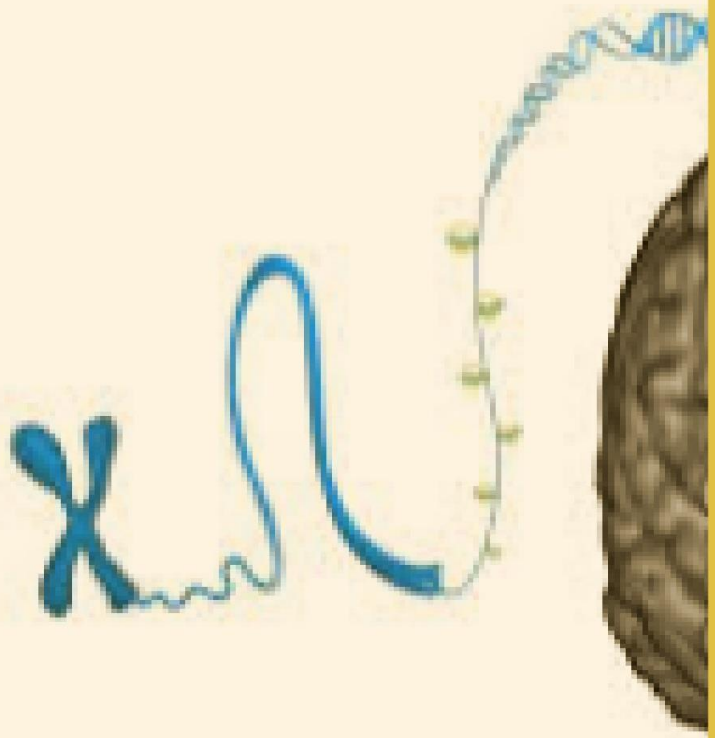

GENETIC PREDICTORS OF RESPONSE TO PHARMACOTHERAPY IN MOOD DISORDERS

STEPS ON THE PATH TO PERSONALIZED PSYCHIATRY

AZMERAW TAYELGN AMARE

“Genetic predictors of response to pharmacotherapy in patients with mood disorders” PhD thesis of Azmeraw T. Amare, discipline of Psychiatry in the School of Medicine, the University of Adelaide, Australia.

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Genetic predictors of response to pharmacotherapy in patients with mood disorders

Steps on the path to personalised psychiatry

PhD thesis

To obtain the degree of Doctor of Philosophy (PhD) in Medicine at the University of Adelaide

By

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Born on 15th September 1984 in Ethiopia

Supervisors

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July 2018

Adelaide, Australia

Declaration

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Azmeraw T. Amare

Dedication

This work is dedicated to my beloved wife, Digisie Mequanint Jemere, my sweet daughter Natanim Azmeraw, dear Dad, dear Mam, and the rest of my family for their support and input into my life.

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Chapter 1

General introduction

History of mood disorders

Psychiatric care has existed for thousands of years and has connections to ancient philosophy, culture and religion[1]. According to testimonies of the ancient Egyptians, the Bible (including sacred texts from Jews and Christians covering the old and new testaments), and the Jewish Torah, mental health disorders were attributed to the actions of evil spirits resulting from divine punishment[2]. Ancient Greek and Roman philosophers introduced more natural and rational explanations of mental illnesses during the era of Hippocrates (460–357 BC), Aristotle (384–322 BC), and Galen (131–201 AD). In the history of medicine, this period defined the theory of 'humorism' in which a deficiency or an excess of any of the four essential humors (yellow bile, black bile, blood and phlegm) were associated with impaired physical and mental health equilibrium[3]. Thus, treatments were directed at restoring the imbalance. Hippocrates, the 'father of modern medicine', postulated that mental disorders were linked to an excess of black bile that he thought lead to mood darkening through an impact on the brain[1]. Modern thinking about mood disorders can be traced back to ancient thoughts of mental health illness. The concepts of 'melancholia' and 'mania' recognised in modern psychiatry were introduced in the past[4]. Until recently, Greek and Roman thoughts of melancholia persisted in European and Arabic medicine[1,5].

Today's psychiatric care relies on well-standardised clinical guidelines developed based on scientific research. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5), published by the American Psychiatric Association (APA)[6], and the International Statistical Classification of Diseases and Related Health Problems 10th revision (ICD-10), produced by the World Health Organization (WHO)[7], offer standard criteria for classification and diagnosis of mood disorders. Scientists of the present era have made progress towards understanding the biological basis of mood disorders[8-13] and current research in psychiatry

tends to focus on identification of biological markers for psychiatric disorders and treatment efficacy through investigation of genomes (DNA), epigenomes, transcriptomes (RNA), proteomes, metabolomes, microbiomes, connectomes, and brain neuroimaging of patients. Results of such studies will further reshape our understanding of the definition and classification of psychiatric diseases.

Epidemiology and classification of mood disorders

Mood disorders refer to a cluster of psychiatric disorders characterised by a core symptom of mood disturbance. In DSM-5, mood disorders are replaced by separate sections of bipolar disorders (BPD) and depressive disorders, each with diverse subtypes (Figure 1.1). Major depression and BPD are amongst the most disabling mental health disorders worldwide[9,11-13] with a lifetime prevalence of ~12%[14] and 1%[15]-4.4%[16], respectively. While women are more likely to be affected by a depressive disorder, having a double fold of risk compared to men [17,18], the lifetime prevalence of BPD is stable across gender[17]. BPD has an early age of onset, typically between 15 - 20 years[16], and age of onset for depression ranges from adolescence to the early 40s[19]. Based on the 2013 global burden of diseases study by the Institute for Health Metrics and Evaluation at the University of Washington, major depressive disorder (MDD) alone caused an estimated 61.6 million years of life lived with disability worldwide, accounting for 8.1% of the total years lived with disability (YLDs) and for 2.5% of the total disability-adjusted life years[10-12]. BPD contributed an additional 9.9 million years of life lived with disability[9]. The WHO predicted depression will be the leading cause of disability by 2020 worldwide[19].

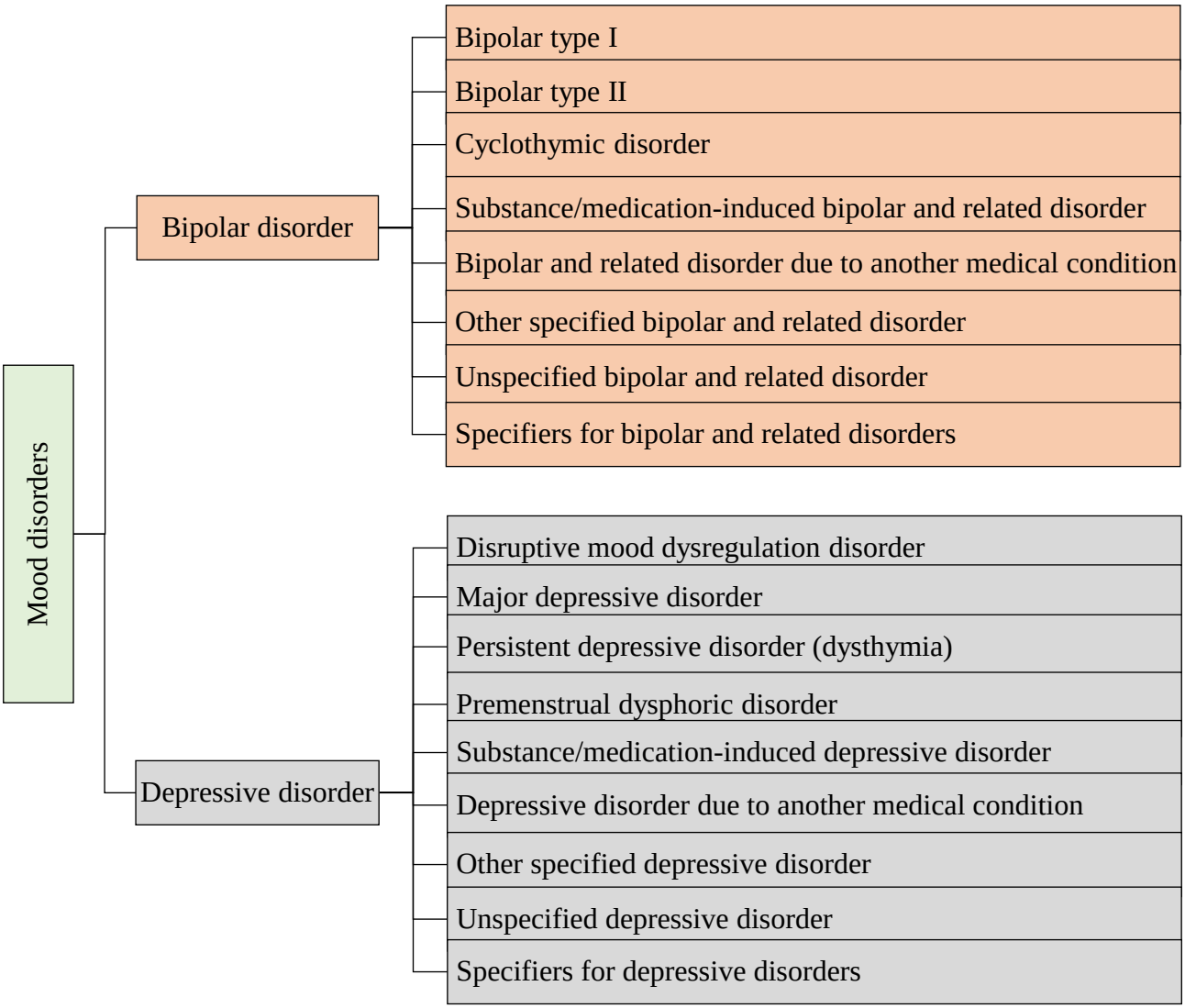

Figure 1.1. Classification and subtypes of mood disorders according to DSM-5

Bipolar and related disorders

The defining features of bipolar disorder are cycles of abnormally elevated mood (mania) and low mood (depression)[6] (Figure 1.2). Common subtypes of BPD include bipolar type I (BPD I), bipolar type 2 (BPD II),cyclothymic disorder, bipolar and related disorders due to another medical condition, substance/medication-induced bipolar and related disorder, other specified

bipolar and related disorder, unspecified bipolar and related disorder, and specifiers for bipolar and related disorders[6].

Individuals with BPD I have had one or more manic episode lasting at least one week, present most of the day, nearly every day with or without a depressive episode[6]. Patients with BPD II have at least one hypomanic episode lasting for four or more consecutive days and present most of the day and at least one major depressive episode. Cyclothymic disorder is a chronic, fluctuating mood disturbance involving several periods of hypomanic and depressive symptoms[6]. Substance/medication-induced bipolar and related disorder begins with use, intoxication and withdrawal effects of specific substances, such as cocaine, dexamethasone, that are assumed to cause bipolar mood symptoms. Bipolar and related disorder due to another medical condition is defined by the presence of manic or hypomanic episode associated with onset of a medical illness, especially in chronic medical conditions. Bipolar diagnosis often involving a short-duration of hypomania that does not meet specific criteria for bipolar I, bipolar II, or cyclothymic disorder are classified under the unspecified bipolar and related disorder category.

Depressive disorder

The main feature of all subtypes of depressive disorders is the presence of a persistent feeling of low mood, accompanied by cognitive and somatic symptoms that significantly affect the individual's daily function (Figure 1.2). The different subtypes of depression include disruptive mood dysregulation disorder, MDD, persistent depressive disorder (dysthymia), premenstrual dysphoric disorder, substance/medication-induced depressive disorder, depressive disorder due to another medical condition, other specified depressive disorder, unspecified depressive disorder and specifiers for depressive disorders.

Clinical diagnosis of MDD is based on the presence of five (or more) symptoms of either depressed mood or loss of interest and pleasure (anhedonia) on most days for at least two weeks, plus additional symptoms such as weight loss, changes in appetite, insomnia or hypersomnia, psychomotor agitation or retardation, loss of energy or fatigue, feelings of worthlessness, poor concentration and suicidal thoughts[6]. In persistent depressive disorder (dysthymia), depressed mood symptoms are chronic and usually occur for most of the day for at least two years in adults, or at least one year for children and adolescents. MDD may precede dysthymia and individuals whose symptoms meet MDD criteria for two years are given the diagnosis of dysthymia as well as MDD. Premenstrual dysphoric disorder is characterised by mood swings that occur repeatedly during the premenstrual period and remits around the onset of menses or shortly thereafter. These symptoms may be accompanied by behavioral and physical symptoms. Substance/medication-induced depressive disorder begins during, or soon after substance intoxication or withdrawal, or after exposure to a medication where the individual develops prominent and persistent depressed mood. Depressive disorder due to another medical condition is thought to be related to the direct physiological effects of another medical condition and essential features include presence of a prominent and persistent period of depressed mood[6].

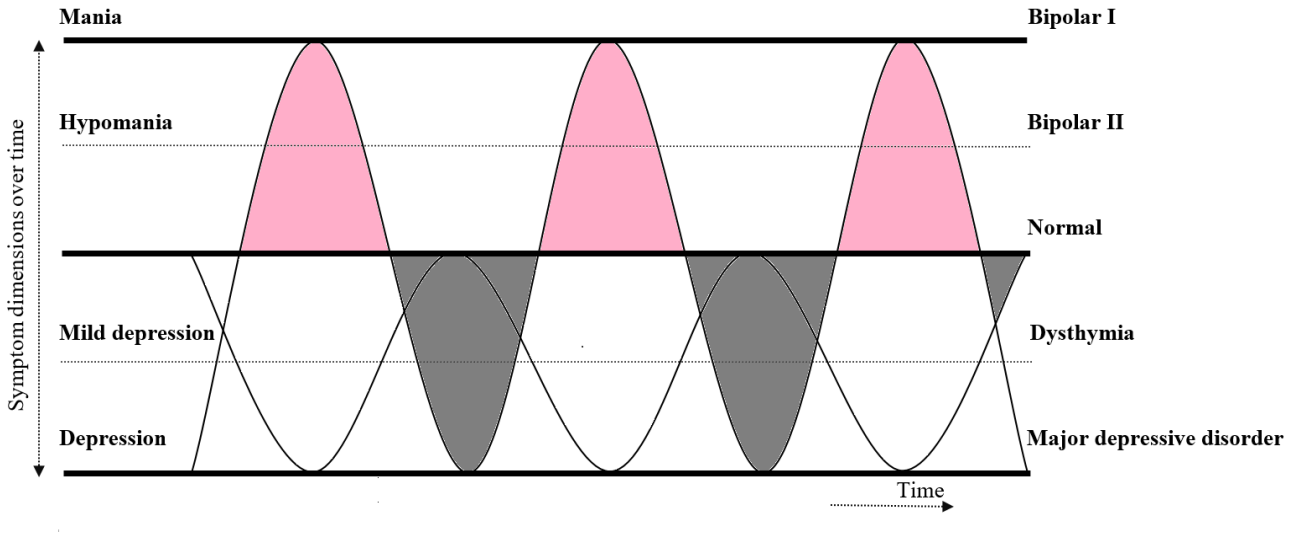

Figure 1.2. Symptom dimensions of mood changes over time for bipolar and depressive disorders

Pharmacotherapy in mood disorders

Lithium

According to the 'Big Bang theory', lithium was one of the elements created early during formation of the universe some 14 billion years ago along with hydrogen, helium and beryllium[20]. Lithium was discovered in 1800 by a Brazilian chemist named Jose Bonifacio de Andrada e Silva (1763-1838) in the form of lithium aluminium tectosilicate (petalite) on a small island of Utö, Sweden. In 1817, a Swedish chemist, Johan August Arfwedson (1792 – 1841) isolated lithium as a pure element form while working on petalite ore[21]. Lithium is the lightest element and is shiny-white in color. It is highly reactive to oxygen and has similar chemical properties to sodium and potassium. Because it was found in certain rocks, the Greek name '*lithos'*, was given, meaning a stone. Lithium is found in human and other animals in a small quantity and its compounds have very important medical and industrial applications[21]. Although a trace amount of lithium is present in biological systems of the human body, it seems to have an important biological function. As a medication, lithium is

available in the form of salt compounds and is used to treat psychiatric and other medical disorders at therapeutic doses of about 100 times natural daily intake. Studies found lithium treatment reduces the risk of dementia[22] and naturally occurring lithium in drinking water is associated with lower incidence of dementia[23].

The modern history of lithium began in 1948 when an Australian psychiatrist, Dr. John Cade (1912-1980), revealed the efficacy of lithium as a mood stabiliser[24]. While he was a World War II prisoner of the Japanese military, he observed strange, vacillating behaviour displayed by some fellow inmates. He suspected a toxin in the prisoner's urine may be affecting their brains. After the war, he performed an experiment to test the hypothesis that a toxin in urine affects brain and behaviour. In his crude experiment, urine of mentally ill patients was injected into guinea pigs and Cade found that guinea pigs injected with urine (uric acid) from mentally ill patients died earlier than those injected with urine from healthy individuals. Later, Cade extended his experiment to investigate the effect of uric acid at different concentration gradients. In an attempt to create a concentration gradient and increase water solubility of uric acid, lithium carbonate was added to uric acid producing a solution of lithium urate that was injected into guinea pigs. During this process, Cade realised that guinea pigs injected with lithium urate were calmer and less responsive to external stimuli compared to those that had less/no lithium solution[21,24]. This finding ultimately led to the conclusion that lithium may be a treatment for bipolar disorder. As Cade's findings appeared highly promising, he ingested lithium salts himself to ensure safety in humans, followed by a small trial using lithium citrate and lithium carbonate in patients diagnosed with mania and dementia[21,24].

Cade's discovery initiated a series of clinical studies that confirmed the anti-manic, anti-suicidal, anti-aggression properties of lithium salts[24]. Today, lithium is a first line drug for treatment of bipolar disorder and is widely recommended by several guidelines[25]. Although lithium is among the most effective drugs for patients with mood disorders, it is not without controversy. Studies revealed that up to 30% of patients are only partially responsive, and more than a quarter show no clinical response at all[26,27]. A significant proportion of patients who use lithium develop common side effects — thirst and excessive urination, nausea, diarrhea, weight gain, cognitive impairment, sexual dysfunction and tremor[21,28]. Lithium intoxication following long-term use of lithium may lead to severe adverse effects on the kidneys, thyroid and parathyroid glands[28]. Response to lithium varies considerably across individuals, but there is no biomarker to identify treatment responders and non-responders. Studies have shown that lithium treatment response is a strongly inheritable trait and genetic markers for lithium response have recently been identified[27,29].

Selective serotonin reuptake inhibitors

The monoamine hypothesis articulated 60 years ago proposed that depression is the result of a depleted level of neurotransmitters, such as serotonin, norepinephrine, and dopamine, in the central nervous system[30,31]. This appears to be supported by the pharmacological mechanisms of antidepressants[32]. Neurotransmitters influence multiple complex processes, including cognition, memory, learning, mood, affection, emotion, reward, appetite and sleep[33].

Serotonin (5-hydroxytryptamine, 5-HT) is perhaps one of the best-known and well-studied neurotransmitters primarily produced by enterochromaffin cells from an essential amino acid (tryptophan). Serotonin modulates neuron signaling at synapses in the nervous system and serotonin receptors are major targets of antidepressants, such as selective serotonin reuptake

inhibitors (SSRIs)[34]. SSRIs belongs to the class of drugs that inhibit reabsorption of serotonin from the synaptic cleft leading to an increased length of time that serotonin is available in the synaptic cleft to repeatedly stimulate postsynaptic receptors yielding increased neuronal firing. A preliminary report regarding the first SSRI drug LY110140 (fluoxetine) was published in 1974[35]. Fluoxetine was introduced into the market in 1988 after approval by the United States Department of Health Food and Drug Administration (FDA)[36,37]. Today, dozens of SSRIs are available for treatment of patients with depression and those approved by the FDA include citalopram, escitalopram, fluoxetine, paroxetine and sertraline[8].

Response to pharmacotherapy in the treatment of mood disorders

Although lithium and SSRIs are the first line pharmacotherapic alternatives for treatment of BPD and depression, respectively, the rate of therapeutic response is unsatisfactory and shows high variability between individuals. These medications are effective only in a subset of patients or produce partial responses, and are often associated with treatment side effects. It is estimated that a third of bipolar patients are excellent responders to lithium[38,39]. In our recent study conducted by the International Consortium on Lithium Genetics (ConLi^{+}Gen), we found that only 27.2% of patients have a good response to lithium[40]. Similarly, patients with depression who have been treated with SSRIs reported unsatisfactory rate of response and poor remission. In the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study only 36.8% achieved remission after 12-14 weeks of first-line therapy with SSRIs and results were even worse when treatment follow-up was longer[41].

Among other factors, genetic variations contribute to a significant proportion of inter-individual variation to drug response with ranging from a positive treatment response to a

potentially life-threatening adverse drug reaction. The therapeutic action of lithium and SSRIs is thought to be mediated at least partially mediated by genetic factors.

Pharmacogenomics research in mood disorders and response prediction

In the early 1960s, Werner Kalow introduced the concept of pharmacogenetics in his book 'Heredity and the response to drugs'[42]. Since then, a number of pharmacogenetic studies have been conducted aiming to understand genetic variations that influence the absorption and distribution of drugs and mediate their mechanisms of actions, ultimately determining the efficacy/response to pharmacotherapy. Early approaches were based on candidate gene approaches and completion of the Human Genome Project has allowed a genome-wide approach (pharmacogenomics) to study how whole-genome genetic variation influences response to drug treatments[43].

With advancement in genomic research and high-throughput technologies, there is growing interest to apply pharmacogenomic evidence to personalise treatments and maximise the efficacy of currently available drugs. The goal of pharmacogenomics is to discover the genetic determinants of treatment response and use this information to predict how patients will respond to different drugs allowing psychiatrists to determine the right medications for patients, thus leading to 'personalised psychiatry'. In spite of research progress in pharmacogenomics, clinical application of research findings is slow and requires development of genetic methods. The major challenges we face as we attempt to translate pharmacogenomics to patient care is that response to treatment is a very complex trait, accounted for by several genetic variants each contributing to a small proportion of the total variance. Thus, successful translation of pharmacogenomic evidence requires methods, such as polygenic score modeling, that can capture the effects of whole genome single nucleotide

polymorphisms (SNPs). In this project, we carried out genome-wide analysis and polygenic score modeling to identify genetic predictors of response to lithium and SSRIs in patients with mood disorders.

Outline and aim of the thesis

The main aim of this PhD research was to investigate the genetic (polygenic) predictors of treatment response to pharmacotherapy in patients with mood disorders. Especially, I was interested in identifying genetic (polygenic) predictors and biological pathways associated with treatment response to lithium and SSRIs. We studied the influence of genetic loading for psychiatric and somatic traits on treatment response to mood stabilisers. Genetic research approaches, such as polygenic score analysis, candidate gene method, genome-wide association study, functional pathway and network analysis, were implemented. Each of the methods is presented in detail in each chapter.

This PhD thesis consists of eight chapters. C**hapter 1** provides a general introduction to the classification, historical background and pharmacotherapy of mood disorders. In **chapter 2**, a review of pharmacogenomic studies in the treatment of mood disorders is presented — with a focus on lithium and SSRIs. Here, I systematically review candidate genes associated with treatment response to lithium and SSRIs and then propose potential strategies and opportunities for combining clinical data with omics information (DNA, RNA, proteins and microbiomes) in order to provide personalised psychiatric care. In **chapter 3,** I studied cardiometabolic disease genes that have overlapping effects on mood disorders. I also assessed the role of these genes and associated biological pathways on treatment response to lithium and SSRIs. In **chapter 4**, I showed the association of polygenic score for schizophrenia and the human leukocyte antigen (HLA) and inflammation genes with

response to lithium in bipolar affective disorder. Association of polygenic score for major depressive disorder and depressive symptoms with response to lithium in patients with bipolar disorder is described in **chapter 5**. In **chapter 6**, I investigated the association of polygenic scores for personality traits and response to SSRIs in patients with a major depressive disorder. In **chapter 7**, I explored the association of obesity and coronary artery disease genes with response to SSRIs in treatment of major depression. In **chapter 8**, I provide an overview of general discussion on our findings, future perspectives and conclusions.

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Name of Principal Author (Candidate)	Azmeraw T. Amare		
Contribution to the Paper	Conceived the research concept and design, performed a literature review, made an interpretation of the results and prepared the first draft of the manuscript. Made manuscript submission, prepared a reply letter for reviewers and finalised the manuscript based on reviewer feedback.		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	3/04/2018

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	A/professor Klaus Oliver Schubert		
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Contribution to the Paper	Critical revision of the manuscript, overall guidance and supervision. As a corresponding author, he made communications with journal editors.		
Signature		Date	3/04/2018

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Chapter 2

Pharmacogenomics in the treatment of mood disorders: Strategies and opportunities for personalised psychiatry

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ABSTRACT

Personalised medicine (personalised psychiatry in a specific setting) is a new model towards individualised care, in which knowledge from genomics and other omics pillars (microbiome, epigenomes, proteome and metabolome) are combined with clinical data to guide efforts to new drug development and targeted prescription of existing treatment options. In this review, we summarise pharmacogenomic studies in mood disorders that may lay a foundation towards personalised psychiatry. In addition, we discuss possible strategies to integrate data from omics pillars as a future path to personalised psychiatry.

So far, the progress of uncovering single nucleotide polymorphisms (SNPs) underpinning treatment efficacy in mood disorders (e.g. genes associated with SSRIs or lithium treatment response in patients with bipolar disorder and major depressive disorder) are encouraging, but not adequate. Genetic studies have pointed to a number of SNPs located at candidate genes that possibly influence response to; a) antidepressants *COMT*, *HTR2A*, *HTR1A*, *CNR1*, *SLC6A4*, *NPY*, *MAOA*, *IL1B*, *GRIK4*, *BDNF*, *GNB3*, *FKBP5*, *CYP2D6*, *CYP2C19*, and *ABCB1*; b) mood stabilisers (lithium) *5-HTT*, *TPH*, *DRD1*, *FYN*, *INPP1*, *CREB1*, *BDNF*, *GSK3β*, *ARNTL*, *TIM*, *DPB*, *NR3C1*, *BCR*, *XBP1*, and *CACNG2*.

Given this evidence, we suggest three alternative and complementary strategies to implement the existing knowledge from pharmacogenomics. The first strategy can be to implement diagnostic, therapeutic or prognostic genetic testing based on candidate genes or gene products. The second alternative is an integrative analysis (systems genomics approach) of omics data obtained from the different pillars of omics investigation, including genomics, epigenomes, proteomics, metabolomics and microbiomes. The main goal of systems

genomics is an identification and understanding of biological pathways, networks and modules underlying drug response.

The third strategy refers to the development of multivariable diagnostic or prognostic algorithms (tools) by combining individual's genomic information (polygene information) with other predictors (e.g. omics pillars, neuroimaging, and clinical characteristics) to finally predict therapeutic outcomes.

An integration of molecular science with that of current clinical practice is the way forward to drug discoveries, novel therapeutic approaches and to characterise psychiatric disorders leading to a better predictive, preventive and personalised medicine (PPPM) in psychiatry. With future advances in omics technology and methodological developments for data integration, the goal of PPPM in psychiatry is promising.

Keywords: Personalised psychiatry, lithium, SSRIs, pharmacogenomics, depression, bipolar disorder, predictive, preventive and personalised medicine (PPPM).

INTRODUCTION

Mood disorders in psychiatry encompass bipolar disorder (BPD), which is characterised by alternating episodes of elevated mood (mania) and depression, and a major depressive disorder (MDD), which is defined by symptoms associated with pervasively low mood. The causes of mood disorders are complex and involve the interplay between genetic predisposition and non-genetic biological, psychological and social factors. Both MDD and BPD are highly heritable, and genetic factors contribute 31-42% of disease risk in MDD[1] and 59% - 85% in BPD[2,3]. Moreover, patients with MDD and BPD often show overlapping clinical features[4]. It is estimated that about 47% of genetic risk factors of MDD are shared with BPD[5]. There is also a shared genetic risk between mood disorders and other psychiatric and medical morbidities[5-7]. Environmental risk factors, such as childhood abuse (physical, sexual or psychological), are also frequently reported to be associated with both disorders[8].

Candidate gene and genome-wide association studies (GWAS) have identified a list of candidate genes for MDD[9-12] and BPD[13-16]. To date, GWAS approaches have identified eighteen loci contributing to risk of MDD[10-12] and over eight loci associated with BPD[13-17], including shared genetic loci located in the *CACNA1C, CACNB2, AS3MT, ITIH3* and *CCDC68* genes[7]. An extension of GWAS approaches, such as polygenic score analysis[18], bivariate restricted maximum likelihood (REML) in genome-wide complex trait analysis (GCTA)[5] and linkage disequilibrium (LD) score regression, have strongly suggested that mood disorders are polygenic in nature, in that multiple genetic variants interacting with environmental factors contribute to development of the diseases[18].

Pharmacogenomics in the treatment of mood disorders After successful completion of the Human Genome Project there was an expectation that new genetic discoveries would rapidly

and fundamentally improve medical care. For example, it was hoped that pharmacological treatment response and treatment-associated side effects would become more predictable from patient genetic signatures. However, progress to date has been much slower than initially expected. Part of this delay relates to the fact that an individual's response to pharmacotherapy is multifactorial and involves multiple genes that in turn interact with numerous environmental factors[19]. This is a challenge, especially in the pharmacogenomics of psychiatric drugs, where underlying traits are extraordinarily complex and heterogeneous. Moreover, it is unclear whether existing knowledge from pharmacogenomics has yet the necessary and sufficient information for clinical tests and applications. Nevertheless, knowledge of pharmacogenomics is continuing to expand and in the future it may be possible to integrate genomic data with other biological and clinical information to support decision-making in psychiatric care.

In this review, we discuss recent discoveries in the pharmacogenomics of mood disorders, mainly focusing on treatment response to selective serotonin reuptake inhibitors (SSRIs) in MDD, and lithium treatment response in BPD. We also discuss methodological strategies to integrate genetic evidence into clinical care and suggest future directions towards personalised psychiatry.

Key concepts, definitions and principles of pharmacogenomics

Pharmacogenomics focuses on the study of whole genome genetic variation for its effect on pharmacological treatment outcomes, such as therapeutic efficacy and medication side effects. The human genome is composed of roughly 3.1 billion nucleotide bases. The 1000 Genomes Project recently sequenced the genomes of 2,504 individuals representing over 26 population groups and reported a total of over 88 million genetic variants, of which 84.7

million are single nucleotide polymorphisms (SNPs), 3.6 million are short insertions/deletions (indels) and the remaining 60,000 are structural variants[20]. The human genome has around 30,000 genes and every individual inherits two copies of most genes, one from each parent.

Single nucleotide polymorphisms

Although the DNA of two individuals is roughly 99 percent identical, over 84 million genetic variations occur at the nucleotide level across the human genome[20]. Genetic variants found in more than 1% of the population are called polymorphisms. The most abundant type of genetic polymorphisms, found in more than 5% of the human population are called common SNPs. It is recognised that roughly 54% of SNPs are located within the coding region of genes that determine the structure of the gene product (protein)[21].Thus, a sequence variation within these regions may result in alterations in the encoded protein, which in turn may have an effect on phenotypes (e.g. treatment response). Pharmacogenomics studies how a person's genetic makeup at the nucleotide level influences their reaction to medications. It also investigates how genetic variations interact with environmental determinants (microbiome, diet, age, lifestyle and state of physical health) to influence an individual's response to drugs.

Objectives of pharmacogenomics

In the context of psychiatry, the aim of pharmacogenomics is the identification of genes associated with treatment response to psycho-pharmacotherapeutic agents, with an ultimate goal of implementing this information to improve treatment outcomes.

The current psychiatric assessment and decision-making process for diagnosis and treatment is primarily dependent on clinical experience and professional judgment of psychiatrists, and

no known biological marker is yet available to perform either a diagnostic or a prognostic test. The treatment response of patients with mood disorders treated by the current approaches of psycho-pharmacotherapy vary widely between individual patients and is unsatisfactory in many cases[22-24]. For instance, in patients with MDD, the treatment efficacy of selective serotonin reuptake inhibitors (SSRIs), the most commonly used first-line pharmacological agent[25], varies between 48% to 64%[22,23] and reported remission rates are as low as 23.5%[23,26]. Similarly, patients treated with lithium in BPD are only partially responsive and more than a quarter have no clinical response at all[24]. Thus, an application of findings from pharmacogenomics in mood disorders may help to adjust pharmacotherapy to improve efficacy and reduce the risk of side effects. The key goals of pharmacogenomics include the following:

Improving patient care and safety: One objective of pharmacogenomics and genetic testing is to identify patients likely to respond to treatment and experience adverse drug reactions. Genetic testing may highlight to psychiatrists which patients need particularly careful monitoring and could guide the switching of medications as appropriate.

Improving health care costs and efficiency: The resources, including drugs and human resources that are invested in patient care, can be optimised by prescribing the right drug for the right patient at the right time.

Individualised adjustment and selection of drugs: An understanding of differences in the genetic make-up of patients may help to select the right drug for the right patient and allow adjustment of drug dosage according to the likelihood that they will have a favorable response to a lower risk of side effects. Pharmacogenomic agents that show proven efficacy and safety with minimal cost as evidenced by clinical trials can be considered as the right

drugs. In recent years, pharmacogenomic discoveries and related tests allowed drug selection to be more individualised. This included dosage adjustment, so that maximum efficacy could be achieved with minimal side effects as opposed to the traditional 'one size fits all' model of drug selection, which is less useful.

Current state of pharmacogenomic studies in mood disorders

In the following section, we summarise some recent findings from larger scale pharmacogenomic studies in mood disorders, namely on the genomics of SSRIs treatment response in MDD and of lithium treatment in BPD.

SSRI treatment for major depressive disorder

Studies using twin, candidate gene and genome-wide association study (GWAS) designs suggest that antidepressant treatment response is substantially influenced by genetic factors[23,27-34]. Although quantitative genetic studies estimated that the genetic basis of antidepressant (e.g. SSRIs) treatment response accounts for up to 42% of individual variation, progress in uncovering the particular genetic polymorphisms underpinning treatment response has been slow[35]. As described in Table 1, candidate gene studies have pointed to a number of genes and SNPs that may influence antidepressant treatment outcomes[23,27-33], including polymorphisms within the *COMT*[27,36], *HTR2A*[28,37,38], *HTR1A*[29], *CNR1*[29], *SLC6A4*[30], *NPY*[31], *MAOA*[32,36], *IL1B*[33,36], *GRIK4*[39], *BDNF*[36,37], *GNB3*[36], *FKBP5*[37], *CYP2D6*[38,40], *CYP2C19*[38,40] and *ABCB1*[37,41] genes (Table 1). For example, a negative influence of higher activity COMT 158val/val genotype on antidepressant treatment response was shown[27]. In the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Study, MDD patients who were homozygous for the A allele at rs7997013 within the *HTR2A* gene

demonstrated an 18% better response to citalopram treatment compared to those homozygous for the G allele[42].

Unfortunately, GWASs have been underpowered to conclusively discover the genetic polymorphisms involved in antidepressants treatment outcomes. To date, several efforts in GWAS of antidepressants reported only suggestive genetic regions and the findings were scarcely replicated in subsequent studies. The first GWAS involving a total of 339 depressed patients from the Munich-Antidepressant-Response-Signature (MARS) project and a replication analysis using data from the psychiatric hospital of the Ludwig Maximilians University (LMU, n=361) and STAR*D (n=832) reported two suggestive SNPs rs6989467 in the *CDH17* gene associated with partial response ($p = 7.6 \times 10^{-7}$) and rs1502174 ($p = 8.5 \times 10^{-5}$) in the *EPHB1* gene associated with response and remission to antidepressants[43].

The second GWAS on treatment response to the SSRI antidepressant citalopram studied 883 treatment responders and 608 non-responders derived from STAR*D in USA[44]. This study reported three SNPs rs6966038 in the *UBE3C* gene ($p = 4.65 \times 10^{-7}$), rs6127921 in the *BMP7* gene ($p = 3.45 \times 10^{-6}$) and rs809736 in the *RORA* gene ($p = 8.19 \times 10^{-6}$) that showed suggestive association with treatment response and remission[44]. A similar GWAS on the patterns of treatment response using STAR*D subjects having sustained (n = 869) versus unsustained (n = 247) patterns and a replication attempt at the Genome-Based Therapeutic Drugs for Depression (GENDEP, n=585) found that the strongest suggestive association was detected at rs10492002 ($p = 4.5 \times 10^{-6}$) in the *ACSS3* gene[45]. A GWAS on susceptibility to antidepressant side effects on STAR*D (n = 1,439) identified ten linked SNPs in *SACM1L* gene ($p = 4.98 \times 10^{-7}$) associated with bupropion side effects on sexual dysfunction[46]. Similarly, two SNPs rs17135437 ($p = 3.27 \times 10^{-8}$) within *EMID2* gene and intergenic SNP

rs16965962 ($p = 3.22 \times 10^{-7}$) exhibited positive association with the effects of citalopram on vision/hearing[47].

The third GWAS on antidepressant efficacy using patients treated with escitalopram (n = 394) and nortriptyline (n = 312) from the GENDEP study revealed rs2500535 ($p = 3.56 \times 10^{-8}$) in the *UST* gene associated with nortriptyline response and the rs1126757 ($p = 2.83 \times 10^{-6}$) in the *IL11* gene associated with citalopram response[48]. A further meta-analysis of the three GWASs on antidepressant treatment response from MARS, STAR*D, and GENDEP, which included 2,256 participants, was unable to detect SNPs at a genome-wide level of significance[49].

The fourth GWAS on response to serotonergic and noradrenergic antidepressants (n = 1,790) from the Novel Methods leading to New Medications in Depression and Schizophrenia (NEWMEDS) consortium found no SNP associated with antidepressant response, even after a meta-analysis of results from NEWMEDS and STAR*D (n=2897)[34].

The fifth most recent pharmacogenomics study on SSRIs response reported by the International SSRIs Pharmacogenomics Consortium (ISPC) was also unsuccessful to discover genetic variants associated with SSRIs response and remission in MDD patients after four weeks of treatment[23]. Similarly, polygenic approaches that tried to elucidate overall genome-wide genetic influences on antidepressant treatment response in MDD failed to predict antidepressant efficacy in GENDEP (n = 736) or STAR*D (n = 1409) studies[50,51]. A possible explanation for the difficulty in uncovering genetic variants associated with SSRIs treatment response is that treatment response is clouded by the clinical heterogeneity of MDD as a DSM-defined disorder. A second possible explanation is that SSRIs treatment response likely represents a complex, highly polygenic trait, influenced by a large number of genes

with small effects and interacting environmental factors. Increasing the sample size for current pharmacogenomics studies seems the only available option to be successful in this process, as previously observed in MDD GWASs[11]. Genetic variants associated with MDD were undetectable for smaller sample sizes (<200,000)[11]. The identification of additional variants could be made easier through further advances in sequencing technologies, improved analysis methods and a more homogeneous definition of clinical (sub-) phenotypes.

Table 1: Overview of genetic variants and candidate genes associated with antidepressants treatment response in patients with MDD

Candidate genes	**Coded proteins**	**Pharmacogenomic of antidepressants (description)**
IL1B **Interleukin-1 beta**	*IL1B* is one of eight other interleukin-1 family genes that encodes interleukin-1 cytokine proteins. These and other related proteins produced by activated macrophages are important mediators of inflammatory response throughout the body, including in the central nervous system (CNS)[52] .	Single nucleotide polymorphisms (SNPs) rs16944, rs116343 in the *IL1B* gene and their interaction with childhood maltreatment influences the effect of antidepressants in patients with MDD[53] and increase the risk of non-remission after antidepressant treatment in patients with MDD[33].
FKBP5 **FK506 binding protein 5**	Encodes a protein that is a member of the immunophilin family proteins involved in immune-regulation and related cellular processes. This protein binds to the immunosuppressant proteins such as FK506 and rapamycin[52].	A genetic variant rs1360780 in the *FKBP5* gene predicts remission to antidepressant treatment[54] and SNP rs352428 might influence SSRIs treatment outcomes in MDD patients[55].
CNR1 **Cannabinoid receptor 1**	Encodes cannabinoid receptor-1, one of the two receptors for cannabinoid — psychoactive ingredients of marijuana[52].	The *CNR1* gene polymorphisms rs806368 and rs806371 have shown a significant effect on the clinical response to SSRI (citalopram)[56]. A SNP rs1049353 may

		confer an increased risk for antidepressant treatment resistance[29].
NPY **Neuropeptide Y**	Encodes a neuropeptide that is widely expressed in the CNS and is involved in several physiological processes, including stress response and circadian rhythms[52].	*NPY* gene variation rs16147 was associated with a slow response and remission to antidepressant treatment[57].
ABCB1 **ATP-binding cassette subfamily B member 1**	The protein encoded by the *ABCB1* gene is a member of ATP-binding cassette (ABC) transporters — superfamily proteins involved in multidrug resistance. ABC proteins transport molecules across extra- and intra-cellular membranes and functions as a transporter in the blood-brain barrier[52].	In patients with MDD, *ABCB1* polymorphisms (rs1045642, rs2032582, and rs1128503) were associated with adjustment to antidepressant dosages to achieve remission[58]. Genotyping for rs2032583 and rs2235015 (TT/GG) may be used for clinical application to optimise antidepressant treatment[59]. Antidepressant treatment outcome was significantly associated with rs203258, rs2235015[60], rs2032588[61] and C3435T[62] polymorphisms. Moreover, a significant association was found between SSRIs-related adverse drug effects and SNPs rs2032583 and rs2235040[63].
BDNF **Brain-derived neurotrophic factor**	Encodes a member of the nerve growth factor family of proteins that promote neuronal survival in the adult brain. *BDNF* is involved in stress response and in the biology of mood disorders[52].	Genetic polymorphisms *BDNF* G196A[64], rs908867[65] did influence antidepressant treatment outcome. For example, *BDNF* G196A polymorphism in part determined the antidepressant effect of both milnacipran and fluvoxamine[64], and the *BDNF* Val66Met was associated with SSRI treatment resistance[66] and may be connected with lithium prophylaxis[67].
GRIK4 **Glutamate ionotropic receptor kainate-type subunit 4**	Encodes a glutamate-gated ionic channel family protein that collectively functions as excitatory neurotransmitters in the CNS[52].	The *GRIK4* SNPs rs12800734[28] and rs1954787 showed a strong association with antidepressant treatment outcome (remission and response)[39].

