRKCA*	rs9895580	
GRIA1	rs7714428	*GRM5*	rs11020781	*PRKCA*	rs9896549	
GRIA1	rs7727515	*GRM5*	rs11020938	*PRKCA*	rs9896922	
GRIA1	rs7735696	*GRM5*	rs11021034	*PRKCA*	rs9897124	
GRIA1	rs778816	*GRM5*	rs11021035	*PRKCA*	rs9898205	
GRIA1	rs812389	*GRM5*	rs11021132	*PRKCA*	rs9900205	
GRIA1	rs889060	*GRM5*	rs11021266	*PRKCA*	rs9900353	
GRIA1	rs9324751	*GRM5*	rs1116378	*PRKCA*	rs990082	
GRIA1	rs9686702	*GRM5*	rs11606431	*PRKCA*	rs990083	
GRIA2	rs17035916	*GRM5*	rs11828996	*PRKCA*	rs9900927	
GRIA2	rs17035959	*GRM5*	rs12290410	*PRKCA*	rs9901804	
GRIA2	rs4392549	*GRM5*	rs12787863	*PRKCA*	rs9903792	
GRIA3	rs10521718	*GRM5*	rs12802146	*PRKCA*	rs9904266	
GRIA3	rs11260421	*GRM5*	rs1391873	*PRKCA*	rs9908167	
GRIA3	rs12396914	*GRM5*	rs1391874	*PRKCA*	rs9908953	
GRIA3	rs12557782	*GRM5*	rs1391875	*PRKCA*	rs9911259	
GRIA3	rs2157272	*GRM5*	rs1391878	*PRKCA*	rs9912468	
GRIA3	rs2353721	*GRM5*	rs1391954	*PRKCA*	rs9915719	
GRIA3	rs4825476	*GRM5*	rs1499026	*PRKCB*	rs1013316	
GRIA3	rs4825836	*GRM5*	rs1499030	*PRKCB*	rs1014632	
GRIA3	rs498126	*GRM5*	rs1499185	*PRKCB*	rs10852256	
GRIA3	rs503118	*GRM5*	rs1499188	*PRKCB*	rs10852263	
GRIA3	rs550640	*GRM5*	rs1508688	*PRKCB*	rs11074583	
GRIA3	rs5910006	*GRM5*	rs16914662	*PRKCB*	rs11074598	

GRIA3	rs5911548	*GRM5*	rs16914739	*PRKCB*	rs120908	
GRIA3	rs5911590	*GRM5*	rs16915130	*PRKCB*	rs12932420	
GRIA3	rs5911622	*GRM5*	rs17184781	*PRKCB*	rs1468130	
GRIA3	rs593293	*GRM5*	rs1846442	*PRKCB*	rs1557671	
GRIA3	rs5956542	*GRM5*	rs1846443	*PRKCB*	rs16940401	
GRIA3	rs637574	*GRM5*	rs1903848	*PRKCB*	rs16973180	
GRIA3	rs652582	*GRM5*	rs1903851	*PRKCB*	rs16973277	
GRIA3	rs6608067	*GRM5*	rs2047512	*PRKCB*	rs174828	
GRIA3	rs6608068	*GRM5*	rs2221108	*PRKCB*	rs17809041	
GRIA3	rs6608087	*GRM5*	rs2387412	*PRKCB*	rs17810486	
GRIA3	rs687577	*GRM5*	rs2892290	*PRKCB*	rs195996	
GRIA3	rs695214	*GRM5*	rs308879	*PRKCB*	rs196004	
GRIA3	rs7052603	*GRM5*	rs308880	*PRKCB*	rs198142	
GRIA3	rs7058062	*GRM5*	rs4107263	*PRKCB*	rs198182	
GRIA3	rs762993	*GRM5*	rs4121738	*PRKCB*	rs198200	
GRIA3	rs970665	*GRM5*	rs4488199	*PRKCB*	rs198201	
GRIA3	rs970666	*GRM5*	rs4604887	*PRKCB*	rs198202	
GRIA3	rs989638	*GRM5*	rs4604888	*PRKCB*	rs1989647	
GRIA3	rs989639	*GRM5*	rs4753731	*PRKCB*	rs2188355	
GRIA4	rs10502061	*GRM5*	rs640211	*PRKCB*	rs2188356	
GRIA4	rs10502062	*GRM5*	rs6483397	*PRKCB*	rs2188360	
GRIA4	rs10502063	*GRM5*	rs6483399	*PRKCB*	rs2238493	
GRIA4	rs10750733	*GRM5*	rs7121916	*PRKCB*	rs2340988	
GRIA4	rs10791767	*GRM5*	rs719182	*PRKCB*	rs2470688	
GRIA4	rs10791773	*GRM5*	rs723859	*PRKCB*	rs2560402	
GRIA4	rs10895849	*GRM5*	rs7396702	*PRKCB*	rs2560406	
GRIA4	rs10895850	*GRM5*	rs7396713	*PRKCB*	rs375624	
GRIA4	rs10895875	*GRM5*	rs7483940	*PRKCB*	rs377133	
GRIA4	rs11226828	*GRM5*	rs7935922	*PRKCB*	rs3785380	
GRIA4	rs11226856	*GRM5*	rs7943357	*PRKCB*	rs3785394	
GRIA4	rs1267444	*GRM5*	rs8181526	*PRKCB*	rs3785403	
GRIA4	rs17104596	*GRM5*	rs904462	*PRKCB*	rs381901	
GRIA4	rs17104715	*GRM5*	rs961689	*PRKCB*	rs3826260	
GRIA4	rs17104747	*GRM5*	rs973716	*PRKCB*	rs400066	
GRIA4	rs17104835	*GRM5*	rs982010	*PRKCB*	rs405322	
GRIA4	rs17482908	*GRM5*	rs992259	*PRKCB*	rs4238948	
GRIA4	rs1938961	*GRM6*	rs17078852	*PRKCB*	rs437545	
GRIA4	rs1939148	*GRM6*	rs17078862	*PRKCB*	rs4787291	
GRIA4	rs1940964	*GRM6*	rs2645341	*PRKCB*	rs4788423	
GRIA4	rs2155045	*GRM7*	rs10222587	*PRKCB*	rs6497704	
GRIA4	rs2186598	*GRM7*	rs10490857	*PRKCB*	rs7192816	
GRIA4	rs495498	*GRM7*	rs10510353	*PRKCB*	rs7196543	
GRIA4	rs505829	*GRM7*	rs10510354	*PRKCB*	rs7197785	
GRIA4	rs515803	*GRM7*	rs10510356	*PRKCB*	rs7404005	

