Pharmacogenetics of psychotropic drugs and genetic influences on adverse drug reactions

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I, Isabelle Austin-Zimmerman, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the work.

Abstract

In this thesis, I investigate the impact of genetic variation on adverse drug reactions to psychotropic medications, with a focus on the metabolic and sleep related side effects of psychotropic drugs. In addition to reviewing published literature, I have considered this research topic in three main ways. Chapter one is a systematic review and meta-analysis of the impact of CYP2D6 genetic variation on antipsychotic-induced hyperprolactinaemia and weight gain, which are a relatively common but understudied adverse-drug reactions. Chapters two, three and four are based on data from UK Biobank, where I have conducted a hypothesis-driven analyses of known pharmacogenes and their association with two common adverse drug reactions: increased diabetes risk and sleep disturbance. In working on this thesis, two key limitations became apparent. Firstly, inconsistencies in genotyping and phenotyping make some findings difficult to interpret. Secondly, the nature of my analysis using cross-sectional UK Biobank data makes it difficult to draw firm conclusions on the causal direction of any observations. Chapter five aim to address these limitations. Here, I describe the set-up of a clinical study to assess pharmacogenetic interventions in a psychiatric patient population. Although only pilot data is available, due to a pause in recruitment during the Covid-19 pandemic, I describe the scientific rationale for the study and outline the work conducted to set-up and gain ethical approvals for the study. In addition, I outline my contribution to drafting clinical guidelines for the implementation of pharmacogenetic testing in the NHS.

Impact Statement

This thesis aimed to establish the extent to which genetic variation contributes to therapeutic response to antidepressants and antipsychotics. Pharmacogenetics is a well-studied field, and there are already examples of pharmacogenetic interventions as standard of care within the NHS. In psychiatry, however, previous studies have been hindered by small sample sizes and inconsistent genotyping. There is evidence that genetic variation in certain genes can alter serum drug levels. Establishing the clinical significance of these changes is more challenging. Improving treatment response is a vital goal in psychiatry given the high side-effect burden, rates of treatment failure, and low adherence.

This thesis starts by investigating the available literature for two important gene-drug interactions: CYP2D6 variation and antipsychotic-induced hyperprolactinaemia and weight gain. The overwhelming finding in both meta-analyses was that the existing literature is too limited and heterogeneous to establish a clear relationship between gene and drug reaction. By elucidating some of the gaps in the existing literature, I was able to design subsequent analyses that could attempt to address these.

Chapter 2 uses UK Biobank data to consider the relationship between CYP2C19 and CYP2D6 metabolic phenotype groups and several adverse drug reactions. This had not been done before due to the challenge of assigning CYP450 phenotypes on a large scale. This chapter demonstrates a simple and accurate method to group participants to their appropriate metabolic phenotype groups. The gene-drug analyses presented in this chapter serve as a proof-of-concept, and the major finding was that more refined analyses (and, where possible, single drug analyses) are essential given the complexity of these gene-drug interactions. Chapters 3 and 4 do just this. Chapter 3 investigates diabetes risk in greater detail. Indeed, this chapter does reveal more clear results, and adds support to existing and emerging clinical guidelines suggesting that dose or drug alterations may be necessary for certain patients, considering their CYP450 genotype. Chapter 4 also considers a specific

adverse-drug reaction, sleep disturbance, and its relationship to CYP450 genotype. The findings show a clear role for CYP450 activity in mediating risk for sleep-related adverse reactions to certain drugs.

The final chapter of this thesis sets out the design and set-up of a clinical trial to investigate a pharmacogenetic intervention in psychiatry. This involves offering genetic testing so that patients and clinicians can adjust antipsychotic or antidepressant treatment following evidence-based guidelines. Although recruitment was interrupted during the Covid-19 pandemic, the impact of this work is clear. This will be the largest study of its type in the UK and will help establish the future of pharmacogenetic testing for psychiatry patients within the UK National Health Service (NHS). The early data available demonstrates that clinicians are highly interested in this question. The direct impact of this clinical work is already apparent through my contribution to writing the first NHS guidelines for pharmacogenetic testing for four antidepressants, in collaboration with Genomics England and NHS England. This work is set to become the first pharmacogenomics NHS service in the UK covering mental health, cardiovascular and cancer drugs.

Publication list

- Calafato MS*, **Austin-Zimmerman I***, Thygesen JH, Sairam M, Metastasio A, Marston L... Bramon E (2020). **The effect of CYP2D6 variation on antipsychotic-induced hyperprolactinaemia: a systematic review and meta-analysis**. Pharmacogenomics J, 20(5), 629-637. doi:10.1038/s41397-019-0142-9

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- Bhat A, Irizar H, Thygesen JH, Kuchenbaecker K, Pain O, Adams RA, Zartaloudi E, Harju-Seppänen J, **Austin-Zimmerman I**, Wang B, Muir R, Summerfelt A, Du XM, Bruce H, O'Donnell P, Srivastava DP, Friston K, Hong LE, Hall MH, Bramon E. **Transcriptome-wide association study reveals two genes that influence mismatch negativity**. Cell Rep. 2021 Mar 16;34(11):108868. doi: 10.1016/j.celrep.2021.108868.

- **Austin-Zimmerman I**, Wronska M, Wang B, Irizar H, Thygesen JH, ... Bramon E (2021)/ **The influence of CYP2D6 and CYP2C19 genetic variation on diabetes mellitus risk in people taking antidepressants and antipsychotics** Genes (Basel). 2021 Nov 3;12(11):1758. doi: 10.3390/genes12111758.