GNB3 **G protein subunit beta 3**	Encodes a beta subunit of the G protein beta family of G proteins (guanine nucleotide-binding proteins) that help to integrate signals between receptor and effector proteins[52].	*GNB3* C825T polymorphism influences the efficacy of antidepressants in the treatment of MDD[68-72].
HTR1A **5-hydroxytryptamine receptor 1A**	Encodes a receptor for serotonin that belongs to the 5-hydroxytryptamine receptor subfamily[73]. Serotonin is a neurotransmitter with several roles in the brain and body, and it is assumed to regulate feelings of happiness and well-being[52].	Patients with BPD who carries a 5-HT1A*C/C genotype showed a better response to an antidepressant (fluvoxamine)[74]. SNPs rs10042486, rs1364043, rs6295 within the *HTR1A* gene were significantly associated with antidepressant response[75].
HTR2A **5-hydroxytryptamine receptor 2A**	Encodes one of the receptors for serotonin.	Polymorphisms in 5HT-2A receptor rs17288723[28], rs7997012[76,77], rs9534505[78], rs6311, rs6313, rs7997012 and rs1928040[77,79] were significantly associated with therapeutic response or remission to antidepressants[77].
SLC6A4 **Solute carrier family 6 member 4**	Encodes a membrane protein that transports serotonin from synaptic spaces into presynaptic neurons, and terminates the action of serotonin and recycles it[52].	SNPs within *SLC6A4* were associated with antidepressant response[80-83], 5-HTTLPR pre-treatment genotyping might help to predict treatment remission[84]. Patients with 5-HTTLPR L/L or STin2 12/12 genotype experienced better clinical response to SSRI treatment[85]. A high-affinity antidepressant-binding site was found within the serotonin transporter (hSERT) protein[86]. SNP rs8076005[87] may be a modulator of antidepressant response. Moreover, the *GNB3*, *HTR2A* and *SLC6A4* genes may act in an interactive manner to influence antidepressant treatment outcome[71].

COMT **Catechol-O-methyltransferase**	Encodes the COMT enzyme protein that breakdowns neurotransmitters, such as dopamine, epinephrine, and norepinephrine. This enzyme might be involved in the metabolism of drugs[52].	The Val (108/158) Met variation of the *COMT* gene is among the most studied polymorphisms associated with response to antidepressants treatment[27,88,89] or electroconvulsive therapy[90] in patients with MDD.
CYP2C19 **Cytochrome P450 (CYP) family 2 subfamily C member 19**	Encodes a member of the CYP superfamily of enzymes (monooxygenases) known to metabolise several drugs, including psychiatric drugs. CYP enzymes together with the permeability glycoproteins (PGP) play a role in eliminating drugs from the brain and body[52].	Genetic variants within this gene are associated with variable ability to metabolize drugs and genotyping for these variants may help to classify individuals as poor or extensive drug metabolisers. *CYP2C19* contributes to the clearance of many antidepressants[91] and an amino acid residue 72 plays a key role in the metabolism of antidepressants by limiting the binding affinities of CYP2C9[92].
CYP2D6 **Cytochrome P450 family 2 subfamily D member 6**	A highly polymorphic gene that encodes an enzyme in the CYP superfamily[52].	Polymorphisms in the *CYP2D6* gene may influence metabolism and antidepressant response[93-95]. Similar to other CYP enzymes, *CYP2D6* may result in a different ability to metabolise drugs (poor or ultrarapid).
MAOA **Monoamine oxidase A**	One of two genes that encode mitochondrial enzymes, which catalyses degradation of amines (dopamine, norepinephrine and serotonin).	Genetic variants of the *MAOA* gene may influence antidepressant treatment response in patients with MDD[32,96].

Lithium treatment for bipolar disorder

Lithium is a first-line mood stabiliser introduced by the Australian psychiatrist John Cade in 1949[97]. Since then, it has been used as a first-line treatment of BPD and as prophylaxis to prevent recurrence[98]. The rate of lithium treatment response[99] and prophylactic efficacy[100] in BPD is relatively high compared to placebo. Yet, data has shown that a substantial proportion

of patients hardly achieve acceptable levels of lithium response with significant inter-individual variation between treatment responders and non-responders[24]. While clinical studies report a combination of demographic and clinical characteristics as potential predictors of treatment response in mood disorder patients[101], genetic factors are also highly involved in lithium treatment response with a potential interplay between genetic and environmental factors[24,102].

Pharmacogenomic studies that aimed to identify genetic variants associated with lithium treatment responses, efficacy, tolerability and safety in patients with BPD have identified novel SNPs located in protein-coding genes. Candidate gene studies have reported several polymorphisms associated with lithium treatment response located in the *5-HTT*[103,104], *TPH*[105], *DRD1*[106], *FYN*[107], *INPP1*[108], *CREB1*[109], *BDNF*[67], *GSK3β*[110], *ARNTL*[111], *TIM*[111], *DPB*[112], *NR3C1*[113], *BCR*[114], *XBP1*[115], *CACNG2*[116] genes (see Table 2).

To date, three GWASs have successfully identified SNPs associated with lithium treatment response in patients with BPD. A GWAS by the International Consortium on Lithium Genetics (ConLi^{+}Gen), incorporating over 2,500 bipolar patients from Europe, USA, Asia and Australia, found a single locus of four linked SNPs on chromosome 21[24]. Another GWAS by the Taiwan Bipolar Consortium involved 294 bipolar patients of Han Chinese descent and identified two SNPs in high linkage disequilibrium located in the introns of *GADL1* gene[117]. A third GWAS that involved 3,874 patients with BPD using subjectively (self-reported) and objectively defined (clinically documented) lithium response data from Sweden and the United Kingdom revealed a SNP within the *PLET1* gene associated with lithium-responsive patients in BPD[118]. However, in-depth follow-up functional characterisation is required before these associations can be harnessed to improve the

understanding of molecular mechanisms underlying the therapeutic effects of lithium. Findings to date are inconsistent across studies and are far from being helpful in everyday patient care[24].

It is interesting to note that some candidate genes reported in the pharmacogenomic studies of mood disorders have overlapping effects on response to antidepressants in MDD and lithium treatment response in BPD. For instance, polymorphisms in the *SLC6A4* gene were associated with antidepressant response or remission[81-85,87]. Similar *SLC6A4* variants have shown an association with lithium treatment outcomes in patients with BPD[104,114].

Table 2: Overview of genetic variants and candidate genes associated with lithium response in patients with bipolar disorder

Candidate genes	**Coded proteins**	**Pharmacogenomics of mood stabilisers, lithium (description)**
XBP1 **X-box bind protein 1**	Encodes a protein that regulates MHC class II genes by binding to an X-box (a promoter element) and it is involved in endoplasmic reticulum stress response[52].	Polymorphisms in *XBP1* (-116C/G) showed significant association with prophylactic treatment response to mood stabilisers - valproate[119] and lithium[115] in patients with BPD.
INPP1 **Inositol polyphosphate-1-phosphatase**	Encodes inositol polyphosphate-1-phosphatase, one enzyme involved in phosphatidylinositol signaling pathways[52].	A polymorphism in *INPP1* C973A might be associated with efficacy of lithium[108]. Significant interactions were also found between lithium response and *INPP1* SNP rs206472[120].
CREB1 **cAMP responsive element binding protein 1**	Encodes a transcription factor (a member of binding proteins) that induces transcription of genes in response to hormonal stimulation of the cAMP pathway[52].	Variations in the *CREB1* gene were associated with lithium response[121].

GSK3B **Glycogen synthase kinase 3 beta**	Encodes serine-threonine kinase, a protein belonging to the glycogen synthase kinase subfamily involved in energy metabolism and neuronal cell development[52].	*GSK3B* genetic variants may underlie therapeutic response to lithium[122,123] and patients with TT genotype at rs334558 had a poorer response to lithium treatment[123].
NR3C1 **Nuclear receptor subfamily 3 group C member 1**	Encodes a glucocorticoid receptor that binds to glucocorticoid responsive genes to activate their transcription. This protein is involved in inflammatory responses[52].	NR3C1 polymorphisms (rs6198, rs6191, rs6196, rs258813, rs33388) have shown a significant association with lithium treatment response in patients with BPD[113].
DRD1 **Dopamine receptor D1**	Encodes dopamine receptor D1, the most abundant dopamine receptor in the CNS. D1 receptors regulate neuronal growth and development. This protein mediates emotion processing and behavioral response[52].	*DRD1* gene genotype has been associated with poorer prophylactic effect of lithium[106].
FYN **FYN proto-oncogene, Src family tyrosine kinase**	Encodes a tyrosine kinase oncogene family protein implicated in the control of cell growth[52].	*FYN* gene polymorphism rs3730353 was associated with prophylactic response to lithium in bipolar patients[107].
Circadian clock genes ***TIMELESS*** Timeless circadian clock ***PER3*** Period circadian clock 3 ***CLOCK*** Circadian locomotor output cycle kaput ***ARNTL*** Aryl hydrocarbon receptor nuclear translocator-like	*TIMELESS, PER3, CLOCK* and *ARNTL* are members of the Period family of genes mainly involved in the control of circadian pattern. They also control cell survival after damage or stress, locomotor activity, metabolism and behavior[52].	Polymorphism in the circadian clock genes may be associated with lithium treatment and prophylactic response in bipolar illness[111].

TPH1 **Tryptophan hydroxylase-1**	Encodes a member of the aromatic amino acid hydroxylase family protein, involved in biosynthesis of serotonin[52].	TPH variants are associated with the prophylactic efficacy of lithium in mood disorders[105].
SLC6A4 **Solute carrier family 6 member 4**	Encodes a membrane protein that transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons[52].	Possible association between *SLC6A4* variants and lithium treatment outcomes in patients with BPD[104,114].
CACNG2 **Calcium voltage-gated channel auxiliary subunit gamma 2**	Encodes a type I transmembrane AMPA receptor regulatory protein (TARP) that regulates channel gating of AMPA receptors and mediate fast neurotransmission in excitatory synapses[52].	*CACNG2* gene polymorphisms (rs2284017, rs2284018, rs5750285) were associated with response to lithium treatment[116].
ASIC2 **Acid-sensing ion channel subunit 2**	Encodes a member of the degenerin/epithelial sodium channel superfamily that may play a role in neurotransmission[52].	*ASIC2* (*ACCN1*) gene is a potential candidate gene for response to lithium treatment in bipolar patients[124].
GADL1 **Glutamate decarboxylase-like 1**	Encodes a protein that catalyses decarboxylation of aspartate, cysteine sulfinic acid and cysteic acid to beta-alanine, hypotaurine and taurine, respectively[52,125].	Two linked SNPs, rs17026688 and rs17026651, mapped to *GADL1* gene showed associations with lithium response in patients with BPD[117].

Polygenic approach to pharmacogenomics in mood disorders

Genome-wide association studies have consistently demonstrated that psychiatric disorders are highly polygenic, with a large number of genetic variants of small effect increasing the susceptibility to the disorders[126]. Similar polygenicity is expected in drug-response phenotypes in mood disorders[23,127]. One approach that can assist in translating genomic knowledge into clinical applications is the polygenic score (PGS) method proposed by the International Schizophrenia Consortium[128]. This method quantifies the aggregate effect of genome-wide genetic variants, including those polymorphisms that showed only a marginal

association with treatment-associated phenotype. Polygenic modeling has an advantage over commonly used cross-trait methods, such as LD score regression method or the cross-trait restricted maximum likelihood (REML) approach[5], in that, it is suitable for clinical application and epidemiological interpretation. An accurate and successful polygenic model may assist early screening for disease risk, clinical diagnosis and in the prediction of treatment response and disease prognosis. Thus, polygenic profiling may help to stratify patients by status of drug response and help to choose or refine treatments. Moreover, PGS might be used as a quantitative measure of genetic load that can be tested for its association with endo-phenotypic measures, such as plasma level of drugs or half-life. PGS associations can also be translated into odds ratios by comparing highest versus lowest quartile (decile) groups, which makes clinical interpretations more simplified. Whether these PGSs have the potential to be applied to every day clinical care depends on their ability to differentiate patients into categories of treatment responders versus non-responders or remitters versus non-remitters. More research is required to determine whether this is the case and future studies should consider related variables, such as multi-trait PGSs analysis or interaction analyses with clinical and environmental factors, i.e., PGS x PGS x environmental interactions.

The success of PGSs is likely to depend on three important factors: a) power of initial GWAS studies, b) homogeneity of the population, and c) sample size. It has been shown that polygenic score is likely to be useful when it is population-specific (in a homogeneous population) and when the initial GWAS is successful.

An additional implication of using a PGS is that it provides platforms for understanding convergence in co-morbid diseases and their influence on treatment outcomes. A recent

review of GWAS and candidate gene studies provided evidence for a cross-disorder genetic overlap between mood disorders and cardiometabolic abnormalities. This evidence indicated a genetic basis for the impact that medical comorbidities have on treatment outcomes in mood disorders[6]. PGS analysis can be applied in such cases to estimate the extent of genetic overlap as well as to identify polygenic predictors[6].

Challenges of pharmacogenomics studies in mood disorders

Pharmacogenomics studies in mood disorders have specific challenges in addition to those common to other genetic studies. Generally, accumulating an adequate number of patients, precisely defining drug response phenotypes and addressing analytic issues of large data are challenges to successful accomplishment of pharmacogenomic studies. Additional challenges specific to mood disorders include heterogeneity of disorders, often overlapping with other medical and psychiatric morbidities, which may result in a heterogeneous treatment outcome (see Box 1). Until more stratified diagnosis and treatment are established, the only way to improve the power of existing pharmacogenomic studies in mood disorders is increasing sample size and optimising study designs to involve more severe cases. Data from large samples of patients treated with a single treatment option is also very rare, and dosage of drugs also vary, further complicating efforts to study the pharmacogenomics of specific drugs. Moreover, patients with mood disorders are often treated with a combination of psychological and pharmacological treatment alternatives, and it is sometimes difficult to rule out whether patients are benefiting from pharmacological or non-pharmacological treatment, especially when treatment effect is low. Designing a large pharmacogenomics study is very costly adding clinical trial requirements to genotyping costs. As the cost of sequencing and

genotyping continue to decline, every effort should be made to increase sample sizes of current projects in order to carry out comprehensive pharmacogenomic studies.

Box 1: Challenges of pharmacogenomics studies in mood disorders

Potential challenges	**Description**
Establishing a hard-end definition of treatment outcome (response and remission)	Mood disorders are highly heterogeneous and there are no objective criteria to define diagnosis of disorders and treatment outcomes. Assessment tools applied in diagnosis and treatment follow-up of mood disorders have poor sensitivity and specificity.
Optimising sample size	Underpower is the major bottleneck for success in pharmacogenomic studies of mood disorders and global collaboration is required to improve existing efforts.
Bioinformatic tools for integrative analysis	To provide an evidence-based decision about a patient, complete evaluation of data obtained from different pillars of omics investigation, including genomics, epigenomes, proteomics, metabolomics and microbiomes is required. Advanced bioinformatics tools are required to perform integrative analysis.
Replication of findings and moving to clinical application	Findings from candidate gene studies and GWASs are rarely replicated and translating findings is inadequate.

Generally, when we plan pharmacogenomic studies in mood disorders, particular care needs to be exercised during: a) choosing the patient; b) designing the study and estimating sample size and c) planning statistical analysis and replication. Appropriate sample size to design a

pharmacogenomics study depends on the expected effect sizes that genetic variants have on treatment outcome, as well as on the number of independent tests (number of variants) to be tested for association with treatment outcome. Thus, sample size of a study is dictated by study design. Candidate gene studies include fewer variants than GWASs; therefore, candidate gene studies can enroll a much smaller number of participants. Nonetheless, GWASs require hundred-thousands to millions of individuals to detect the effect of genetic variants with a small effect — odds ratios from 1.1 to 2[129]. It is unlikely that individual clinical trials in a single country will achieve such a sample size, and therefore, optimising the design of pharmacogenomic studies requires global collaboration. To be successful, collaborations among geneticists, methodologists, pharmacologists, clinicians and pharmaceutical companies should be encouraged and cross-consortia partnerships may be required. Some examples of global collaboration efforts in the pharmacogenomics studies of mood disorders are described below. These can be future opportunities for pharmacogenomic studies in mood disorders.

International collaboration as an opportunity to pharmacogenomic studies in mood disorders

An international collaboration between scientists in academia, as well as with pharmaceutical industries, provides excellent opportunities for pharmacogenomic studies in mood disorders. In the following section, we introduce some of these initiatives.

a) Collaborations to study the pharmacogenomics of antidepressants

Clinical trials and international consortia with available GWAS data in the field of antidepressant response include the International SSRIs Pharmacogenomics Consortium

(ISPC) study[23], Depression and Sequence of Treatment (DAST) Study, the NEWMEDS consortium[34,50,130], the Pharmacogenomics Research Network Antidepressant Medication Pharmacogenomics (PGRN-AMPS) Study[131] , the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Study[132], GENDEP project and the MARS project[50,133,134].

International SSRI Pharmacogenomics Consortium (ISPC) study

The ISPC is an international collaboration of experts established with the aim to discover genetic variants responsible for SSRIs treatment outcomes in MDD patients. The first genome-wide association study by this group was published in 2015[23]. This group has data on 998 MDD patients collected from different countries including in Europe, USA and Asia. The genotyping for the ISPC sample was performed at the RIKEN Center for Integrative Medical Sciences (Yokohama, Japan). Demographic and clinical data for the ISPC are available at the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB) www.pharmgkb.org. The depressive symptom measure in the ISPC data was the 17-item Hamilton Depression Rating Scale. Further details are available elsewhere[23].

Depression and Sequence of Treatment (DAST) study

The DAST study is a prospective naturalistic treatment study of 746 inpatients diagnosed with a major depressive disorder of adult age (>18yrs of age) collected at the Department of Psychiatry and Psychotherapy at the University of Münster, Germany[27,33,50]. MDD was diagnosed using a structured clinical diagnostic interview (SCID). Treatment response and remission were assessed using the Hamilton Depression Rating Scale (HAM-D) as the primary treatment outcome measure administered on a weekly basis at least for six weeks duration by trained psychiatrists and psychologists. Patients are of Caucasian ancestry and

treatment selection was made by clinicians and included flexible antidepressant dosage and agents for augmenting[27,33,50].

Pharmacogenomics Research Network Antidepressant Medication Pharmacogenomics Study (PGRN-AMPS)

The PGRN-AMPS is a single-arm clinical trial focused on the response of MDD patients to citalopram or escitalopram over eight weeks of treatment. The study involved 530 participants with nonpsychotic MDD aged between 18 and 84 years, who were recruited from the inpatient and outpatient practices of the Department of Psychiatry and Psychology at Mayo Clinic in Rochester, Minnesota. Depression severity in this cohort was assessed using the 16-item Quick Inventory of Depressive Symptomatology (QIDS-C16). Details on the PGRN-AMPS can be found elsewhere[131].

*Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study*

The STAR*D study is a multilevel clinical trial of outpatients with nonpsychotic MDD funded by the National Institute of Mental Health. The STAR*D initially enrolled 4,041 MDD patients aged between 18 and 75 years[132], who received the SSRI citalopram in the first 12 to 14 weeks (level 1). Clinical data for STAR*D was collected using the QIDS-C16 scale. Data on covariates such as age, gender and on the specific SSRIs medications were also gathered, and details can be found elsewhere[23,131,135].

The Genome-based Therapeutic Drugs for Depression (GENDEP) project

The GENDEP project involved 868 Caucasian ancestry patients aged 18 to 75 years and is a 12 week multicentre partly-randomised pharmacogenetics trial with two active treatments (a SSRI-escitalopram and a tricyclic antidepressant-nortriptyline)[49].The GENDEP research project is an integrated project involving scientists from ten countries, including Germany,

UK, Ireland, Belgium, Italy, Sweden, Denmark, Poland, Slovenia and Croatia. Investigators at the UK Medical Research Councils (MRC) Social, Genetic and Developmental Psychiatry Centre (SGDP) at the Institute of Psychiatry, Kings College London, lead the project. The diagnosis of depression was per the ICD-10/DSM-IV criteria and the primary treatment outcome measure was the Montgomery-Åsberg Depression Rating Scale (MADRS).

Munich Antidepressant Response Signature (MARS) project

The MARS project (http://www.mars-depression.de) is a prospective naturalistic study of 842 inpatients aged 18 to 75 years admitted to hospitals in southern Germany for the treatment of MDD or BPD. Diagnoses are based on DSM-IV criteria and a clinical interview by trained psychiatrists[50,133,134]. This project was initiated in 2000 by the Max Planck Institute of Psychiatry (MPI-P, Munich, Germany), and longitudinal data were collected from three clinical sites in southern Bavaria (MPI-P, Munich; Bezirkskrankenhaus Augsburg; Klinikum Ingolstadt). All patients are of Caucasian ancestry, and treatment was selected by clinicians and included flexible antidepressants dosage and agents for augmenting. The Hamilton Depression Rating Scale (HAM-D) was the primary treatment outcome measure, which was administered weekly by trained psychiatrists and psychologists[50,133,134].

The Genetic and Clinical Predictors of Treatment Response in Depression (GENPOD) study

The GENPOD study was a multicentre RCT conducted in Bristol, Birmingham, and Newcastle, UK involving 601 (men n=161, women n=347) aged 18-74 years recruited in primary care who had an ICD-10 diagnosis of MDD who were randomised to either a SSRI (citalopram) or a NARI (reboxetine)[136].

Novel Methods Leading to New Medications in Depression and Schizophrenia (NEWMEDS) consortium

NEWMEDS is an international consortium of research academic-industry collaboration aiming to find new methods for the development of drugs for schizophrenia and depression. Individual clinical trials currently part of the NEWMEDS consortium (http://www.newmeds-europe.com) include three studies from academic institutions (GENDEP, n=868; GENPOD, n=601; and GODS, n=131) and two studies by the European Federation of Pharmaceutical Industries and Associations (Pfizer, n=355, and GlaxoSmithKline, n=191). In all, NEWMEDS has enrolled 2,146 individuals (941 men and 1,205 women) diagnosed with MDD according to the ICD-10 and DSM-IV, with data on the prospective outcome of treatment with norepinephrine reuptake inhibitors (NRIs) or selective serotonin reuptake inhibitors (SSRIs)[34,50,130].These patients underwent 6 to 12 weeks of treatment with either SSRIs (citalopram, escitalopram, sertraline, paroxetine, fluoxetine) or NRIs (reboxetine, nortriptyline)[34,50,130].

The Geneva Outpatient Depression Study (GODS)

The GODS study[137] is a partly randomised trial led by researchers at Geneva University Department of Psychiatry, which examined the efficacy of four antidepressants (clomipramine, paroxetine, nefazodone, and venlafaxine) in a cohort of 131 subjects (53 men and 78 women) with MDD patients aged 18-65 years[34,137].

Pfizer: Pharmaceutical Company

The study by Pfizer involved a total of 355 MDD patients from eight clinical facilitates primarily conducted a double-blind, placebo-controlled trial, with follow-up at 6 to 8 weeks. The patients in the treatment groups had sertraline, fluoxetine or paroxetine[34].

Glaxo Smith Kline (GSK): Pharmaceutical Company

The samples for the GSK study were derived from two randomised studies conducted from January 2003 to June 2004 in the United States to compare the efficacy of antidepressants and the effect of bupropion and escitalopram on sexual functioning in outpatients with depression (n=210)[34,138].

b) Collaborations to study the pharmacogenomics of lithium

The International Consortium on Lithium Genetics (ConLi^{+}Gen)

ConLiGen consortium is the largest gathering in the genetics of lithium to date that aims to identify genetic polymorphisms associated with lithium treatment response in BPD, as well as genetic determinants of adverse events that may result from lithium treatment. The ConLi^{+}Gen Consortium (www.ConLiGen.org) is an initiative by the National Institute of Mental Health (NIMH) and the International Group for the Study of Lithium-Treated Patients (IGSLI) (www.IGSLI.org)[98]. The ConLiGen has compiled genetic and clinical data from 3,193 patients with BPD who had undergone lithium treatment in Europe, USA, Asia and Australia[24]. The first GWAS was published in 2016[24]. ConLiGen has continued to invite researchers from around the world, including from developing countries, to join the current efforts of increasing sample size to adequately represent the patient population.

Strategies and future paths to personalised psychiatry

The terms precision, personalised and individualised medicine are often used interchangeably, and refer to the treatment of patients based on individual characteristics including genomic information[139,140]. The European Association for Predictive, Preventive and Personalised Medicine (EPMA) promotes this integrative concept of medicine[141-144] and describes Predictive, Preventative and Personalised Medicine (PPPM) as the medicine of the

future. PPPM is one of the main strategies in 'Horizon 2020'[142-144]. Horizon 2020 is the biggest European Union Research and Innovation programme with a funding budget of nearly €80 billion over 2014 to 2020.

With pharmacogenomics being one focus in the process towards personalised psychiatry, we suggest three alternative and complementary strategies to implement knowledge generated from genetic studies. The first strategy is to design a therapeutic or diagnostic genetic testing scheme based on candidate genes or gene products — testing their interactions with psychopharmacological drugs and predict treatment outcomes. The second strategy refers to an integrative analysis of omics data obtained from the different pillars of omics investigation including genomics, epigenomes, proteomics, metabolomics, and microbiomes. Biologists have labeled this procedure as 'systems genomics (genetics)' approach. The main goal of systems genomics is an identification and understanding of biological pathways, networks and modules affected by underlying complex genetic traits. These pathways could be used as drug targets and may guide the efforts to new drug development. The third approach may be to develop multivariable prediction algorithms for diagnostic or prognostic purposes.

Strategy 1: Genetic testing based on candidate genes or gene products

The clinical utility of pharmacogenomics evidence and subsequent genetic testing in psychiatric care is an ongoing process given that studies are generating new evidence. The current approach of genetic testing is based on 'targeted genotyping' of genetic variations in genes involved in the metabolism of many drugs, including psychiatric medications. An example of pharmacogenomic testing in psychiatry is the genotyping of drug-metabolising enzyme genes, known as cytochromes P450 (CYP)[145-147]. Variations within the CYP genes are extensive and have long been known to affect the metabolism of several drugs, including

antidepressants and lithium. Hence, the CYP genes have been extensively studied in psychiatry. There are now commercially available gene-chips to genotype these genetic variations for patients who are receiving antidepressants or mood stabilisers[148]. For example, a commercial pharmacogenetics test (GeneSight) helps to identify individuals who are either poor metabolisers or ultrarapid metabolisers for over 55 neuropsychiatric drugs based on genotype of the CYP (*CYP2D6, CYP2C19, CYP2C9* and *CYP1A2*)[38,148]*, SLC6A4, HTR2A* and *ABCB1* genes[38].

Strategy 2: Integrative analysis — systems genomics approach

Systems genomics — broadly called systems biology — is a global perspective of understanding the mechanisms underlying complex traits[149,150]. It involves a range of experimental and statistical modeling techniques aimed to integrate broad data from genomes (DNA), epigenomes, transcriptomes (RNA), proteomes, metabolomes, and their interactions with the environment, such as with microbiomes[149,150] (see Figure 1). Systems genomics can be applied in drug-response phenotypes of mood disorders — to identify biological pathways, networks and modules that may ultimately be used as targets for new drug development. This approach is complementary to other strategies suggested in our review, but specifically aimed to understand molecular underpinnings of complex traits, rather than the development of diagnostic or prognostic algorithms (tools) for immediate application. The statistical techniques advanced from a single phenotype-single data approach (single omics data) to meta-dimensional and multistage analysis (multiple omics approaches). The analysis methods include, among others, expression quantitative trait loci (eQTL), pathway analysis, Bayesian networks, evolutionary computing methods, symbolic regression and artificial neural networks (ANNs). These data integration techniques can be categorised into two approaches,

multi-staged analysis and meta-dimensional analysis approach. In multi-staged modeling, analysis is divided into different steps to test the associations between different data types and phenotypes, allowing for only two different scales at a time (i.e. continuous and categorical scales). Conversely, meta-dimensional modeling combines multiple data types and all scales simultaneously to identify complex, meta-dimensional models with multiple variables from different data types. Software tools have been developed to implement several techniques of multi-staged, meta-dimensional analysis and a combination of these techniques. Details of these methods are available elsewhere[149,150].

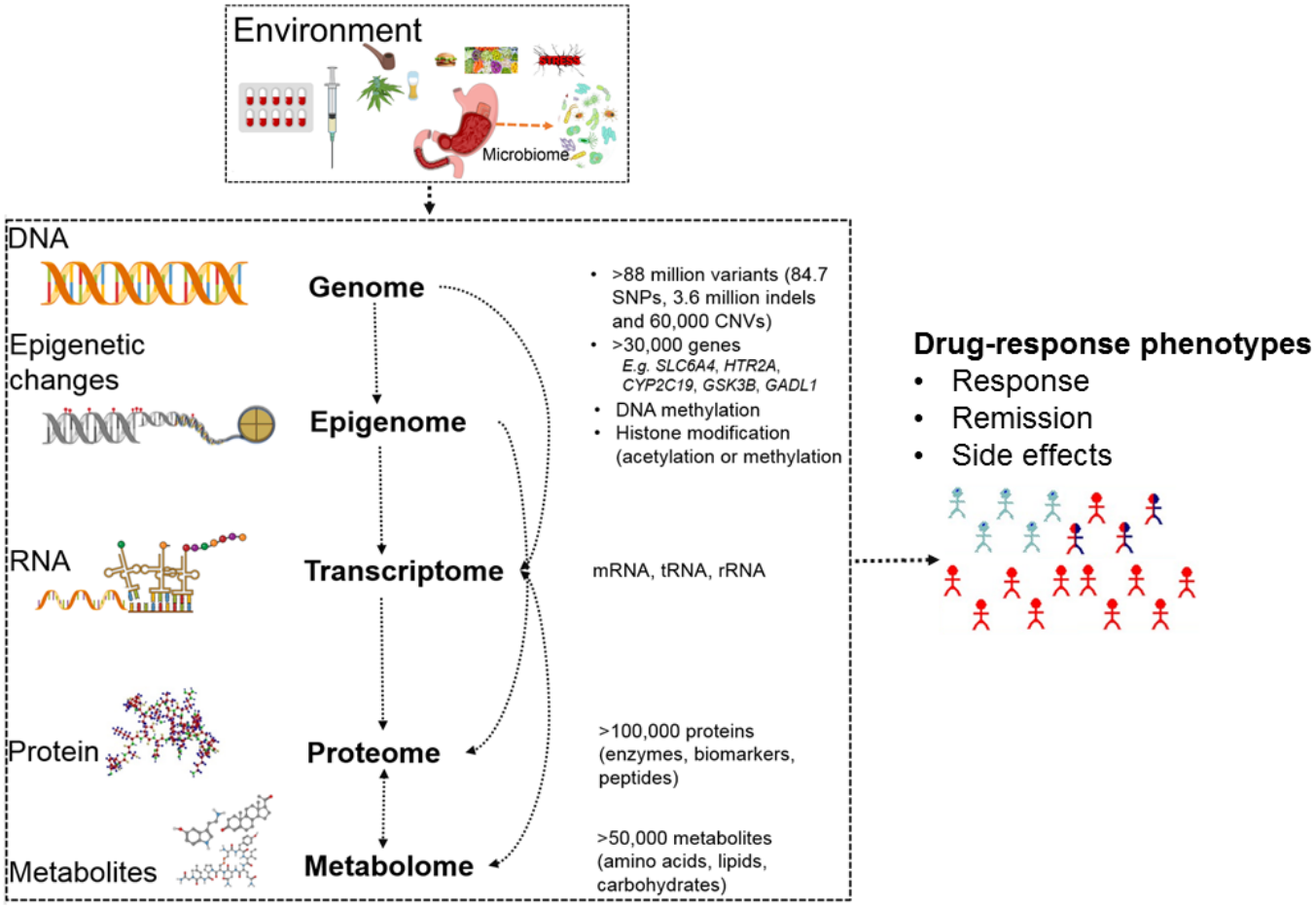


Figure 1: An overview of the biological data that need to be integrated into the systems genomics approach to investigate pathways in complex traits (e.g. treatment response)

Strategy 3: Development of prognostic models

While efforts continue to improve the power of existing GWAS studies, we are also beginning to see the opportunities and challenges in implementing genomic information to personalised psychiatric care. Until all genetic discoveries are successful and validated with high confidence, the clinical utility of genetic variants requires careful implementation and integration of innovative algorithms that capture overall variance in a complex trait (e.g. treatment response) accounted by genome-wide genetic variants. In this regard, the polygenic modeling method allows capturing of the contribution of genome-wide SNPs to complex phenotypes, such as pharmacogenomic traits. Clinical translation of polygenic findings may be challenging, but future advances in sequencing technologies and novel statistical genetics analysis methods may improve the predictive power of polygenic scores. In the future, every individual's genotype data may be readily available and improved algorithms that can combine the genetic effects of every polymorphism, including from rare genetic variants, may be developed. Until then, the goal should be identification of the remaining genetic variants for treatment response and implement at least a semi-powerful polygenic approach. For better use of the current scientific output, prognostic models combining data from biological, clinical, psychosocial and sociodemographic predictors could be developed and tested.

Prognostic models developed based on several patient characteristics[151-153], including genetic information, are a cornerstone of precision medicine. Despite limited available information, structural and functional networks in the brain[151], including neuronal connections[152], microbiome[153], epigenetic mechanisms[154,155] and changes in gene expression[156], are all associated with drug efficacy and could be considered as additional markers to predict

treatment response in a clinically meaningful way. The capacity to combine all potential genetic and non-genetic factors that possibly affect treatment response to psycho-pharmacotherapy determines the future path to personalised psychiatry.

The concept behind the development of prognostic models is to estimate the probability that a patient with a given set of characteristic (predictors) will respond favorably or poorly to pharmacological treatment. Such models are developed based on patient baseline characteristics likely to be associated with prognosis of the disorders and efficacy of drugs (see Figure 2 and Box 2). These predictors are usually selected based on biological evidence, clinical experience and findings from the literature. Given these characteristics, prognostic models allow estimation of the probability that a patient will favorably respond or poorly react to the drugs. Once such models are developed, their predictive accuracy should be evaluated before they are applied in clinical practice. This process is known as model validation and it involves calibration assessment (testing agreement between observed treatment outcomes and predictions) and discrimination assessment (evaluation of the model's capability to discriminate between responders and non-responders). Models are usually validated both internally (for example, using bootstrapping) and externally using data from another patient population not used for model development.

Box 2: Approaches in prognostic model development and validation

1) Stepwise model development

As an example, we present the principle statistical procedure to construct a prognostic model for mood disorders.

*Treatment outcome***:** Response to treatment (favourable versus poor)

Predictor variables: Age, sex, genetic predictors (e.g. polygenic score for potential predictors), disease severity and other clinical characteristics of the disease at baseline, including psychosocial variables, and co-morbidities (see Figure 2)

A prognostic model estimates the patient's probability to respond to treatments given a set of predictor variables at the baseline. The appropriate statistical modeling choices for binary outcomes such as treatment response can be:

a) *A binary logistic regression model (binary outcome)*: This model takes the forms

$$\text{Probability of response } = \frac{\exp^{(\text{patient's response score})}}{1 + \exp^{(\text{patient's response score})}}$$

Where,

$$\text{Patient's response score} = \text{intercept} + \beta 1\text{age} + \beta 2\ \text{sex} + \beta 3\ \text{genetic score} + \beta 4\ \text{illness severity at baseline} + \ldots).$$

The beta's (β_1 to β_n) are regression coefficients for each predictor variable.

b) *Survival analysis using Cox proportional hazards model*, predicts treatment outcomes (e.g. response) at varying time points. This model has the form

$$\text{hi}\,(t) = \text{ho}(t) * \exp^{[\text{intercept} + \beta 1\text{age} + \beta 2\ \text{sex} + \beta 3\ \text{genetic score} + \beta 4\ \text{illness severity at baseline} + \ldots]}$$

Where, hi (t) is the expected hazard (response) for individual 'i' at time t, and ho (t) is the baseline hazard and represents the hazard when all of the predictors (or independent variables) are equal to zero.

External validation

Models developed in the above procedure should be externally validated using a new group of patients treated with the same drug to predict treatment outcomes using similar predictors. Regression coefficients estimates (from the model development phase) are used to predict the probability of patients' response in the validation sample. The agreement between the predicted and

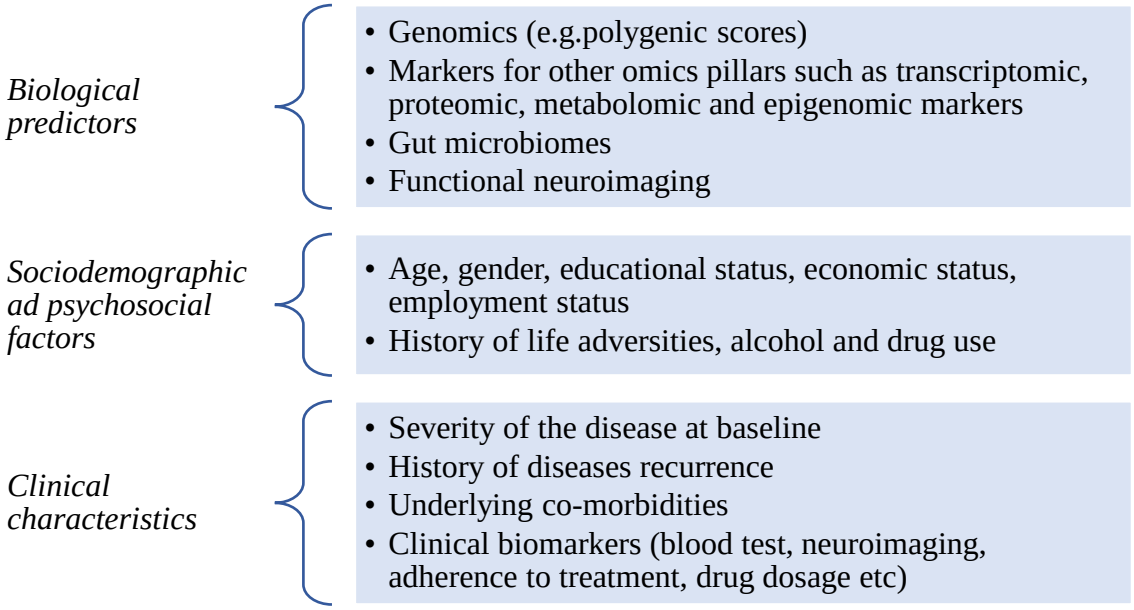
observed outcomes is assessed, i.e. the model is assessed for its performance, for example, calibration and discrimination.

The use of external cohorts increases the generalisability of the model. In case no external patient sample is not available, a bootstrap validation may be used.

2) ***Machine learning*** is a recently evolving method, which often overlaps with the above statistical approaches. Essentially, a machine learning explores the data structure using provided instructions (algorithms) to detect patterns of statistical regularity across the data to make meaningful classifications and build prediction models. Supervised machine learning approaches are often suggested as a powerful tool in medicine. Several methods in machine learning use a multivariate approach to the entire dataset and are able to handle interactions[157,158].

Given the complex nature of predictors for treatment response in mood disorders, a broader understanding of potential predictors may enhance the predictive capacity of diagnostic or prognostic algorithms (tools). Essentially, predictor variables should be collected in a wider scope including the characteristics that showed weak associations. As shown below, the potential predictor variables in mood disorders can be broadly categorised as biological predictors, clinical characteristics, sociodemographic factors and psychosocial factors. Although a detailed review of each potential predictor is beyond the scope of this article, the specific biomarkers in each category are usually selected based on biological evidence, clinical experience and findings from the literature. Once this information is collected, statistical techniques, such as machine learning, can be applied to produce a final model with the best predictors[159]. During the data mining process, all potential predictors will be pooled and variables that better improve the model will be finally filtered and remained in the model, as measured by, for example, area under the ROC curve (see Figure 2). Application of

machine learning approaches to predict treatment response has shown promising results to predict treatment outcomes (e.g. in antidepressants)[160].Analysis of STAR*D data has identified over 25 variables most predictive of antidepressant treatment outcome with an accuracy ranging from 59.6% to 64·6%[160]. A similar approach using peripheral gene expression data in major depression identified a panel of 13 genes which predicted citalopram treatment response with a 76–79% accuracy[161]. These findings are encouraging, but they reported low predictive accuracy indicating that an additional or stronger predictor variable(s) are required. This is the stage at which emerging predictors, such as genomic information, can help to improve the performance of existing models.