GRIA4	rs545184	*GRM7*	rs10510364	*PRKCB*	rs8047317	
GRIA4	rs552767	*GRM7*	rs10510370	*PRKCB*	rs8051531	
GRIA4	rs601480	*GRM7*	rs1066658	*PRKCB*	rs8051700	
GRIA4	rs602098	*GRM7*	rs1083801	*PRKCB*	rs8055831	
GRIA4	rs610160	*GRM7*	rs1106486	*PRKCB*	rs8056879	
GRIA4	rs622178	*GRM7*	rs11131064	*PRKCB*	rs8063762	
GRIA4	rs631002	*GRM7*	rs11131069	*PRKCB*	rs886114	
GRIA4	rs632100	*GRM7*	rs11131078	*PRKCB*	rs9806904	
GRIA4	rs638099	*GRM7*	rs1143740	*PRKCB*	rs9925126	
GRIA4	rs646007	*GRM7*	rs1155966	*PRKCB*	rs9930905	
GRIA4	rs658965	*GRM7*	rs11706732	*PRKCB*	rs9938298	
GRIA4	rs661329	*GRM7*	rs11708019	*PRKCB*	rs9940479	
GRIA4	rs668120	*GRM7*	rs11710946	*PRKCD*	rs13084863	
GRIA4	rs682708	*GRM7*	rs11711367	*PRKCD*	rs1483185	
GRIA4	rs7102566	*GRM7*	rs11713266	*PRKCD*	rs17052826	
GRIA4	rs7122727	*GRM7*	rs11716647	*PRKCE*	rs10166692	
GRIA4	rs748353	*GRM7*	rs11717471	*PRKCE*	rs10179954	
GRIA4	rs994575	*GRM7*	rs11717750	*PRKCE*	rs10188306	
GRIK1	rs1016700	*GRM7*	rs11915789	*PRKCE*	rs10189339	
GRIK1	rs1029224	*GRM7*	rs11918634	*PRKCE*	rs10201978	
GRIK1	rs11088125	*GRM7*	rs12489041	*PRKCE*	rs10202504	
GRIK1	rs11701584	*GRM7*	rs12494654	*PRKCE*	rs1020445	
GRIK1	rs12627201	*GRM7*	rs12497688	*PRKCE*	rs10210199	
GRIK1	rs16985084	*GRM7*	rs12637466	*PRKCE*	rs10211547	
GRIK1	rs1735072	*GRM7*	rs13070476	*PRKCE*	rs10490341	
GRIK1	rs2142161	*GRM7*	rs13072518	*PRKCE*	rs10490342	
GRIK1	rs2244881	*GRM7*	rs1351938	*PRKCE*	rs10865208	
GRIK1	rs2249140	*GRM7*	rs1356268	*PRKCE*	rs11125038	
GRIK1	rs2252974	*GRM7*	rs1485167	*PRKCE*	rs11125041	
GRIK1	rs2268206	*GRM7*	rs1485171	*PRKCE*	rs11125048	
GRIK1	rs2832417	*GRM7*	rs1485172	*PRKCE*	rs11690929	
GRIK1	rs2832442	*GRM7*	rs1485174	*PRKCE*	rs11691705	
GRIK1	rs2832445	*GRM7*	rs1499079	*PRKCE*	rs11903923	
GRIK1	rs2832448	*GRM7*	rs1499161	*PRKCE*	rs12464563	
GRIK1	rs2832449	*GRM7*	rs1499199	*PRKCE*	rs12465869	
GRIK1	rs2832457	*GRM7*	rs1499204	*PRKCE*	rs12616328	
GRIK1	rs2832464	*GRM7*	rs1504047	*PRKCE*	rs12712963	
GRIK1	rs2832465	*GRM7*	rs1532544	*PRKCE*	rs13003856	
GRIK1	rs2832473	*GRM7*	rs1605705	*PRKCE*	rs13016869	
GRIK1	rs2832477	*GRM7*	rs162802	*PRKCE*	rs13036100	
GRIK1	rs2832488	*GRM7*	rs17046231	*PRKCE*	rs13404973	
GRIK1	rs2832489	*GRM7*	rs17046322	*PRKCE*	rs13405086	
GRIK1	rs2832493	*GRM7*	rs17047073	*PRKCE*	rs13424270	
GRIK1	rs2832494	*GRM7*	rs17047149	*PRKCE*	rs1375054	