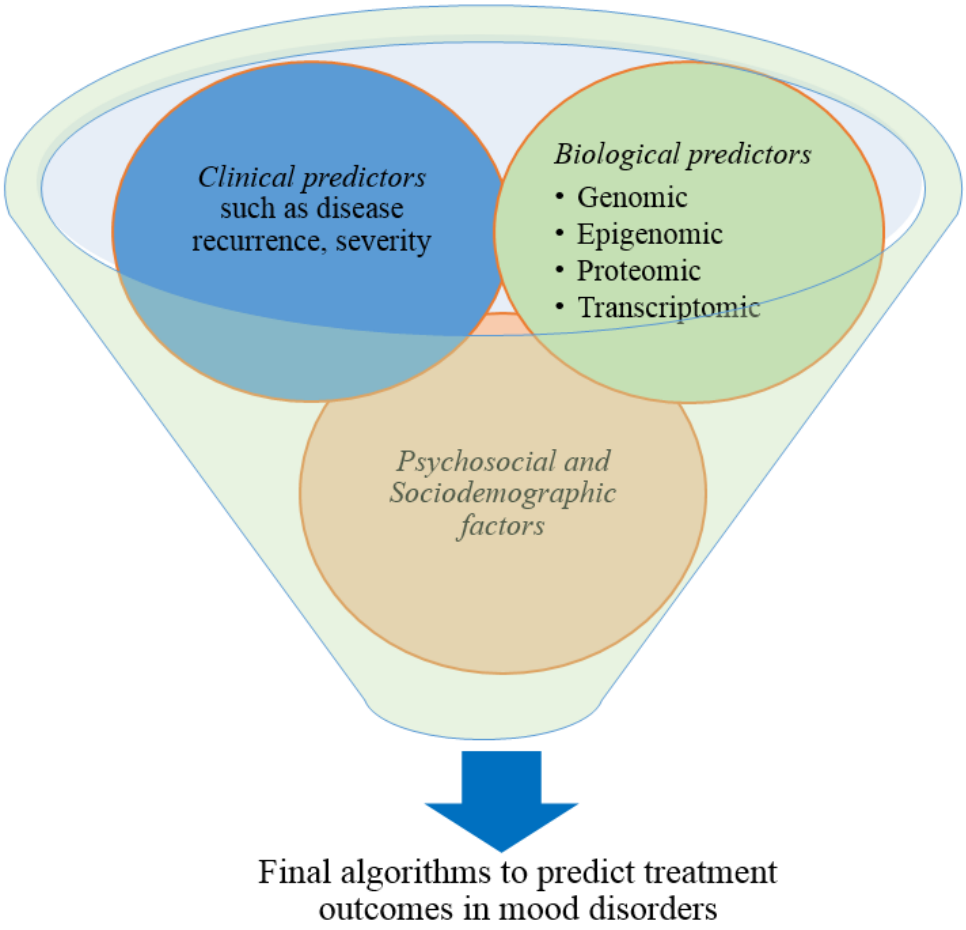


Figure 2: Overview of how potential predictors can be pooled into a statistical model during the development of algorithms to predict treatment outcomes (e.g. treatment response)

Conclusions and expert recommendations

In conclusion, a number of pharmacogenomic studies have been conducted to uncover genes associated with treatment response to mood stabilisers and antidepressants. Indeed, the findings from candidate gene and GWAS were encouraging, but not adequate in terms of their power to identify the expected number of genetic variants. Strong international collaboration between scientists in academia and the pharmaceutical industry are important to improve the power of the existing GWAS studies, and to achieve the goals of personalised psychiatry.

Findings from pharmacogenomic studies have the potential to improve psychiatric care and many advances are expected in the near future with improvements in the definition of clinical phenotypes, advancement in sequencing technology and better statistical tools to analyse a broad range of data. The integration of molecular science with that of traditional clinical practice is the way forward to drug discoveries, novel therapeutic approaches and to characterise disorders. Suggested strategies to translate current knowledge into clinical practice includes genetic testing, integrative analysis (systems genomics approach) and the development of multivariable diagnostic or prognostic algorithms (tools) to predict therapeutic outcomes. The integration of omics data with clinical variables could lead to a better predictive, preventive and personalised medicine (PPPM) in psychiatry, for example, helping to distinguish patients with favorable response to pharmacological treatment. Omics studies, so far, were inadequate and had limited power. Further studies should build on existing efforts of international collaborations to increase sample size and identify additional biological markers — then data integration and implementation into standard clinical decision-making will be realised in the future. This would be a major step towards PPPM in psychiatry. While we are still in the early stages of this 'revolution', significant scientific innovation gives the field hope to shape the future of psychiatric medicine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 3

The genetic overlap between mood disorders and cardiometabolic diseases: A systematic review of genome-wide and candidate gene studies.

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ABSTRACT

Meta-analyses of genome-wide association studies (meta-GWASs) and candidate gene studies have identified genetic variants associated with cardiovascular diseases, metabolic diseases and mood disorders. Although previous efforts were successful for individual disease conditions (single disease), limited information exists on shared genetic risk between these disorders. This article presents a detailed review and analysis of cardio-metabolic diseases risk (CMD-R) genes associated with mood disorders. Firstly, we reviewed meta-GWASs published until January 2016, on the diseases "type-2 diabetes, coronary artery disease, hypertension" and for risk factors "blood pressure, obesity, plasma lipid levels, insulin and glucose-related traits". We then searched the literature for published associations of these CMD-R genes with mood disorders. We considered studies that reported a significant association of at least one CMD-R gene and "depression" OR "depressive disorder" OR "depressive symptoms" OR "bipolar disorder" OR "lithium treatment response" OR "serotonin reuptake inhibitors treatment response". Our review revealed 24 potential pleiotropic genes likely to be shared between mood disorders and CMD-Rs. These genes include *MTHFR*, *CACNA1D*, *CACNB2*, *GNAS*, *ADRB1*, *NCAN*, *REST*, *FTO*, *POMC*, *BDNF*, *CREB*, *ITIH4*, *LEP*, *GSK3B*, *SLC18A1*, *TLR4*, *PPP1R1B*, *APOE*, *CRY2*, *HTR1A*, *ADRA2A*, *TCF7L2*, *MTNR1B*, and *IGF1*. Pathway analysis of these genes revealed significant pathways: corticotrophin-releasing hormone signaling, AMPK signaling, cAMP-mediated or G-protein coupled receptor signaling, axonal guidance signaling, serotonin or dopamine receptors signaling, dopamine-DARPP32 feedback in cAMP signaling, circadian rhythm signaling and leptin signaling. Our review provides insights into the shared biological mechanisms of mood disorders and cardio-metabolic diseases.

INTRODUCTION

Major depressive disorder (MDD), bipolar disorder (BPD), coronary artery diseases, type 2 diabetes and hypertension are amongst the major causes of disability, morbidity and mortality worldwide[1,2]. While each of these conditions independently represents a major burden facing health-care systems[1-3], their co-occurrence (co-morbidity) aggravates the situation and represents a challenge in psychosomatic medicine[4]. Epidemiologically, MDD and BPD are bi-directionally associated with cardio-metabolic diseases[5,6]. A similar pattern of association has been shown in the relationship between pharmacological treatment of mood disorders and cardio-metabolic diseases. For instance, the use of antidepressants and mood stabilisers is associated with increased risk of cardio-metabolic abnormalities[7] and cardiac medications may increase the risk of mood disorders[8]. One explanation for these relationships could be the presence of pleiotropic (common) genes and shared biological pathways that function as a hub to link the disorders. Potential common biological mechanisms underlying mood disorders and cardio-metabolic disease comorbidity have been proposed, including altered circadian rhythms[9], abnormal hypothalamic-pituitary-adrenal axis (HPA axis) function[10], imbalanced neurotransmitters[11] and inflammation[6]. However, the molecular drivers of these commonly affected mechanisms remain poorly understood.

The genetics of mood disorders and cardio-metabolic diseases

Major depression, bipolar disorder and cardio-metabolic diseases are highly heritable and are caused by a combination of genetic and environmental factors. Genetic factors contribute to 31-42% in MDD[12], 59% - 85% in BPD[13,14], 30-60% in coronary artery

diseases[15], 26-69% in type 2 diabetes[16,17], 24-37% in blood pressure[18], 40–70% in obesity[19] and 58-66% in serum lipid level[20]. Moreover, twin studies have revealed relatively modest genetic co-heritabilities (genetic correlations) between mood disorders and different cardio-metabolic abnormalities suggesting the influence of pleiotropic genes and shared biological pathways among them. For instance, the genetic correlation of depression with hypertension is estimated to be 19%, and for depression and heart disease is about 42%[21]. The genetic correlation of depressive symptoms with plasma lipids level ranges from 10% to 31%[22], and 12% of the genetic component for depression is shared with obesity[23]. Furthermore, gene-environment interactions can contribute to the cardio-metabolic and mood disorders link. The interactions of genetic factors with stress, physical exercise, diet and lifestyle influence the progression and pathogenesis of both cardio-metabolic and mood disorders (Figure 1)[24-26]. These factors may, for example, modulate expression of genes involved in cardio-metabolic pathways and a variety of pathways in the brain. Although at infancy stage, the 'microbiome' era has revealed a range of complex interactions between environmental factors, genes and diseases[27].

In the last decade, substantial amounts of univariate (single disease) meta-analyses of genome-wide association studies (meta-GWASs) and candidate gene studies have been published. Indeed, meta-GWASs and candidate gene studies have successfully identified a considerable list of candidate genes for major depressive disorder[28], bipolar disorder[29], coronary artery diseases[30], type 2 diabetes[31], hypertension[26], obesity[32], plasma lipids level[33], insulin and glucose traits[31,34], and blood pressure[26,35].

Despite the potential significance of studying pleiotropic genes and shared biological pathways, previous meta-GWAS and candidate gene studies were entirely focused on a single phenotype approach (single disease). A recent analysis of SNPs and genes from the NHGRI GWAS catalogue[36] has shown 16.9% of genes and 4.6% of SNPs have pleiotropic effects on complex diseases[37]. Considering such evidence, we hypothesised that common genetic signatures and biological pathways mediate mood disorders to cardio-metabolic disease relationships. Additionally, these genes and their signaling pathways can influence response to treatments in mood disorder patients (Figure 1). In this review, we systematically investigated the risk of cardio-metabolic disease (CMD-R) genes that are possibly associated with mood disorder susceptibility and with treatment response to MDD and BPD. We performed pathway and gene network analyses of these genes to provide additional insights into the common pathways and biological mechanisms regulating mood disorders and CMD-Rs. Understanding of these common pathways may provide new insights and novel ways for diagnosis and treatment of comorbid cardio-metabolic and mood disorders.

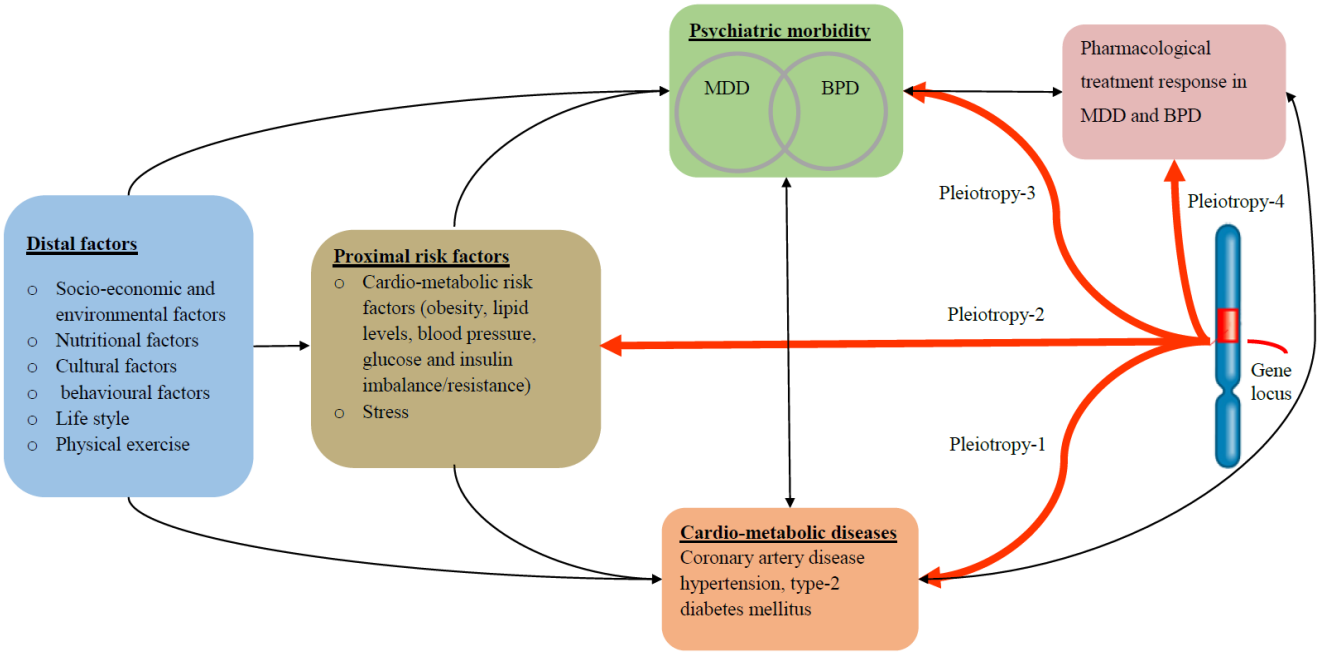

Figure 1. Schematic model for potential pleiotropic effects of a shared gene locus associated with mood disorders and cardio-metabolic diseases[5,6,26,38-40]. The distal and proximal factors were obtained from the literature, and the World Health Organization (WHO) often uses the classification. Distal factors refer to those factors that require an intermediate factor to cause disease, while proximal factors can directly cause disease. The red bold lines represent the pleiotropic effect of a genetic locus on cardio-metabolic diseases and associated risk factors, MDD, BPD and treatment response in MDD and BPD. The bi-directional arrows indicate bi-directional epidemiological relationships. MDD: major depressive disorder, BPD: bipolar disorder.

METHODS AND MATERIALS

SEARCH STRATEGY

Step 1: Identification of candidate genes for cardio-metabolic diseases

We carried out a systematic search of candidate genes for cardio-metabolic diseases and associated risk factors. The National Human Genome Research Institute (NHGRI) GWAS catalogue[36], Westra et al. (2013)[41] and Multiple Tissue Human Expression Resource (MuTHER)[42] databases were used to identify CMD-R genes. We reviewed meta-GWA study papers published until January 2016 for the diseases "type 2 diabetes" OR "coronary artery disease" OR "hypertension" and (or) for the risk factors "blood pressure" OR "obesity or body mass index (BMI) " OR "plasma lipid levels (high-density lipoprotein, low-density lipoprotein, triglycerides, total cholesterol)" OR "insulin and glucose-related traits (fasting glucose, fasting insulin, fasting proinsulin, insulin sensitivity, insulin resistance-HOMA-IR, beta cell function-HOMA-β and glycated haemoglobinA1C-HbA1C)".

All GWAS significant SNP ($P<5x10^{-8}$) information (lead SNPs, reported genes, author (s), PubMed ID, date of publication, journal, discovery and replication sample sizes) was downloaded from the GWAS catalogue database. Additional information about the effect of lead SNPs on nearby gene expression (cis-eQTLs) was collected from their respective publications. For SNPs with no cis-eQTL information in their respective publications, we performed expression quantitative trait loci (cis-eQTL) analysis to verify the functional relationship between reported genes and lead SNPs using two publicly available databases: Westra et al. (2013)[41] and MuTHER[42]. A CMD-R gene was considered as a candidate gene

if, 1) at least one of the lead SNPs is located within or nearby to the gene, and 2) it is functionally relevant to influence at least one of the CMD-Rs as evidenced by gene expression analyses. We took the identified CMD-R genes forward for the second literature review, as described below.

Step 2: Exploration of the role of cardio-metabolic genes in mood disorders

In the second systematic review, we conducted a literature search in PubMed (MEDLINE database) for genome-wide association, candidate gene, or gene expression analysis studies published in the fields of mood disorders and pharmacogenetics of mood disorders until January 2016. This step of the literature search was performed using the SNIPPER tool (see web resources and tools). We considered studies that reported at least one of the CMD-R genes in "depression" OR "depressive disorder" OR "depressive symptoms" OR "MDD" OR "bipolar disorder" OR "mood disorder" OR "lithium treatment response", OR "selective serotonin reuptake inhibitor (SSRI) treatment response". A prior literature search implemented before the final review found that the majority of genetic studies on treatment response to antidepressants and mood stabilisers were on lithium and SSRIs. As a result, the literature search on the pharmacogenomics of mood disorders was limited to these predominant treatments.

Inclusion criteria: General inclusion criteria of genetic studies that involve individuals of all ages and both sexes was implemented. The pharmacogenomics studies were restricted to only lithium or SSRIs treatment response in mood disorders.

Exclusion criteria: Pharmacogenomic studies that used SSRIs or lithium for treatment of psychosis, anxiety disorders, obsessive-compulsive disorder, post-traumatic stress disorder

were excluded. We also excluded genetic studies that investigated drug-induced side effects of mood disorders.

BIOLOGICAL PATHWAY AND NETWORK ANALYSIS

Potential pleiotropic genes were further explored to identify the most enriched canonical pathways and visualise gene networks using QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity). For analysis, all 24 potential pleiotropic genes were entered as input into the software. IPA compares the proportion of input genes mapping to a biological pathway to reference genes in the ingenuity databases. The significance of overrepresented canonical pathways was determined using the right-tailed Fisher's exact test later adjusted for multiple testing using the Benjamini-Hochberg (BH) method[43]. Significance levels were determined at BH adjusted p-value <0.01. A gene network that connects the input genes with MDD, BPD and cardio-metabolic disorders was also generated.

Web resources and tools

GWAS Catalogue: https://www.ebi.ac.uk/gwas/home

Westra et al. blood eQTL browser: http://genenetwork.nl/bloodeqtlbrowser/

MuTHER eQTL resource, http://www.muther.ac.uk/

SNIPPER tool v1.2: http://csg.sph.umich.edu/boehnke/snipper/

QIAGEN's Ingenuity® Pathway Analysis: www.qiagen.com/ingenuity

RESULTS

Characteristics of meta-GWA studies for cardio-metabolic disorders

Literature searches in the GWAS catalogue yielded 153 meta-GWA studies for CMD-Rs: 38 studies for type 2 diabetes, 17 studies for coronary artery disease, 15 studies for hypertension and blood pressure, 26 studies for obesity and BMI, 37 studies for lipids and 20 studies for glucose and insulin traits (Figure 2). As shown in Figure 2, meta-GWA studies reported 1047 lead SNPs and 682 nearby genes. Of these, 123 genes were functionally relevant to cardio-metabolic diseases and associated risk factors, as confirmed by gene expression analysis (cis-eQTLs). These genes were reviewed for their association with mood disorders and pharmacogenetics of mood disorders. Twenty-four of 123 CMD-R genes have been implicated in mood disorders, and we named these genes the cardio-metabolic mood disorders hub (CMMDh) genes.

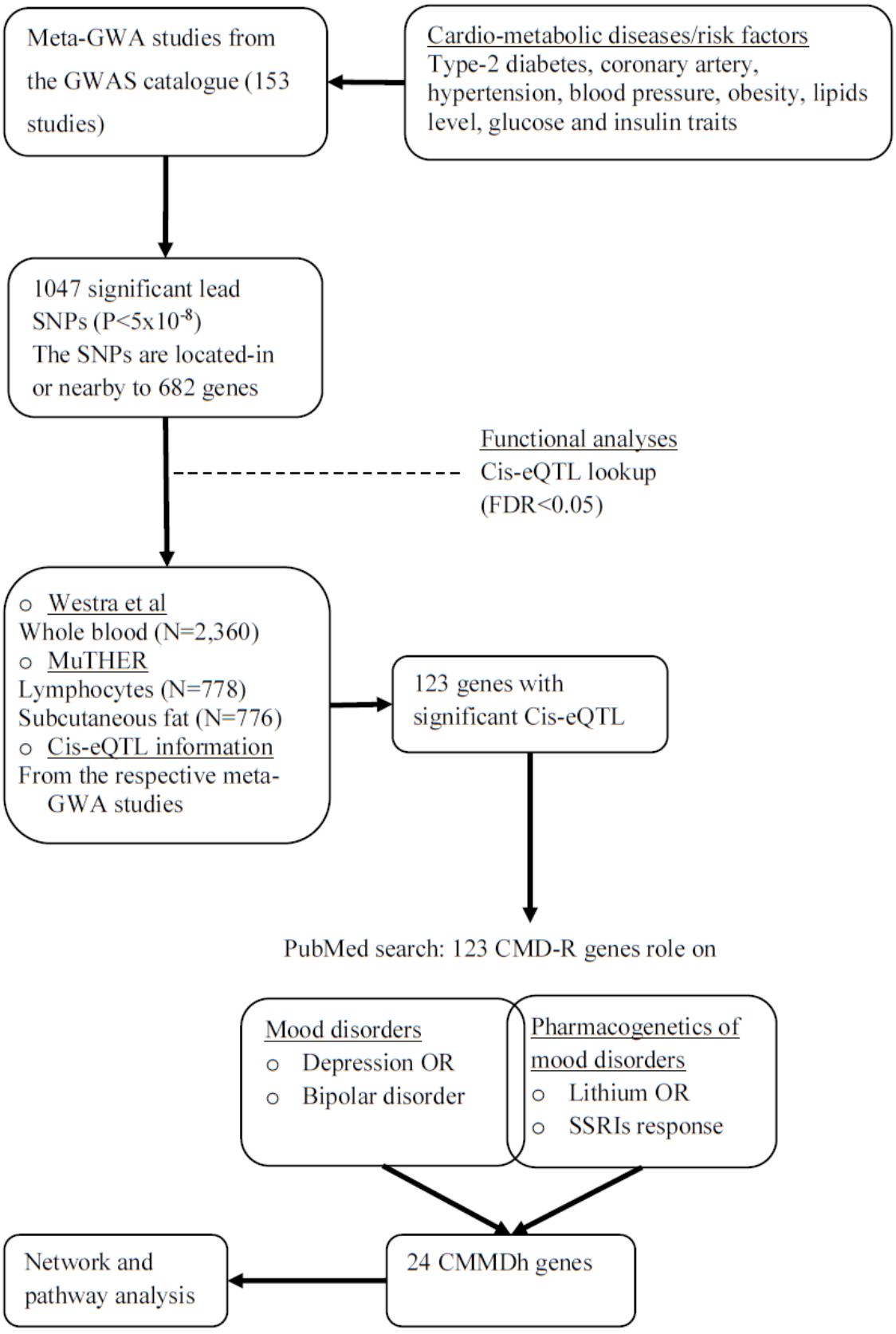
Meta-GWA studies from the GWAS catalogue (153 studies)
Cardio-metabolic diseases/risk factors
Type-2 diabetes, coronary artery, hypertension, blood pressure, obesity, lipids level, glucose and insulin traits
1047 significant lead SNPs (P<5x10-8)
The SNPs are located-in or nearby to 682 genes
Functional analyses
Cis-eQTL lookup
(FDR<0.05)
Westra et al
Whole blood (N=2,360)
MuTHER
Lymphocytes (N=778)
Subcutaneous fat (N=776)
Cis-eQTL information
From the respective meta-GWA studies
123 genes with significant Cis-eQTL
PubMed search: 123 CMD-R genes role on
Mood disorders
Depression OR
Bipolar disorder
Pharmacogenetics of mood disorders
Lithium OR
SSRIs response
24 CMMDh genes
Network and pathway analysis

Figure 2: Flowchart shows the stages of literature search and evaluation of candidate pleiotropic genes for CMD-Rs and mood disorders. CMD-R genes refer to genes in which CMD-R lead SNPs are located in or nearby and their expression is influenced by the respective lead SNPs (cis-eQTL).
Meta-GWAS: Meta-analysis of Genome-Wide Association Studies, GWAS: Genome-Wide Association CMD-R: Cardio-Metabolic Diseases and associated Risk factors, MuTHER: Multiple Tissue Human Expression Resource, CMMDh: Cardio-Metabolic Mood Disorders hub genes, Cis-eQTL: Cis (nearby) gene expression quantitative trait loci.

Table 1 summarises the 24 CMMDh genes and specific genetic variants across mood disorders and cardio-metabolic diseases. These genes are *MTHFR*, *CACNA1D*, *CACNB2*, *GNAS*, *ADRB1*, *NCAN*, *REST*, *FTO*, *POMC*, *BDNF*, *CREB*, *ITIH4*, *LEP*, *GSK3B*, *SLC18A1*, *TLR4*, *PPP1R1B*, *APOE*, *CRY2*, *HTR1A*, *ADRA2A*, *TCF7L2*, *MTNR1B*, and *IGF1* (for further details see Table 1). These genes were over-represented in the following biological pathways: corticotrophin-releasing hormone signaling *BDNF*, *CREB1*, *GNAS*, *POMC*; AMPK signaling *ADRA2A*, *ADRB1*, *CREB1*, *GNAS*, *LEP*; cAMP-mediated and G-protein coupled receptor signaling *ADRA2A*, *ADRB1*, *CREB1*, *GNAS*, *HTR1A*; axonal guidance signaling *BDNF*, *GNAS*, *GSK3B*, *IGF1*; serotonin and dopamine receptors signaling *GNAS*, *HTR1A*, *SLC18A1*, *PPP1R1B*; dopamine-DARPP32 feedback in cAMP *PPP1R1B*, *CACNA1D*, *CREB1*, *GNAS*; leptin signaling *GNAS*, *LEP*, *POMC*; and the circadian rhythm signaling *CRY2*, *CREB1* (Table 2 and Figure 3).

Table 1: An overview of the 24 CMMDH genes shared between mood disorders and cardio-metabolic diseases

Pleiotropic genes	**The function of the coded protein**	**Polymorphisms associated with**	
		Cardio-metabolic disorders(lead SNP)	**Mood disorders (description)**
MTHFR	Part of the process to build amino-acids and to form vitamin folate	Blood pressure rs17367504-G/A[44]	The common *MTHFR* C677T was associated with depression[45], and BPD[46]. *MTHFR* gene polymorphisms interaction with childhood trauma increases the risk for depression[47].
CACNA1D	Mediate the entry of calcium ions into excitable cells	Blood pressure and hypertension rs9810888-G/T[48]	Rare variants in the calcium channel genes(*CACNA1B, CACNA1C, CACNA1D, CACNG2*) contribute to BPD[49] and may influence treatment response to lithium[50].
CACNB2	Mediate the entry of calcium ions into excitable cells	Blood pressure rs4373814-G/C[35] rs12258967-G/C[44] rs11014166-A/T[51]	Polymorphisms in the *CACNB2* gene were implicated in MDD and BPD[52].
GNAS	Control the activity of endocrine glands through adenylate cyclase enzyme	Blood pressure and hypertension rs6015450-G/A[35]	SNPs in the *GNAS* gene were associated with BPD (rs6064714, rs6026565, rs35113254)[53] and may influence antidepressant treatment response[54].
ADRB1	Mediate the effects of epinephrine and norepinephrine	Blood pressure rs2782980-T/C[44]	Gly389 polymorphism of the beta(1)-adrenergic receptor might lead to better response to antidepressant treatment in patients with MDD[55]
REST	Regulate neurogenesis	Coronary artery disease rs17087335-T/G[30]	Reduced expression of *REST* in MDD patients at depressive state[56], and alteration in the expression of the *REST*

			gene was revealed in the brain of women with MDD[57].
LEP	Regulate body weight	Type 2 diabetes rs791595-A/G[58]	SNPs in the leptin gene decreased leptin gene expression and leptin deficiency in serum were related to antidepressant resistance[59]. A significant reduction of mRNA expression was found in the brain of MDD and suicidal patients[60].
ADRA2A	Regulate neurotransmitter release from sympathetic nerves and from adrenergic neurons in the central nervous system	Type 2 diabetes or fasting glucose rs10885122-G/T[31]	*ADRA2A* gene polymorphisms (ADRA2A-1291G-male, ADRB2 Arg-female) were associated with sex-specific MDD[61], predicted antidepressant treatment outcome in MDD[62], and modified the effect of antidepressants for better improvement[63]. However, they increased suicidal ideation during antidepressant treatment[64]. Treatment with lithium produced an overexpression of the *ADRA2A* gene in rats brain [65].
TCF7L2	Regulate blood glucose homeostasis	Type 2 diabetes rs7903146-T/C[66,67] Fasting glucose, proinsulin, insulin levels, or insulin resistance rs7903146-T/C[68,69] rs4506565-T/A[31,34]	Genome-wide association study of BPD in European Americans identifies a new risk allele(rs12772424-A/T) within the *TCF7L2* gene[70]
HTR1A	Receptor for serotonin	Fasting insulin or insulin resistance	Variants in the *HTR1A* gene (rs6295, rs878567) were related to MDD and

		rs16891077-A/G[71]	BPD[72,73]. A significant decrease in *HTR1A* mRNA levels in the brain of patients with MDD and BPD was found[74]. Other polymorphisms (5-HT1A-1019G, Gly272Asp) in this gene were associated with antidepressant treatment response in MDD[75-77] and in BPD[76]. Increased DNA methylation in the promoter region of the *HTR1A* gene was also observed in patients with BPD [78].
CRY2	Regulates the circadian clock	Fasting glucose or insulin rs11605924-A/C[31,34]	Polymorphisms in *CRY2* gene were significantly associated with MDD [79] and BPD[79,80].
MTNR1B	Participate in light-dependent functions in the retina and brain. May be involved in the neurobiological effects of melatonin	Type 2 diabetes or plasma glucose level rs3847554-C/T[34] rs10830962-C/G[81] rs2166706-T/C[82] rs10830963-G/C[31] rs1387153-T/C[83,84]	Gałecka et al. 2011 reported the significance of the *MTNR1B* gene polymorphism (rs4753426) for recurrent MDD[85]. Additional SNP on the *MTNR1B* gene(rs794837) increased mRNA level in MDD patients[85].
IGF1	Involved in mediating body growth and development	Fasting insulin, fasting glucose, or glucose homeostasis rs35767-G/A[31], rs35747-G/A[34]	An elevated level of IGF-I was associated with MDD and antidepressant treatment response[86]. A long-term deficiency of IGF-1 in adult mice induced depressive behaviour[87]. Polymorphisms in the *IGF1* gene increased BPD risk[88]. An over-expression of *IGF1* gene of BPD patients who respond well to lithium treatment was also reported[89].

FTO	Regulates energy homeostasis, contributes to the regulation of body size and body fat accumulation	Obesity rs7185735-G/A[32,90] Type 2 diabetes rs9936385-C/T[66] HDL or triglycerides rs1121980-A/G[33]	The *FTO* gene variant (rs9939609-A/T) was associated with depression[91]. Other variants of the *FTO* gene were involved in the mechanism underlying the association between mood disorders and obesity[92].
POMC	Maintain the body's energy balance and control sodium in the body	Obesity (BMI) rs713586-C/T[93] rs1561288-T/C[94] rs10182181-G/A[90]	Genetic variants in this gene were involved in treatment response to SSRIs (escitalopram or mirtazapine) in MDD patients[95].
ITIH4	Involved in inflammatory responses	Obesity (BMI) rs2535633-G/C[96]	Genetic variants located in the regions of *ITIH1*, *ITIH3*, *ITIH4* genes were associated with BPD[29], and suicidal attempt in BPD patients[97].
TLR4	Pathogen recognition and activation of innate immunity	Obesity(BMI) rs1928295-T/C[32]	The mRNA levels of the *TLR3* and *TLR4* genes were increased in depressed suicidal patients[98]. *TLR4* gene expression was related to the severity of major depression[99].
BDNF	Promotes the survival of nerve cells	Obesity(BMI) rs2030323-C/A[32,90] rs925946-T/G[100] rs10767664-A/T[93]	The Val66Met polymorphism was associated with depressive disorder [101], BPD [102] and suicidal behavior in depressed and BPD patients[103,104]. It was also associated with SSRIs (escitalopram) response in depressed patients[105]. A significantly decreased expression of the *BDNF* gene was observed in the lymphocytes and platelets of depressed patients[106]. Treatment-responsive depressive

			patients have also shown a decreased mRNA levels of the *BDNF* gene[107].
CREB1	Involved in different cellular processes including the synchronisation of circadian rhythmicity and the differentiation of adipose cells	Obesity rs17203016-G/A[32]	SNPs within this gene were associated with MDD risk in women[108] and antidepressants treatment resistance in MDD patients[109]. An interaction of *CREB1* gene variants with *BDNF* variants predicted response to paroxetine[110]. The *CREB1* gene variants(rs6785, rs2709370) increased BPD susceptibility[111] and other SNPs on *CREB1* were suggested for BPD and lithium response[112].
NCAN	Modulation of cell adhesion and migration	Total cholesterol rs2304130-G/A[113] LDL cholesterol rs16996148-G/T[114] rs10401969-C/T[115] Triglycerides rs17216525-T/C[115] rs16996148-G/T[114]	A SNP (rs1064395) in *NCAN* gene was found to be a risk factor for BPD in the European population[116]. This SNP might result in a structural change of the brain cortex folding[117]
GSK3B	Energy balance, metabolism, neuronal cell development, and body pattern formation	HDL cholesterol rs6805251-T/C[33]	Higher *GSK3B* activity was observed in MDD patients with severe depressive episode[118]. Polymorphisms of this gene (rs334555, rs119258668, rs11927974) were implicated in MDD[119]. In addition, rare variants in *GSK3B* gene increased BPD risk[120,121]. The *GSK3B* is a target gene for several mood stabilisers including lithium[122,123].

SLC18A1	Accumulate and transport neurotransmitters	Triglycerides rs9644568-A/G[124] rs79236614-G/C[125] rs326-A/G[126]	Variations in the *SLC18A1* (rs988713, rs2279709, Thr136Ser) gene confer susceptibility to BPD[127].
PPP1R1B	A target for dopamine	HDL cholesterol rs11869286-G/C[33]	*DARPP-32* decreased in the prefrontal cortex of BPD patients[128], increased expression was also shown in BPD[129].
APOE	Maintaining normal levels of cholesterol	HDL, LDL or total cholesterol rs4420638-A/G[33] rs1160985-C/T[130] rs519113-C/G[131]	Genetic variation in the *APOE* gene contributed to depressive symptoms[132].

Abbreviations: CMD-R: Cardio-metabolic diseases and related risk factors; **SNP:** Single nucleotide polymorphism; **HDL:** High-density lipoprotein; **LDL:** Low-density lipoprotein; **BPD:** bipolar disorder; **MDD:** Major depressive disorder

We also performed a gene network analysis of the CMMDh genes to mood disorders and cardio-metabolic diseases. Based on the network analysis, CMMDh genes were centrally involved in the link between mood disorders and cardio-metabolic diseases. For instance, *ADRB1* and *ADRA2A* genes linked the four most common cardio-metabolic disorders (coronary diseases, hypertension, diabetes, obesity) with BPD and depressive disorder. The *CACNB2* and *CACNA1D* genes have shown network with coronary diseases, hypertension, diabetes, BPD and depression. Similarly, other CMMDh genes acted as a hub between at least one of the cardio-metabolic disorders and BPD and depression (Figure 3).

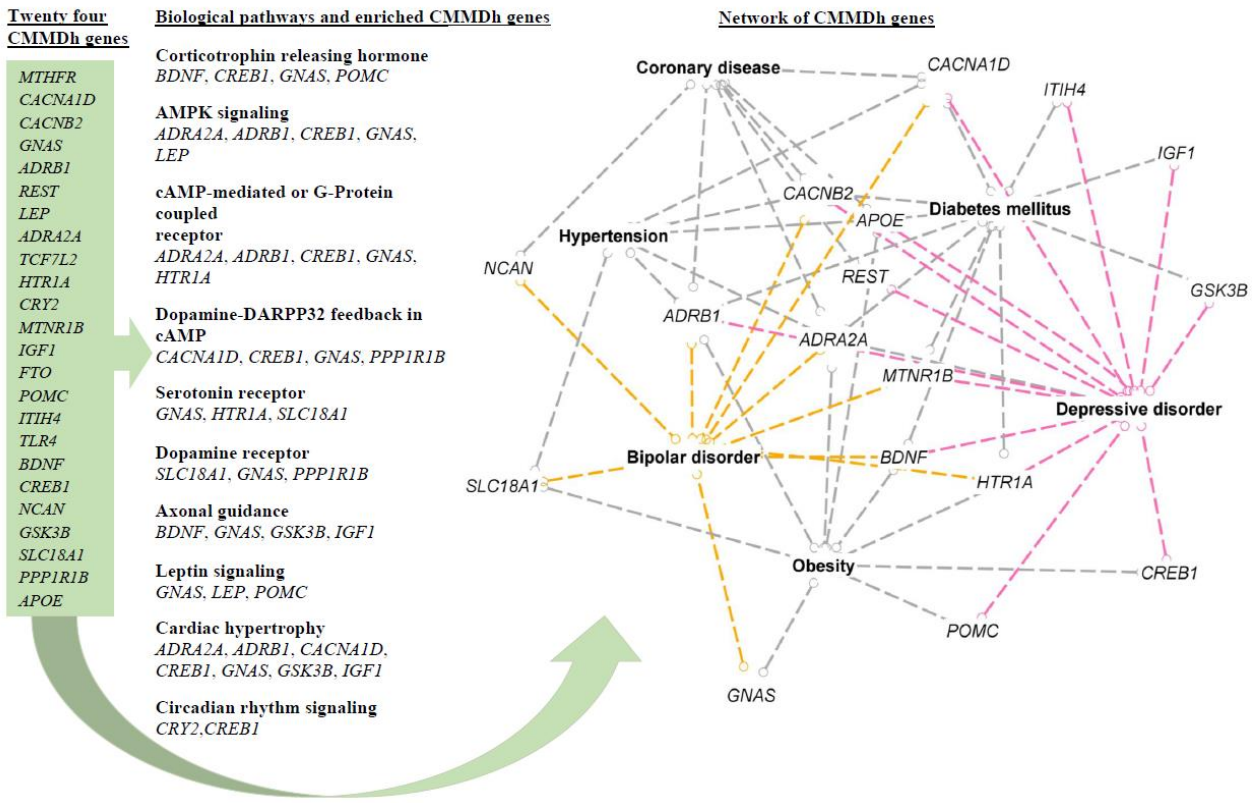

Figure 3: List of 24 CMMDh genes (left), genes enriched to the top canonical signaling pathways (middle) and the network of these genes with mood disorders and CMD-Rs (right). At the right, this figure illustrates ingenuity IPA-generated network of CMMDh genes with coronary artery diseases, hypertension, diabetes mellitus, obesity, depressive disorder and bipolar disorder. Coloured dotted lines highlight CMMDh genes related to bipolar disorder (orange) and depression (red).

DISCUSSION

This first cross-disorder review systematically evaluated candidate pleiotropic genes and biological pathways likely to be shared with mood disorders, cardiovascular diseases and metabolic disorders. We revealed 24 cardiovascular and metabolic disease genes implicated in depression, bipolar disorder or both. These genes belong to interrelated signaling

pathways important in the hypotheses of both cardio-metabolic diseases and mood disorders: corticotrophin-releasing hormone signaling, AMPK signaling, cAMP-mediated and G-protein coupled receptor signaling, axonal guidance signaling, serotonin and dopamine receptors signaling, dopamine-DARPP32 feedback in cAMP signaling, leptin signaling and circadian rhythm signaling.

Corticotropin-releasing hormone (CRH) signaling is one of the top canonical pathways that may underlie the link between CMD-Rs and mood disorders. This pathway comprises of CRH, CRH receptors (CRHR1, CRHR2) and other CRH-related peptides. It is the principal regulator of the hypothalamic–pituitary–adrenal (HPA) axis. There are consistent findings in the literature that support the role of HPA axis dysregulation in mediating the risk of mood disorders and cardiovascular outcome[133]. Our analysis found enriched CMMDh genes in CRH signaling pathways (*BDNF*, *CREB1*, *GNAS, and POMC*). Genetic variants of the genes for BDNF, CREB1, GNAS and POMC are associated with MDD[101,108], BPD[53], obesity[32,93], blood pressure and hypertension[35,44]. The genes belong to the group of stress-responsive genes, and their activity could be modulated through activation of the HPA-axis. In animal studies, the expression of *BDNF*[134] and *CREB1*[135] genes were dysregulated by chronic stress. It is, therefore, possible that an interaction of *BDNF*, *CREB1*, *GNAS* and *POMC* genes with exposure to chronic stress or traumatic life events increase the risk of cardio-metabolic and mood disorders either simultaneously, or through mediating factors. The CRH signaling pathway is the principal regulator of stress responses[136]. Following an exposure to stress, the hypothalamus releases CRH, stimulating secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. This, in turn, stimulates the adrenal gland to produce glucocorticoids (principally cortisol). Cortisol will

then act on several organs including the brain through its receptors[136]. In acute conditions, the production of cortisol helps the body to fight pathogens (stress) and alleviate inflammation. However, when stressors are long-lasting (chronic) they can cause cortisol receptor resistance and failure of the HPA-axis negative feedback mechanism. This increases the duration and chronicity of inflammation, and a failure to down-regulate the inflammatory response. Ultimately, failure in HPA-axis processes may cause dysfunction in the brain and the body, causing both somatic diseases and brain disorders. Stress can either originate from the external environment as chronic extrinsic stress (CES) or within the internal body system as chronic intrinsic stress (CIS). Both CES and CIS can influence CRH pathway genes mainly through gene expression and DNA methylation mechanisms[137].

In relation to stress, there are two possibilities to explain mood disorders to cardio-metabolic disease association. The first is that the human body system may consider mood disorders or CMD-Rs as CIS and then dysregulate the HPA-axis through CRH signaling pathways. Given that mood disorders tend to have an earlier age of onset compared to most CMD-Rs[138], they might be the primary CIS to induce cardio-metabolic outcomes through CRH signaling mechanism. Another possibility is that CES and CIS interact with CRH signaling genes to cause both CMD-Rs and mood disorders. In either of the conditions, CRH signaling genes interact with stressors to cause a dysfunction in the HPA-axis.

The second main canonical pathway is the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway. This pathway regulates intercellular energy balance. It inhibits or induces ATP consuming and generating pathways as needed. The pathway is especially important for nerve cells, as they need more energy with small energy reserves[139].

Abnormalities in the pathway can disturb normal brain functioning. In animal studies, Zhu et al. (2014) showed chronically stressed mice developed symptoms related to mood and metabolic abnormalities, such as significant weight gain, heightened anxiety and depressive-like behavior. They also reported decreased levels of phosphorylated AMP-activated protein kinase α (AMPKα), confirming the involvement of the AMPK pathway and its regulatory genes in metabolic disorders and depression[140]. Recent studies also reported activation of the AMPK pathway in rat hippocampus after ketamine treatment exerting rapid antidepressant effect[141]. Major contributing CMMDh genes enriched in the AMPK pathway are *ADRA2A*, *ADRB1*, *LEP*, *CREB1* and *GNAS*. Variations in one or more of these genes can influence the activity of the AMPK pathway, subsequently impairing energy homeostasis in the brain and possibly in other cells[139]. This could later cause energy shortages for the brain and somatic cells. Since brain cells are the most vulnerable units that require a substantial amount of energy supply, any energy shortage would first severely affect the brain. Symptoms of mood change, such as depressive behavior, could emerge during this process. Moreover, AMP activation, for instance during stress, could induce insulin resistance promoting metabolic syndrome, i.e. obesity, diabetes and cardiovascular diseases[142,143]. Hence, it is very likely that inappropriate activation of the AMPK pathway can imbalance the energy needs of cells and be a cause of mood disorders and cardio-metabolic diseases.

Axonal guidance signaling was also among the top overrepresented canonical pathways. The pathway is essentially related to neuronal connections formed by the extension of axons, which migrate to reach their synaptic targets. Axon guidance is an important step in neural development. It allows growing axons to stretch and reach the next target axon to

form the complex neuronal networks in the brain and throughout the body. The patterns of connection between nerves depend on the regulated action of guidance cues and their neuronal receptors that are themselves encoded by axonal guidance coding genes. Activation of specific signaling pathways can promote attraction or repulsion and affect the rate of axon extension. One important observation in the axonal guidance pathway is the role of calcium and voltage-dependent calcium channels. The pathway is regulated by the entrance of calcium through the plasma membrane and release from intracellular calcium store. Calcium has been implicated in controlling axon outgrowth[144]. CMMDh genes overrepresented in the axonal guidance-signaling pathway include the *BDNF, GNAS, GSK3B* and *IGF1* genes. Mutant axonal guidance genes followed by abnormal axon guidance and connectivity could cause a disorder primarily in the brain and subsequently to the peripheral organs[145].

Other strong candidate mechanisms underlying mood disorders and cardio-metabolic diseases are the serotonin and dopamine receptors signaling pathways. The serotonin pathway is mainly regulated by serotonin and its receptors are known as 5-hydroxytryptamine (5-HT) receptors. Serotonin is a monoamine neurotransmitter synthesised in the central nervous system and its signaling modulates several physiological processes, including regulation of appetite, mood and sleep, body temperature and metabolism. The *SLC18A1*, *HTR1A* and *GNAS* genes are among the CMMDh genes involved in the serotonin receptor-signaling pathway. The *SLC18A1* gene encodes for the vesicular monoamine transporter that transports for monoamines. Its function is essential to activity of the monoaminergic systems that have been implicated in several human neuropsychiatric disorders[146]. The *HTR1A* gene encodes a receptor for serotonin and

belongs to the 5-hydroxytryptamine receptor subfamily. Dysregulation of serotonergic neurotransmission has been suggested as a contributor to the pathogenesis of mood disorders[72,73] and it is implicated in the action of selective serotonin reuptake inhibitors[75-77]. Animal studies have consistently demonstrated the influence of the serotonin pathway on both mood disorders and cardio-metabolic disorders. Ohta et al. (2011) have previously revealed a converge in insulin and serotonin-producing cells that can lead to metabolic diseases (diabetes) and mood disorders[147]. The products of insulin-producing cells (beta-islet cells) are involved to express the genes that synthesise serotonin, and serotonin also plays a role in the synthesis of insulin in beta-islet cells[147].

The dopamine receptor pathway, centrally regulated by dopamine, also appears to underlie the relationship between mood disorders and cardio-metabolic diseases. Dopamine serves as a chemical messenger in the nervous system and its signaling plays important roles in processes of emotion, positive reinforcement, motivation and movement, and in the periphery as a modulator of renal, cardiovascular and the endocrine systems[148]. The *SLC18A1* and *GNAS* genes are among the CMMDh genes that belong to this pathway. The dopamine-signaling pathway further induces dopamine-DARPP32 feedback in cAMP signaling. The central regulator of this pathway is the *PPP1R1B* gene that encodes a bifunctional signal transduction molecule called the dopamine and cAMP-regulated neuronal phosphoprotein (DARPP-32). Other CMMDh genes in the pathway include *CACNA1D*, *CREB1* and *GNAS*. The *CACNA1D* gene encodes the alpha-1D subunit of the calcium channels that mediate entry of calcium ions into excitable cells. Calcium channel proteins are involved in a variety of calcium-dependent processes, including hormone or neurotransmitter release and gene expression[149].

Overall, genes that encode for molecules involved in HPA-axis activity, circadian rhythm, inflammation, neurotransmission, metabolism and energy balance were found to play a central role to link mood disorders with cardio-metabolic diseases. It is also worth noting the gene-environment interaction that might contribute to the diseases.

IMPLICATIONS OF THE REVIEW FINDINGS

Knowledge of genes and molecular pathways shared between mood disorders and cardio-metabolic disorders have several important implications for future research and clinical practice. It is expected that increasing sample size, and consequently increasing power, will identify many more relevant genes in the near future. Here we identify four implications of our findings.

Firstly, the identification of shared molecular pathways implicated in disease susceptibility supports a growing evidence base for cross-diagnostic treatment paradigms. Shared molecular pathways could help to explain recent findings of reduced cardiovascular mortality[150], or improved diabetic control[151], in MDD patients treated with SSRIs. Secondly, further exploration of overlapping molecular pathophysiology has the potential to unveil novel targets for drug development and may give clues for the re-purposing of existing medications.

Thirdly, cardio-metabolic disorders are associated with an increased risk of poor response to standard treatments in mood disorders[39,40]. Genetic profiling for cardio-metabolic risk and stratified diagnosis of patients may help to classify treatment responders and treat them accordingly, thereby reducing the costs of ineffective exposure to medicines for individuals and for society. Early identification of at-risk individuals would also guide practitioner's

treatment recommendations, which may involve alternative somatic (e.g. electroconvulsive therapy, repetitive transcranial magnetic stimulation, ketamine) or specific psychological therapies as first or second line treatments.

Fourthly, studying the mechanisms of pleiotropic genes and shared pathways of mood disorders and somatic diseases could help untangle the clinical and genetic heterogeneity that characterises these illnesses. It is possible that a "cardio-metabolic" endophenotype exists among mood disorder patients that may be identifiable through genetic profiling using polygenic scores or analysis of blood protein biomarkers. Preliminary evidence for such a phenotype, approximating the concept of "atypical depression", characterised by increased appetite, weight gain and increased need for sleep, is emerging[152,153]. Working towards personalised care that allows for precise diagnostic, treatment and prevention strategies, research could then focus on genetically stratified patient cohorts instead of the very diverse patient pool currently diagnosed with MDD or BPD. There is a growing consensus that such stratification approaches have the potential to substantially improve the quality of mental health research and mental health care over the coming decades[154].

Our review has limitations. Perhaps the most fundamental limitation was that almost all of the reviewed studies were performed in a univariate manner (single disease approach). Essentially, multivariate models, such as principal component analyses, multivariate mixed models and multivariate regression analyses are regarded as statistically powerful to perform cross-disorder analyses and identify pleiotropic genes. Unlike the multivariate approach, a univariate analysis investigates the association between a genetic variant and a single phenotype, aimed to identify genetic variants for individual diseases. Secondly, the

review included studies that reported positively associated genes, and neither negative findings nor inconsistent evidence was assessed. We also found limited replication in some candidate genes, thereby demonstrating the necessity of future confirmatory studies. Thirdly, only meta-GWA studies were reviewed for the CMD-Rs and we implemented somewhat less stringent criteria for the genetic studies of mood disorders. Genome-wide association studies for mood disorders have been less successful, mainly due to inadequate sample size and the phenotypic heterogeneity of the disorders. For this reason, the inclusion criteria for studies in these disorders was less strict. Hence, our review should be viewed as complementary to future mood disorder to cardio-metabolic diseases gene investigation, providing an initial thorough summary of potential pleiotropic genes. Further population or case-control studies are necessary to confirm our proposed findings.

CONCLUSION

Our review revealed potential pleiotropic genes and biological pathways that are likely to be shared between mood disorders and cardio-metabolic diseases. While the review provides some insight into common mechanisms and the role of pleiotropic genes, in-depth understanding of how these genes (and possibly others) mediate the association between mood disorders and cardio-metabolic diseases requires future comprehensive cross-disorder research in large-scale genetic studies. This will enable us to better understand why patients suffer from multiple diseases, and how multi-morbidities influence pharmacological treatment response to diseases.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Contribution to the Paper	Conceived the research concept and design, perform the data analysis, made an interpretation of the results and prepared the first draft of the manuscript. Made manuscript submission, prepared a reply letter for reviewers and finalised the manuscript based on reviewer feedback.		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
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Chapter 4

Association of polygenic score for schizophrenia and HLA antigen and inflammation genes with response to lithium in bipolar affective disorder: A genome-wide association study.

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Key Points

Question: Does a polygenic score for schizophrenia (SCZ) predict response to lithium in patients with bipolar affective disorder (BPAD)? What are the molecular drivers of this association?

Findings: We found an inverse association between genetic loading for SCZ risk variants and response to lithium in bipolar patients. Genetic variants in the HLA region and the antigen presentation pathway point to the molecular underpinnings of SCZ and lithium treatment response.

Meaning: In patients with BPAD, an assessment of a polygenic load for SCZ risk variants may assist in conjunction with clinical data to predict whether they would respond to lithium treatment.

ABSTRACT

Importance: Lithium is a first-line mood stabiliser for the treatment of bipolar affective disorder (BPAD). However, the efficacy of lithium varies widely, with a non-response rate of up to 30%. Biological response markers and predictors are lacking.

Objective: Genetic factors are thought to mediate lithium treatment response, and the previously reported genetic overlap between BPAD and schizophrenia (SCZ) led us to test whether a polygenic score (PGS) for SCZ could predict lithium treatment response in BPAD. Further, we explored the potential molecular underpinnings of this association.

Design: Weighted SCZ PGSs were computed at different p-value thresholds (P_T) using summary statistics from a genome-wide association study (GWAS) of 36,989 SCZ cases, and genotype data for BPAD patients from the Consortium on Lithium Genetics (ConLi$^+$Gen). For functional exploration, we performed a cross-trait meta-GWAS and pathway analysis, combining GWAS summary statistics on SCZ and lithium treatment response.

Setting: International multicenter GWAS.

Participants: Patients with BPAD who had undergone lithium treatment were genotyped and assessed for long-term treatment response (n=2,586).

Main outcome measures: Treatment response to lithium was defined on both the categorical and continuous scales using the ALDA score. The effect measures include odds ratios (ORs) and the proportion of variance explained (R^2), and a significant association was determined at $p<0.05$.

Results: The PGS for SCZ was inversely associated with lithium treatment response ($p=8x10^{-5}$), at $P_T <5x10^{-2}$. Patients with BPAD who had a low polygenic load for SCZ responded better to lithium, with ORs for lithium response ranging from 3.46 [95%CI: 1.42-8.41 at 1st decile] to 2.03 [95%CI: 0.86-4.81 at the 9th decile], compared to patients in the 10th decile of SCZ risk. In the cross-trait meta-GWAS, 15 genetic loci that may have overlapping effects on lithium treatment response and susceptibility to SCZ were identified. Functional pathway and network analysis of these loci point to the HLA complex and inflammatory cytokines.

Conclusions and Relevance: The study provides, for the first-time, evidence for a negative association between high genetic loading for SCZ and poor response to lithium in patients with BPAD. These results suggest the potential for translational research aimed at personalised prescribing of lithium.

Keywords: lithium treatment, schizophrenia, bipolar disorder, polygenic score, pharmacogenomics, immune genes, HLA, TNFα, cytokines

INTRODUCTION

Bipolar affective disorder (BPAD) is a severe and often disabling psychiatric condition, characterised by recurrent dysregulation of mood with episodes of mania and depression. With an early disease onset and an estimated lifetime prevalence of 1%[1] to 4.4%[2], BPAD is associated with high personal impairment and societal costs, accounting for 9.9 million years of life lived with disability worldwide[3], increased all-cause mortality, and risk of suicide[4]. The etiology of BPAD is complex, and both genetic and environmental factors contribute to the pathogenesis of the disorder[5]. The estimated heritability of BPAD ranges from 60% to 85%[6], and candidate gene[7] and genome-wide association studies (GWASs)[8-12] have successfully identified genetic loci implicated in the illness.

Lithium's mood stabilising properties were discovered in 1949[13]. Lithium has retained a status as the 'gold standard' mood stabiliser[14,15], possessing unique protective effects against both manic and depressive episodes[16], as well as for suicide prevention[17]. Consequently, lithium is recommended as first-line maintenance treatment for BPAD by several clinical practice guidelines[18-21]. However, there is significant inter-individual variation between lithium treatment responders and non-responders. About 30% of patients are only partially responsive, and more than a quarter show no clinical response at all[22]. While clinical studies report a combination of demographic and clinical characteristics as potential predictors of treatment response[23] genetic factors also appear to be highly involved[22,24-26]. So far, three

GWASs have successfully identified single nucleotide polymorphisms (SNPs) associated with lithium treatment response in BPAD pointing to different genetic loci[22,27,28].

To improve understanding of the molecular mechanisms underlying the therapeutic effects of lithium, alternative genomic approaches that can complement GWAS deserve consideration. One such approach is polygenic analysis, which quantifies the combined effects of genetic variants across the whole genome on a given clinical outcome, computed as a weighted summation of effect sizes of multiple independent polymorphisms. An accurate and successful polygenic model may assist early screening for disease risk, clinical diagnosis and the prediction of treatment response and prognosis. In the current study, we aimed to investigate whether BPAD patients with high trait genetic susceptibility for schizophrenia (SCZ), expressed by their SCZ polygenic score (PGS), would respond better or more poorly to lithium compared to BPAD patients with a low PGS for SCZ. Additionally, we set out to explore the genetic and molecular underpinnings of any identified association between SCZ and lithium treatment response.

A number of previous observations motivated this approach. First, there is increasing evidence for a substantial genetic overlap between BPAD and SCZ. The Psychiatric Genomics Consortium (PGC) estimated a shared genetic variation of ~68%, which is the highest among all pairs of psychiatric diagnoses[29], and several shared risk genes and shared biological pathways associated with both disorders have been identified[30,31,32]. Second, despite these genetic and molecular commonalities, lithium is not an effective medication

for people suffering from SCZ[33], and increased SCZ trait loading in those with BPAD might be expected to serve as a predictor for poor treatment response. An earlier family study found an association between family history of schizophrenia and poor response to lithium[34]. Third, during acute illness episodes, BPAD and SCZ are often difficult to distinguish clinically because of overlapping psychotic symptoms such as hallucinations, delusions, and disorganization, as well as some common behavioral disturbances such as irritability or anger[35]. Aiming to predict response to lithium, which could potentially confer advantages for patients and their treating physicians[36], we sought to evaluate the aggregated effect of genome-wide SNPs for SCZ on lithium treatment response in BPAD using a PGS approach that was based on the results of the largest SCZ GWAS to date[37]. Further, in order to explore potential genetic and molecular drivers of any detected association, we carried out a cross-trait GWAS meta-analysis, combining the summary statistics from the largest available GWAS for both SCZ[37] and lithium response[22].

METHODS AND MATERIALS

Study Sample: The International Consortium on Lithium Genetics (ConLi$^+$Gen)

The ConLi$^+$Gen Consortium (www.ConLiGen.org) is an initiative by the National Institute of Mental Health (NIMH) and the International Group for the Study of Lithium-Treated Patients (IGSLI) (www.IGSLI.org) that was established with the aim of discovering genetic variants responsible for lithium treatment response in BPAD[38]. The ConLi$^+$Gen study involved patients with BPAD from Europe, South America, USA, Asia and Australia[22] who

had been treated with lithium at some stage since diagnosis. The first GWAS based on this initiative was published in 2016[22]. For the current study, genetic and clinical data collected from 2,586 patients with BPAD who were part of the ConLi^{+}Gen consortium were analysed[22,38]. A series of quality control procedures were implemented on the genotype data before and after imputation, as described below.

In the present study, we first tested whether a PGS for SCZ predicts lithium treatment response in patients with BPAD — 79% of cases had BPAD type I, and 21% BPAD type II[39]. In a second step, we applied a cross-trait GWAS meta-analysis approach to identify individual genetic variants shared between the two traits — SCZ and lithium treatment response. In a third step, we characterised the genetic variants identified in the second step and explored the shared biological pathways underlying genetic susceptibility to SCZ and treatment response in BPAD. We built the PGS using the discovery GWAS effect estimates (logs of odds ratio) of 36,989 SCZ cases[37] and the targeted genetic data (n=2,586) from the International Consortium on Lithium Genetics (ConLi^{+}Gen)[22]. Cross-trait meta-analysis and pathway analysis were based on GWAS summary statistics from GWASs of SCZ[37] and lithium treatment response from ConLi^{+}Gen[38]. Overlapping SNPs that met genome-wide significance in the meta-GWAS were subsequently analysed for biological context using the Ingenuity® Pathway Analysis platform (IPA®).

Target outcome

Lithium treatment outcome was assessed using the Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder scale, also known as the ALDA scale[40,41]. The ALDA scale quantifies symptom improvement over the course of treatment (A score, range 0 – 10), which is then weighted against five criteria (B score) that assess confounding factors, each scored 0, 1, or 2. The total score is calculated by subtracting the total B score from the A score, and negative scores are set to zero[22]. We employed a categorical and a continuous outcome for lithium response. The categorical (i.e., good versus poor) response to lithium was defined based on the total score as a cut-off score of 7, in which patients with a total score of 7 or higher were categorised as 'responders'. The ALDA score on subscale-A was used as a continuous outcome after excluding individuals with a total B score greater than 4 or who had missing data on the totals of ALDA subscale-A or B[22].

Genotyping and quality control

The genome-wide genotypes, as well as clinical and demographic data, were collected by 22 participating sites. Quality control (QC) procedures were implemented using PLINK[42]. Samples with low genotype rates <95%, sex inconsistencies (X-chromosome heterozygosity), and genetically related individuals were excluded. We also excluded SNPs that had a poor genotyping rate (<95%), an ambiguity (A/T and C/G SNPs), a low minor

allele frequency (MAF<1%), or that showed deviation from Hardy-Weinberg Equilibrium ($p<10^{-6}$).

Imputation

The genotype data passing QC were imputed on the Michigan server[43] (https://imputationserver.sph.umich.edu) separately for each genotype platform using the 1000 Genomes Project Phase 3 (Version 5) reference panel. During the imputation process, we used the European reference panel for all samples except for those from Japan and Taiwan, for which the East Asian reference population was used. After excluding the low-frequency SNPs (MAF<10%), low-quality variants (imputation INFO < 0.9), and indels, the imputed dosages were converted to best guess genotypes. The subsequent polygenic analyses were performed using the best guess genotypes.

Discovery GWAS summary data

The PGSs were calculated using the approach previously described by the International Schizophrenia Consortium[44]. This method requires discovery and target datasets. The discovery data, which refers to the GWAS summary statistics-effect sizes (beta, a log of odds ratio), were obtained from a previously published SCZ GWAS[37] that was publicly available for download by the Psychiatric Genomics Consortium (PGC) http://www.med.unc.edu/pgc/, accessed on March 18, 2017.

Polygenic scoring

Quality-controlled SNPs were clumped for linkage disequilibrium based on GWAS association p-value informed clumping using $r^2 = 0.1$ within a 250-kb window to create a SNP-set in linkage equilibrium using PLINK software run on Linux (*plink --clump-p1 1 --clump-p2 1 --clump-r2 0.1 --clump-kb 250*). Then, SNPs up to ten p-value thresholds ($<1x10^{-4}$, $<1x10^{-3}$, <0.01, <0.05, <0.1, <0.2, <0.3, <0.4, <0.5, <1) were selected to compute the SCZ PGSs in the ConLi$^+$Gen sample. A genome-wide weighted SCZ PGS for each participant was calculated at each p-value threshold (P_T) as the sum of independent SNPs genotype dosage (from 0 to 2) of the reference allele in the ConLi$^+$Gen genotype data, multiplied by effect sizes on the SCZ GWAS for the reference allele, estimated as log (OR) divided by the total number of SNPs in each threshold.

STATISTICAL ANALYSES

For statistical analyses, we applied PGS association analyses, cross-trait meta-GWAS and Ingenuity Pathway Analysis (IPA) of the cross-trait findings. The details of each analysis are described below.

Polygenic score association analysis

Once the PGSs were constructed, the association of the PGSs at each P_T and lithium treatment response was evaluated using regression models. While a binary logistic regression was implemented for the categorical outcome (response versus non-response), a

linear regression was applied to lithium treatment response on the continuous scale. Using the PGS at the most significant threshold ($P_T < 5x10^{-2}$), we divided the study samples into ten deciles, ranging from the lowest polygenic load (1st decile) to the highest polygenic load (10th decile). Then, we compared BPAD patients with lower polygenic load (1st to 9th deciles) for SCZ with patients with the highest polygenic load (10th decile), to quantify the effect of SCZ polygenic load on lithium treatment outcomes.

To control for confounding effects, PGS association analyses were adjusted for the covariates age, gender, genotyping platforms and 7 principal components (PCs). The analyses were performed using R for Statistical Computing and PLINK 1.9 for Linux[42]. Prediction accuracy, the percentage of variance in lithium response accounted for by the PGS at each P_T, were estimated as the variance explained by the full model including each PGS and covariates minus the variance explained by the model including only covariates.

Statistical significance was determined at $p < 0.05$ after adjusting for covariates.

Cross-trait meta-analysis of genome-wide association studies

Biologically, a significantly associated PGS implies that genetic factors influencing the two traits are overlapping. Thus, further analyses were performed to identify genetic polymorphisms likely to increase the susceptibility to SCZ and also influence treatment response to lithium in patients with BPAD. We performed cross-trait meta-analyses by combining the summary statistics for GWAS on lithium response from the ConLi+Gen[22] and

GWAS on SCZ from the PGC[37]. We applied both the O'Brien's (OB) method and the direct Linear Combination of dependent test statistics (dLC) approach[45,46], which were implemented in the C^{++} eLX package. Briefly, the OB and dLC approach combine univariate meta-GWAS summary statistics (beta coefficients or Z-scores) at each SNP[45,46]. Further details are available elsewhere[45,46].

Ingenuity® Pathway Analysis (IPA®)

To characterise the potential biological significance of the SNPs discovered from cross-trait meta-analyses, we performed analyses using QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, CA, USA, www.qiagen.com/ingenuity).

To prepare the input genes for IPA, we followed a three-step bioinformatics approach:

Step 1: We defined tagSNPs that are in high linkage disequilibrium (LD: $r^2>0.5$) and within a $\pm$ 500-kb region with the meta-GWAS significant SNPs (gSNPs) using the genetic catalog of the 1000 Genomes project phase 3, October 2014 release[47].

Step 2: The gSNPs and tagSNPs from step 1 were mapped to the genes in which they are located. This generated a list of hosting genes (hGenes).

Step 3: We performed an expression quantitative trait loci (eQTL) lookup in three databases, searching for any nearby genes (eGenes) whose expression was associated with each of the

gSNPs and tagSNPs from step 1. These databases contained the results of eQTL-mapping studies from blood and brain tissues:

1) Westra et al[48] at FDR<0.05 http://genenetwork.nl/bloodeqtlbrowser/,

2) Almanac (Braineac)[49] at $p<1x10^{-5}$ http://www.braineac.org/, and

3) Genotype-Tissue Expression (GTEx) data release V6p (dbGaP Accession phs000424.v6.p1) accessed from the GTEx Portal on February 8, 2017, at https://www.gtexportal.org/home/.

Finally, the combined list of hGenes and eGenes was used as input into the IPA software after removing gene duplicates. IPA compares the proportion of input genes mapping to a biological pathway to the reference genes list in ingenuity databases. The significance of overrepresented canonical pathways and functional networks is determined using the right-tailed Fisher's exact test and later adjusted for multiple testing using the Benjamini-Hochberg (BH) method[50]. Significant results were determined at BH adjusted P-value <0.01.

RESULTS

Sample characteristics

In total, 3,193 patients, with BPAD who had undergone lithium treatment and had available genotype and clinical data, participated in the study. After QC, 2,586 patients remained for

analysis, of whom 2,366 were of European ancestry and the remainder Asian. The mean (sd) age of all patients combined was 47.2 (13.9) years and 2,052 (62.7%) were female. In all, 704 (27.2%) had a good response to lithium treatment (ALDA score ≥7). The mean (sd) ALDA score for all participants was 4.9 (3.1) (Table 1).

Table 1: Characteristics of patients with BPAD and outcomes with lithium treatment

Patient characteristics	**Categorical outcome[a] Good versus poor response**	**Continuous scale[b] ALDA score on subscale A**
BPAD patients (N)	2,586	2,244
Responders, N (%)	704 (27.2%)	-
Age at interview, mean (s.d)	47.2 (13.9)	47.4 (13.9)
Sex, women, N (%)	1,478 (57.2%)	1,291 (57.5%)
ALDA scale A score, mean (s.d)	6.2 (3.0)	6.3 (3.0)
ALDA scale total B mean (s.d)	2.5 (1.7)	2.1 (1.2)
ALDA scale total mean (s.d)	4.1 (3.2)	4.5 (3.1)

BPAD: Bipolar affective disorder; [a]Total ALDA score ≥7 was defined as good response; [b]Subjects with total B score >4 or who had missing data on the total scores on ALDA subscale A or B were excluded.

Associations of SCZ PGS with lithium treatment response in BPAD patients

At the most significantly associated p-value threshold ($P_T < 5x10^{-2}$), the PGS for SCZ was strongly associated with lithium treatment response in BPAD ($p=8x10^{-5}$) for the categorical outcome on the ALDA scale (Figure 1), explaining 0.8% of variance. For the continuous

outcome (total score on the ALDA subscale-A), the direction of association was congruent with the finding on the categorical outcome, but was not statistically significant ($p>0.05$). It is also important to note that the relationship between the PGS for SCZ and the total score on the ALDA subscale-A deviates from linearity; thus, the continuous scale might be a less powerful and less suitable measure to represent lithium treatment response in a linear model. The association results of categorical and continuous outcomes at each threshold level are detailed in Figure 1. In each threshold, a lower polygenic load for SCZ was associated with a favorable lithium treatment response in patients with BPAD (Figure 1A).

Table 2 shows the odds ratios (OR) for the association between lithium treatment response in BPAD and SCZ PGS in deciles, comparing the response status of patients in the low polygenic load categories (1^{st} to 9^{th} deciles) with patients in the highest polygenic load category for SCZ (in the 10^{th} decile). BPAD patients who carry a lower polygenic load for SCZ have higher odds of favorable lithium treatment response, compared to patients carrying a high polygenic load. In other words, the OR of favorable treatment response decreased as the genetic load for SCZ increased, ranging from an OR 3.46 [95%CI: 1.42-8.41] at 1^{st} decile to OR 2.03 [95%CI: 0.86-4.81] at the 9^{th} decile, compared to the reference SCZ PGS at the 10^{th} decile (Table 2). There was a highly significant linear trend in the odds of lithium treatment response across the deciles (Figure 1B).

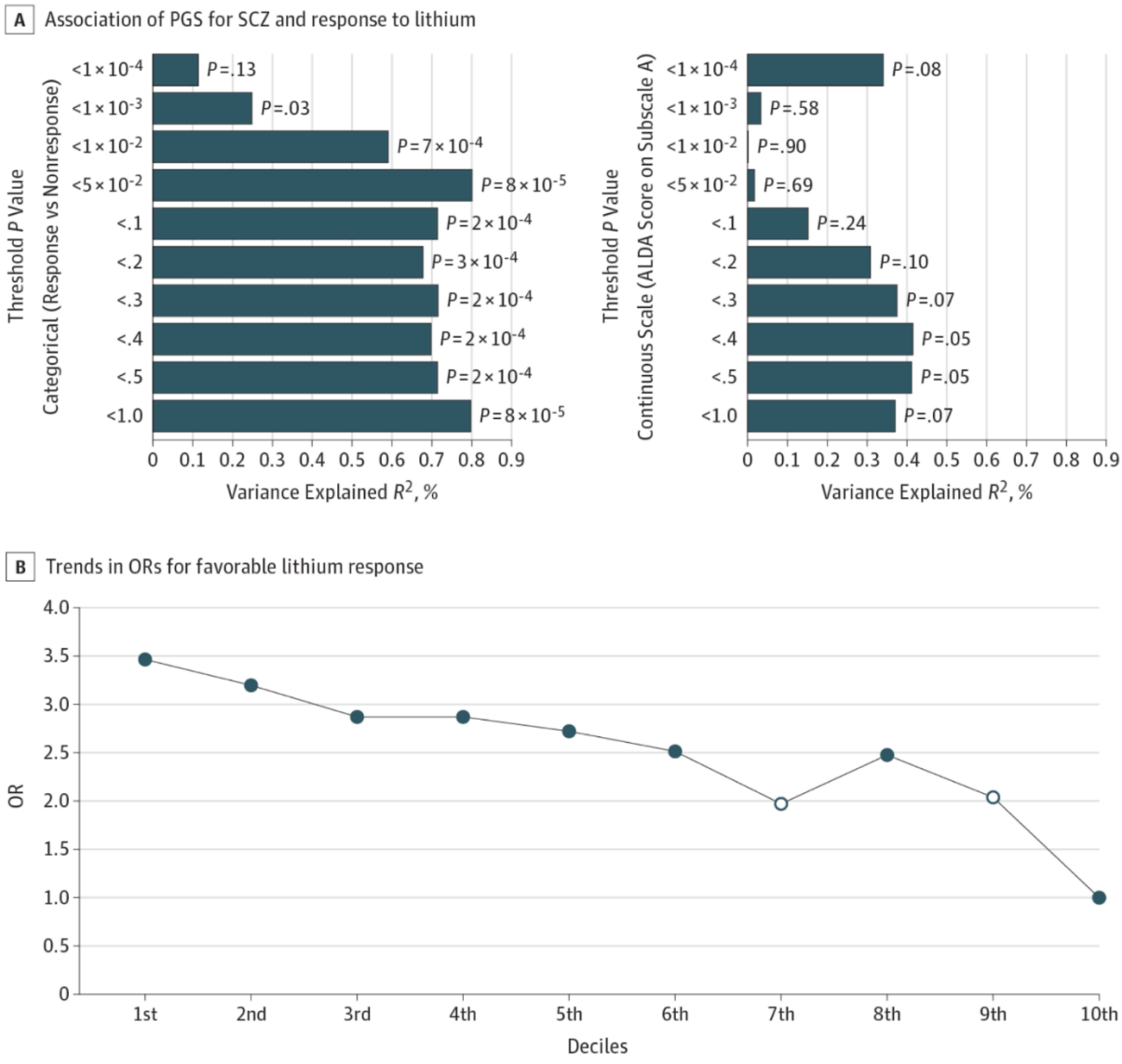


Figure 1: A, The association of PGS for SCZ and lithium treatment response defined as a categorical and continuous scale, at different SCZ genome-wide association study (GWAS) P-value thresholds. The x-axis refers to the percentage of variance in treatment response to lithium accounted for by the PGSs of SCZ at a particular P-value threshold. On the y-axis, plotted from top to bottom, are the GWAS P-value thresholds used to group single-

nucleotide polymorphisms for PGSs. On the right of each bar are the P values of the association between the PGS for SCZ and lithium treatment response.

B, Trends in the odds ratios (ORs) for favorable treatment response to lithium for patients with BPAD in the low SCZ deciles (first to ninth) compared with patients in the highest SCZ PGS decile (10th), estimated at the most significant P-value thresholds ($P < 5 \times 10^{-2}$) (n = 2586). The open circles on the line plot indicate that the association is not statistically significant at that particular decile. ALDA indicates Retrospective Criteria of Long-term Treatment Response in Research Subjects with Bipolar Disorder.

Table 2: The odds ratios of favorable lithium treatment response (categorical outcome) in patients with BPAD, comparing the response status of patients in the low PGS decile for SCZ with patients with the highest polygenic load for SCZ (10^{th} decile).

SCZ PGS in categories (deciles)	**Patients with BPAD (n=2,586)**		
	[a]R/N	unadjusted OR (95% CI)	[b]Adjusted OR (95% CI)
1st lowest score	83/175	1.97 (1.32-2.96)	3.46 (1.42-8.41)
2nd	80/179	1.86 (1.24-2.79)	3.19 (1.32-7.74)
3rd	78/180	1.80 (1.20-2.71)	2.87 (1.18-6.95)
4th	76/184	1.72 (1.14-2.59)	2.86 (1.18-6.91)
5th	76/180	1.76 (1.17-2.64)	2.71 (1.12-6.55)
6th	67/194	1.44 (0.95-2.18)	2.50 (1.03-6.05)
7th	58/200	1.21 (0.79-1.85)	1.97 (0.81-4.79)
8th	75/184	1.70 (1.13-2.55)	2.47 (1.03-5.96)
9th	61/198	1.28 (0.84-1.95)	2.03 (0.86-4.81)
10th highest score	50/208	1 (reference)	1 (reference)

The reference decile (10^{th} decile) is the PGS category with the highest polygenic load for schizophrenia at P_T <$5x10^{-2}$.

[a]R/N: number of lithium responders versus non-responders; [b] adjusted for age, sex, genotyping platform and 7-principal components. SCZ: schizophrenia, PGS: polygenic score, OR: odds ratio.

Cross-trait meta-analysis of GWAS for lithium treatment response in BPAD and GWAS for SCZ

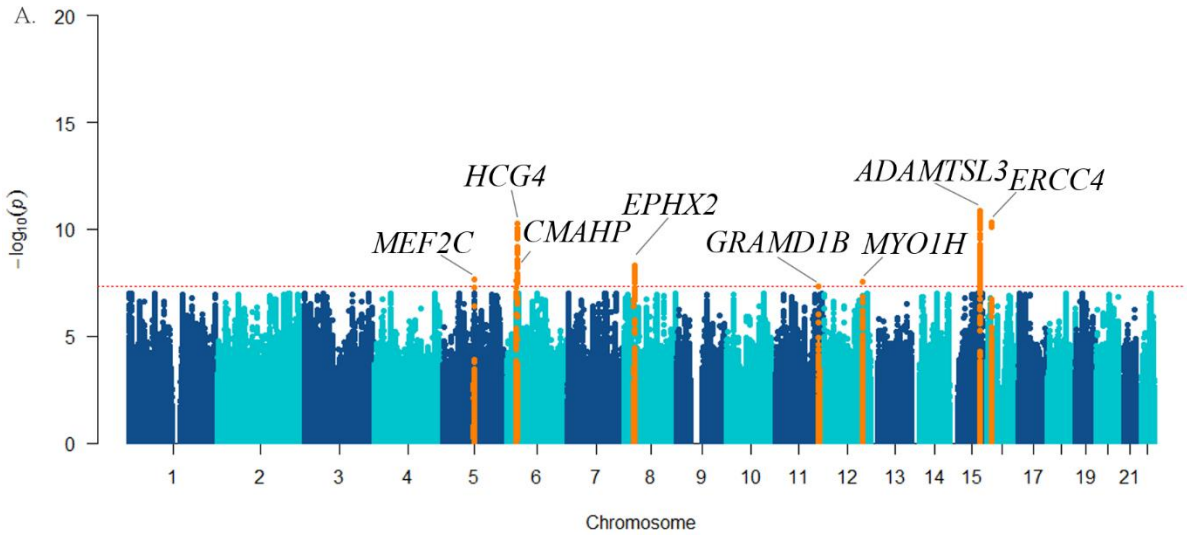
Subsequent to PGS analysis, we performed a SNP-based cross-trait meta-analysis by combining summary statistics for GWASs on: 1) SCZ and lithium treatment response in the categorical outcome; and 2) SCZ and lithium treatment response in the continuous outcome. This meta-analysis yielded 15 loci with p-values below the genome-wide significance level ($p<5x10^{-8}$). The top six loci and closest genes were: rs144373461, *HCG4*; rs66486766, *ADAMTSL3*; rs7405404, *ERCC4;* rs142425863, *HCG4*; rs3919583, *CCNH* and rs59724122, *EPHX2* (Table 3, Figure 2A and 2B).

Table 3: Loci resulting from cross-trait meta-analysis of GWAS on lithium treatment response in BPAD patients and GWAS on SCZ.

SNP	**CHR**	**BP**	**A1**	**A2**	**SCZ[¥]**	**Lithium**	**Cross-trait[§]**	**ED**	**Nearby gene**
rs324899	5	87915582	A	G	$5.82x10^{-7}$	$4.63x10^{-3}$	$2.28x10^{-8}$	--	*MEF2C*
rs6942227	6	25177508	A	G	$9.86x10^{-8}$	$8.45x10^{-3}$	$2.53x10^{-8}$	+-	*CMAHP*
rs142425863	6	29751753	T	C	$2.50x10^{-10}$	$9.92x10^{-3}$	$5.13x10^{-11}$	--	*HCG4*
rs59724122	8	27424696	T	C	$2.22x10^{-8}$	$7.21x10^{-3}$	$5.16x10^{-9}$	-+	*EPHX2*

rs61123830	11	123392846	A	G	$2.85x10^{-6}$	$2.60x10^{-3}$	$4.53x10^{-8}$	--	*GRAMD1B*
rs7959663	12	109884367	C	G	$4.74x10^{-5}$	$2.06x10^{-4}$	$2.79x10^{-8}$	--	*MYO1H*
rs66486766	15	84806060	A	G	$1.07x10^{-10}$	$4.95x10^{-3}$	$1.38x10^{-11}$	--	*ADAMTSL3*
rs7405404	16	13749859	T	C	$3.93x10^{-10}$	$5.27x10^{-3}$	$4.62x10^{-11}$	++	*ERCC4*
rs6728642	2	97607071	A	G	$1.10x10^{-4}$	$1.34x10^{-4}$	$4.81x10^{-8}$	--	*FAM178B*
rs62200793	2	185750642	T	C	$1.70x10^{-7}$	$5.45x10^{-3}$	$1.40x10^{-8}$	++	*ZNF804A*
rs7588746	2	200986345	A	G	$2.08x10^{-7}$	$6.33x10^{-3}$	$3.91x10^{-8}$	+-	*MAIP1*
rs3919583	5	86947591	A	C	$4.18x10^{-6}$	$2.65x10^{-4}$	$4.54x10^{-9}$	--	*CCNH*
rs144373461	6	29751005	A	C	$8.30x10^{-17}$	$3.93x10^{-3}$	$1.28x10^{-17}$	--	*HCG4*
rs209474	6	32924584	A	G	$7.49x10^{-7}$	$3.41x10^{-3}$	$2.20x10^{-8}$	--	*HLA-DMA*
rs1521470	7	45646852	A	G	$2.41x10^{-6}$	$3.92x10^{-4}$	$3.23x10^{-8}$	+-	*ADCY1*
rs79403677	14	35539131	T	G	$2.91x10^{-7}$	$2.04x10^{-3}$	$1.92x10^{-8}$	+-	*FAM177A1*

[¥]P-value $<1x10^{-2}$ and [§]cross-trait P-value $<5x10^{-8}$. A1, effect allele; A2, another allele; Effect direction (ED): the effect of the SNPs on schizophrenia and lithium treatment response oriented to the effect allele (A1). Nearest genes were based on refseq genes (build 37).



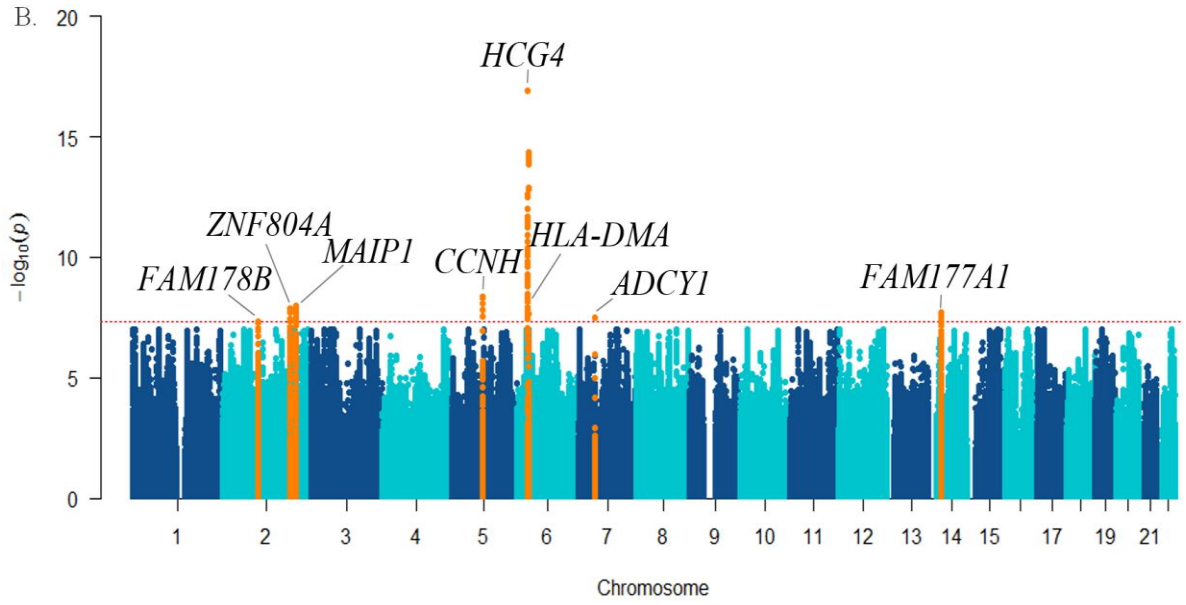


Figure 2: Manhattan plot showing the result of a cross-trait meta-analysis of GWASs on SCZ and the GWASs on lithium treatment response ALD in a; **A)** categorical scale, **B)** continuous scale highlighting the loci that showed genome-wide significance (orange), and the nearest genes (top). The −log10 (cross-trait p-value) is plotted against the physical position of each SNP on each chromosome. The threshold for genome-wide significance (cross-trait p-value<5x10-8) is indicated by the red dotted horizontal line.