GRIK1	rs307943	*GRM7*	rs17047199	*PRKCE*	rs1448219	
GRIK1	rs363442	*GRM7*	rs17047734	*PRKCE*	rs1522987	
GRIK1	rs363457	*GRM7*	rs17234886	*PRKCE*	rs17033965	
GRIK1	rs363495	*GRM7*	rs17235018	*PRKCE*	rs17034571	
GRIK1	rs363497	*GRM7*	rs17288561	*PRKCE*	rs17034573	
GRIK1	rs363501	*GRM7*	rs17655560	*PRKCE*	rs17034576	
GRIK1	rs363515	*GRM7*	rs17664833	*PRKCE*	rs17034610	
GRIK1	rs363516	*GRM7*	rs17665113	*PRKCE*	rs17034641	
GRIK1	rs363526	*GRM7*	rs17673467	*PRKCE*	rs17034719	
GRIK1	rs363528	*GRM7*	rs17694650	*PRKCE*	rs17799476	
GRIK1	rs363529	*GRM7*	rs17697853	*PRKCE*	rs1868271	
GRIK1	rs363566	*GRM7*	rs17717959	*PRKCE*	rs1868272	
GRIK1	rs388700	*GRM7*	rs17723289	*PRKCE*	rs1868273	
GRIK1	rs398989	*GRM7*	rs17751439	*PRKCE*	rs1868390	
GRIK1	rs402280	*GRM7*	rs17824866	*PRKCE*	rs1868392	
GRIK1	rs406191	*GRM7*	rs1857697	*PRKCE*	rs1966813	
GRIK1	rs408151	*GRM7*	rs1872394	*PRKCE*	rs1982366	
GRIK1	rs408302	*GRM7*	rs1872397	*PRKCE*	rs2005271	
GRIK1	rs415336	*GRM7*	rs1872400	*PRKCE*	rs2029087	
GRIK1	rs441426	*GRM7*	rs1876614	*PRKCE*	rs2034360	
GRIK1	rs453238	*GRM7*	rs1878164	*PRKCE*	rs2053797	
GRIK1	rs455354	*GRM7*	rs1909397	*PRKCE*	rs2204204	
GRIK1	rs455804	*GRM7*	rs1963265	*PRKCE*	rs2218660	
GRIK1	rs456784	*GRM7*	rs2069062	*PRKCE*	rs2255091	
GRIK1	rs458470	*GRM7*	rs2116711	*PRKCE*	rs2255094	
GRIK1	rs459617	*GRM7*	rs2133450	*PRKCE*	rs2278777	
GRIK1	rs460583	*GRM7*	rs2291867	*PRKCE*	rs2285024	
GRIK1	rs462393	*GRM7*	rs2324122	*PRKCE*	rs2345178	
GRIK1	rs463021	*GRM7*	rs2875257	*PRKCE*	rs2345625	
GRIK1	rs463360	*GRM7*	rs329044	*PRKCE*	rs2345955	
GRIK1	rs463479	*GRM7*	rs332938	*PRKCE*	rs2594491	
GRIK1	rs464576	*GRM7*	rs340657	*PRKCE*	rs2595213	
GRIK1	rs464668	*GRM7*	rs340659	*PRKCE*	rs2595222	
GRIK1	rs467028	*GRM7*	rs3749380	*PRKCE*	rs2711286	
GRIK1	rs467155	*GRM7*	rs3749448	*PRKCE*	rs2711292	
GRIK1	rs7277926	*GRM7*	rs3792452	*PRKCE*	rs2711293	
GRIK1	rs7283968	*GRM7*	rs3792460	*PRKCE*	rs2711295	
GRIK1	rs932250	*GRM7*	rs3804850	*PRKCE*	rs281469	
GRIK2	rs10457942	*GRM7*	rs3804857	*PRKCE*	rs281471	
GRIK2	rs12182308	*GRM7*	rs3804867	*PRKCE*	rs281472	
GRIK2	rs12192251	*GRM7*	rs3804883	*PRKCE*	rs281508	
GRIK2	rs12193068	*GRM7*	rs3804886	*PRKCE*	rs2881068	
GRIK2	rs13195038	*GRM7*	rs3804904	*PRKCE*	rs3754566	
GRIK2	rs13203156	*GRM7*	rs3804906	*PRKCE*	rs3754568	

GRIK2	rs1338167	*GRM7*	rs3804928	*PRKCE*	rs3768747	
GRIK2	rs1361450	*GRM7*	rs3804945	*PRKCE*	rs3768751	
GRIK2	rs1415483	*GRM7*	rs3828472	*PRKCE*	rs3795863	
GRIK2	rs17054628	*GRM7*	rs3846161	*PRKCE*	rs3820732	
GRIK2	rs17054632	*GRM7*	rs3864076	*PRKCE*	rs3886870	
GRIK2	rs1832413	*GRM7*	rs4095095	*PRKCE*	rs4557033	
GRIK2	rs1832415	*GRM7*	rs4143516	*PRKCE*	rs4952771	
GRIK2	rs1890278	*GRM7*	rs4441639	*PRKCE*	rs4952787	
GRIK2	rs2245817	*GRM7*	rs456835	*PRKCE*	rs4952805	
GRIK2	rs2248660	*GRM7*	rs4686101	*PRKCE*	rs4953254	
GRIK2	rs2255496	*GRM7*	rs4686102	*PRKCE*	rs4953262	
GRIK2	rs2518150	*GRM7*	rs4686119	*PRKCE*	rs4953269	
GRIK2	rs2518203	*GRM7*	rs4686145	*PRKCE*	rs4953273	
GRIK2	rs2518204	*GRM7*	rs4686146	*PRKCE*	rs4953279	
GRIK2	rs2518296	*GRM7*	rs4686148	*PRKCE*	rs4953288	
GRIK2	rs2518313	*GRM7*	rs576913	*PRKCE*	rs4953290	
GRIK2	rs2579938	*GRM7*	rs6443090	*PRKCE*	rs4953305	
GRIK2	rs2749052	*GRM7*	rs6443099	*PRKCE*	rs4953321	
GRIK2	rs2749054	*GRM7*	rs6443100	*PRKCE*	rs505310	
GRIK2	rs2764226	*GRM7*	rs6764411	*PRKCE*	rs556650	
GRIK2	rs2782892	*GRM7*	rs6769814	*PRKCE*	rs563601	
GRIK2	rs2782920	*GRM7*	rs6771606	*PRKCE*	rs612717	
GRIK2	rs2786239	*GRM7*	rs6772333	*PRKCE*	rs637889	
GRIK2	rs2787566	*GRM7*	rs6775424	*PRKCE*	rs6544843	
GRIK2	rs2852571	*GRM7*	rs6777701	*PRKCE*	rs6544873	
GRIK2	rs2852584	*GRM7*	rs6777970	*PRKCE*	rs656823	
GRIK2	rs2852604	*GRM7*	rs6778030	*PRKCE*	rs666334	
GRIK2	rs2852605	*GRM7*	rs6781223	*PRKCE*	rs667747	
GRIK2	rs4112793	*GRM7*	rs6782528	*PRKCE*	rs6705717	
GRIK2	rs4839801	*GRM7*	rs6799329	*PRKCE*	rs6719779	
GRIK2	rs4840195	*GRM7*	rs6801970	*PRKCE*	rs6725257	
GRIK2	rs4840196	*GRM7*	rs6803027	*PRKCE*	rs6732900	
GRIK2	rs4840197	*GRM7*	rs6808554	*PRKCE*	rs6742742	
GRIK2	rs4840202	*GRM7*	rs6810141	*PRKCE*	rs6743119	
GRIK2	rs6905533	*GRM7*	rs712775	*PRKCE*	rs6746467	
GRIK2	rs6918619	*GRM7*	rs712777	*PRKCE*	rs6748375	
GRIK2	rs6919217	*GRM7*	rs712779	*PRKCE*	rs6750700	
GRIK2	rs6928180	*GRM7*	rs712782	*PRKCE*	rs6751349	
GRIK2	rs7749722	*GRM7*	rs712785	*PRKCE*	rs6760363	
GRIK2	rs7754131	*GRM7*	rs7340751	*PRKCE*	rs753572	
GRIK2	rs9322608	*GRM7*	rs752300	*PRKCE*	rs7557421	
GRIK2	rs9322609	*GRM7*	rs7621537	*PRKCE*	rs7558342	
GRIK2	rs9377274	*GRM7*	rs7622749	*PRKCE*	rs7563379	
GRIK2	rs9390757	*GRM7*	rs7628504	*PRKCE*	rs7573407	