To characterise the functional implications of these loci, we undertook IPA pathway analysis using query gene inputs generated from results of cross-trait and eQTL analyses. The IPA pathway analysis found significantly represented IPA® canonical pathways, the top five being: *Antigen Presentation Pathway*, *OX40 Signaling Pathway*, *Autoimmune Thyroid Disease Signaling*, *Cdc42 Signaling*, and *B Cell Development* (Table 4). These

pathways were predominantly identified on the basis of several HLA genes — *HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-DOB*, *HLA-DPB1*, *HLA-F*, *HLA-G*, *PSMB9* and *TAP2*.

Table 4: The top canonical signaling pathways enriched for genes identified in cross-trait meta-analyses

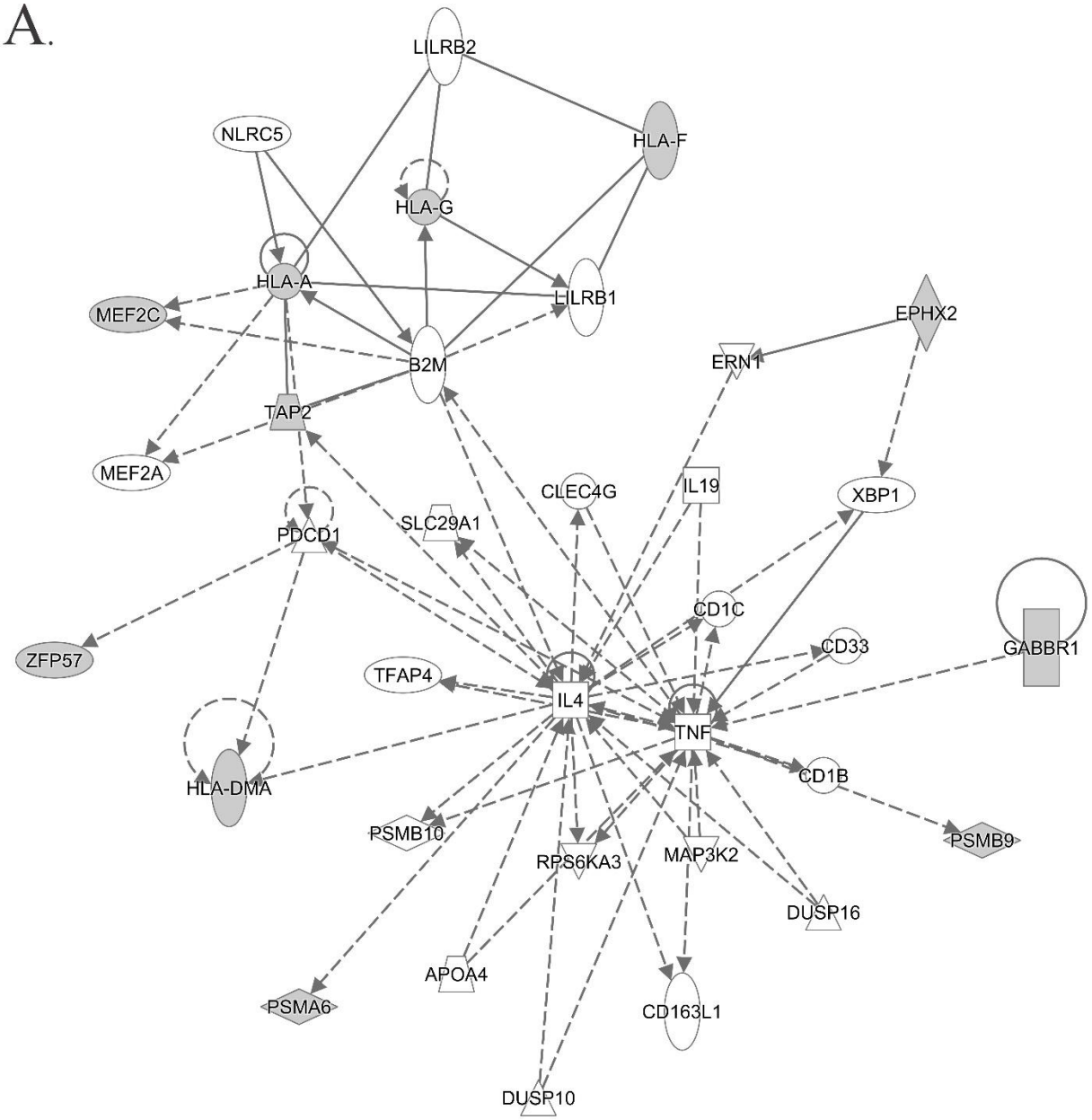
Ingenuity Canonical Pathways	**Enriched genes**	**P-values** [a]
Antigen Presentation Pathway	*HLA-DPB1*, *HLA-A*, *TAP2*, *HLA-DMA*, *HLA-DMB*, *HLA-G*, *HLA-DOB*, *PSMB9*, *HLA-F*	7.94×10^{-16}
OX40 Signaling Pathway	*HLA-DPB1*, *HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-G*, *HLA-DOB*, *HLA-F*	4.47×10^{-10}
Autoimmune Thyroid Disease Signaling	*HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-G*, *HLA-DOB*, *HLA-F*	2.29×10^{-9}
Cdc42 Signaling	*HLA-DPB1*, *HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-G*, *HLA-DOB*, *HLA-F*	1.07×10^{-7}
B Cell Development	*HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-DOB*	1.55×10^{-6}
Nur77 Signaling in T Lymphocytes	*HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-DOB*	1.82×10^{-5}
Calcium-induced T Lymphocyte Apoptosis	*HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-DOB*	2.95×10^{-5}
Th1 Pathway	*HLA-DPB1*, *HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-DOB*	3.63×10^{-5}
Th2 Pathway	*HLA-DPB1*, *HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-DOB*	6.03×10^{-5}
T Helper Cell Differentiation	*HLA-A*, *HLA-DMA*, *HLA-DMB*, *HLA-DOB*	4.79×10^{-5}

[a] P-values were adjusted by Benjamini & Hochberg (BH) method[50]. The top canonical pathways and enriched genes are determined at BH adjusted P-value <0.01. The P-value reflects the likelihood that the association between a set of input genes and given canonical pathways are statistically significant.

OX40-is a member of the tumour necrosis factor receptor (TNFR) -superfamily; **Cdc42-** Cell division control protein 42 homolog is a protein involved in regulating signaling pathways that control cellular functions including cell morphology, cell migration, endocytosis and cell cycle progression; **Nur77** is a member of nuclear receptor family involved in mediating inflammatory responses and it also induces apoptosis; **Th1/Th2** are pathways related to type 1 and type 2 T helper cells that play a vital role in the adaptive immune system. These pathways regulate immune responses by releasing T cell cytokines.

The IPA® network analysis revealed two relevant functional networks. As shown in Figure 3A and 3B, the top two networks indicate that tumor necrosis factor alpha (TNFα), interleukin-4 (IL-4) and interferon gamma (IFNγ) might represent important functional molecular nodes in the interaction between lithium response and SCZ.

A.


LILRB2
NLRC5
HLA-F
HLA-G
HLA-A
LILRB1
MEF2C
EPHX2
ERN1
B2M
TAP2
MEF2A
CLEC4G
IL19
XBP1
PDCD1
SLC29A1
CD1C
GABBR1
ZFP57
CD33
TFAP4
IL4
TNF
HLA-DMA
CD1B
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PSMB9
RPS6KA3
MAP3K2
DUSP16
APOA4
PSMA6
CD163L1
DUSP10

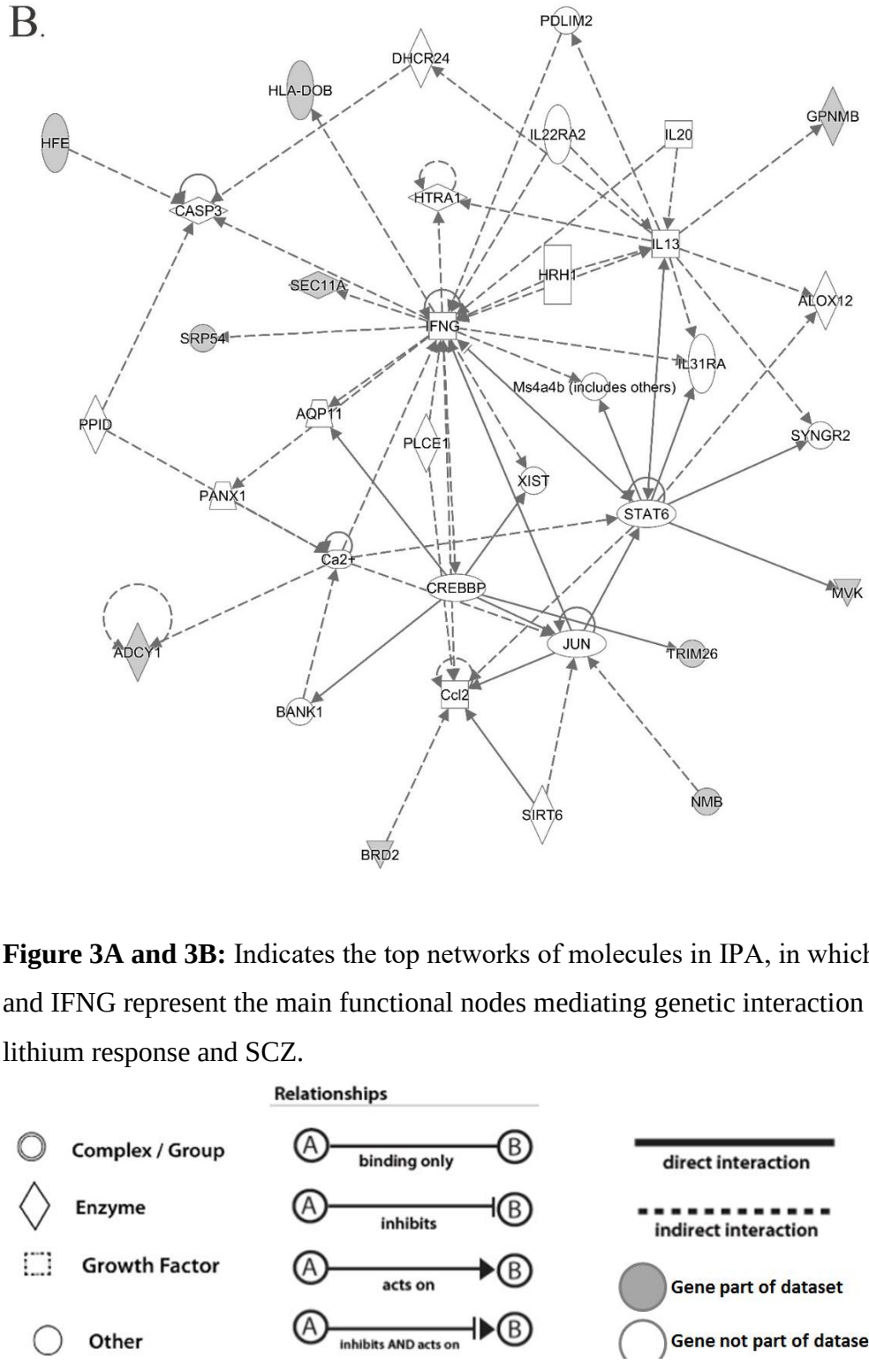

Figure 3A and 3B: Indicates the top networks of molecules in IPA, in which TNFα, IL-4 and IFNG represent the main functional nodes mediating genetic interaction between lithium response and SCZ.

DISCUSSION

The present study reports two main findings. First, using PGS methodology, we demonstrate an inverse association between genetic loading for SCZ risk variants and long-term therapeutic response to lithium in patients with BPAD on the categorical outcome of the ALDA scale. Second, we show in cross-trait meta-GWAS and pathway analyses that genetic variants in the HLA region, antigen presentation pathway and inflammatory cytokines, such as TNF-α, IL-4 and IFNγ, could have a biological role in lithium treatment response in BPAD.

These findings are consistent with previous clinical and epidemiological studies of lithium response. Lithium is not an effective medication for people suffering SCZ spectrum disorders[51,33]. Moreover, lithium may be deleterious for patients with SCZ because of their greater liability to developing lithium-induced neurotoxicity, even at modest doses and blood levels[51,52]. The severity of psychotic symptoms in bipolar patients was inversely associated with lithium treatment response[53]. Similarly, slow resolution of psychosis in response to lithium treatment during acute manic episodes has been shown to predict poorer overall response to the drug[54]. Amongst patients with BPAD, those with a family history of SCZ show a poorer response to lithium compared to those with a family history of BPAD[55]. Our findings may provide insight into the genetic architecture underlying these clinical observations.

In SCZ to lithium response cross-trait GWAS meta-analyses, 15 genetic loci located within protein-coding genes that appear to have overlapping effects on SCZ risk and response to lithium treatment in BPAD were identified. Only one of these genes, type 1 adenylyl cyclase *(ADCY1)* had previously been directly implicated in genetic studies of both SCZ[56] and lithium treatment response[26].

The most significant finding of the cross-trait GWAS and the SNPs from the post-GWAS functional analyses suggest that the HLA system could be implicated in genetic susceptibility to SCZ and lithium treatment response. The HLA region is the most robust genetic finding in SCZ[57] and could be marking a schizophrenia-type pathogenesis that is associated with lithium non-response. While extensive LD in the HLA region, and the fact that non-HLA genes are embedded within it, could compromise the biological precision of our pathway analysis, some previous studies have linked HLA surface protein composition to lithium responsiveness in BPAD[58-60]. Lithium exposure of human monocytes and mouse microglia *in vitro* resulted in increased expression of complement component 3 (C3), an HLA protein, which in turn was driven by the inhibition of glycogen synthase kinase-3 (GSK-3)[61]. Inhibition of GSK-3 is to date the most comprehensively documented molecular effect of lithium in neurons, glia and peripheral immune cells[62,63]. Whether these effects are in some way compromised by decreased neuronal complement component 3 (C3) expression associated with SCZ risk variants in the HLA region[57], and whether such mechanisms play a role in lithium's clinical efficacy, needs to be explored in future studies.

Further, network analyses of genes from our meta-GWAS findings implicated TNFα, IL-4, and IFN-γ as central functional nodes, suggesting that the negative interaction between lithium response and genetic predisposition for SCZ could be mediated by mechanisms implicating these inflammatory cytokines, which is also supported by a growing body of evidence describing aberrant inflammatory processes in patients with first-episode psychosis[64] and SCZ[65]. Previous studies have reported modulatory effects of lithium treatment on these cytokines and underscore the possibility that mechanisms involving inflammatory cytokines might play a role in mediating therapeutic effects of lithium in patients with BPAD[66-73].

Our findings have important implications for treatment of BPAD and for future research. We show, for the first time, that genetic characterisation has the potential to aid stratification of bipolar patients into lithium responders and non-responders prior to treatment initiation. Our study also supports the idea that responsiveness to lithium could represent a true psychiatric endophenotype beyond current nosology[74]. Findings underscore the importance of careful assessments of patient family psychiatric histories in the context of treatment selection. In schizoaffective disorder, which remains challenging clinically due to a lack of specific effective treatments[75], determination of SCZ PGS might aid the choice of mood stabilising agents. In order to achieve full clinical translation, PGS analyses could be combined with other biological and clinical predictors in prognostic algorithms.

This study has limitations that should be noted. First, the polygenic load for SCZ accounted for only a modest percentage (~1%) of observed variation in lithium treatment response in patients with BPAD. While this is in line with previous reports on the effects of PGSs on complex clinical phenotypes, such as SCZ and BPAD[76], the significance of this finding at clinical- and population-levels needs to be further explored. Second, lithium response in our study was assessed using the ALDA scale, which is a retrospective measure. In order to substantiate our findings further, prospective studies are required that can measure clinical responses to lithium prospectively. Third, while our strategy for exploring the biological context of our genetic findings can point towards avenues for future research, it is not designed to provide definitive mechanistic answers. Hypothesis-driven experiments are required to follow up on these leads.

In conclusion, we demonstrated for the first time that lower SCZ loading is strongly associated with better lithium response in patients with BPAD. Follow-up functional analyses implicate genes that code for the immune system, including the HLA complex and inflammatory cytokines. For future clinical translation, a high genetic loading for SCZ risk variants could be used in conjunction with clinical parameters to predict the likelihood of non-response to lithium treatment in BPAD.

Conflict of interest

All authors declare that they have no competing financial interests apart from those disclosed in the article.

Web resources

The URLs for data presented herein are as follows:

PGC-Psychiatric Genomics Consortium: schizophrenia, GWAS data,

http://www.med.unc.edu/pgc/downloads

Blood eQTL browser: http://genenetwork.nl/bloodeqtlbrowser

The Brain eQTL Almanac (Braineac): http://www.braineac.org/

The Genotype-Tissue Expression (GTEx): http://www.gtexportal.org/home/.

Tools

OB and dLC methods in eLX package:

https://sites.google.com/site/multivariateyihsianghsu/.

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Overall percentage (%)	75%		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Chapter 5

Association of polygenic score for major depressive disorder and depressive symptoms with response to lithium in patients with bipolar disorder

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ABSTRACT

Background: Lithium is a first line medication for bipolar disorder (BIP), but only ~30% of patients respond optimally to the drug. Since genetic factors are known to mediate lithium treatment response, we asked whether polygenic susceptibility to the spectrum of depression traits is associated with treatment outcomes in patients with BIP. In addition, we explored the potential molecular underpinnings of this relationship.

Methods: Weighted polygenic scores (PGSs) were computed for major depressive disorder (MDD) and depressive symptoms (DS) for BIP patients from the Consortium on Lithium Genetics (ConLi^{+}Gen; n=2,586). Lithium treatment outcome was assessed using the ALDA scale. Summary statistics from genome-wide association studies (GWAS) in MDD (130,664 cases and 330,470 controls) and DS (n=161,460) were used for PGS weighting. Associations between PGSs, depression traits and treatment response were assessed by binary logistic regression. For functional analysis of findings, we performed a cross-trait meta-GWAS, followed by Ingenuity® Pathway Analysis.

Outcomes: BIP patients with a low polygenic load for depressive traits are more likely to respond well to lithium, compared to patients with high polygenic load (MDD: OR 1.64 [95%CI: 1.26-2.15], lowest vs highest PGS quartiles; DS: OR 1.53 [95%CI: 1.18-2.00]). Associations were significant for type 1, but not type 2 BIP. Functional characterization implicated insulin-related pathways, mitogen-activated protein-kinase (MAPK) signaling, and miRNA expession.

Interpretation: Genetic susceptibility to depression in BIP type 1 patients lower their odds of responding optimally to lithium. Our findings support the emerging concept of a lithium-responsive biotype in BIP.

Keywords: lithium treatment, major depressive disorder, depressive symptoms, depressive traits, bipolar disorder, polygenic score, pharmacogenomics, voltage-gated potassium channel, insulin, MAPK.

INTRODUCTION

Bipolar disorder (BIP) is a chronic and severe psychiatric illness characterized by episodic, abnormal manic and depressive mood states. An estimated 48.8 million people are affected by BIP globally[1]. The disorder accounts for 9.9 million years of life lived with disability worldwide[1], and substantially increases all-cause mortality and risk of suicide[2].

Amongst available treatments, lithium is regarded as a gold standard by several clinical guidelines[3,4] and has been recently shown to be particularly effective preventing rehospitalisation[5]. Lithium protects against both manic and depressive illness phases, and has demonstrated protective effects against suicide[6-8]. However, not all patients with BIP fully benefit from lithium, and only about 30% show full response to the drug[6-8]. In current psychiatric practice, no biological or clinical markers exist that could reliably predict responsiveness to lithium[9], and prescribing cannot be targeted to patients who benefit most while avoiding side effects and sub-optimal treatment for poor responders[10 11 12,13]. In order to develop objective response markers and to move towards personalized prescribing of lithium for BIP patients, a better understanding of the biological mechanisms underlying lithium response is urgently required.

Genetic variation is recognized as an important mediator in response to long term lithium treatment response of BIP[6,14,15]. Additionally, several psychiatric and physical traits have been shown to impact lithium treatment response [16 17 18]. We have recently demonstrated that high genetic loading for schizophrenia (SCZ) risk variants in people with BIP substantially decreases

the likelihood of favourable response to lithium, suggesting that polygenic score (PGS) analysis of such traits could be useful in predicting treatment outcomes[15]. Other psychiatric traits that could contribute to genetic prediction include major depressive disorder (MDD) and depressive symptoms (DS) on the assumption BIP and MDD show 47% genetic overlap[19-21], and with shared risk genes and biological pathways described[21,22]. Further, lithium can be effective as an augmentation strategy in MDD patients who have experienced an insufficient response to first-line antidepressants[23,24], and is protective against further MDD episodes after symptom remission has been achieved[20,25]. Moreover, a large observational study based on the Finnish registry showed that lithium is the most effective agent preventing rehospitalization in MDD[25]. However, the genetic and molecular underpinnings of these complex interactions between BIP, MDD, and therapeutic response to lithium are not well understood and require further exploration.

In the current study, we tested whether BIP patients with a high genetic susceptibility for depression (MDD and DS), expressed by their PGS for these traits, would respond better or worse to lithium than BIP patients with a low genetic loading [26 27]. To explore potential genetic and molecular drivers of any detected polygenic association, we carried out a cross-trait GWAS meta-analysis, combining the summary statistics from the largest available GWASs for MDD[26] and DS[27] with response to lithium treatment in patients with BIP[6]. Overlapping SNPs that met genome-wide significance in the meta-GWAS were subsequently analyzed for biological context using the Ingenuity® Pathway Analysis platform (IPA®).

METHODS AND MATERIALS

Discovery GWAS summary data sets

The polygenic score and cross-trait meta-analysis for this study were based on genetic data from the International Consortium on Lithium Genetics (ConLi^{+}Gen)[6], and the summary statistics of the three largest GWASs available for MDD[26], DS[27] and treatment response to lithium in patients with BIP[6].

Major depressive disorder

The most recent GWAS meta-analysis of 9.6 million SNPs (Psychiatric Genomics Consortium-PGC; http://www.med.unc.edu/pgc/), obtained from 7 cohorts (deCODE, Generation Scotland, GERA, iPSYCH, UK Biobank, CONVERGE and 23andMe) containing 130,664 MDD cases and 330,470 healthy controls, identified 44 independent loci that reached the criteria for statistical significance. Details on this study are available elsewhere[26].

Depressive symptoms

The GWAS on DS (N = 161,460) used data from the PGC, the UK Biobank (UKB), the Resource for Genetic Epidemiology Research on Aging (GERA) Cohort and the Social Science Genetic Association Consortium (SSGAC) https://www.thessgac.org/. The summary statistics were made publically available for scientific usage[27].

Lithium treatment response in BIP

The summary GWAS on lithium treatment response was produced through a combined analysis of 2,563 patients collected by 22 participating sites from the International Consortium on Lithium Genetics (ConLi^{+}Gen). http://www.conligen.org/. In our analysis, we used the data analyzed on the categorical scale for lithium response[6].

Target Study Sample

For the PGS analysis, clinical data on lithium treatment response and genetic information were obtained for n=2,586 patients from the International Consortium on Lithium Genetics (ConLi^{+}Gen; www.ConLiGen.org)[3,6,15]. A series of quality control procedures were implemented on the genotype data before and after imputation as described below.

Target outcome

Lithium treatment response was assessed using the validated "Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder" scale, also known as the ALDA scale [8,28,29].This scale quantifies symptom improvement over the course of treatment (A score, range 0–10), which is then weighted against five criteria (B score) that assess confounding factors[6]. Patients with a total score of 7 or higher are categorized as "good responders", and the remainder were categorized as poor responders[6,29]. In addition to the ALDA scale scores, information on covariates such as age and gender was collected, as described in detail elsewhere[6].

Genotyping and quality control

The genome-wide genotypes, as well as clinical and demographic data, were collected by 22 participating sites. Quality control (QC) procedures were implemented on the genotype data using PLINK, version 1.09 prior to imputation[30]. Samples with low genotype rates <95%, sex inconsistencies (based on X-chromosome heterozygosity), and one of a pair of genetically related individuals were excluded. SNPs were excluded based on the following criteria: a poor genotyping rate (<95%), strand ambiguity (A/T and C/G SNPs), a low minor allele frequency

(MAF<1%), or those deviated from genotype frequency expectations under the Hardy-Weinberg Equilibrium ($p<10^{-6}$).

Imputation

The genotype data passing QC were imputed on the Michigan server[31] (https://imputationserver.sph.umich.edu) separately for each genotype platform using reference data from the 1000 Genomes Project Phase 3 (Version 5). The European reference panel was used for all the samples except for those from Japan and Taiwan, for which the East Asian reference population data was used. After excluding low-frequency SNPs (MAF<10%); low-quality variants (imputation INFO < 0.9); and indels, the imputed dosages were converted to best guess genotypes. The subsequent polygenic analyses were performed using these best guess genotypes.

STATISTICAL ANALYSES

Polygenic score (PGS) association analysis

PGSs were calculated using the approach previously described by the International Schizophrenia Consortium[32]. Prior to PGS computation, independent SNPs were identified through a clumping procedure implemented in PLINK software, version 1.09 run on Linux[30]. Quality-controlled SNPs were clumped for linkage disequilibrium based on GWAS association p-value informed clumping at $r^2 = 0.1$ within a 250- kilobase window to create a SNP-set in linkage equilibrium (*plink --clump-p1 1 --clump-p2 1 --clump-r2 0.1 --clump-kb 250*). Polygenic risk scores were calculated for MDD and DS in the ConLi$^+$Gen sample at 10 p-value thresholds ($<1x10^{-4}$, $<1x10^{-3}$, <0.01, <0.05, <0.1, <0.2, <0.3, <0.4, <0.5, <1). PGS for each patient was calculated at each p-value threshold (P_T) as a weighted sum of allelic counts (from 0 to 2) for the

reference alleles across independent SNPs on a genome-wide scale. The weights were effect sizes estimated as beta or log (odds ratio) obtained from the GWASs of MDD[26] and DS[27]. Here, each of the ten PGS analysis were non-independent, i.e. the PGS at the 2nd p-value threshold had all SNPs from the 1st p-value threshold, next the PGS at the 3rd p-value threshold had all SNPs from the 2nd threshold and finally the PGS at the 10th threshold included all the SNPs from 1st to 9th threshold. Therefore, no correction was assumed for multiple testing.

Once the PGSs were constructed, a binary logistic regression model was applied to evaluate the association of the PGSs for MDD and DS calculated with lithium treatment response at each P_T. Using the PGS at the most optimal thresholds, we divided the study samples into quartiles, ranging from the lowest polygenic load (1st quartile) to the highest polygenic load (4th quartile). Then, we compared BIP patients in the lower polygenic load quartiles (1st, 2nd and 3rd quartiles) with patients in the highest polygenic load quartile (4th quartile), to quantify the effect of MDD and DS polygenic load on lithium treatment response. Associations were considered significant at $p < 0.05$ after adjusting for covariates.

The PGS association analyses were adjusted for the covariates age, gender, genotyping platform, and 7 principal components (PCs) from genotype-derived ethnicity estimates. The analyses were performed using R for Statistical Computing and PLINK, version 1.09 for Linux[30]. Prediction accuracy, the percentage of variance in lithium response accounted for by the PGS at each P_T, was estimated as the variance explained by the full model including each PGS and covariates minus the variance explained by the model including only covariates.

Cross-trait meta-analysis of genome-wide association studies

Having identified a significant polygenic association that indicated the presence of genetic overlap, we conducted two cross-trait meta-analyses of GWASs to identify genetic polymorphisms that were likely to increase the susceptibility to both MDD and DS as well as influence lithium treatment response in patients with BIP. The cross-trait meta-analyses were performed by combining the summary statistics for GWAS on lithium response [6] and GWAS on MDD[26] and DS[27]. We applied the O'Brien's (OB) method and the direct Linear Combination of dependent test statistics (dLC) approach[33,34], which are implemented in the C^{++} eLX package. The OB and dLC approach follow an inverse-variance meta-analysis method. It directly combines univariate meta-GWAS summary data (Z-scores) for each SNP (as in meta-analyses) considering the correlation within the univariate test statistics and estimated variances between the traits[33,34]. The OB method is more powerful when the summary statistics are homogeneous (not very different) and in a similar direction, while dLC is more powerful when the test statistics are either heterogeneous or in opposite directions. Because they often vary based on the sign of the Z-scores, the p-value on either of the two tests could be used to determine statistical significance. Further details are available in previous publications[33,34].

In this cross-trait meta-analysis, Z-scores on the GWAS for lithium response [6] were combined with the scores for MDD[26] and DS[27] for each SNP. Each analysis generated 2 test statistics and associated p-values, one for the OB method and one for the dLC method. Statistical significance of the cross-trait association was determined at ($p< 5\times10^{-8}$) based on the smaller of the two p-values as they vary by the sign of the Z-scores. For each cross-trait meta-analysis, only one

independent lead SNP per locus was reported. Nearby SNPs in LD (r^2>0.1) with the lead SNP were considered dependent and belonging to the same locus.

Ingenuity® Pathway Analysis (IPA®)

To characterize the biological context of the discovered SNPs from the cross-trait meta-analyses, we implemented a functional analysis using QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, CA, USA, www.qiagen.com/ingenuity). We followed a three-step bioinformatics approach to prepare the input genes for IPA.

Step 1: We defined tagSNPs that were in high linkage disequilibrium (LD: r^2>0.5) and within a ± 500-kb region with the meta-GWAS significant SNPs (gSNPs) using the genetic catalog of the 1000 Genomes project phase 3, October 2014 release[35].

Step 2: The gSNPs and tagSNPs from step 1 were mapped to the genes in which they were located. This step generated a list of hosting genes (hGenes).

Step 3: We performed an expression quantitative trait loci (eQTL) lookup in 3 databases, searching for any nearby genes (eGenes) whose expression was associated with each of the gSNPs and tagSNPs from step 1. These databases contained the results of eQTL-mapping studies from blood and/or brain tissues: 1) Westra et al[36] at FDR<0.05 http://genenetwork.nl/bloodeqtlbrowser/, 2) Almanac (Braineac)[37] at $p<1x10^{-5}$ http://www.braineac.org/, and 3) Genotype-Tissue Expression (GTEx) at false discovery rate (FDR) threshold of ≤0.05[38] data release V7 (dbGaP Accession phs000424.v7.p2) accessed from the GTEx Portal on December 21, 2017, at https://www.gtexportal.org/home/.

Finally, the combined list of hGenes and eGenes were used as input into the IPA software after removing gene duplicates. The IPA compares the input genes with the ingenuity knowledge

databases in order to identify Upstream Regulators, enriched pathways, and functional networks[39]. The IPA analysis was performed using the right-tailed Fisher's exact test and later adjusted for multiple testing using the Benjamini-Hochberg (BH) method[40]. Significant results were determined at BH adjusted P-value ≤ 0.01.

RESULTS

Sample characteristics and lithium treatment response rates

After QC, 2,586 patients (3,193 before QC) remained for analysis. While 2,366 were of European ancestry, the remaining were of Asian ancestry. In all, 704 (27.2%) responded to lithium treatment (ALDA score ≥7). Detailed sample and demographics details have been described previously[15].

MDD and DS PGS are associated with lithium treatment response in BIP

Associations between the PGSs for MDD and DS with lithium treatment response were found at various p-value thresholds. The strongest association were found for MDD (p= 0.0003) at $P_T < 5x10^{-2}$, $R^2 = 0.7\%$ and for DS (p= 0.0003) at $P_T < 1x10^{-2}$, $R^2 = 0.7\%$) (Figure 1).

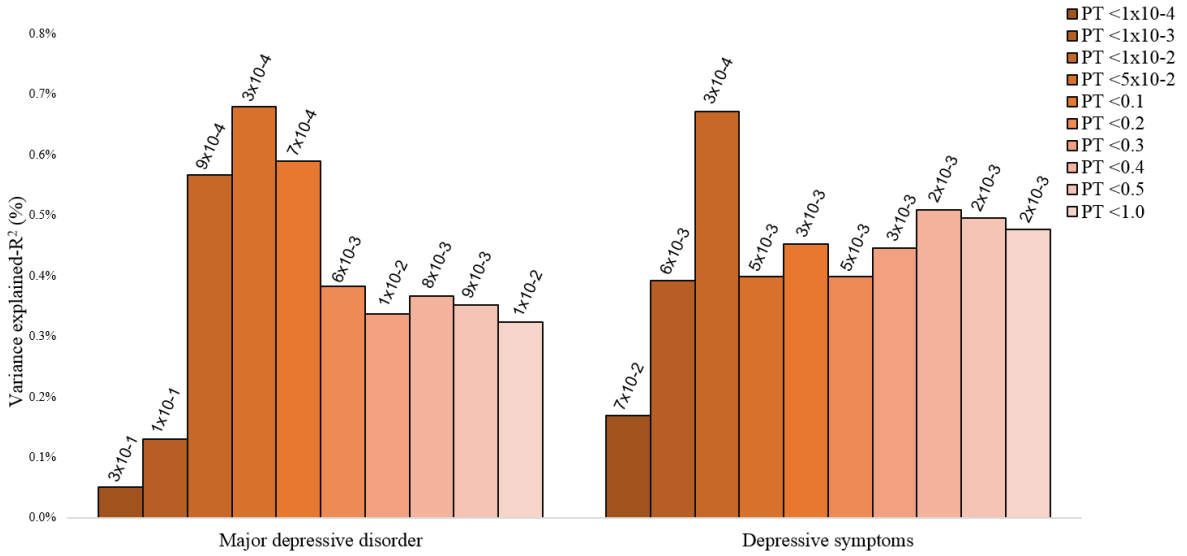


Figure 1: The association of PGS for depression traits (MDD and DS) and lithium treatment response at different GWAS p-value thresholds. The y-axis refers to the percentage of variance in treatment response to lithium accounted for by the PGSs for depressive traits at particular P-value thresholds. On the x-axis, are the GWAS P-value thresholds used to select single-nucleotide polymorphisms for the PGSs. On the top of each bar are the p-values for the association between the PGSs for depressive traits and lithium treatment response.

High genetic loadings for MDD and DS are associated with poorer response to lithium in BIP

We divided the study population into quartiles, according to their polygenic loading for MDD and DS, respectively. As shown in Figure 2 and Table 1, BIP patients who carry a lower polygenic load (1st quartile) for MDD or DS have higher odds of favorable lithium treatment response, compared to patients carrying a high polygenic load (4th quartile). The odds ratio (OR) of favourable response for patients in the 1st quartile compared with those in the 4th quartile was

1.64 [95%CI: 1.26-2.15] for MDD PGS, and 1.53 [95%CI: 1.18-2.00]) for DS PGS (Table 1 & Figure 2).

Table 1: Odds ratios of favorable lithium treatment response in patients with BIP, comparing the response status of patients in the low PGS quartile for MDD and DS with patients with the highest polygenic load for MDD/DS (4th quartile).

Categories PGS quartiles	Patients with BIP (n=2,586)		
	Responders/ Non-responders	unadjusted OR (95% CI)	¥Adjusted OR (95% CI)
MDD			
1st lowest score	197/449	1.56 (1.21-2.00)	1.64 (1.26-2.15)
2nd	202/444	1.62 (1.26-2.07)	1.60 (1.22-2.08)
3rd	163/485	1.19 (0.92-1.54)	1.20 (0.91-1.57)
4th highest score	142/504	1 (reference)	1 (reference)
DS			
1st lowest score	195/452	1.50 (1.17-1.93)	1.53 (1.18-2.00)
2nd	199/447	1.55 (1.21-1.99)	1.53 (1.18-1.99)
3rd	166/481	1.20 (0.93-1.55)	1.25 (0.96-1.64)
4th highest score	144/502	1 (reference)	1 (reference)

Legend Table 1: The reference quartile (4th quartile) is the PGS category with the highest polygenic load for MDD/DS at the most significant threshold.

¥adjusted for age, sex, genotyping platform and 7-principal components. MDD: Major depressive disorder, DS: Depressive symptoms, PGS: polygenic score, OR: odds ratio

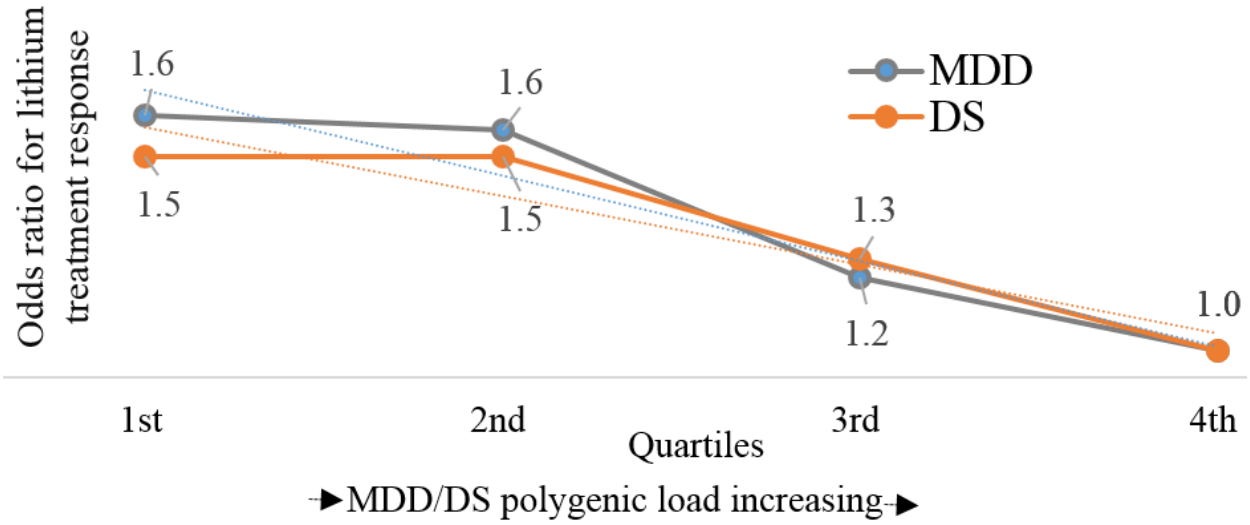


Figure 2: Odds ratios (ORs) for favorable treatment response to lithium for patients with BIP in the low depression polygenic load quartiles (1st to 3rd), compared with patients in the highest depression polygenic load quartile (4th), estimated at the most significant p-value thresholds (n = 2586).

Inverse associations between depression PGS and lithium response are significant in BIP Type 1, but not BIP type 2

In order to explore whether the associations between MDD/DS PGSs and lithium response are specifically driven by BIP type 1 or type 2, we analyzed these diagnostic subgroups separately (type 1 n=2,044; type 2 n=506). Inverse effects for MDD and DS PGS remained statistically significant for BIP type 1 patients. In contrast, no significant associations were established for BIP type 2 (Table 2). For this group, we saw an opposite trend for MDD PGSs, i.e. higher MDD loading was associated with *better* response to lithium.

Table 2: The association of PGS for depression traits (MDD and DS) and lithium treatment response in patients with BIP type 1 versus BIP type 2 at different GWAS p-value thresholds (P_T).