GRIK2	rs9390779	*GRM7*	rs7635212	*PRKCE*	rs7577273	
GRIK2	rs9399726	*GRM7*	rs7650218	*PRKCE*	rs7582320	
GRIK2	rs9404115	*GRM7*	rs779694	*PRKCE*	rs7593255	
GRIK2	rs9404120	*GRM7*	rs779699	*PRKCE*	rs7593800	
GRIK2	rs9404125	*GRM7*	rs779705	*PRKCE*	rs7598174	
GRIK2	rs9485549	*GRM7*	rs779733	*PRKCE*	rs7602129	
GRIK2	rs9498701	*GRM7*	rs779741	*PRKCE*	rs7604415	
GRIK2	rs954765	*GRM7*	rs779742	*PRKCE*	rs878812	
GRIK3	rs1032722	*GRM7*	rs779749	*PRKCE*	rs884400	
GRIK3	rs11263956	*GRM7*	rs781393	*PRKCE*	rs935656	
GRIK3	rs1160752	*GRM7*	rs908465	*PRKCE*	rs935672	
GRIK3	rs12133182	*GRM7*	rs9311976	*PRKCE*	rs938661	
GRIK3	rs12736276	*GRM7*	rs951557	*PRKCG*	rs443239	
GRIK3	rs16823702	*GRM7*	rs9812630	*PRKCH*	rs10134093	
GRIK3	rs3738085	*GRM7*	rs9814809	*PRKCH*	rs10483742	
GRIK3	rs3767066	*GRM7*	rs9814881	*PRKCH*	rs10483743	
GRIK3	rs3767101	*GRM7*	rs9819314	*PRKCH*	rs1088673	
GRIK3	rs4652931	*GRM7*	rs9819987	*PRKCH*	rs11621346	
GRIK3	rs4653233	*GRM7*	rs9823996	*PRKCH*	rs11621877	
GRIK3	rs471710	*GRM7*	rs9826424	*PRKCH*	rs12590817	
GRIK3	rs476894	*GRM7*	rs9836538	*PRKCH*	rs12881922	
GRIK3	rs480495	*GRM7*	rs9837019	*PRKCH*	rs12883769	
GRIK3	rs484017	*GRM7*	rs9837834	*PRKCH*	rs17098278	
GRIK3	rs493243	*GRM7*	rs9837989	*PRKCH*	rs17098279	
GRIK3	rs499230	*GRM7*	rs9849147	*PRKCH*	rs17098351	
GRIK3	rs501832	*GRM7*	rs9860274	*PRKCH*	rs17098356	
GRIK3	rs507460	*GRM7*	rs9860560	*PRKCH*	rs17098382	
GRIK3	rs517969	*GRM7*	rs9870018	*PRKCH*	rs17098514	
GRIK3	rs522481	*GRM7*	rs9870241	*PRKCH*	rs17098542	
GRIK3	rs527442	*GRM7*	rs9870680	*PRKCH*	rs1957907	
GRIK3	rs527631	*GRM7*	rs9875041	*PRKCH*	rs2181987	
GRIK3	rs535620	*GRM7*	rs989126	*PRKCH*	rs2184634	
GRIK3	rs535876	*GRM7*	rs9990013	*PRKCH*	rs4281621	

A4.2 Single SNP Association (BD-AUD)

Chromosome	SNP	Minor allele	Odds ratio	t-statistic	p-value
2	rs2594491	C	0.6677	-3.066	0.002172
16	rs3104704	T	0.5604	-2.986	0.002823
16	rs1875205	C	0.6155	-2.864	0.004178
7	rs2299556	G	1.549	2.668	0.007641
14	rs1956177	T	1.464	2.565	0.01032

7	rs1419442	A	1.425	2.556	0.01058
3	rs1392366	C	1.468	2.49	0.01276
2	rs4953262	A	1.375	2.456	0.01403
7	rs2299546	A	1.403	2.451	0.01425
11	rs7925270	A	1.394	2.438	0.01475
7	rs2299536	A	1.377	2.415	0.01576
5	rs6898504	C	0.6967	-2.409	0.01601
14	rs1998056	G	1.37	2.406	0.01613
21	rs2832448	C	1.419	2.393	0.01673
5	rs1463747	C	0.7258	-2.388	0.01695
3	rs17047149	G	0.08411	-2.374	0.01759
6	rs10457942	A	0.6329	-2.37	0.01777
2	rs667747	T	0.4858	-2.369	0.01784
11	rs973716	T	0.6356	-2.368	0.01788
21	rs2244881	C	1.41	2.351	0.01874
16	rs2352740	A	1.366	2.337	0.01944
21	rs2832449	T	1.403	2.318	0.02043
5	rs1493389	T	0.5659	-2.308	0.02097
5	rs2452801	C	0.7271	-2.308	0.02099
11	rs10790400	T	1.354	2.292	0.0219
14	rs11160168	T	1.392	2.275	0.02289
6	rs12192251	G	0.6276	-2.268	0.02332
5	rs1973372	A	0.5783	-2.242	0.02495
2	rs563601	A	1.37	2.238	0.02525
2	rs17034641	T	1.555	2.233	0.02552
4	rs17035959	C	1.364	2.23	0.02577
16	rs2239340	A	0.7105	-2.227	0.02593
16	rs2188360	T	0.7211	-2.22	0.0264
11	rs6589833	C	1.35	2.214	0.0268
16	rs3785394	C	1.343	2.203	0.02759
16	rs6416623	T	1.342	2.2	0.02778
4	rs17035916	T	1.357	2.186	0.0288
5	rs17518831	T	0.748	-2.18	0.02929
7	rs2188187	C	1.365	2.163	0.03053
4	rs2107172	C	1.323	2.16	0.03076
9	rs16920504	A	0.6944	-2.144	0.03203
2	rs17034719	G	2.182	2.141	0.03229
7	rs2237790	G	0.7192	-2.139	0.0324
7	rs6975798	A	0.7561	-2.118	0.03414
2	rs637889	C	1.336	2.109	0.03495
5	rs2398660	G	0.7506	-2.098	0.03593
16	rs7404005	A	0.7251	-2.093	0.03636
2	rs2218660	C	1.344	2.064	0.03904
5	rs17114836	C	0.607	-2.063	0.03911

14	rs12437224	A	1.443	2.061	0.03932
11	rs7116106	A	1.316	2.04	0.04137
6	rs362851	G	0.7757	-2.036	0.04172
7	rs4141410	G	0.7615	-2.035	0.04182
16	rs195996	A	1.301	2.026	0.04279
6	rs9380406	T	1.306	2.022	0.04316
2	rs2711295	T	1.63	2.015	0.04385
4	rs11936376	A	1.352	2.015	0.04391
16	rs4782271	G	1.392	2.003	0.04518
6	rs4112793	C	0.459	-1.99	0.04659
17	rs7225164	A	0.7666	-1.976	0.04816
7	rs952912	G	1.365	1.967	0.04916

A4.3 Single SNP Association (BD)

Chromosome	SNP	Minor allele	Odds Ratio	t-statistic	p-value
5	rs812389	G	1.565	3.112	0.001858
9	rs10980210	G	2.381	3.077	0.002093
12	rs16909348	A	0.3075	-3.003	0.002672
4	rs4027073	C	1.5	2.867	0.00414
9	rs10816924	A	1.676	2.843	0.004462
2	rs7565427	A	0.4937	-2.833	0.004615
2	rs10865208	G	1.45	2.742	0.006098
16	rs2239340	A	1.501	2.738	0.006186
17	rs16959593	T	1.823	2.716	0.006607
3	rs9823996	G	1.463	2.702	0.006895
4	rs13142954	A	1.494	2.692	0.007092
12	rs11055557	A	1.408	2.564	0.01035
16	rs8044064	A	1.422	2.54	0.01108
16	rs16973277	C	0.4203	-2.498	0.01249
12	rs1805543	C	1.391	2.462	0.01381
6	rs6557123	G	0.7005	-2.454	0.01412
16	rs1900718	G	0.7173	-2.441	0.01466
3	rs779749	T	0.6986	-2.435	0.01489
12	rs1362816	T	1.641	2.429	0.01515
2	rs2595222	T	0.7095	-2.425	0.01532
6	rs10457871	G	0.7054	-2.418	0.01561
2	rs13003856	C	0.711	-2.416	0.01571
16	rs4998386	T	0.5173	-2.407	0.01609
3	rs1155966	G	1.73	2.402	0.01631
14	rs10498642	G	1.592	2.389	0.01692
4	rs3815072	C	1.483	2.386	0.01705
16	rs9924226	G	0.717	-2.353	0.01861
5	rs4958351	T	0.7059	-2.35	0.01878

3	rs1963265	T	1.396	2.344	0.01906
2	rs4952771	T	1.389	2.314	0.02069
3	rs6771606	G	1.406	2.304	0.02125
16	rs2238493	G	0.5248	-2.291	0.02199
3	rs1106486	C	0.7152	-2.261	0.02376
3	rs6769814	G	1.375	2.259	0.02391
5	rs10070447	T	0.7165	-2.256	0.02408
17	rs17604987	A	0.6752	-2.246	0.02471
16	rs7190716	A	0.5398	-2.246	0.02473
11	rs646543	G	0.672	-2.245	0.02476
17	rs850622	T	0.6758	-2.243	0.02488
11	rs719182	T	1.449	2.238	0.0252
16	rs10852263	T	0.655	-2.226	0.02603
4	rs17024387	T	1.367	2.208	0.02724
11	rs7128009	T	0.6636	-2.207	0.02728
3	rs3804945	T	0.7287	-2.2	0.02781
2	rs6751349	A	0.5969	-2.195	0.02818
16	rs7205594	T	0.732	-2.188	0.0287
3	rs13070476	C	0.6768	-2.175	0.02961
16	rs11644367	T	0.7418	-2.173	0.02975
6	rs9485549	G	1.917	2.171	0.02989
16	rs2267786	A	1.372	2.167	0.03023
16	rs6497732	T	1.339	2.158	0.03092
2	rs6732900	T	0.7351	-2.156	0.03107
3	rs7628504	G	0.68	-2.153	0.03135
12	rs12422385	G	1.592	2.145	0.03193
12	rs10492136	C	0.5461	-2.13	0.03315
3	rs3792460	C	1.365	2.128	0.03331
7	rs2299233	C	0.7451	-2.126	0.03353
16	rs13336632	C	0.5846	-2.123	0.03372
16	rs2267772	C	0.733	-2.113	0.03459
2	rs7573407	T	0.7391	-2.11	0.03483
16	rs13331514	T	0.5867	-2.11	0.03486
16	rs7188291	G	0.5872	-2.103	0.0355
9	rs7022772	A	0.7042	-2.102	0.03553
3	rs1351938	A	0.7502	-2.102	0.03558
16	rs17671033	T	0.5879	-2.098	0.0359
12	rs10845863	T	2.245	2.096	0.03605
12	rs1805199	T	0.6647	-2.096	0.03612
16	rs1070476	A	1.324	2.084	0.03719
12	rs11055628	A	1.363	2.078	0.03771
14	rs7159519	T	1.329	2.076	0.03793
16	rs10518151	G	0.5917	-2.075	0.03796
16	rs1448262	T	0.5911	-2.075	0.03799