MDD PGS		**BIP type 1**		**BIP type 2**	
P_T	No of SNPs	p-value	R^2 (%)	P-value	R^2 (%)
$P_T < 1x10^{-4}$	852	$1.83x10^{-1}$	0.1%	0.43	0.2%
$P_T < 1x10^{-3}$	2987	$1.09x10^{-1}$	0.2%	0.98	0.0%
$P_T < 1x10^{-2}$	11892	$4.34x10^{-4}$	0.8%	0.85	0.0%
$P_T < 5x10^{-2}$	31712	$2.94x10^{-5}$	1.2%	0.50	0.1%
$P_T < 0.1$	47716	$8.86x10^{-5}$	1.0%	0.50	0.1%
$P_T < 0.2$	70784	$1.08x10^{-3}$	0.7%	0.39	0.2%
$P_T < 0.3$	88552	$2.61x10^{-3}$	0.6%	0.40	0.2%
$P_T < 0.4$	102503	$1.33x10^{-3}$	0.7%	0.33	0.2%
$P_T < 0.5$	114071	$1.72x10^{-3}$	0.6%	0.35	0.2%
$P_T < 1.0$	147596	$2.97x10^{-3}$	0.6%	0.37	0.2%
DS PGS		**BIP type 1**		**BIP type 2**	
P_T	No of SNPs	p-value	R^2 (%)	p-value	R^2 (%)
$P_T < 1x10^{-4}$	229	$4.08x10^{-2}$	0.3%	$2.67x10^{-1}$	0.3%
$P_T < 1x10^{-3}$	1352	$1.18x10^{-2}$	0.4%	$1.08x10^{-1}$	0.7%
$P_T < 1x10^{-2}$	8057	$7.26x10^{-3}$	0.5%	$6.26x10^{-2}$	0.9%
$P_T < 5x10^{-2}$	25918	$5.51x10^{-2}$	0.2%	$4.68x10^{-2}$	1.0%
$P_T < 0.1$	41865	$1.46x10^{-1}$	0.1%	$4.86x10^{-2}$	1.0%
$P_T < 0.2$	65634	$1.54x10^{-1}$	0.1%	$5.51x10^{-2}$	0.9%
$P_T < 0.3$	83845	$1.99x10^{-1}$	0.1%	$5.48x10^{-2}$	0.9%
$P_T < 0.4$	98217	$1.61x10^{-1}$	0.1%	$5.16x10^{-2}$	1.0%
$P_T < 0.5$	109888	$1.95x10^{-1}$	0.1%	$4.96x10^{-2}$	1.0%
$P_T < 1.0$	143345	$2.16x10^{-1}$	0.1%	$4.42x10^{-2}$	1.0%

Cross-trait meta-analysis of GWAS on lithium treatment response and GWAS on MDD and depressive symptoms yields 7 significant loci

Subsequent to the PGS analysis, we performed a SNP-based cross-trait meta-analysis by combining the summary statistics for the GWASs on: 1) MDD and lithium treatment response; and 2) DS and lithium treatment response — with the aim of identifying individual genetic variants implicated in the genetic susceptibility to both depression traits and lithium treatment response. These analyses yielded 7 loci with p-values below the genome-wide significance level

($p<5x10^{-8}$). These loci, and their nearby genes, were rs2327713: *PUM3* [OMIM: 609960], rs7134419: *KSR2* [OMIM: 610737], rs59659806: *RASGRP1* [OMIM: 603962], rs7405404: *ERCC4* [OMIM: 133520], rs11657502: *MYO18A* [OMIM: 610067], rs8099160: *DCC* [OMIM: 120470], rs6066909: *ARFGEF2* [OMIM: 605371] (Figure 3, Table 3)

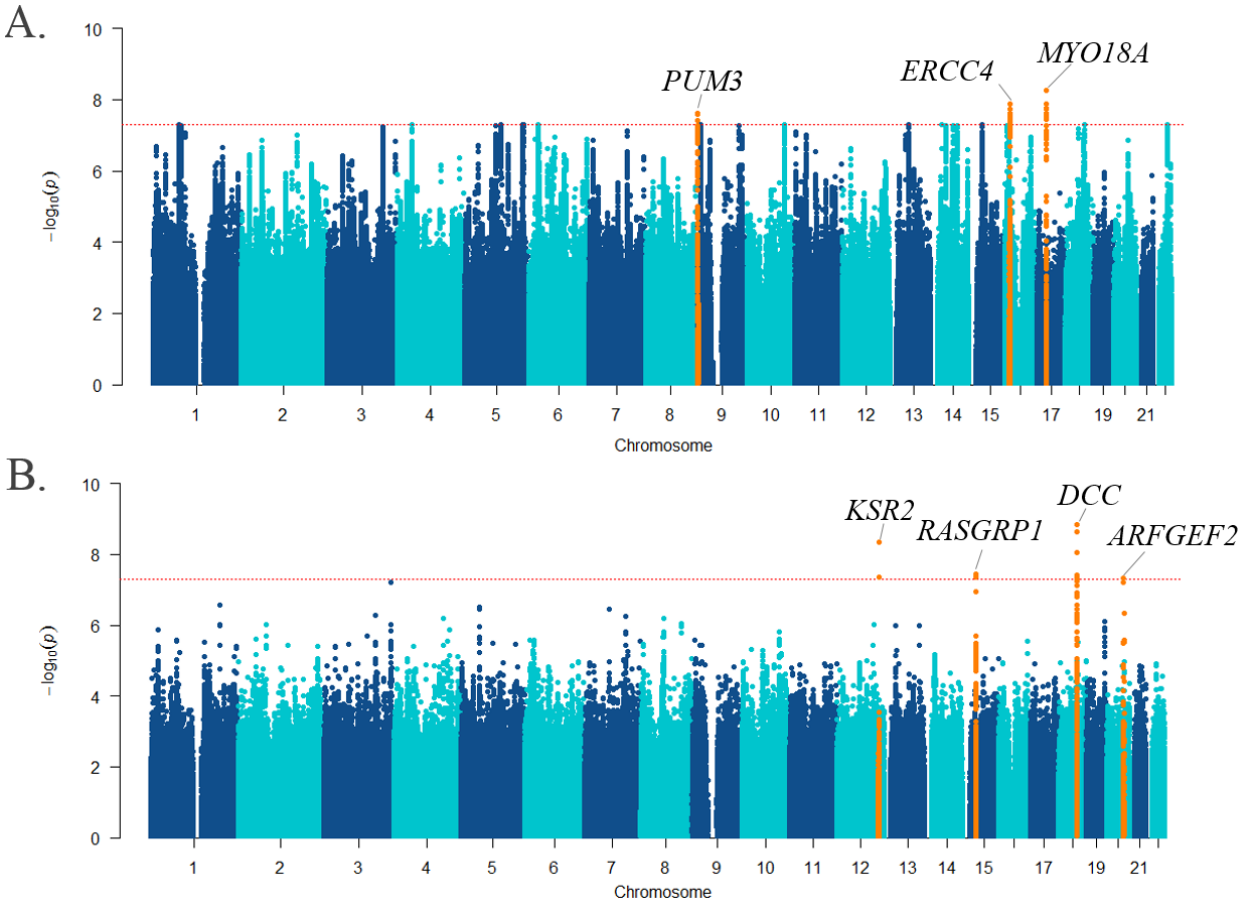


Figure 3: Manhattan plot of the cross-trait meta-analyses of GWASs on lithium treatment response and A) MDD; and B) DS. The loci that showed genome-wide significance are highlighted (orange), and their nearest genes indicated (top). The −log10 (cross-trait p-value) is plotted against the physical position of each SNP on each chromosome. The threshold for

genome-wide significance (cross-trait p-value<$5x10^{-8}$) is indicated by the red dotted horizontal line.

Table 3: Loci resulting from a cross-trait meta-analysis of GWASs for lithium treatment response in patients with BIP patients and GWAS for MDD and DS (cross-trait p<$5x10^{-8}$).

SNP	CHR	BP	A1	A2	GWAS P-value			ED	Nearest gene
					MDD	Lithium	Cross-trait		
rs2327713	9	2974953	T	C	$3.19 x10^{-8}$	$3.81 x10^{-2}$	$2.35 x10^{-8}$	+-	*PUM3*
rs7405404	16	13749859	T	C	$1.91 x10^{-7}$	$5.27 x10^{-3}$	$1.33 x10^{-8}$	++	*ERCC4*
rs11657502	17	27490977	A	G	$9.87 x10^{-7}$	$4.53 x10^{-4}$	$1.35 x10^{-8}$	+-	*MYO18A*
					DS				
rs7134419	12	118383133	A	C	$9.39 x10^{-8}$	$5.64 x10^{-1}$	$4.34 x10^{-8}$	+-	*KSR2*
rs59659806	15	38919964	T	C	$6.01 x10^{-7}$	$5.12 x10^{-2}$	$3.58 x10^{-8}$	--	*RASGRP1*
rs8099160	18	50752610	A	G	$2.68 x10^{-8}$	$4.67 x10^{-1}$	$1.42 x10^{-9}$	++	*DCC*
rs6066909	20	47529913	T	C	$9.23 x10^{-7}$	$2.21 x10^{-1}$	$4.66 x10^{-8}$	++	*ARFGEF2*

A1, effect allele; A2, alternative allele; CHR: chromosome; BP: position in base-pairs based on build 37; ED: Effect direction, +, increased susceptibility to MDD/DS or positive effect on lithium treatment response oriented to the effect allele (A1); -, decreased susceptibility to MDD/DS or negative effect on lithium treatment response oriented to the effect allele (A1); Nearest genes were based on RefSeq genes (build 37).

Functional and biological characterization of genes associated with BIP lithium non-response and MDD/depressive symptoms

To characterize the functional implications of the SNPs identified by cross-trait meta-analysis, we first explored the functional genetic context of these variations by examination of SNPs with high linkage disequilibrium, characterizing of their nearby hosting genes, and eQTL lookup from published databases. This approach yielded a list of 39 genes with potential functional significance. Second, we investigated the biological roles of these 39 genes using IPA® analysis.

IPA® identified cellular development and cellular growth and proliferation as the top cellular functions associated with the 39 genes. Cardiovascular disease and cardiovascular system development and function were the disorder and physiological system with the highest associations. These associations were driven by only a handful of genes, including micro RNA (miR) -144, miR-451, regulatory factor X3 (*RFX*), phosphatidylinsositol-3,4,5-trisphosphate dependent Rac exchange factor 1 (*PREX1*), and RAS guanyl releasing protein 1 (*RASGRP1*).

The top IPA®-identified functional networks containing dataset genes pointed to 'hub' functions for insulin, ERK, JNK (network 1), ELAV like RNA binding protein 1 (ELAV1)(network 2), and nuclear RNA export factor 1 (NXF1) (network 3), [Figures 4 A-C]. The IPA® top hits for upstream regulators were potassium voltage-gated channel subfamily A member 1 (KCNA1) and leucine-2-alanine encephalin, both of which influence the dataset gene potassium voltage-gated channel subfamily B member 1 (KCNB1) (Table 4).

Table 4: Top upstream regulators for genes identified in the cross-trait meta-analyses

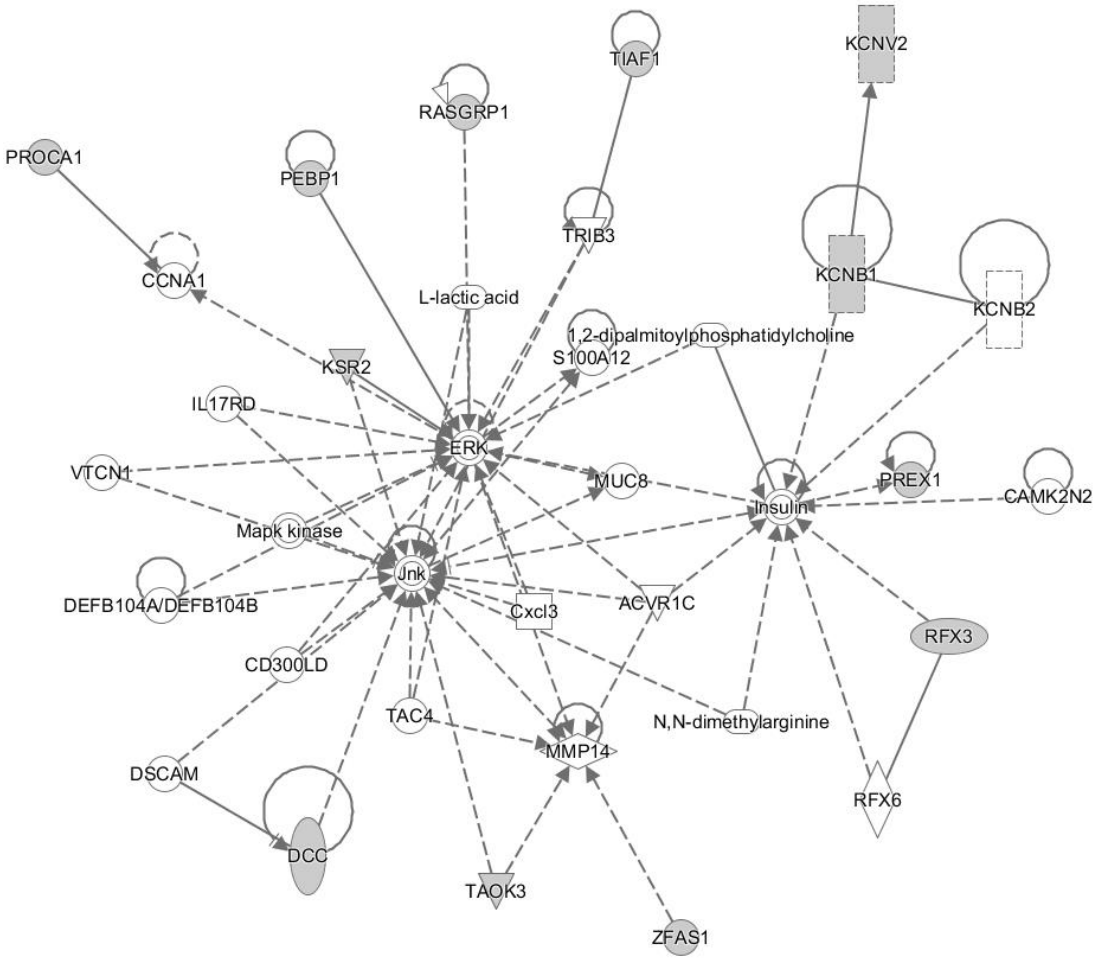
Upstream Regulator	**Molecule type**	**P-value of overlap**	**Target molecules in dataset**	**Pathway for the regulator**
KCNA1	ion channel	9.31X10-4	KCNB1	Voltage-gated potassium channel (function)
leucine-2-alanine enkephalin	chemical drug	2.79X10-3	KCNB1	opioid peptide (selective delta opioid receptor agonist) with analgesic properties
rubitecan	chemical drug	3.72X10-3	PEBP1	A semisynthetic agent with antitumor and antiviral properties

CAMKK2	kinase	7.43X10-3	KCNB1	AMPK Signaling; Calcium Signaling; Dopamine-DARPP32 Feedback in cAMP Signaling
EGR2	transcription regulator	8.69X10-3	FLOT2, TAOK1	Adipogenesis pathway
DAB1	other	8.35X10-3	DCC	Reelin Signaling in Neurons

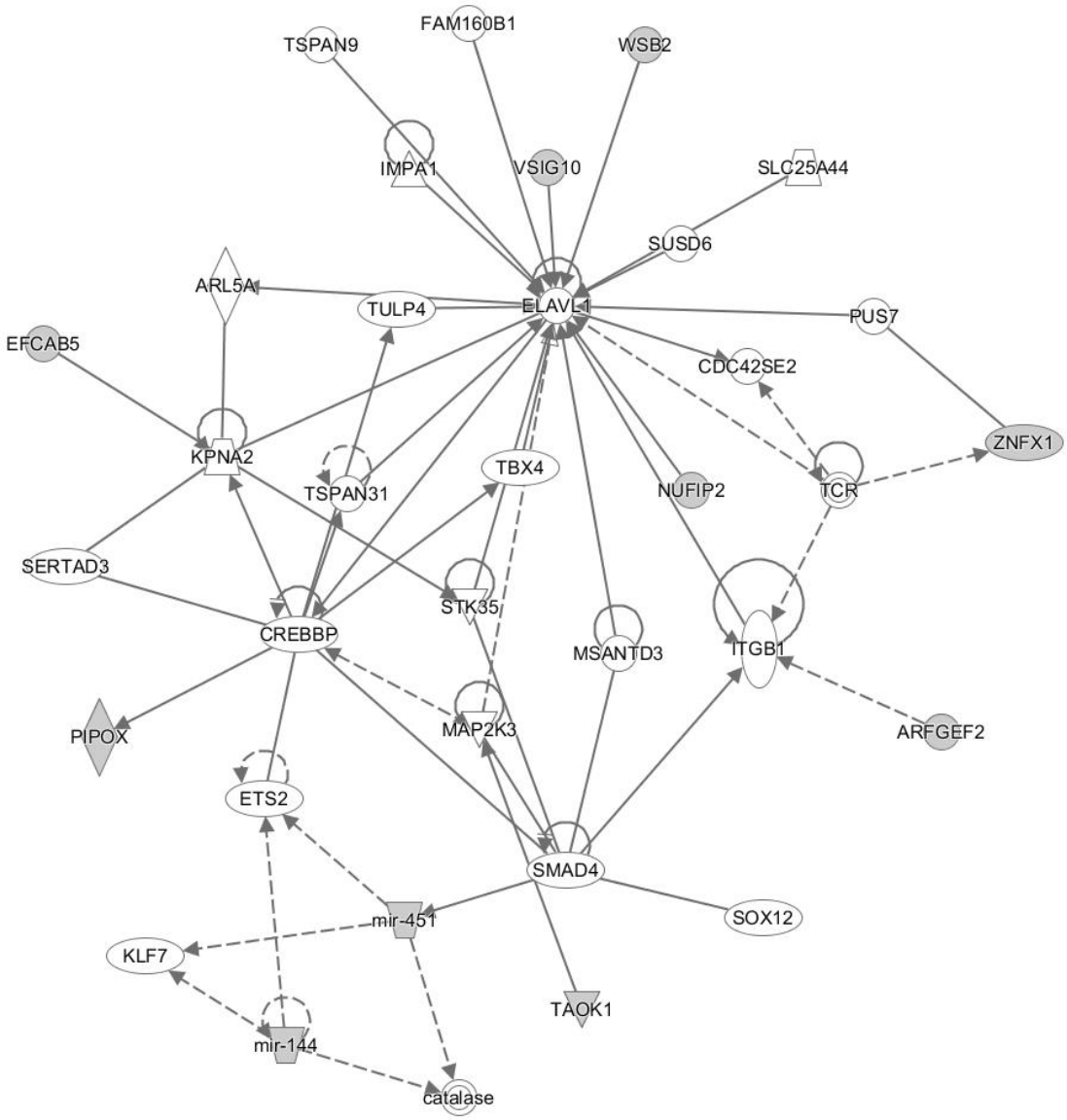
Legend: [a]P-values were adjusted by Benjamini & Hochberg (BH) method[40].The p-value of overlap was determined at BH adjusted p-value ≤0.01.

Figure 4: Top IPA® functional network of genes associated with lithium response in BIP and MDD/depressive symptoms.

A) Network-1



B) Network-2


FAM160B1
TSPAN9
WSB2
IMPA1
VSIG10
SLC25A44
SUSD6
ARL5A
TULP4
ELAVL1
PUS7
EFCAB5
CDC42SE2
ZNFX1
KPNA2
TBX4
TSPAN31
NUFIP2
TCR
SERTAD3
STK35
CREBBP
MSANTD3
ITGB1
PIPOX
MAP2K3
ARFGEF2
ETS2
SMAD4
SOX12
mir-451
KLF7
TAOK1
mir-144
catalase

C) Network-3

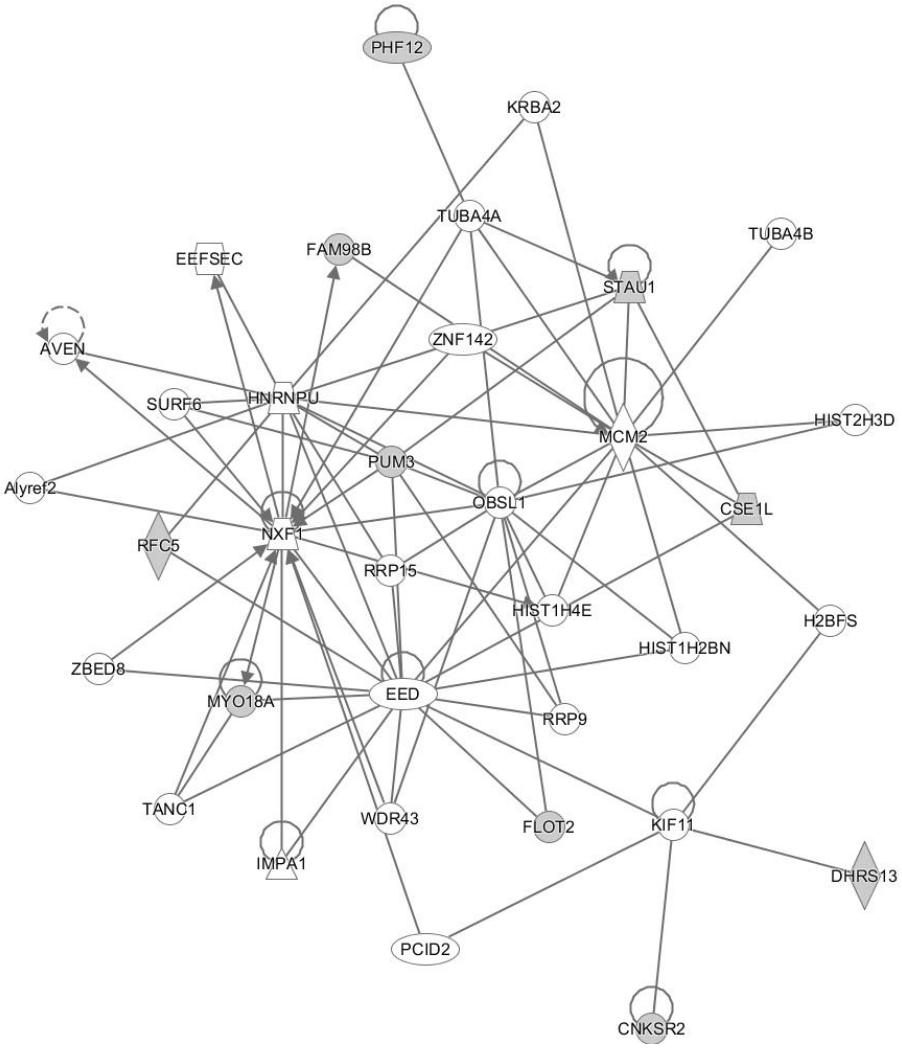


Figure 4 legend: IPA generates the network using a proprietary algorithm, and included genes that could contribute to the network, even if they were not contained in the original dataset.

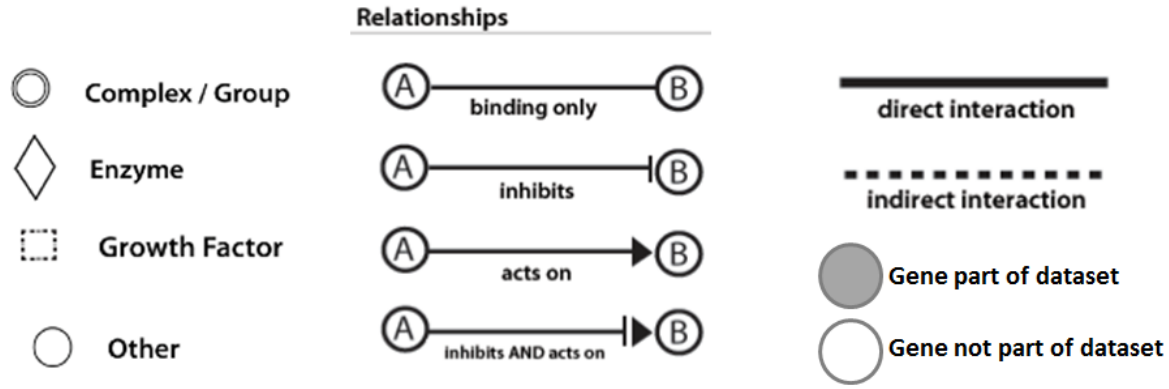


Table 5: Molecules within the three-top significant functional networks

Molecules in Network	Score	Focus Molecules
1, 2-dipalmitoylphosphatidylcholine, ACVR1C, CAMK2N2, CCNA1, CD300LD, Cxcl3, DCC, DEFB104A/DEFB104B, DSCAM, ERK, IL17RD, Insulin, Jnk, KCNB1**, KCNB2,** KCNV2**,** KSR2**, L-lactic acid, Mapk kinase, MMP14, MUC8, N, N-dimethylarginine,** PEBP1, PREX1, PROCA1, RASGRP1, RFX3, **RFX6, S100A12, TAC4,** TAOK3, TIAF1**, TRIB3, VTCN1,** ZFAS1	27	12
ARFGEF2**, ARL5A, catalase, CDC42SE2, CREBBP,** EFCAB5**, ELAVL1, ETS2, FAM160B1, IMPA1, ITGB1, KLF7, KPNA2, MAP2K3,** mir-144, mir-451**, MSANTD3,** NUFIP2, PIPOX**, PUS7, SERTAD3, SLC25A44, SMAD4, SOX12, STK35, SUSD6,** TAOK1**, TBX4, TCR, TSPAN9, TSPAN31, TULP4,** VSIG10**,** WSB2, ZNFX1	21	10
Alyref2, AVEN, CNKSR2**,** CSE1L, DHRS13**, EED, EEFSEC,** FAM98B, FLOT2**, H2BFS, HIST1H2BN, HIST1H4E, HIST2H3D, HNRNPU, IMPA1, KIF11, KRBA2, MCM2,** MYO18A**, NXF1, OBSL1, PCID2,** PHF12, PUM3, RFC5**, RRP9, RRP15,** STAU1, **SURF6, TANC1, TUBA4A, TUBA4B, WDR43, ZBED8, ZNF142**	21	10

Legend: The molecules represented in **bold** are derived from the cross-trait meta-GWAS (Table 1) and post-GWAS analysis. The p-score is calculated by IPA, and estimates, the probability of finding twelve (group 1), ten (group 2) or ten (group 3) more focus molecules in a network of 39 molecules randomly selected from IPA's Global Molecular Network. The p-score = −log10 (p-value); the p-value is calculated by Fisher's exact test.

DISCUSSION

Our study represents the first direct evidence of an association between a genetic predisposition for depression and poorer response to lithium treatment in patients with BIP. Using PGS analyses of genetic variants related to MDD and DS, we found that BIP patients with high genetic loading for these variants were about 1.6 times less likely to have favourable long-term outcomes following lithium treatment compared to BIP patients with very low MDD/DS loading. Analysis of diagnostic subgroups revealed that these associations are driven by patients with BIP type 1, but not BIP type 2, in our cohort. To explore which genes might functionally drive these effects, we carried out a cross-trait meta-analysis of lithium response and MDD/DS. Pathway analyses of variants associated with both traits implicated insulin regulation, the ERK and JNK signalling pathways, and the micro RNAs miR-144 and miR-451.

Our findings could form part of a genetic explanation for previous clinical observations in relation to depression and lithium response in BIP. Lithium's polarity index is higher than 1, meaning that it is more effective preventing manic than depressive episodes[41,42]. Hence, lithium responders may more likely belong to the subgroup of patients with manic predominant polarity, as opposed to depressive predominant polarity[43]. For example, Kessing et al. (2011) reported that excellent lithium responders were characterized by a manic but not depressive polarity of the index episode[44]. Kleindienst et al. (2005) described an episodic illness pattern of 'mania-depression-interval' as a predictor for good response, whereas a 'depression-mania-interval' predicted poorer outcomes[45]. Inter-episode residual mood symptoms, as opposed to full remission[7,8,46], a rapid cycling pattern[45,46], and a history of mixed episodes[47,48] have also been

described as predictors of poor response. Together, these studies have raised the notion that lithium responsiveness could be associated with a 'core' bipolar phenotype in the *Kraepelinian* form of manic depression[48,49]. The current findings, together with our previous finding of an inverse association of lithium response and schizophrenia PGS in BIP[50], provide genetic support to this concept.

In our cohort, BIP type 2 patients differed from type 1 patients with regards to the depression PGS on lithium response association. A non-significant trend for *improved* lithium treatment response was found in type 2 patients with high MDD PGS. Genetically, there are differences between type 1 and type 2 cohorts [51], and type 2 patients show substantially higher genetic overlap with MDD[52]. Lithium's effectiveness as an adjunct antidepressant treatment for people with treatment-resistant MDD is well established[53-59], and our finding raises the intriguing possibility of MDD-specific mechanisms of action, which are different from the mechanisms underlying the more 'anti-manic' response in BIP type 1.

Our cross-trait GWAS meta-analysis yielded 7 loci that exceeded a genome-wide significance level of 5×10^{-8}. Amongst the nearby genes of these loci, the *DCC* gene and its encoded netrin 1 receptor has previously been shown to play an important role in mediating axonal growth in developing human brain[60,61]. Genetic variation within the *DCC* gene has previously been shown to be associated with depressive symptoms[27].

Further functional characterization by IPA® suggested that genes regulating insulin homeostasis could be important mediators of the MDD-lithium relationship (Figure 4). These genes included regulating factor X3 (RFX3), Phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger

1 (PREX 1), and potassium voltage-gated channel subfamily B member 1(KCNB1). Interestingly, previous clinical studies have shown that BIP patients with impaired glucose tolerance or diabetes mellitus type 2 are over 8-times less likely to benefit from lithium and have an overall less favourable illness course [18].

Functionally, the genes *RFX3*, *PREX1*, and *KCNB1* are involved in insulin regulation in various ways. The transcription factor RFX3 is required for the differentiation and function of insulin-producing, mature pancreatic beta cells, and regulates the beta-cell promotor of the glucokinase gene[62]. Interestingly, *RFX3* variants were also implicated in a recent GWAS examining sleeplessness/insomnia[63], a condition with aetiological relationships to BIP[64], and depression[65]. Variants of the PREX1 gene on chromosome 20q12-13.1were associated with increased risk of diabetes mellitus type 2 and increased BMI in a cohort of European Americans[66], through mechanisms are yet insufficiently understood[67]. Variants of the KCNB1 gene in humans are associated with increases of waist to hip ratio, fasting insulin, and triglycerides, as well as decreased insulin sensitivity[68,69]. Mechanistically, the KCNB1-encoded Kv2.1 and other voltage-gated potassium channels (Kv's) important for the fine-tuning of the release of cellular insulin and other hormones or neurotransmitters, and have both inhibitory (through re-polarization of the membrane potential[70]) and stimulating (through interaction with the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex)[71-74] effects on exocytotic mechanisms. Interestingly, a previous study in rats suggested that treatment with lithium directly stimulates the expression of SNARE protein in brain tissue[75].

Genetic variations in genes regulating ERK and JNK expression were identified as additional contributors to the effects of MDD PGS on lithium response. Belonging to a family of protein kinases in the mitogen-activated protein kinases (MAPK) pathway, these molecules are highly interactive with insulin-signaling mechanisms. Interestingly, previous evidence has indicated that MAPK and insulin signaling could be activated by lithium, to enhance insulin-stimulated glucose transport and glycogen synthesis[76]. Additionally, lithium is known to stimulate MAPK mediated neurite growth, neuronal survival, and neurogenesis[77], and to regulate circadian rhythms[78]. Therefore, it is possible that variation in MAPK-associated genes interferes with these potentially therapeutic effects of lithium.

MicroRNAs (miRNAs) regulate messenger RNA (mRNA) translation in a sequence-specific manner and are emerging as critical regulators of central nervous system plasticity. We found that genetic effects on miR-144 and miR-451 expression could play a role in mediating lithium response in BIP. Previous animal studies have shown that lithium treatment in vivo induces changes in miRNA expression, specifically miR-144[79]. It is possible that variations of miNA genes lower their contribution to lithium's therapeutic mechanisms.

The main limitation of our study is that PGSs for MDD and DS explain only a small proportion of the variance in lithium treatment response (about 2%), and have on their own no utility as clinical tests. Our cross-trait analyses provides a clue for a potential genetic overlap; howevere, no formal pleiotropy analyses was employed to confidently conclude about the effect of each genetic variant on the phenotypes tested. In addition, our pathway analysis findings have not been validated with experimental procedures in cellular models. The centrality of insulin-

associated pathways in our findings could be a result of high representation of these genes within curated tools such as IPA®. However, these tools are powerful for hypothesis generation and indicate plausible molecular targets to be tested. Since our sample size already detected significant effects, it is likely that in the future, an increased sample size will further improve the predictive power of PGSs[80].

In conclusion, we demonstrated that high genetic loadings for MDD and DS are predictive of unfavourable long-term response to lithium in patients with BIP type 1, but not type 2. Our study underscores the potential of PGS analysis to contribute to predictive models for medication response in psychiatry, and to uncover novel molecular pathways that drive these effects. While our findings, in isolation, are not yet ripe for clinical applications, they could serve as a component of multimodal predication models incorporating clinical and other biological data. The results of our study have implications for the conceptualization of BIP and MDD as diagnostic entities, suggesting that a distinct biotype of lithium responsiveness exists within the bipolar spectrum.

Conflict of interest

All authors declare that they have no competing interests.

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Chapter 6

Association of the polygenic scores for personality traits and response to SSRIs in patients with major depressive disorder

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ABSTRACT

Studies report a strong genetic correlation between the Big Five personality traits and major depressive disorder (MDD). Moreover, personality traits are thought to be associated with response to antidepressant treatment that might partly be mediated by genetic factors. In this study, we examined whether polygenic scores (PGSs) derived from the Big Five personality traits predict treatment response and remission in patients with MDD who were prescribed selective serotonin reuptake inhibitors (SSRIs). In addition, we performed meta-analyses of GWASs on these traits to identify genetic variants underpinning the cross-trait polygenic association.

The PGS analysis was performed using data from two cohorts: the Pharmacogenomics Research Network Antidepressant Medication Pharmacogenomic Study (PGRN-AMPS, n=529) and the International SSRI Pharmacogenomics Consortium (ISPC, n=865). The cross-trait GWAS meta-analyses were conducted by combining GWAS summary statistics on SSRIs treatment outcome and on the personality traits.

Results showed that the PGS for openness and neuroticism were associated with SSRIs treatment outcomes at $p< 0.05$ across P_T thresholds in both cohorts. A significant association was also found between the PGS for conscientiousness and SSRIs treatment response in the PGRN-AMPS sample. In the cross-trait GWAS meta-analyses, we identified eight loci associated with a) SSRIs response and conscientiousness near *YEATS4* gene, and b) SSRI remission and neuroticism 7-loci near *PRAG1*, *MSRA*, *XKR6*, *ELAVL2*, *PLXNC1*, *PLEKHM1* and *BRUNOL4* genes.

An assessment of a polygenic load for personality traits may assist in conjunction with clinical data to predict whether MDD patients might respond favorably to SSRIs.

Keywords: Pharmacogenomics, polygenic score, personality traits, major depression, antidepressants, SSRIs

INTRODUCTION

Major depressive disorder (MDD) is the most common and disabling mental health disease worldwide[1,2] with a lifetime prevalence of ~12%[3]. Studies estimated a 61.6 million years of life lived with disability caused by MDD accounting for 2.5% of the total disability-adjusted life years (DALY) and for 8.1% of the total years lived with disability (YLDs) resulted from all diseases[2,4].

Selective serotonin reuptake inhibitors (SSRIs) are commonly used as the first-line pharmacological treatment for MDD[5]. However, treatment efficacy with SSRIs varies widely among individual patients and is inadequate in many cases. Clinical response rates range from 48% to 64%[6,7] and reported remission rates are as low as 23.5%[7,8]. To improve this situation, an investigation of the biological and psychosocial factors that drive heterogeneity in treatment outcomes is necessary.

There is growing evidence from genetic studies that antidepressant treatment response is substantially influenced by genes[7,9-17]. A study involving nearly 3,000 MDD patients estimated that genetic factors explain 42% of the differences in the level of treatment response[18]. A number of genes and single nucleotide polymorphisms (SNPs) that could influence antidepressant treatment outcomes have been reported, including polymorphisms within the *COMT*[9], *HTR2A*[10], *HTR1A*[11], *CNR1*[11], *SLC6A4*[12], *NPY*[13], *MAOA*[14] and *IL1B*[15] genes. A pharmacogenomic study on SSRIs response by the International SSRIs Pharmacogenomics Consortium (ISPC) identified several SNPs with a suggestive association after four weeks of treatment, including the

neuregulin-1 gene, which is involved in many aspects of brain development, such as neuronal maturation[7].

In addition to genetic factors, multiple demographic, clinical and psychological predictors of SSRI response in MDD have been identified, collectively explaining 5-15% of variance in treatment outcomes[19-23]. Among psychological predictors, personality traits defined by the Five Factor Model of Personality (Big Five: extraversion, agreeableness, conscientiousness, neuroticism, openness)[24] have previously been reported to influence antidepressant treatment response and remission[25-29]. Of these, neuroticism is a frequently reported predisposing factor for depression and was shown to negatively affect antidepressants treatment response[30,31]. In a recent study, MDD patients resistant to antidepressants were more likely to report high clinical scores for neuroticism, but low scores for openness, conscientiousness and extraversion[26]. In a large study of patients with MDD (n = 8,229), pre-existing personality dysfunction was associated with poor response to antidepressants[27]. Further, some studies have suggested that SSRIs have a direct positive impact on scores for neuroticism or extraversion in MDD patients, and that part of the antidepressant effect might be explained through these adjustments[28,29,32,33]. Moreover, shared genes are thought to play a key role in the association between personality factors and MDD[34]. For example, studies have estimated the genetic correlation between MDD and neuroticism at 55% to 75%[35,36]. However, no previous work has directly addressed the question whether there is a genetic relationship between the Big Five personality traits and SSRI treatment response and remission in MDD.

It has been shown that the genetic architecture of personality traits is highly polygenic, in which several genes of small effect contribute to the overall phenotype[35,37]. Thus, a polygenic score (PGS) analysis approach proposed by the schizophrenia consortium[38], and later applied in several studies[16,39], is potentially powerful to investigate the genetic influence of each of the Big Five personality traits on antidepressant treatment outcomes. A PGS for each of the Big Five personality traits quantifies the combined effects of genetic variants across the whole genome, computed as a weighted summation of effect sizes obtained from GWASs. A successful multi-trait polygenic model may assist early screening of disease risk, clinical diagnosis and prediction of treatment response and prognosis[38,39].

Implicitly, one could also interpret a polygenic association as a biological relationship partly explained by the role of shared genes and common molecular mechanisms. With this in mind, we conducted GWAS meta-analyses by combining GWAS summary statistics on the Big Five personality traits and SSRIs treatment outcome to identify shared genes involved in the cross-trait association.

METHODS AND MATERIALS

The characteristics of clinical and genetic data, as well as sources of the GWAS summary statistics used in our analysis, are described below.

Study samples

Pharmacogenomics Research Network Antidepressant Medication Pharmacogenomic Study (PGRN-AMPS)

The PGRN-AMPS is a clinical trial on the response to escitalopram or citalopram of 529 MDD patients over eight weeks of treatment. The baseline and follow-up assessment of depression severity were performed using the 16-item Quick Inventory of Depressive Symptomatology (QIDS-C16)[40].

International SSRI Pharmacogenomics Consortium (ISPC) study

The ISPC is an international consortium established to discover genes that are responsible for SSRIs treatment response in patients with MDD. For our study, we used data from 865 MDD patients recruited in the USA, Germany, Thailand, Taiwan and Japan who received SSRI treatment. The 17-item Hamilton Depression Rating Scale was used as a measurement tool to assess and follow-up treatment progress[7].

Genotyping and quality control

The genotype and clinical data for the PGRN-AMPS were available via a controlled access system at the database of Genotypes and Phenotypes: dbGaP; http://www.ncbi.nlm.nih.gov/gap and ISPC data were obtained from the ISPC consortium[7].

For genotype data of both samples, we implemented quality control (QC) steps using PLINK [41] and samples with low genotype rates <95%, sex inconsistencies (X-chromosome heterozygosity), and genetically related individuals were excluded. We also excluded SNPs that

had poor genotyping rate <95%, an ambiguity (A/T and C/G SNPs), a minor allele frequency (MAF≤1%) or showed deviation from Hardy-Weinberg Equilibrium ($p<10^{-6}$).

Imputations

Genotype data passing QC criteria were imputed in the Michigan server https://imputationserver.sph.umich.edu[42], separately for each study samples using 1000 Genomes project reference panel.

After excluding low-frequency SNPs (MAF<10%) and poor-quality variants (imputation INFO < 0.9) and indels, the imputed dosages were converted to best guess genotypes. The subsequent PGS analyses were performed using the best guess genotypes.

GWAS summary statistics data

The PGSs were calculated using the approach previously described by the International Schizophrenia Consortium[43]. This method requires an estimated effect size for each SNP to compute a weighted PGS. The effect estimates (betas) for this study were the summary statistics obtained from previously published GWASs on extraversion, openness, agreeableness, conscientiousness[37] and neuroticism[35]. The data were publicly available for download at http://www.tweelingenregister.org/GPC/ and http://www.thessgac.org/data, respectively. The effect size estimates for each SNP — quantified as beta was extracted from the download file and used to compute weighted PGS in the PGRN-AMPS and ISPC cohorts.

Definition of SSRI treatment outcomes

Treatment response and remission to SSRIs were defined after four weeks of treatment follow-up of MDD patients in both cohorts. In addition, PGS associations were evaluated at eight weeks in PGRN-AMPS. While treatment response was determined as a $\geq$50% reduction from baseline in the HRSD-17 or QIDS-C16 total scores, SSRI treatment remission was defined as achieving a HRSD-17 score $\leq$7 or a QIDS-C16 score $\leq$5 at four or eight weeks of treatment.

Data on the covariates — age, gender and type of SSRI medication, were also collected and details can be found in earlier publications[7,40,44].

Statistical Analyses

Polygenic score computation and association analyses

The PGSs were computed for each of the Big Five personality traits using imputed genetic data weighted by GWAS summary statistics of the respective personality traits, separately for the two cohorts: PGRN-AMPS (n=529) and ISPC (n=865) (Table 1 and Figure 1). First, quality-controlled SNPs were clumped for linkage disequilibrium (LD) using genome-wide association p-value informed clumping with $r^2 = 0.1$ in a 250-kb window to create an independent SNP-set using PLINK software run on Linux. Next, weighted PGSs were calculated for each individual at a given p-value threshold (P_T) as a weighted sum of allele dosages (0, 1 or 2). The weighting was performed by multiplying the dosage of each effect increasing allele by its effect size derived from the GWAS summary statistics (β- coefficient), then divided by the total number of SNPs in each threshold. The PGS was computed at a range of p-value thresholds ($<1x10^{-2}$, $<5x10^{-2}$, <0.1,

<0.2, <0.3, <0.4, <0.5, <1.0) separately for each of the two cohorts. Logistic regression modeling was carried out adjusting for common covariates such as age, sex and cohort-specific covariates including four principal components (PCs) in the PGRN-AMPS and 'study sites' in the ISPC. A statistically significant association was determined at $p < 0.05$ across the P_T in both study samples. Prediction accuracy, percentage of variance explained, Nagelkerke R^2, by the PGSs were calculated as the Nagelkerke R^2 of the full model with PGS and covariates minus the Nagelkerke R^2 of the model with only covariates. To determine the effect of high or low polygenic load on treatment outcomes, we grouped study subjects into PGS quartiles (Q_1-Q_4) at the most significant GWAS p-value thresholds. Then, we estimated the odds of treatment response/remission status in groups of MDD patients with high polygenic load (Q_2 to Q_4) compared to patients in the lowest PGS quartile (Q_1).