7	rs13240504	T	1.467	2.071	0.03838
16	rs9922383	T	0.592	-2.07	0.03848
17	rs8069859	G	1.404	2.068	0.03859
12	rs2160402	A	0.5558	-2.064	0.03904
17	rs203457	A	0.7426	-2.062	0.03918
2	rs6719779	G	1.332	2.061	0.03928
17	rs119672	G	0.7443	-2.05	0.04036
5	rs4958676	A	0.6749	-2.048	0.04055
4	rs17484259	A	0.6656	-2.046	0.04071
4	rs11936376	A	0.6876	-2.036	0.04173
17	rs2108978	T	0.7463	-2.031	0.04226
16	rs971035	C	1.342	2.009	0.04451
17	rs7209653	G	0.7235	-2.005	0.04491
4	rs7684265	G	0.5778	-2.002	0.04532
21	rs363497	G	0.2403	-1.988	0.04684
3	rs3804904	A	1.331	1.973	0.04854
16	rs11644461	C	1.307	1.968	0.04901
7	rs10488509	G	1.316	1.968	0.04912
2	rs7563379	C	0.7564	-1.967	0.04914

A4.4 Gene-Based Results (BD-AUD)

Gene	NSNP	NSIG	ISIG	EMP1	SNPS
PRKCZ	8	0	0	1	NA
GRIK3	34	0	0	1	NA
PRKAR2A	1	0	0	1	NA
PRKCD	3	0	0	1	NA
CAMK2A	7	0	0	1	NA
GRM6	3	0	0	1	NA
SYNGAP1	1	0	0	1	NA
AKAP12	19	0	0	1	NA
CAMK2B	2	0	0	1	NA
GRM3	31	0	0	1	NA
AKAP9	15	0	0	1	NA
PRKAR2B	10	0	0	1	NA
IDO1	1	0	0	1	NA
SLC1A1	26	0	0	1	NA
PALM2-AKAP2	40	0	0	1	NA
PRKCQ	3	0	0	1	NA
GRIA4	43	0	0	1	NA
AKAP3	4	0	0	1	NA
GRIN2B	90	0	0	1	NA
AKAP6	57	0	0	1	NA
PRKCH	33	0	0	1	NA

AKAP13	82	0	0	1	NA
AKAP10	6	0	0	1	NA
AKAP1	12	0	0	1	NA
PRKCA	80	0	0	1	NA
PRKAR1A	4	0	0	1	NA
GRIK5	1	0	0	1	NA
PRKCG	1	0	0	1	NA
MAPK1	10	0	0	1	NA
POMC	4	0	0	1	NA
UCN	0	0	0	1	NA
CRH	0	0	0	1	NA
CRHR2	9	0	0	1	NA
UCN3	1	0	0	1	NA
UCN2	2	0	0	1	NA
CRHR1	14	0	0	1	NA
AVP	0	0	0	1	NA
AVPR1B	0	0	0	1	NA
CRHBP	1	0	0	1	NA
FKBP5	9	0	0	1	NA
MC2R	6	0	0	1	NA
NR3C2	51	0	0	1	NA
AKAP11	2	0	0	1	NA
AKAP14	1	0	0	1	NA
AKAP4	0	0	0	1	NA
AKAP5	0	0	0	1	NA
AKAP7	21	0	0	1	NA
AKAP8	1	0	0	1	NA
ARC	0	0	0	1	NA
CREB1	3	0	0	1	NA
DICER1	2	0	0	1	NA
DUSP1	0	0	0	1	NA
GLS	3	0	0	1	NA
GRIA3	31	0	0	1	NA
GRIN2C	2	0	0	1	NA
GRIN3B	0	0	0	1	NA
GRM2	0	0	0	1	NA
MAPK3	0	0	0	1	NA
PRKACA	0	0	0	1	NA
PRKACB	13	0	0	1	NA
PRKACG	0	0	0	1	NA
STX1A	0	0	0	1	NA
SYP	0	0	0	1	NA
GRIK2	58	3	2	0.5219	rs10457942|rs4112793
PRKCI	5	1	1	0.028	rs1392366

GRIA1	80	6	2	0.2885	rs1463747|rs1493389
GRIN3A	30	1	1	0.331	rs16920504
GRIA2	3	2	1	0.06055	rs17035959
GRM7	170	1	1	0.468	rs17047149
CAMK2G	5	1	1	0.1688	rs17631000
SERPINA6	10	4	3	0.1023	rs1956177|rs1998056|rs12437224
CAMK2D	39	1	1	0.4019	rs2107172
DLG4	1	1	1	0.0259	rs2239340
GRM8	103	7	5	0.2676	rs2299556|rs1419442|rs2299546|rs2237790|rs6975798
GRIN2D	4	1	1	0.1483	rs2380121
PRKCE	129	5	5	0.2728	rs2594491|rs4953262|rs667747|rs17034641|rs563601
GRIK1	64	4	2	0.495	rs2832448|rs307943
GRIN2A	111	5	3	0.1824	rs3104704|rs2352740|rs4782271
GRM1	43	1	1	0.4751	rs362851
PRKCB	62	5	2	0.475	rs3785394|rs2188360
NR3C1	37	2	2	0.3114	rs6898504|rs2398660
GRIK4	57	4	1	0.2089	rs7925270
GRM4	10	1	1	0.2788	rs9380406
GRM5	67	1	1	0.193	rs973716

A4.5 Gene-Based Results (BD)

SET	NSNP	NSIG	ISIG	EMP1	SNPS
PRKCZ	8	0	0	1	NA
GRIK3	34	0	0	1	NA
PRKCD	3	0	0	1	NA
CAMK2A	7	0	0	1	NA
GRM6	3	0	0	1	NA
SYNGAP1	1	0	0	1	NA
GRM4	10	0	0	1	NA
CAMK2B	2	0	0	1	NA
GRM3	31	0	0	1	NA
PRKAR2B	10	0	0	1	NA
IDO1	1	0	0	1	NA
GRIN3A	30	0	0	1	NA
PRKCQ	3	0	0	1	NA
CAMK2G	5	0	0	1	NA
GRIA4	43	0	0	1	NA
AKAP3	4	0	0	1	NA
PRKCH	33	0	0	1	NA
AKAP13	82	0	0	1	NA
PRKAR1A	4	0	0	1	NA
MAPK1	10	0	0	1	NA

UCN	0	0	0	1	NA
CRH	0	0	0	1	NA
CRHR2	9	0	0	1	NA
UCN3	1	0	0	1	NA
UCN2	2	0	0	1	NA
CRHR1	14	0	0	1	NA
AVP	0	0	0	1	NA
AVPR1B	0	0	0	1	NA
CRHBP	1	0	0	1	NA
FKBP5	9	0	0	1	NA
MC2R	6	0	0	1	NA
NR3C1	37	0	0	1	NA
SERPINA6	10	0	0	1	NA
AKAP11	2	0	0	1	NA
AKAP14	1	0	0	1	NA
AKAP4	0	0	0	1	NA
AKAP5	0	0	0	1	NA
AKAP7	21	0	0	1	NA
AKAP8	1	0	0	1	NA
ARC	0	0	0	1	NA
CREB1	3	0	0	1	NA
DUSP1	0	0	0	1	NA
GLS	3	0	0	1	NA
GRIA2	3	0	0	1	NA
GRIA3	31	0	0	1	NA
GRIN2C	2	0	0	1	NA
GRIN2D	4	0	0	1	NA
GRIN3B	0	0	0	1	NA
GRM1	43	0	0	1	NA
GRM2	0	0	0	1	NA
MAPK3	0	0	0	1	NA
PRKACA	0	0	0	1	NA
PRKACB	13	0	0	1	NA
PRKACG	0	0	0	1	NA
PRKCI	5	0	0	1	NA
DICER1	2	1	1	0.0334	rs10498642
PRKCE	129	8	5	0.4766	rs10865208|rs2595222|rs4952771|rs6751349|rs6719779
PALM2-AKAP2	40	2	2	0.0202	rs10980210|rs10816924
GRM8	103	1	1	0.7598	rs13240504
GRIN2B	90	6	5	0.2278	rs16909348|rs11055557|rs1362816|rs12422385|rs10492136
PRKCA	80	2	2	0.2753	rs16959593|rs8069859

PRKCB	62	3	2	0.3047	rs16973277|rs10852263
AKAP10	6	5	1	0.05	rs17604987
AKAP9	15	2	2	0.1377	rs2299233|rs10488509
GRIK1	64	1	1	0.7188	rs363497
CAMK2D	39	2	2	0.3754	rs3815072|rs7684265
NR3C2	51	1	1	0.0262	rs4027073
GRIK4	57	2	1	0.4171	rs646543
AKAP12	19	2	1	0.1124	rs6557123
SLC1A1	26	1	1	0.4113	rs7022772
AKAP6	57	1	1	0.7121	rs7159519
GRM5	67	1	1	0.3766	rs719182
AKAP1	12	1	1	0.2987	rs7209653
POMC	4	1	1	0.0117	rs7565427
GRIN2A	111	9	5	0.3287	rs8044064|rs1900718|rs4998386|rs2267786|rs6497732
GRIA1	80	4	3	0.1453	rs812389|rs4958351|rs4958676
GRIK2	58	1	1	0.6026	rs9485549
GRM7	170	9	5	0.441	rs9823996|rs779749|rs1155966|rs6771606|rs13070476