Cross-trait meta-analyses of genome-wide association studies

In cross-trait meta-analyses, we applied the O'Brien's (OB) method and the direct Linear Combination of dependent test statistics (dLC) approach[45-47], which are implemented in the C^{++} eLX package. Briefly, the OB method and the dLC approach help to combine GWAS effect estimates of genome-wide SNPs, obtained from univariate GWASs and generated two test statistics and associated p-values — one for the OB method and one for the dLC method. More details can be found elsewhere[45,46]. The eLX package is available at https://sites.google.com/site/multivariateyihsianghsu/.

Here, GWAS on personality traits that have shown a significant association in the PGS analysis were combined with GWAS on SSRIs treatment outcome. The GWAS summary statistics on

SSRIs treatment response[7] were combined with those on i) conscientiousness[34] and ii) openness personality[34]. Similarly, the GWAS summary statistics on SSRIs treatment remission[7] was meta-analysed with i) openness personality[34] and iv) neuroticism[35].

Statistical significance was determined based on the smaller of OB or dLC p-values. A significant association was determined if (1) p-value for cross-trait meta-analysis reached genome-wide significance ($p < 5\times10^{-8}$) and (2) univariate GWAS effects were at least nominally significant ($p< 0.05$). For each cross-trait meta-analysis, only one lead SNP per locus was reported. Nearby SNPs in LD ($r^2>0.1$) with the lead SNP were considered dependent and belonging to the same locus.

RESULTS

Patient characteristics and treatment outcomes

In this study, we analysed data from 1,394 MDD patients who had SSRI treatment divided into PGRN-AMPS (n=529) and ISPC (n=865) samples. The average age of the patients was 42.2 years and the majority (64.3%) were female (Table 1).

Of all patients, 622 (46.8%) were classified as treatment responders with slight variation across the study samples, 44.4% in the PGRN-AMPS and 48.1% in the ISPC. Remission rates were 27.6% and 26.1% in the PGRN-AMPS and ISPC samples, respectively. The rate of remission combined across the two studies was 26.7% (Table 1).

Table 1: Baseline characteristics of MDD patients and their treatment outcomes with selective serotonin reuptake inhibitors after four weeks of follow-up

Patient characteristics	PGRN-AMPS N=529	ISPC N=865	Total N=1,394
Responders, N (%)	206 (44.4)	416 (48.1)	622 (46.8)
Remitters, N (%)	128 (27.6)	226 (26.1)	354 (26.7)
Age, mean (s.d)	39.6 (13.7)	43.7 (14.7)	42.2 (14.5)
Sex, female, N (%)	335 (63.3)	561 (64.9)	896 (64.3)

PGRN-AMPS: Pharmacogenomics Research Network Antidepressant Medication Pharmacogenomics Study; ISPC: International SSRI Pharmacogenomics Consortium study.

Association of PGS for the Big Five personality traits with SSRIs treatment outcomes

PGSs were computed for each of the Big Five personality traits, and we investigated their association with two SSRI treatment outcomes — response and remission, after four weeks (PGRN-AMPS and ISPC) and eight weeks (PGRN-AMPS) of treatment.

After four weeks of treatment, genetic predisposition to openness, conscientiousness and neuroticism were associated with SSRIs treatment response and remission at ($p<0.05$) across PT thresholds, in at least one of the two assessed cohorts (Figures 1A-1C). Genetic loading for openness was associated with response and remission in both cohorts (Figures 1A1 and 1A2). An elevated PGS for conscientiousness was associated with treatment response, but not remission, in the PGRN-AMPS sample only (Figure 1B). A PGS association for neuroticism with remission, but not treatment response, was shown in both cohorts (Figure 1C). The PGSs for extraversion and agreeableness were associated with neither response nor remission.

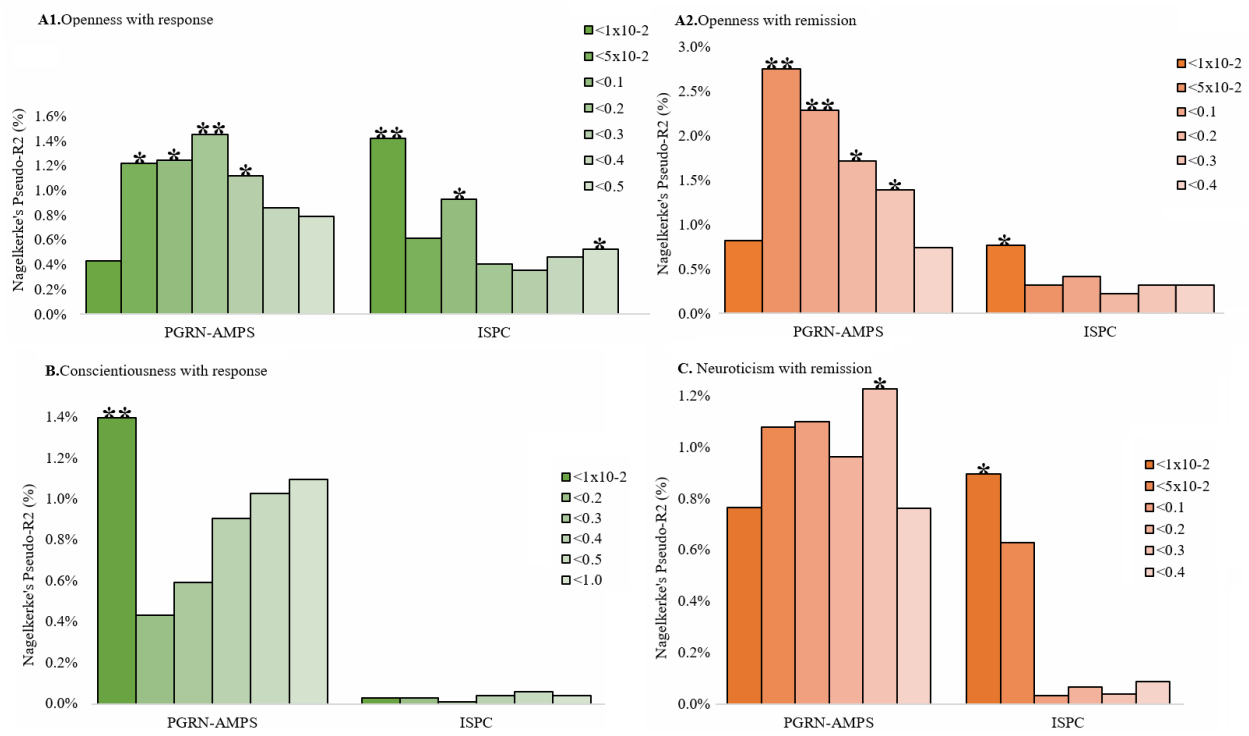

Figure 1: Bar graphs (A-C) show association of PGSs for the Big Five personality traits with SSRIs response or remission at different p-value thresholds (P_T) after four weeks of treatment in PGRN-AMPS (n=529) and ISPC (n=865) samples.

Figure 1 legends: Y-axis (Nagelkerke's pseudo-R^2) refers to percentage of variance in SSRIs treatment response/remission accounted for the PGSs of the Big Five personality traits at a particular P_T in each sample. On the x-axis, plotted from left to right, are the GWAS P_T for personality traits used to group SNPs for the PGSs. The *sign on the top of each bar signifies statistical significance of the PGS association as *p < 0.05, **p < 0.01, ***p < 0.001.

Abbreviations: PGRN-AMPS: Pharmacogenomics Research Network Antidepressant Medication Pharmacogenomic Study; ISPC: International SSRI Pharmacogenomics Consortium study; SNP: single nucleotide polymorphism; PGS: polygenic score; SSRIs: selective serotonin reuptake inhibitors.

We also assessed the level of observed variation in SSRI treatment outcomes accounted for by the Big Five personality traits, and found that personality traits at the most significant thresholds explained a considerable amount of variance in treatment outcomes. For example, the PGS for openness accounted for ~1.5% of observed variation in SSRIs treatment response and ~2.8% of variance in remission. The PGS for neuroticism explained ~1.5% of variance in remission and the PGS for conscientiousness contributed to ~1.5% of variability in SSRI treatment response.

The status of treatment response and remission for patients in personality trait quartiles Q_2 to Q_4 was compared with those in the lowest personality trait PGS quartile (Q_1) (Figure 2). Our analysis revealed that MDD patients with a high polygenic load for openness personality had initially poorer remission and response rates at four weeks of treatment, with Q_4 versus Q_1 odds ratios ranging from 0.30 (ISPC: 95%CI, 0.15-0.59) to 0.52 (PGRN-AMPS: 95%CI, 0.29-0.90) (Figures 2A1 and 2A2, green and brown graphs). After longer treatment duration, we observed a reverse effect. Here, a higher polygenic load for openness was associated with a better SSRI treatment response at eight weeks in the PGRN-AMPS, with an odds ratio of 1.58 (95%CI, 1.10-2.90) (Figures 2A1 and 2A2, blue graphs).

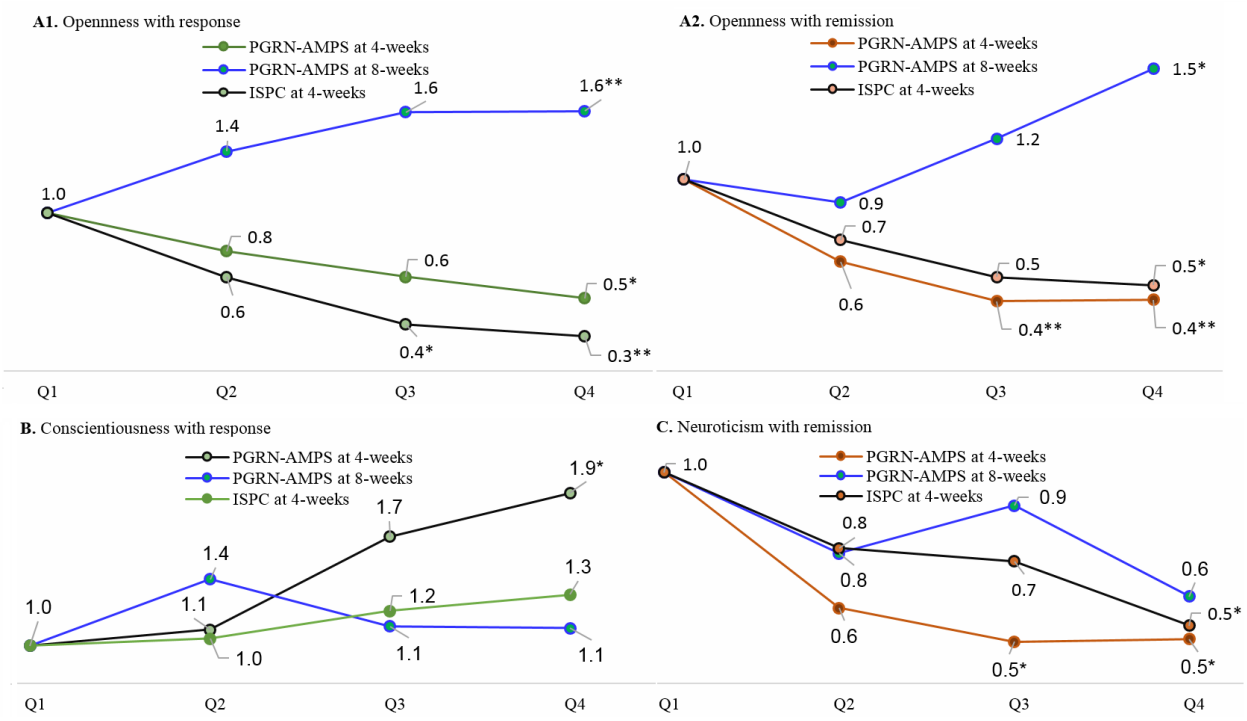

Figure 2: Line plots represent odds ratios (ORs) for favorable SSRI treatment response or remission in patients with MDD with a high personality polygenic load (Q_2, Q_3 and Q_4), compared to patients with lowest polygenic load (Q_1), estimated at the most significant p-value thresholds.

Figure 2 legends: Quartile based PGS analyses were performed using data at four weeks in the ISPC and at four and eight weeks in the PGRN-AMPS. The ORs are reported on the lines and the *sign indicates statistical significance of ORs as *p < 0.05, **p < 0.01, ***p < 0.001.

Abbreviations: PGRN-AMPS: Pharmacogenomics Research Network Antidepressant Medication Pharmacogenomic Study; ISPC: International SSRI Pharmacogenomics Consortium study. OR: odds ratio; Q_1: quartile 1; Q_2: quartile 2; Q_3: quartile 3; Q_4: quartile 4; MDD: major depressive disorder.

MDD patients with a higher polygenic load for conscientiousness personality had 1.95 (95%CI, 1.13-3.36) times better SSRI treatment response compared to patients in the lowest PGS, although this association was only significant in the PGRN-AMPS sample at four weeks of treatment (Figure 2B).

Conversely, MDD patients with a higher polygenic load for neuroticism personality had poorer treatment outcomes with SSRIs. After four weeks of treatment, patients in Q_4 based on the PGS for neurotic personality had about 50% lower odds of remission compared to patients in Q_1 with OR ranging from 0.50 (PGRN-AMPS: 95% CI, 0.28-0.90) to 0.54 (ISPC: 95% CI, 0.33-0.89) (Figure 2C). Constantly, results after eight weeks of treatment showed a trend inverse association between the PGS for neurotic personality and SSRIs treatment remission, although this was not statistically significant (Figure 2C).

To reduce the effect of false positive findings, the association p-values were corrected for multiple testing at each P_T for SSRI treatment response and remission using the Benjamini-Hochberg (BH) method. Each p-value was adjusted assuming a conventionally accepted level of 5% false discovery rate (FDR)[48]. After FDR adjustment, associations of the PGS for openness personality with SSRI treatment response remained statistically significant (in the ISPC sample: FDR adjusted p-value=0.02 at $P_T<1x10^{-2}$) and with remission (in the PGRN-AMPS sample: FDR adjusted p-value=0.04 at $P_T<5x10^{-2}$). The PGSs for conscientiousness and neuroticism were not associated with SSRI treatment outcome after implementing the FDR adjusted p-value<0.05.

Cross-trait meta-analyses of genome-wide association studies

For personality traits that showed a significantly associated PGS, cross-trait GWAS meta-analyses were performed by combining summary GWAS data on SSRI treatment outcomes and personality traits. Table 2 and Figure 3 summarise the cross-trait meta-analyses findings, including the list of genetic loci and nearest genes that are potentially overlapping between the traits. At a p-value of $<5x10^{-8}$, we identified eight genetic loci located within or near to protein-coding genes with possible overlapping effects on SSRI treatment outcomes and personality traits. We found: i) one locus associated with conscientiousness and SSRI response near the *YEATS4* gene (Table 2 and Figure 3A), ii) seven loci associated with remission and neuroticism located at or near *PRAG1*, *MSRA*, *XKR6*, *ELAVL2*, *PLXNC1*, *PLEKHM1* and *BRUNOL4* genes (Table 2 and Figure 3B). From the meta-analyses of SSRI treatment outcomes with openness personality, we identified only suggestive evidence at significance $p<1x10^{-6}$ (Table 2).

Table 2: Significant loci resulting from cross-trait meta-analyses of GWASs on SSRI treatment response/remission and GWAS on the Big Five personality traits at univariate GWAS p-value $<5x10^{-2}$ and cross-trait meta-analysis p-value $<5x10^{-8}$.

SNP	**Chr**	**Position Ch37**	**A1**	**A2**	**GWAS p-value for**			**Nearest gene**	**ED**
					SSRIs response (N=865)	**Openness** (N= 260,861)	**Cross-trait**		
rs7555693	1	106838539	A	G	$6.46 x10^{-3}$	$4.49x10^{-5}$	$1.37x10^{-6}$	*PRMT6*	--
rs9321987	6	145030284	A	G	$7.49 x10^{-3}$	$1.06x10^{-5}$	$5.05x10^{-7}$	*UTRN*	--
rs352759	8	15599714	T	A	$6.16 x10^{-4}$	$2.82x10^{-4}$	$5.52x10^{-7}$	*TUSC3*	+-
rs7828021	8	50640014	C	G	$3.68 x10^{-3}$	$2.91x10^{-6}$	$7.43x10^{-8}$	*SNTG1*	--
rs11591827	10	82887882	A	G	$1.87 x10^{-2}$	$4.64x10^{-6}$	$8.70x10^{-7}$	*SH2D4B*	--
rs7189979	16	12630187	C	A	$2.28 x10^{-3}$	$1.66x10^{-5}$	$1.77x10^{-7}$	*SNX29*	+-

						Conscientiousness (N= 260,861)			
rs3825243	12	69750839	A	G	$5.78x10^{-4}$	$1.41x10^{-5}$	$4.04x10^{-8}$	*YEATS4*	--
					SSRIs remission (N=865)	**Neuroticism** (N=170,911)			
rs2979204	8	8298857	T	C	$3.24x10^{-3}$	$5.48x10^{-10}$	$8x10^{-11}$	*PRAG1*	++
rs11990063	8	10165195	T	C	$4.00x10^{-2}$	$6.77x10^{-9}$	$9x10^{-9}$	*MSRA*	--
rs35792458	8	10822431	C	G	$1.00x10^{-2}$	$5.25x10^{-10}$	$1x10^{-12}$	*XKR6*	-+
rs12555870	9	23347724	G	A	$4.00x10^{-2}$	$1.25x10^{-6}$	$1x10^{-8}$	*ELAVL2*	+-
rs4761545	12	94426468	G	T	$2.00x10^{-2}$	$3.54x10^{-7}$	$8x10^{-10}$	*PLXNC1*	++
rs144733372	17	43564222	G	T	$1.00x10^{-2}$	$1.23x10^{-9}$	$3x10^{-11}$	*PLEKHM1*	-+
rs11082011	18	35145122	C	T	$1.00x10^{-2}$	$8.60x10^{-9}$	$4x10^{-8}$	*BRUNOL4*	-+
						Openness (N= 260,861)			
rs55679149	1	89534338	T	C	$2.77x10^{-3}$	$6.31x10^{-5}$	$8.25x10^{-7}$	*GBP1*	+-
rs11728985	4	130036435	T	C	$1.93x10^{-2}$	$1.24x10^{-6}$	$4.03x10^{-7}$	*C4orf33*	-+
rs11155372	6	145019738	T	G	$5.11x10^{-4}$	$5.85x10^{-5}$	$1.28x10^{-7}$	*UTRN*	-+
rs7828021	8	50640014	C	G	$1.23x10^{-2}$	$2.91x10^{-6}$	$4.18x10^{-7}$	*SNTG1*	--
rs1411216	9	24520194	A	G	$7.81x10^{-3}$	$6.88x10^{-6}$	$4.53x10^{-7}$	*CRIPAK*	++
rs7189979	16	12630187	C	A	$2.82x10^{-3}$	$1.66x10^{-5}$	$2.73x10^{-7}$	*SNX29*	+-

A1, effect allele; A2, another allele. ED: Effect direction — represents SNPs effect on SSRI treatment response or remission for the effect allele based on the ISPC GWAS[7] versus its effect on the GWASs of personality traits as listed in the table.

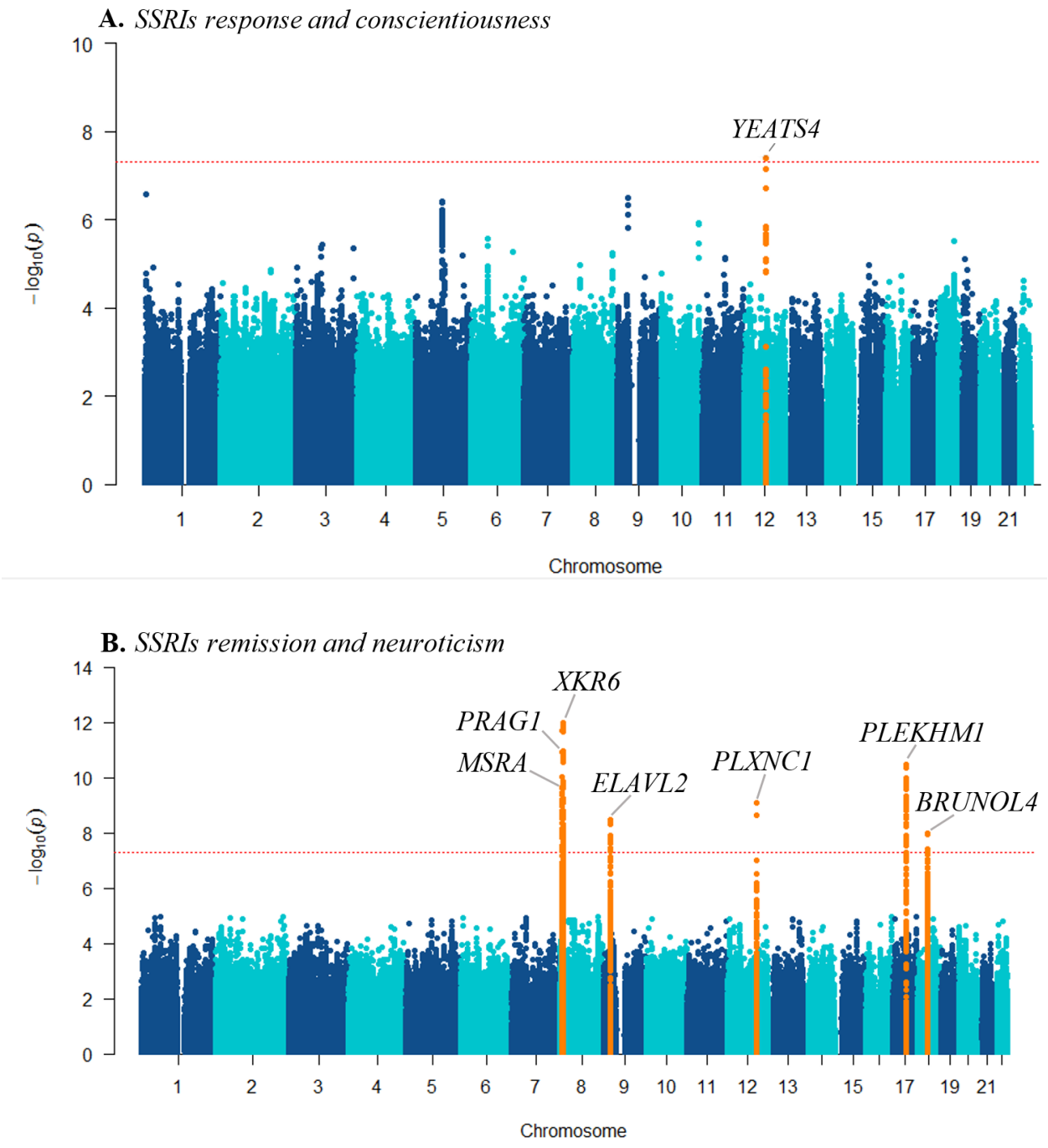

Figure 3: Manhattan plots show results of cross-trait meta-analysis of GWASs on SSRI treatment outcomes (response or remission) with GWASs on: A) Conscientiousness personality

trait; B) Neuroticism personality, highlighting the loci that showed genome-wide significance (orange), and the nearest genes.

Legends: The −log10 (cross-trait p-value) is plotted against the physical position of each SNP on each chromosome. The threshold for genome-wide significance (cross-trait p-value<$5x10^{-8}$) is indicated by the red dotted horizontal line.

DISCUSSION

In this study, we analysed data from 1,394 MDD patients treated with SSRIs and assessed whether it is possible to predict antidepressants treatment outcomes, response and remission, using PGS for the Big Five personality traits. To further validate the PGS association findings and provide additional evidence, cross-trait meta-analyses of GWASs on SSRI treatment outcomes versus GWASs on the Big Five personality traits were performed. Our findings from both analyses found complementary evidence that the association of the Big Five personality traits with SSRIs treatment outcomes is partly genetic.

Among the Big Five personality traits, the PGS for openness, conscientiousness and neuroticism were significantly associated with SSRI treatment outcomes in patients with MDD. A high polygenic load for openness predicted poorer odds of response and remission after four weeks of treatment. However, after eight weeks of treatment, the odds of response and remission was reversed and high loading for openness was associated with favourable outcomes. Patients with a high polygenic load for conscientiousness had a better odds of response to SSRIs after four weeks of treatment, but were neither more nor less likely to have good outcomes after eight

weeks. In contrast, patients who possessed a higher polygenic load for neuroticism risk genetic variants responded poorer to SSRIs treatment at both time points.

The discrepancy between short-term and intermediate-term treatment outcomes in patients with high polygenic loading for openness was unexpected in the context of the previous literature[26,27], and raises the question whether statements about personality impact on SSRI treatment outcomes can be reliably reached on the basis of assessments conducted within the first month. While longitudinal studies of treatment outcomes in MDD suggest that treatment response within the first month occurs for a majority of patients who eventually remit[49], they also indicate that there is a considerable proportion of patients who achieve response and remission after much longer treatment periods[50,51]. In this context, our finding raises the possibility that the different Big Five personality traits could have differential effects on early versus delayed responses to treatment in MDD.

Moreover, inconsistencies in the direction of the relationship between the Big Five personality traits and response to long-term versus short-term treatment to SSRIs might be explained by a psychological theory[52-54]. Studies suggest that antidepressants have a primary effect on emotional processing, providing a platform for long-term cognitive and psychological recovery[52] and the clinical effects of antidepressant treatment may be mediated by early changes in emotional processing[53,54].

In our data, consistency between the outcome parameters, treatment response and remission, was variable. Only the PGS for openness showed a significant association with both treatment response and remission. The PGS for conscientiousness was associated with better treatment

response, but not with remission. The PGS for neuroticism predicted lower odds of treatment remission, but not poorer treatment response. At face value, these findings suggest that openness and neuroticism could play more important roles in predicting ultimate remission from depressive episodes, whereas conscientiousness might drive early treatment effects rather than longer-term outcomes. However, another explanation is that our cohorts might have been underpowered to detect more consistent effects, or that some observed associations were chance findings, perhaps driven by multiple testing. Indeed, only associations of the PGS for openness personality with SSRI treatment response remained statistically significant after FDR adjustment. Therefore, future genetic studies with higher patient numbers are required to confirm our findings.

In all, our genetic findings are in line with previous clinical investigations of the influence of personality characteristics on antidepressant treatment response in MDD. A study in Japan revealed depressed patients resistant to treatment had a higher neuroticism score and lower scores for openness, conscientiousness and extraversion than patients who remitted and healthy controls[26]. In another study, higher clinical scores for openness at baseline were associated with improved treatment response to antidepressants, whereas a higher score for neuroticism was associated with poor treatment outcomes[55]. More generally, poor treatment response was associated with personality dysfunction in a large sample study of more than 8,000 antidepressant-treated adults with MDD[27]. Similarly, a meta-analysis of 34 clinical studies concluded that MDD patients with a comorbid personality disorder had double the risk of overall poor clinical and treatment outcomes, compared to patients no co-occurring personality disorder[56]. Additionally, previous studies have shown genetic correlations between Big Five

personality traits and psychiatric disorders, and the PGS for neuroticism was significantly associated with MDD[57].

Since the PGS association reflects a shared genetic etiology, we applied cross-trait GWAS meta-analyses by combining summary statistics on SSRI treatment outcomes with personality traits, and identified eight overlapping genetic loci. The *YEATS4* gene locus was associated with treatment response to SSRIs and conscientiousness. Previously, a gene expression analysis in depressed patients further replicated in mice found lower levels of *YEATS4* in depressed patients compared to healthy controls. Moreover, expression level of this gene was correlated with the dose of imipramine (a tricyclic antidepressant)[58].

The second gene locus (rs144733372) in *PLEKHM*, which was found in the cross-trait meta-analysis of neuroticism and SSRIs treatment remission, is highly linked (LD: $r^2>0.8$) with several other SNPs located within the *CRHR1* gene. The *CRHR1* gene encodes a G-protein coupled receptor that binds with neuropeptides of the corticotrophin-releasing hormone family, a major regulator of the hypothalamic-pituitary-adrenal pathway[59]. Functional gene polymorphisms in the *CRHR1* gene have been associated with SSRI treatment response[60], and moderate the association of maltreatment with neuroticism[61]. Corticotrophin-releasing hormone signaling has previously been implicated in mood disorders and treatment response to antidepressants[62].

Another gene showing shared associations with SSRI treatment response and neurotic personality is *MSRA*, which has shown the highest levels of expression in brain tissue[63]. Previous studies reported that genetic variants within the *MSRA* gene could be associated with

schizophrenia, bipolar disorder[64,65], executive cognitive function[66], fluid intelligence[65] and self-reported irritable temperament[67].

Further, loci within the *PRAG1* and *PLXNC1* genes have shown overlapping influence on SSRI treatment and neuroticism personality. A genetic polymorphism rs706895C/T within the *FYN* gene belonging to the same family of genes (tyrosine protein kinase family) was significantly associated with personality traits[68]. SNPs within the plexin family gene, *PLXNA2*, have previously been implicated in neuroticism, depression and psychological distress[69].

Overall, these findings lend further weight to our PGS analyses and reinforce the idea that certain gene polymorphisms have a dual impact on personality structure and antidepressant treatment outcomes in MDD. Studying the individual mechanism of each significant genetic locus in relation to antidepressants in future studies might lead to novel insights into the molecular underpinnings of these drugs. In conclusion, our study provides evidence into the potential ability of the PGS for the Big Five personality traits to elucidate shared biological mechanisms and to predict SSRI treatment outcomes. Whether these PGSs could be applied to everyday clinical practice in the future relies on their ability to stratify MDD patients into categories of good treatment responders versus non-responders. Further research is required to determine if this is the case. However, the small effect sizes found in our study give rise to cautious interpretation. In our view, their full clinical value likely lies in their contribution to multi-variable models that also comprise clinical and environmental factors influencing medication response.

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available at http://www.pharmgkb.org/downloads/. The PGRN-AMPS data were obtained through controlled access distributed from the NIH in the dbGaP. http://www.ncbi.nlm.nih.gov/projects/gap/.

The analysis of this study was carried out using the high-performance computational capabilities of the University of Adelaide, Phoenix supercomputer https://www.adelaide.edu.au/phoenix/.

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Overall percentage (%)	85%		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
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Chapter 7

The association of obesity and coronary artery disease genes with response to SSRIs treatment in major depression

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) are first-line antidepressants. However, treatment response during an initial therapeutic trial is often poor and difficult to predict. Heterogeneity of response to SSRIs in depressed patients is partly driven by co-occurring somatic disorders, such as coronary artery disease (CAD) and obesity. In this study, we assessed the association of CAD and obesity with treatment response to SSRIs in patients with MDD using a polygenic score (PGS) approach. We also performed cross-trait meta-analyses to pinpoint genetic variants underpinning the relationship of CAD and obesity with SSRI treatment response.

First, PGSs were calculated at different p-value thresholds (P_T) for obesity measured as body mass index (BMI) and CAD. Next, binary logistic regression was applied to evaluate the association of the PGSs to SSRI treatment response in discovery sample (ISPC, N=865) and replication study (STAR*D, N=1,878) cohorts. Finally, a cross-trait genome wide associated study (GWAS) meta-analysis was performed by combining summary statistics.

Results showed that the PGSs for CAD and obesity were inversely significantly associated with SSRI treatment response at $p<0.05$. In cross-trait meta-analyses, we identified 19 loci associated with SSRIs treatment response and i) obesity 14 loci (top four: *NEGR1*, *CADM2*, *PMAIP1*, *PARK2)* and ii) CAD 5-loci (top four: *PHACTR1*, *CDKN2B*, *ATXN2*, *KCNE2).*

Our findings implicate that genetic variants of CAD and obesity are linked to SSRI antidepressant treatment response. Improved SSRI treatment response might be achieved through stratified allocation of treatment for MDD patients with a genetic risk of obesity/or CAD.

Keywords: Pharmacogenomics, polygenic score, major depression, antidepressants, SSRIs, obesity, body mass index, coronary artery disease, pleiotropy

INTRODUCTION

Major depressive disorder (MDD) and major cardiometabolic disorders (CMDs) contribute to a large proportion of the total disability, morbidity and mortality attributed to non-communicable diseases[1,2]. CMDs, such as coronary artery disease (CAD) and obesity, represent major health issues in patients with MDD[3,4]. In patients with MDD, the co-occurrence of CMDs is high and this co-morbidity is associated with poor health-related quality of life and poorer MDD treatment response. However, the underlying mechanism through which CMDs impact health outcomes of MDD patients remains poorly understood.

Genetic studies suggest that the etiology of MDD and CMDs is contributed by genetic factors, with an estimated heritability of 31-42% in MDD[5], 30-60% in CAD[6] and 40-70% in obesity[7]. Similar studies that aimed to estimate genetic overlap have revealed that depression shares a genetic component of 42% with heart disease[8], 19% with hypertension[9] and 12% with obesity[10]. In our recent systematic review of genome-wide and candidate gene studies, we found 24 shared genes and signaling pathways that could partly account for comorbidity between CMDs and MDD that subsequently may affect treatment response[3], further strengthening the genetic relationship between MDD with CAD and obesity. Because of this biological relationship, the co-morbidity of CAD and obesity with MDD may significantly influence the pharmacological effects of antidepressants.

Selective serotonin reuptake inhibitors (SSRIs) are first-line drugs for treatment of MDD[11]. However, treatment efficacy with SSRIs is often poor, ranging between 48% to 64%[12,13]. Previous research suggests that physical co-morbidities have a potential impact on response to

antidepressants[14-17]. These clinical features are also regarded as mediators of depressive relapses and increased vulnerability to adverse pharmacological effects has been reported in MDD patients with medical co-morbidities who received antidepressant medications[15,17]. Abnormalities in the cardiovascular or endocrine systems of depressed patients have been implicated in negatively influencing the rate of treatment response to antidepressants[15].

Elucidation of the biological mechanisms by which CAD and obesity co-morbidities influence treatment response may provide clues to effectively use medications for MDD patients with physical medical co-morbidities. A technique to examine these biological mechanisms may be an implementation of cross-trait genetic analysis to investigate the molecular drivers (hubs) linking medical co-morbidities and antidepressants treatment response[3]. Because the genetic architecture of CAD and obesity is highly polygenic, the cumulative effect of many genetic variants accounts for their genetic variability. Thus, we sought to determine the aggregated effect of genome-wide single nucleotide polymorphisms (SNPs) for CAD and obesity on SSRI treatment response using a polygenic score (PGS) approach. In this study, we implemented two-step genetic analyses consisting of: a) PGS analyses, and b) cross-disorder GWAS meta-analyses, with the aim to identify shared genes between CAD, obesity and SSRI treatment response in patients with MDD. If replicated, such an approach could shed further light on the genetic factors impacting SSRI treatment response in MDD patients, and the PGS could contribute to development of clinical algorithms for personalised treatment in psychiatry.

METHODS AND MATERIALS

In this study, we assessed whether the PGSs for CAD and obesity predict response to SSRI treatment. The PGS for each trait was constructed using genome-wide genetic data. The

polygenic model uses GWAS summary statistics to construct a weighted PGSs for CAD and obesity. The PGS analysis was initially performed in the International SSRI Pharmacogenomics Consortium (ISPC) study[13], followed by replication analysis in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study[18]. In subsequent analysis, we implemented cross-trait GWAS meta-analyses to identify specific genetic variants with an overlapping role on CAD and obesity and response to SSRIs in patients with MDD.

Study Samples

Discovery study: *International SSRI Pharmacogenomics Consortium (ISPC) study*

The ISPC is an international collaboration of experts established with the aim to discover genetic loci responsible for SSRIs treatment response in patients with MDD[13]. Genotyping was performed at the RIKEN Center for Integrative Medical Sciences (Yokohama, Japan). Baseline and follow-up measurements of depressive symptoms were assessed using the 17-item Hamilton Depression Rating Scale. Association of the PGS for CAD and obesity with SSRI treatment response was first tested in the ISPC sample involving 865 MDD patients who received SSRI treatment. Further details are available elsewhere[13].

Replication study: *Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study*

The PGS association finding in the ISPC was replicated in the STAR*D study, which is the biggest clinical trial on antidepressants. The study initially enrolled 4,041 MDD patients aged between 18 and 75 years, for which data of 1,878 MDD patients who received citalopram in the first 12 to 14 weeks (level 1) were included in our analysis. Data at baseline and treatment follow-up was collected using the 16-item Quick Inventory of Depressive Symptomatology (QIDS-C16)[18,19].

Genotyping and quality control

Genome-wide genotype and clinical data for the ISPC were obtained from the ISPC consortium[13], while data for the STAR*D were available via a controlled access system at the database of Genotypes and Phenotypes: dbGaP; http://www.ncbi.nlm.nih.gov/gap and the National Institute of Mental Health (NIMH) Repository and Genomics Resource, respectively.

For all genotype data, we implemented quality control (QC) procedures using PLINK[20] and samples with low genotype rates <95%, sex inconsistencies (X-chromosome heterozygosity) and genetically related individuals were excluded. We also excluded SNPs that had poor genotyping rate <95%, an ambiguity (A/T and C/G SNPs), a minor allele frequency (MAF) less than 1% or showed deviation from Hardy-Weinberg Equilibrium ($p<10^{-6}$).

Imputations

Genotype data that passed the above QC criteria were imputed in the Michigan server https://imputationserver.sph.umich.edu [21], separately for each study samples using the 1000 Genomes project Phase 3 (Version 5) reference panel. The ISPC data was divided into Europeans and Asians and then imputed separately using the European and East Asian reference populations, respectively. The STAR*D was imputed with a reference population from a mixed ancestry.

After excluding ,low-frequency SNPs (MAF<10%), low-quality variants (imputation INFO < 0.9) and indels, imputed dosages were converted to best guess genotypes. Subsequent PGS analyses were performed using the best guess genotypes.

Discovery GWAS summary data

The PGSs were calculated using the approach previously described by the International Schizophrenia Consortium[22]. This method requires discovery and target datasets. The discovery summary statistics for CAD and obesity were obtained from a previous GWAS by the CARDIoGRAMplusC4D Consortium[23] available at http://www.cardiogramplusc4d.org and by GIANT consortium[24] available at http://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium

SSRI treatment response criteria

SSRIs response in MDD was defined after four weeks of SSRI treatment as a ≥50% reduction from baseline in the HRSD-17 or QIDS-C16. Data on covariates, such as age, gender, and on the specific SSRI medications were also collected and details can be found in previous publications[13,19].

Statistical Analyses

Polygenic score analyses

We computed PGS separately for CAD and obesity using the 1000 genomes imputed data in the ISPC and STAR*D samples and GWAS summary statistics (Table 1, Figure 1 and 2). First, quality-controlled SNPs were clumped for linkage disequilibrium using genome-wide association p-value informed clumping with $r^2 = 0.1$ in a 250-kb window to create a SNP-set in linkage equilibrium using PLINK software run on Linux (PLINK command; *plink–clump-p1 1 –clump-p2 1 –clump-r^2 0.1 –clump-kb 250*). Next, weighted PGSs were calculated for each study subject at a given p-value threshold (P_T) as the sum of weighted allele dosages (0, 1 or 2). The

weighting was accomplished by multiplying the dosage of each effect increasing allele by its effect size derived from the GWAS summary statistics (β- coefficient) then divided by the total number of SNPs. The PGSs were calculated over a range of P_T ($<1x10^{-4}$, $<1x10^{-3}$, $<1x10^{-2}$, $<5x10^{-2}$, <0.1, <0.2, <0.3, <0.4, <0.5, <1.0) separately in the ISPC and STAR*D samples. The major histocompatibility complex region was excluded from the PGS calculation because of its complex linkage disequilibrium structure. Analyses evaluating the association of PGSs with SSRI treatment response were performed using logistic regression. The PGS analyses were adjusted for covariates age, sex and study site in the ISPC and age, sex, 7-PCs in the STAR*D samples. The analyses were performed using R for Statistical Computing and PLINK 1.9 for Linux[20]. A statistically significant PGS association was determined at $p < 0.05$ for across the P_T. To determine the effect of high or low polygenic load on treatment response, we grouped study subjects into PGS quartiles (Q_1-Q_4) at the most significant GWAS p-value thresholds. Then, we estimated the odds of treatment response status in groups of MDD patients in Q_2 to Q_4 compared to groups of patients in the lowest PGS quartile (Q_1). Finally, for prediction accuracy, the percentage of variance explained, Nagelkerke R^2, by the PGSs were calculated as the Nagelkerke R^2 of the full model with PGS and covariates minus the Nagelkerke R^2 of the model with only covariates.

Cross-trait meta-analyses of genome-wide association studies

Cross-trait meta-analyses were performed by combining GWAS summary statistics on response to SSRIs in patients with MDD[13] with GWASs summary statistics on CAD[23] and obesity[24]. In the meta-analyses, we applied the O'Brien's (OB) method and the direct Linear Combination of dependent test statistics (dLC) approach[25,26], which are implemented in the C^{++} eLX package,

available at https://sites.google.com/site/multivariateyihsianghsu/. Briefly, the OB method and the dLC approach[25,26] help combine univariate meta-GWAS data (beta coefficients or Z-scores) of each SNP from the univariate GWASs and generate a cross-trait OB and dLC test statistics and associated p-values. The methods are equivalent to random effects meta-analytic methods. More details can be found elsewhere[25-27]. The above cross-trait meta-analyses generated two test statistics and associated p-values, one for the OB method and one for the dLC method. Statistical significance of cross-trait meta-analyses was determined based on the smaller of the two p-values. Results were considered significant if (1) the p-value for cross-trait meta-analysis reached genome-wide significance ($p < 5\times10^{-8}$) and (2) univariate meta-GWAS effects were at least nominally significant ($p< 0.05$). For each cross-trait meta-analysis, only one lead SNP per locus was reported. Nearby SNPs in LD ($r^2>0.1$) with the lead SNP were considered dependent and belonging to the same locus.

RESULTS

Patient characteristics and treatment response

Data from a total of 2,743 MDD patients in the ISPC (n=865) and STAR*D (n=1,878) who had been treated with SSRIs were analysed. The average age of patients was 42.4 years and a majority of participants (62.7%) were female. Of all patients, 39.7% were classified as treatment responders, with slight variation across study samples (ISPC, 48.1% and STAR*D, 33.4%) (Table 1).

Table 1: Baseline characteristics of MDD patients and treatment response with selective serotonin reuptake inhibitors after four weeks of follow-up

Patient characteristics	**ISPC N=865**	**STAR*D N=1878**	**Total N=2743**
Responders, N (%)	416 (48.1)	509 (33.4)	925 (39.7)
Age, mean (sd)	43.7 (14.7)	42.6 (13.4)	42.4 (13.9)
Sex, female, N (%)	561 (64.9)	1156 (61.6)	1717 (62.7)

ISPC: International SSRI Pharmacogenomics Consortium study; STAR*D: Sequenced Treatment Alternatives to Relieve Depression Study.

The associations of PGSs for CAD and obesity with SSRI treatment response

Figures 1 and 2 show findings from PGS analyses of CAD and obesity with SSRI treatment response. The polygenic load for CAD and obesity (body mass index) were significantly associated with SSRI treatment response. At the most significant thresholds, the PGS for CAD and body mass index accounted for 1.3% and 0.8% of observed variability in treatment response to SSRIs, respectively (Figure 1).

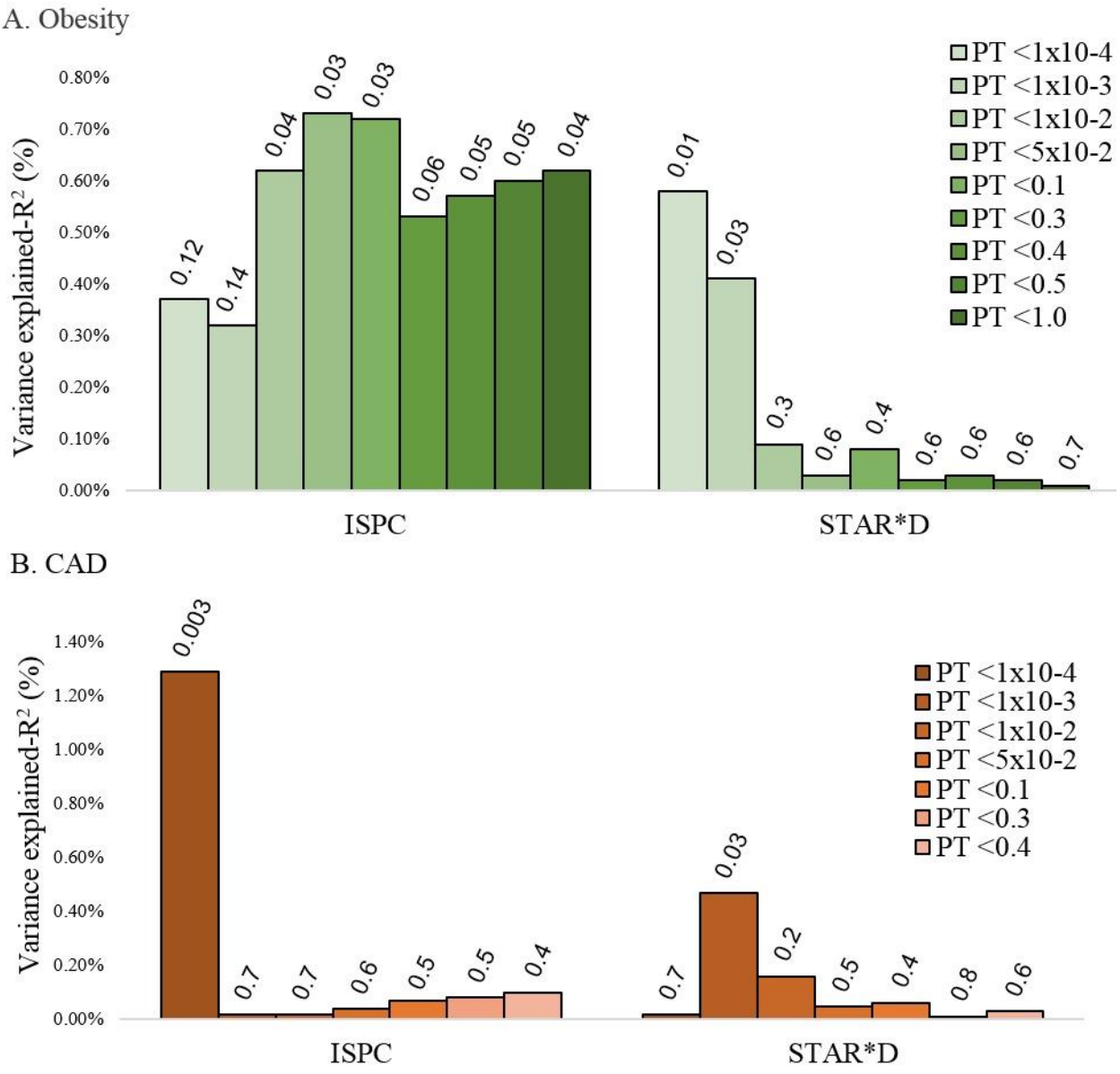


Figure 1: Bar graph shows polygenic scores (PGSs) for CAD and obesity and their significant association with SSRI treatment response at different GWAS p-value thresholds in the discovery (ISPC, N=865) and replication study (STAR*D, N=1878) samples. The y-axis (Nagelkerke's pseudo-R^2) refers to percentage of variance in SSRI treatment response accounted for the PGSs of CAD or obesity at each p-value threshold in each sample. On the x-axis, plotted from left to

right, are the GWAS p-value thresholds used to cluster single nucleotide polymorphisms (SNPs) for the PGSs.

Comparison of treatment response by PGS quartiles showed that MDD patients with a higher polygenetic load for CAD or body mass index tended to have a poorer response to SSRIs than patients in the lowest PGS quartiles. MDD patients in the highest PGS quartile for CAD (Q_4) had an odds ratio (OR) of 0.53 (ISPC: 95% CI, 0.35-0.81 to 0.83; STAR*D: 95% CI, 0.62-1.11) compared to patients in Q_1. Similarly, the OR for patients in Q_4 based on the PGS for obesity was 0.53 (ISPC: 95% CI, 0.32-0.88 to 0.79; STAR*D: 95% CI, 0.58-1.06) compared to patients in Q_1 (Figure 2).

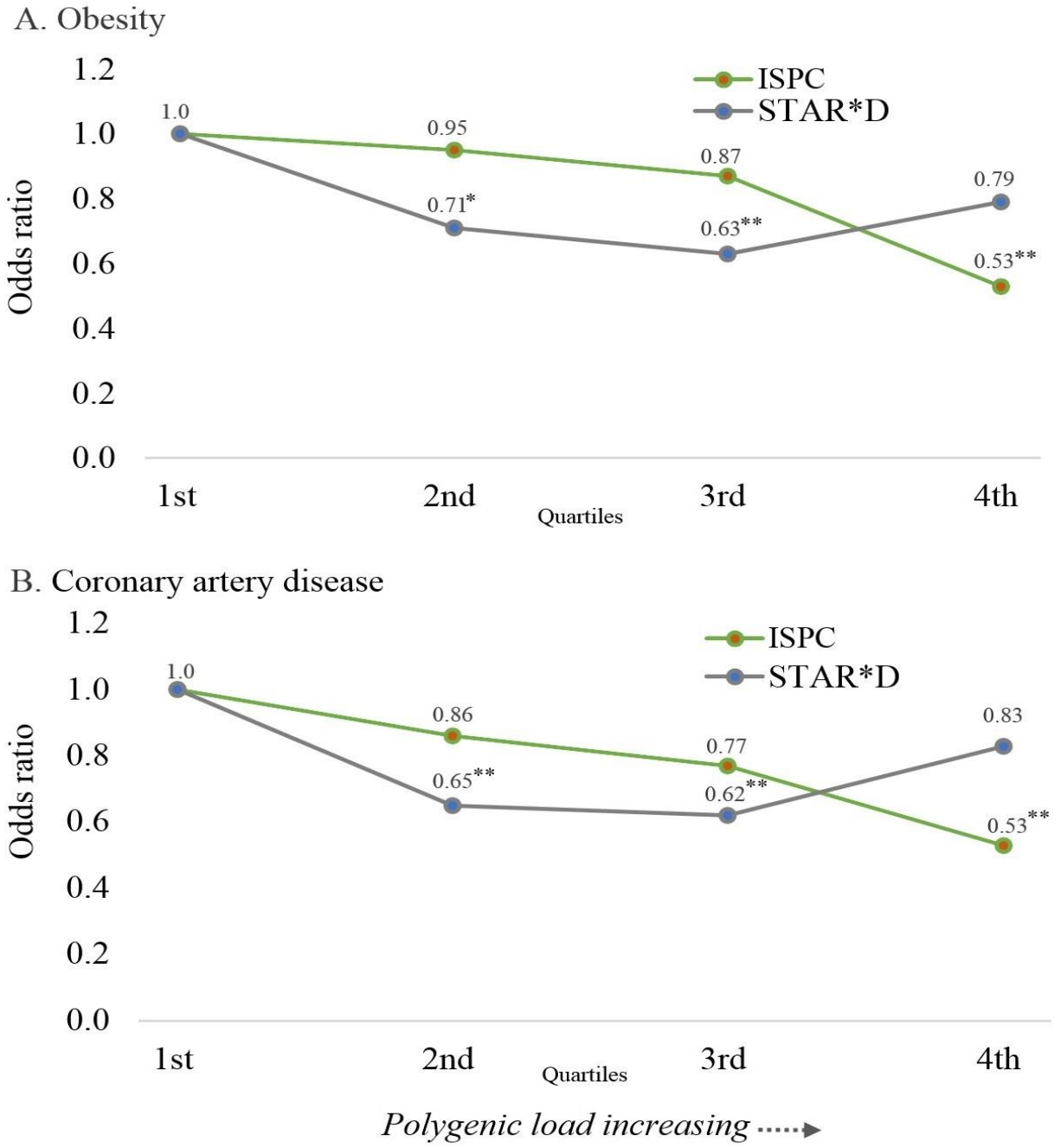


Figure 2: Line plot (right) shows the association of response to SSRIs treatment and the PGS for obesity/CAD, and it represents the odds ratio of response to SSRIs in MDD patients at the high polygenic load quartiles (Q_2-Q_4) compared to samples in the lowest polygenic quartile (Q_1) estimated at the most significant p-value thresholds. The most significant p-value thresholds represent the p-value threshold in which the PGSs showed the most significant association

(lowest association p-value). The effect sizes on the y-axis are estimated in odds ratios and on the x-axis, refers to PGS quartiles (Q_1 to Q_4) for each trait, *p < 0.05, **p < 0.01, ***p < 0.001. ISPC: International SSRI Pharmacogenomics Consortium study; STAR*D: Sequenced Treatment Alternatives to Relieve Depression Study; CAD: coronary artery disease.

Cross-trait meta-analyses of genome-wide association studies

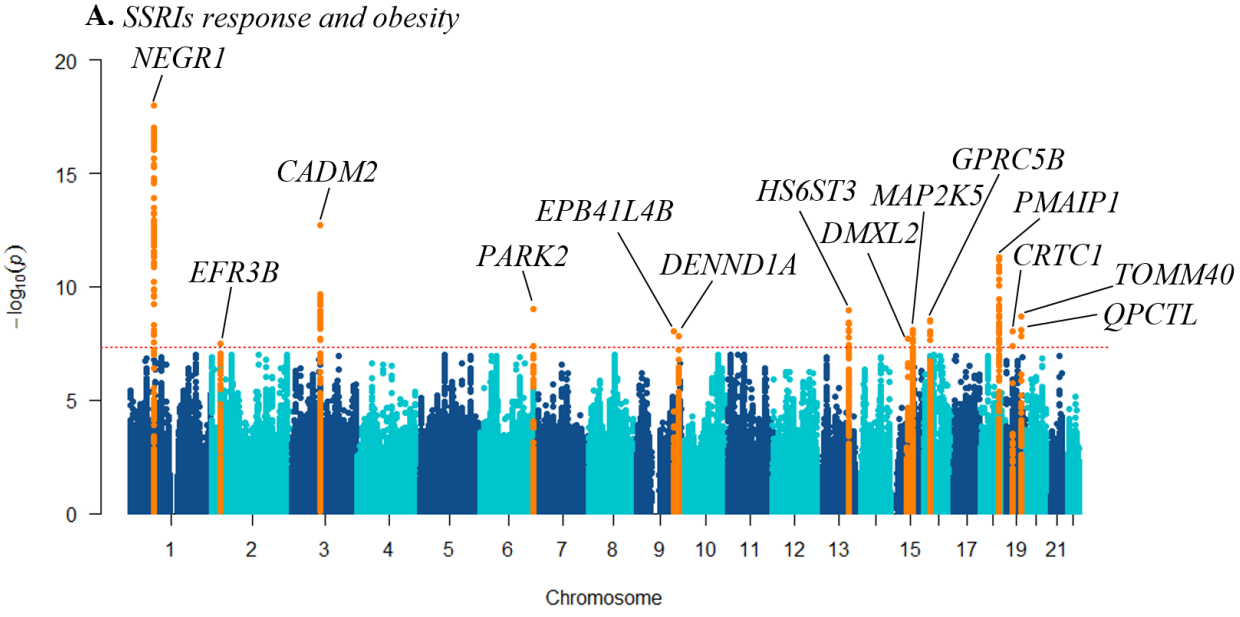
In cross-trait meta-analyses, we combined the GWAS summary statistics on SSRI treatment response and equivalent summary statistics on CAD and obesity. Table 2 and Figure 3 summarise cross-trait meta-analyses findings, including the list of genetic loci and nearest genes that are potentially overlapping between traits. At the level of GWAS significant p-value $<5x10^{-8}$, we identified 19 genetic loci simultaneously associated with SSRI treatment response and BMI 14-loci (top four: *NEGR1*, *CADM2*, *PMAIP1*, *PARK2)* and CAD 5-loci (top four: *PHACTR1*, *CDKN2B, ATXN2*, *KCNE2)* (Table 2 and Figure 3).

Table 2: Significant loci resulting from cross-trait meta-analyses of GWASs on SSRI treatment response in MDD patients with GWASs on coronary artery disease and obesity (univariate GWAS p-value $<5x10^{-2}$ and cross-trait p-value $<5x10^{-8}$).

SNP	Chr	Position Ch37	A1	A2	GWAS p-value for			Nearest gene	ED
					SSRIs response[13]	BMI[24]	Cross trait		
rs975480	1	72517928	G	A	$1.26x10^{-2}$	$4.83x10^{-18}$	$1x10^{-18}$	*NEGR1*	+-
rs551573	2	25305504	G	A	$2.66x10^{-2}$	$5.56x10^{-8}$	$3x10^{-8}$	*EFR3B*	-+
rs7611991	3	85759558	A	G	$3.80x10^{-2}$	$1.42x10^{-10}$	$2x10^{-10}$	*CADM2*	++
rs13191362	6	163033350	G	A	$3.19x10^{-2}$	$1.09x10^{-9}$	$9x10^{-10}$	*PARK2*	--
rs6477694	9	111932342	A	G	$2.42x10^{-2}$	$1.71x10^{-8}$	$9x10^{-9}$	*EPB41L4B*	--
rs1752156	9	126534512	C	T	$3.70x10^{-3}$	$3.17x10^{-7}$	$1x10^{-8}$	*DENND1A*	++
rs1927793	13	96921287	G	C	$1.66x10^{-5}$	$1.72x10^{-6}$	$1x10^{-9}$	*HS6ST3*	-+
rs3736485	15	51748610	G	A	$2.60x10^{-2}$	$4.52x10^{-8}$	$2x10^{-8}$	*DMXL2*	--
rs12905397	15	68034150	G	A	$3.07x10^{-2}$	$1.40x10^{-8}$	$8x10^{-9}$	*MAP2K5*	--

rs6497415	16	19876092	T	C	$2.60x10^{-2}$	$5.47x10^{-9}$	$3x10^{-9}$	*GPRC5B*	--
rs4940929	18	57803890	C	G	$6.38x10^{-3}$	$2.77x10^{-11}$	$5x10^{-12}$	*PMAIP1*	--
rs757318	19	18820308	C	A	$8.88x10^{-3}$	$3.18x10^{-8}$	$1x10^{-8}$	*CRTC1*	++
rs2075650	19	45395619	G	A	$3.42x10^{-2}$	$3.21x10^{-9}$	$2x10^{-9}$	*TOMM40*	--
rs2302593	19	46196634	G	C	$1.06x10^{-2}$	$2.36x10^{-8}$	$8x10^{-9}$	*QPCTL*	--
						CAD[23]			
rs17740744	2	145295362	G	A	$3.62x10^{-2}$	$3.25x10^{-9}$	$3x10^{-9}$	*LINC01412*	++
rs4714990	6	12927845	G	A	$3.00x10^{-2}$	$2.67x10^{-17}$	$2x10^{-17}$	*PHACTR1*	+-
rs13298881	9	22012051	G	A	$3.32x10^{-2}$	$3.75x10^{-10}$	$3x10^{-10}$	*CDKN2B-AS1*	--
rs10774625	12	111910219	A	G	$1.45x10^{-2}$	$2.69x10^{-10}$	$1x10^{-10}$	*ATXN2*	++
rs9978142	21	35652239	A	T	$3.08x10^{-2}$	$3.32x10^{-11}$	$3x10^{-11}$	*KCNE2*	++

A1, effect allele; A2, another allele; obesity measured as body mass index (BMI). ED: effect direction that represents the SNPs effect on SSRIs treatment response for the effect allele based on the ISPC GWAS[13] versus its effect on coronary artery disease (CAD) and obesity.



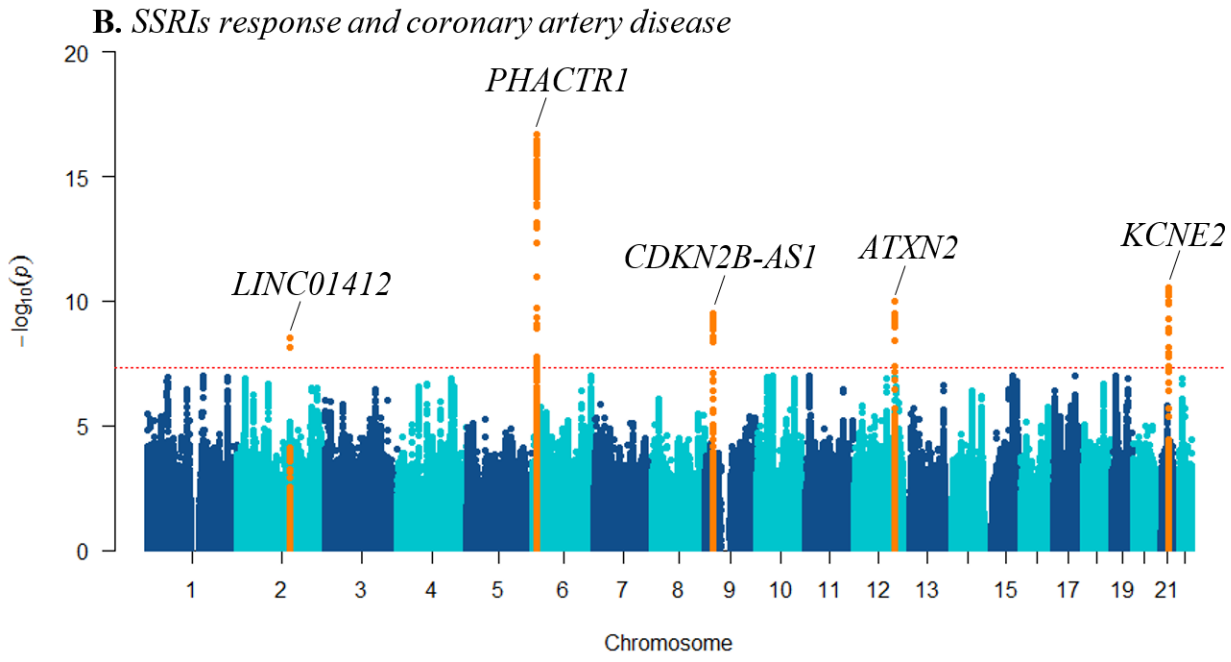


Figure 3: Manhattan plot showing the result of cross-trait meta-analyses of GWASs on SSRI treatment response with GWASs on: a) obesity; b) coronary artery disease, highlighting the loci that showed genome-wide significance (orange), and the nearest genes. The −log10 (cross-trait p-value) is plotted against the physical position of each SNP on each chromosome. The threshold for genome-wide significance (cross-trait p-value<$5x10^{-8}$) is indicated by the red dotted horizontal line.

DISCUSSION

In this study, we calculated PGSs for 2,743 MDD patients who had been treated with SSRIs and reported, for the first time, an association between PGS for CAD and obesity traits and response to SSRIs in depression. Through cross-trait meta-analyses of GWASs on SSRI treatment response versus individual GWASs on CAD and obesity, we identified several genetic loci with overlapping effects on response to SSRIs and CAD and obesity.

Our findings from the PGS analyses and cross-trait meta-analyses were complementary and support that both CAD and obesity may influence SSRI treatment response in patients with MDD. MDD patients with a high polygenic load for CAD or obesity were inversely associated with SSRI treatment response. Based on the findings, a key question was whether the PGSs associations for each of the traits were consistently significant at different thresholds. While significant associations between treatment response and PGS for obesity were observed at several P-value thresholds, the PGSs for CAD were associated with treatment response only at a single P-value threshold. This suggests that the latter finding is less consistent across thresholds. Although replication of the findings in a different cohort supports the possibility of strong effects, another explanation is that our cohorts might be underpowered to detect consistent effects, or that some observed associations were chance findings.

Our results are consistent with previous genetic findings that have pointed to the relationship between antidepressant treatment response and CAD and obesity[28]. Genetically driven somatic factors might predict antidepressant treatment response. Samaan et al. demonstrated that variants within obesity risk genes were associated with MDD, suggesting shared genetic mechanisms between the two traits[28]. Shared genes between MDD and cardiometabolic traits have previously been shown in the relationship between these traits which suggest biological relevance of somatic traits for response to SSRIs in patients with MDD[3]. Clinical studies lend further support to results of our findings in that medical co-morbidities, including cardiovascular and endocrine disorders, have been previously shown to negatively impact antidepressant treatment response in MDD[15], which is in line with our findings.

In support of the PGS findings, cross-trait meta-analyses of GWASs on SSRI treatment response with GWASs on CAD and obesity identified several overlapping genes. For instance, genetic variants of the obesity gene *NEGR1* have effects on brain structure, and may potentially affect efficacy of antidepressants[29]. The *NEGR1* locus (rs975480) is highly linked with other SNPs (rs1486084, rs11209899, rs12036443, rs2821272, rs11209900, rs36047136) associated with expression of the *POMC* gene[30]. Polymorphisms of *POMC* confer vulnerability to MDD and may alter treatment response to antidepressants in MDD patients[31]. Similarly, another obesity gene locus[32], *EFR3B* (rs551573), was associated with expression of the adenylate cyclase 3 (*ADCY3*) gene, accessed at https://gtexportal.org/home/ on 13/04/2017. The *ADCY3* gene is a major component of the CREB pathway that has been implicated in the disease mechanism of MDD[33] and it may affect antidepressant treatment response. The *CRTC1* gene locus[34] was previously reported to play a role in obesity and treatment-resistant depression[35]. Overall, these findings reinforce the idea that specific gene polymorphisms may confer susceptibility to psychiatric and somatic traits further impacting on response to antidepressants in patients with MDD. Studying the individual mechanism of each significant genetic locus in relation to antidepressants is beyond the scope of this study, but the genetic overlap at the SNP level strongly supports the notion of applying PGS profiles of obesity and CAD to implement stratified diagnosis and treatment of MDD with a basic consideration of cardiometabolic comorbidities. The clinical utility of the PGS for CAD and obesity depends on the potential to differentiate MDD patients into categories of responders versus non-responders according to CAD or obesity status. Further research is required to determine if this is the case.

Overall, findings of this study should be best viewed as a starting point to investigate predictors of antidepressant treatment response. Because treatment response was defined after four weeks

of treatment, which is too soon to see full response, we encourage replication of our study in a larger, prospective study with a longer period of treatment follow-up. At this stage, the PGSs for the above traits at the most significant P_T could be incorporated into prediction models in combination with clinical variables to determine if there is an added predictive value together with other clinical and biological factors. It needs to be pointed out that PGSs are currently not yet useful as standalone predictors of antidepressant treatment response.

In conclusion, our study provides first insight into the ability of PGSs of CAD and obesity to elucidate shared biological pathways to predict SSRI treatment response. Future studies need to replicate our findings in larger, prospective and more homogeneous samples. The clinical value of our PGSs likely lies in the contribution to multi-variable models that also comprise clinical and environmental factors influencing the response to these drugs.

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Conflict of interest

VA is a member of the advisory boards (past 5 years) for Astra-Zeneca, Eli Lilly, Lundbeck, Otsuka, Servier, Trommsdorff. BTB is a member of advisory board (past 5 years) of Lundbeck. RW and LW own stock in OneOme LLC. RBA is a stockholder in Personalis Inc. and a paid advisor for Pfizer and Karius. TEK is a paid scientific advisor to the Rxight Pharmacogenetics Program. Other authors declare they have no competing interests.

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Chapter 8

General discussion and future perspectives

General discussion

Historically, two periods of affirmative changes have transformed psychiatric service into a modern form of care. A century ago, the first transformation occurred through introduction of scientific knowledge into teaching and clinical practice of psychiatry. The most transformative changes in psychiatry were the introduction of lithium by Dr. John Cade in 1948[1] and the incidental discovery of the antipsychotic effect of chlorpromazine in the same decade in 1952 by French surgeon Henri Laborit[2], followed by further chemical development of chlorpromazine-related substances leading to tricyclic antidepressants. The second, 21st century transformation is emerging now because of the revolution of scientific advances in genomics, epigenetics, proteomics, microbiomes and neuroimaging technology followed by promotion of precise, personalised psychiatric care. Personalised psychiatry is a new model towards individualised care, in which knowledge from genomics and other omics pillars (microbiome, epigenomes, proteome, and metabolome) are combined with clinical data to guide efforts to new drug development and targeted prescription of existing treatment options[3].

Numerous studies have consistently reported inter-individual differences in response to mood stabilisers[3-12]. While many clinical variables, such as age, gender, disease characteristics and nutrition, have been associated with response to mood stabilisers, inherited variation of drug target genes also contribute to observed variation in response to treatment. Lithium and SSRIs are the most commonly prescribed mood stabilisers for treatment of BPD and depression, respectively. Treatment success rate to these drugs is inadequate and often associated with side

effects. Studies report a large inter-individual variability in lithium and SSRIs treatment response — a major source of variability being due to genetic factors[3,12].

Clearly, an understanding of genetic determinants of response to lithium and SSRIs will enable to identification of patients who respond well or poorly to treatment or develop undesirable side effects. GWAS and candidate gene studies are the most widely used methods in identifying genetic determinants of drug response. To date, such studies have unveiled a number of genetic polymorphisms and provided valuable insight to inter-individual variation to lithium[13-27], and antidepressant response[28-36] and adverse drug reactions[3]. Findings from such studies may be translated to patient care using genotyping technologies through guiding clinicians in selection of the most optimal combination of treatment and drug dosage for individual patients. However, as treatment outcomes are complex traits controlled by several genes, the classic monogenic Mendelian approach of translating genetic evidence into clinical application is challenging. It is also noteworthy that most genetic associations do not reach GWAS significance due to small sample size or marginal effects, but are still important. In such situations, an alternative strategy could be a 'polygenic score method' in which effects of thousands of genetic variants are combined and assessed for their capacity to predict a range of complex diseases and response to pharmacotherapy, including response to lithium and SSRIs. If successful, PGSs could have value as a genetic tool, alongside clinical predictors, in stratifying patients and providing individualised treatment in BPD and MDD, thereby contributing to personalised prescribing in psychiatry.

In the context of psychiatry, it is also important to understand the impact of clinical diagnostic and symptom overlap on treatment response. Given that mood disorders are genetically and clinically overlapping with other psychiatric[37,38] and somatic disorders[39], understanding the

influence of such comorbidities on treatment response is a crucial step to fully address inter-individual treatment variability and discover somatic biomarkers.

This thesis highlights the polygenic (genetic) determinants of response to lithium and SSRIs in patients with mood disorders and suggests possible strategies and opportunities to integrate genomes with patient clinical characteristics to optimise treatment outcomes. Specifically, this study investigated polygenic (genetic) association of SCZ, MDD, depressive symptoms, the big five personality traits, obesity, coronary artery diseases and response to a) SSRIs in patients with MDD, and b) lithium in patients with BPD. Advanced bioinformatic research methodologies, such as PGS analyses, cross-trait genome-wide association, candidate gene analyses, and pathway and gene network analyses approaches, were implemented with the ultimate goal of supporting current effort towards a personalised psychiatry. First, genetic loading aggregated as PGSs were computed for different psychiatric and somatic traits. Then these PGSs were evaluated for association with response to lithium and SSRIs. Genetic and clinical data used for this project were obtained from the following cohorts: International Consortium on Lithium Genetics (ConLi^{+}Gen, N=2,586), International SSRI Pharmacogenomics Consortium (ISPC, N=865), Pharmacogenomics Research Network Antidepressant Medication Pharmacogenomic Study (PGRN-AMPS, N=529) and Sequenced Treatment Alternatives to Relieve Depression (STAR*D, N=1878).

Results presented in this thesis illustrate the significance of using pharmacogenomics to a make a paradigm shift in diagnosis and treatment of mood disorders that ultimately has potential to bring us closer to true personalised psychiatric care. There are three main findings of this thesis. First, through systematic review of literature and follow-up gene function analysis, we confirmed

genetic overlap of cardiometabolic disease with mood disorders and investigated the influence of this overlap on response to lithium and SSRIs.

Second, by applying polygenic score and GWAS analysis methods, we revealed strong evidence to predict lithium response using genetic scores of SCZ[4], MDD and depressive symptoms. Moreover, response to SSRIs was associated with genetic scores of the big five personality traits[40], obesity and coronary artery disease. These findings are interesting, but a major question remains concerning the capacity to predict response to lithium or SSRIs using currently available genetic scores. At this stage, a genetic score for a single trait could not predict more than 2% of variation in response to lithium or SSRIs. To improve treatment response prediction and facilitate clinical uptake it is important to identify multi-phenotype genetic scores and combine these data with patient clinical characteristics.

Third, for traits in which polygenic association was confirmed with lithium or SSRI treatment response, cross-trait GWAS meta-analysis was performed using GWAS summary statistics followed by functional pathway and network analysis. We found several genetic loci, biological pathways and molecular networks involved in susceptibility to psychosomatic disorders and treatment response to mood stabilisers[4].

From our findings it is clear that mood disorders, response mood stabilisers and their cross-trait relationship with cardiometabolic disorders and personality traits is very complex. Understanding of the complex genetic relationship may offer a unique opportunity to characterise pharmacotherapeutic response to lithium or SSRIs and re-classify mood disorders according to treatment response patterns. Moreover, it is likely that response to therapy provides

a more homogeneous cluster of patients, for instance, a lithium responsive phenotype or SSRI responsive phenotype.

Overall, this thesis consists of eight chapters. Chapter 1 provided general introductory background on mood disorders, lithium and SSRIs. In chapter 2, a review of pharmacogenomic studies conducted to date on treatment of mood disorders was presented, with a focus on SSRIs and lithium. Here, we reported a number of genetic polymorphisms located within 15 candidate genes *IL1B, FKBP5, CNR1, NPY, ABCB1, BDNF, GRIK4, GNB3, HTR1A, HTR2A, SLC6A4, COMT, CYP2C19, CYP2D6* and *MAOA* associated with SSRI treatment response. Genetic variants located within 16 candidate genes, *XBP1, INPP1, CREB1, GSK3B, NR3C1, DRD1, FYN, TIMELESS, PER3, CLOCK, ARNTL, TPH1, SLC6A4, CACNG2, ASIC2* and *GADL1*, that may influence treatment response to lithium in patients with BPD was also discussed[3]. The review highlighted challenges, potential strategies and opportunities of combining clinical data with omics information (DNA, RNA, proteins and microbiomes) to provide a personalised psychiatric care[3].

Results presented in Chapter 3 described findings of a systematic review and analysis of cardiometabolic disease genes that show overlapping effects on mood disorders and treatment response to mood stabilisers. This chapter presented 24 genes associated with risk of cardiometabolic diseases associated with mood disorder susceptibility that influence response to lithium and SSRI treatment. The genes included *MTHFR, CACNA1D, CACNB2, GNAS, ADRB1, NCAN, REST, FTO, POMC, BDNF, CREB, ITIH4, LEP, GSK3B, SLC18A1, TLR4, PPP1R1B, APOE, CRY2, HTR1A, ADRA2A, TCF7L2, MTNR1B* and *IGF1*. Pathway analysis of these genes revealed significant pathways: corticotrophin-releasing hormone signaling, AMPK signaling,

cAMP-mediated or G-protein coupled receptor signaling, axonal guidance signaling, serotonin or dopamine receptors signaling, dopamine-DARPP32 feedback in cAMP signaling, circadian rhythm signaling and leptin signaling[39].

Chapter 4 presented the association between polygenic loading for SCZ genetic variants and response to lithium in patients with BPD. Using cross-trait meta-GWAS, 15 genetic loci that may have overlapping effects on lithium treatment response and susceptibility to SCZ were identified. Functional pathway and network analysis of these loci point to the HLA complex and inflammatory cytokines (TNFα, IL-4, IFNγ) as molecular contributors to lithium treatment response in BPAD[4].

In chapter 5, we investigated the association of polygenic score for MDD and depressive symptoms risk variants and response to lithium in patients with BPD. Our findings showed an inverse association. A follow-up cross-trait GWAS and functional analysis of GWASs on MDD and depressive symptoms with GWASs on lithium treatment response revealed genetic loci pointing to the role of sodium-potassium-ATPase channel molecules. In Chapter 6, the association of the PGSs for the big five personality traits and response to SSRIs in patients with MDD was presented. In this study, we examined whether the PGSs derived from the big five personality traits predict treatment response to SSRIs in patinets with MDD. The PGS analysis was performed using data from two cohorts: PGRN-AMPS (n=529) and ISPC (n=865). Results showed that PGS for openness and neuroticism were associated with SSRI treatment response at $p< 0.05$ across P_T thresholds in both cohorts. A significant association was also found between PGS for conscientiousness and SSRI treatment response in the PGRN-AMPS sample. In addition, we performed meta-analyses of GWASs on these traits to identify genetic variants

underpinning cross-trait polygenic association and identified eight genetic loci associated with a) SSRI treatment response and conscientiousness near *YEATS4* gene, and b) SSRI treatment remission and neuroticism at seven loci near *PRAG1*, *MSRA*, *XKR6*, *ELAVL2*, *PLXNC1*, *PLEKHM1* and *BRUNOL4* genes.

In Chapter 7, we explored the association of obesity and coronary artery disease genes with response to SSRI treatment in MDD. Chapter 8 provided a general discussion on findings, future perspectives and conclusions.

Future perspectives

Genetic determinants of response to drugs targeting SZC and MDD remain largely unknown. This will continue to be a broad area of research and it is clinically interesting to identify individuals who respond better to drugs and those who are unlikely to benefit. Early identification of response to drugs would enable clinicians to intervene at early stages of treatment. In this regard, findings of our study may provide evidence and support the path to personalised psychiatry.

In a decade of GWAS studies, multiple SNPs that contribute to MDD, BPD and response to lithium and SSRIs have been identified. However, because the effect size of individual SNPs is modest, they have limited utility for prediction of risk or treatment outcomes. PGS provides a better alternative for aggregating the cumulative impact of several SNPs. In our studies, we demonstrated the likelihood of predicting response to lithium and SSRIs using PGS for different psychosomatic traits, suggesting potential translation of PGSs to clinical use. However, it remains uncertain whether the PGS can perform better, or at least as good as, clinical and other

biological markers. The next step should be to develop a multi-polygenic algorithm and evaluate whether these PGSs can be used for prediction of treatment outcomes. It is also important to note that PGSs represents only genetic factors and other predictors, such as epigenetic, neuroimaging, microbiomes, clinical and psychosocial factors, should be considered during development of prediction algorithms. The capacity to integrate a large number of genetic and non-genetic predictors, including interactions and development of prediction and prognosis models, will realise the potential of personalised medicine. There are several statistical methods to combine multiple predictor variables. Among these, a machine-learning approach is the most recently promoted method because it encompasses a wide-ranging class of algorithms and allows multi-variable interactions for prediction of complex traits.

Conversely, accumulating evidence from GWASs on psychiatric traits releveled an abundant presence of shared genetic influences among psychiatric disorders and between psychiatric and somatic traits. We performed numerous combinations of bivariate GWASs followed by functional pathway and network analysis to uncover the mechanisms of genetic overlap contributing to co-occurrence of both traits. Our studies revealed the role of several genes, biological pathways and molecular networks in response to lithium and SSRI treatment. Replication of findings is the next step prior to a laboratory-based investigation. Better understanding and targeted mechanisms of these genes is to be investigated in the future by implementing an experimental design using animal and cellular models.

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About the author

Azmeraw Tayelgn Amare was born on September 5, 1984, in Ethiopia, Amhara region; Achefer Woreda in a village (Kebele) called Lalibela, Addisbete Gote. Azmeraw was an excellent student who has been performing very well from the early ages of school. He scored 4.0, recorded "A" in all seven subjects in the 12th grade Ethiopian School Leaving Certificate Examination (ESLCE), then joined Debub University (now called Hawassa University) for undergraduate study and graduated in bachelor of health science in 2006. Azmeraw was the first, top student and gold medal among 2006 graduates in the faculty of public health. In 2010, he graduated in master of Public health from the University of Gondar. After graduation, he worked as a lecturer in Debre Birhan University and Bahir Dar University teaching courses such as epidemiology, research methodology and biostatistics for undergraduate medical and health science students.

In 2011, he received a CIUF-CUD Scholarship from the Government of Belgium through the University Commission for Development (CUD) program to study master in public health research methodology (MPHM) coordinated by the Université Libre de Bruxelles (ULB) and Université de Mons (UMONS), Brussels (Belgium) and graduated in 2012. In September 2012, he moved to the Netherlands to study a researcher master in Clinical and Psychosocial Epidemiology (CPE) at the University Medical center Groningen (UMCG) and graduated in 2014. The School of HeAlth REsearch (SHARE) research institute at the University Medical center Groningen sponsored his study. During his training, he got interested in the mechanisms of genetic overlap between psychiatric and somatic disorders. Thus, he performed his master research project on "a bivariate Genome-Wide Association Study (GWAS) of depressive symptoms and plasma tumor necrosis factor alpha (TNF-α) level" supervised by Professor

Harold Snieder and Dr. Behrooz Z. Alizadeh. In summer 2014, he traveled to the United States of America and attended the 19th summer institute statistical genetics courses at the University of Washington in Seattle. Azmeraw found genetic research interesting and decided to pursue PhD in Australia. He designed his PhD project to examine "the Genetic predictors of response to pharmacotherapy in patients with mood disorders focusing on antidepressants and lithium" under the supervision of Professor Bernhard T. Baune and A/Professor Klaus Oliver Schubert. The University of Adelaide granted his PhD project through Adelaide Scholarship international (ASI) program. In 2017, he was awarded an early career researcher travel grant by the World Psychiatric Association (WPA) and attended the WPA XVII World Congress of psychiatry that was held in Berlin, Germany. Additional conferences that he attended during his PhD includes the 2017 Society for Mental Health Research (SMHR) Annual Conference, which was held in Canberra, Australia and the World Psychiatric Association's Thematic Congress held in Melbourne, Australia 2018.

Azmeraw has a research interest in clinical epidemiology, personalized medicine, genetics, genomics, pharmacogenomics and global health. He has published several articles in collaboration with scientists in the field. Azmeraw will continue his research career as a postdoctoral associate at the South Australian Health and Medical Research Institute (SAHMRI), in Australia.

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Publications

During his PhD period, Azmeraw has authored (co) over 40 articles and a book chapter that can be found at https://www.ncbi.nlm.nih.gov/pubmed/?term=Azmeraw+T.Amare

The following are included in this thesis:

1. **Amare AT,** Schubert KO, Hou L, et al. Association of Polygenic Score for Schizophrenia and HLA Antigen and Inflammation Genes With Response to Lithium in Bipolar Affective Disorder: A Genome-Wide Association Study. *JAMA Psychiatry.* 2018;75(1):65-74.
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