- Wang B, Giannakopoulou O, **Austin-Zimmerman I**, Irizar H, Harju-Seppänen J, Zartaloudi E, Bhat A, McQuillin A, Kuchenbäcker K, Bramon E. **Adolescent Verbal Memory as a Psychosis Endophenotype: A Genome-Wide Association Study in an Ancestrally Diverse Sample.** Genes (Basel). 2022 Jan 3;13(1):106. doi: 10.3390/genes13010106.

- Wannasuphoprasit Y, Andersen SE, Arranz MJ, Catalan R, Jurgens G, ... **Austin-Zimmerman, I***, Bramon E* (2021) **CYP2D6 Genetic Variation and Antipsychotic-Induced Weight Gain: a Systematic Review and Meta-Analysis**. Front. Psychol. 2022 Feb 3; 12. doi:10.3389/fpsyg.2021.768748.

- **Austin-Zimmerman I**, Levey DF, Giannakopoulou O, Zhou H, Denaxas S, ... Gelernter J (2021) **Genome-wide association study investigating genetic loci for self-reported sleep duration: a meta-analysis with data from UK Biobank and the Million Veteran Program** [In prep.]

* Indicates joint first or joint senior authorship.

Statement of Contributions

The work presented in this thesis would not have been possible without the valuable contribution of my supervisors and colleagues. This thesis was written entirely by me, and below I outline the input of colleagues to each chapter.

Introduction

This introduction was written and researched independently by me.

Chapter 1

CYP2D6 and antipsychotic-induced hyperprolactinaemia

An early draft of this had been prepared by Stella Calafato, Johan Thygesen and other co-authors when I joined the Bramon group for my PhD. I re-ran the search criteria and identified several additional papers to include. In addition, I was able to obtain unpublished data that allowed the inclusion of three further papers in the meta-analysis, almost doubling the sample size. I re-ran all the analyses and conducted several sub-analyses to consider the influence of diagnosis, sex, and age differences on the results. I re-wrote the introduction, methods, results and discussion to reflect the additional data. I am a first author of the associated paper, now published in The Pharmacogenomics Journal.

CYP2D6 and antipsychotic-induced weight-gain

The design of this study was based on the hyperprolactinaemia study described above. This work formed the basis of an undergraduate dissertation by Yanisa Wannasuphoprasit, for whom I served as supervisor alongside Elvira Bramon. I came up with the search terms and provided the R scripts to conduct the analyses and generate the necessary figures. The words describing this study included in this thesis are my own, but I include some of the tables and figures created by Yanisa, under my supervision. I am joint senior author on the associated paper, under final review in Frontiers in Psychology.

Chapter 2

The application for access to the UK Biobank data were completed by Andrew McQuillin, Elvira Bramon and Johan Thygesen prior to my arrival at UCL. The genetic data from the UK Biobank is managed centrally by a team led by Spiros Denaxas at the UCL Institute of Health Informatics Research. This group performed initial quality control and provided advice and guidance on data extraction. I extracted the necessary genetic and phenotype data from the UK Bioban data, which included identifying all potential drugs of interest from the self-reported data. I designed the method to assign CYP450 genotypes with valuable support from Johan Thygesen and Aritz Irizar. After the initial work on this, I established that it might be possible to include some missing rare variants, and conducted this work with the support of Aritz Irizar. All of the analyses of gene-drug interactions presented in this chapter were conducted independently by me.

Chapter 3

This work used the pre-processed dataset I created and described in chapter 2. I came up with the statistical analysis plan for this study and prepared R scripts for the statistical analysis. Some of the work presented in this chapter contributed to a Masters thesis by Marta Wronska, which I supervised along with Elvira Bramon. Marta adapted my R Scripts to add two additional variables to my pre-processed data: diabetes diagnoses and diabetes treatment. Using this dataset, Marta conducted regression analyses under my close supervision and we discussed the interpretation together, with additional input from Aritz Irizar and Elvira Bramon. Marta conducted valuable background research on diabetes risk in depression, which I added to for the resulting paper and this thesis. I am a first author on the associated paper, which is currently under review.

Chapter 4

This work used the pre-processed dataset I created and described in chapter 2. All statistical analyses and writing in this section of the chapter were conducted independently by me.

Chapter 5

This protocol was written by me and reviewed by Elvira Bramon and by the JRO study manager Suzanne Emerton. The Integrated Research Application System (IRAS) form was prepared by me and reviewed by Elvira Bramon and Suzanne Emerton. Further supporting documents (patient information sheet, informed consent form, GP letter) were prepared by

me based on UCL templates and using existing documents prepared by Andrew McQuillin. I undertook phlebotomoty training and led the sample collection for this study. I was assisted by my colleague Baihan Wang. Until the recruitment was paused as a result of the Covid-19 pandemic, I led the data collection and trial management. This has now been taken over by Eirini Zartaloudi, with whom I worked closely to hand over responsibilities.

Conclusions

The concluding remarks were written and researched independently by me.

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I would first like to thank my primary supervisor, Elvira Bramon, for her excellent guidance throughout this PhD, and for always being so positive and encouraging of my thoughts and ideas. Thanks also to my supervisors Andrew McQuillin and Johan Thygesen, for their invaluable support and advice over the years. Thank you to Karoline Kuchenbaecker, for offering such useful and thoughtful guidance. I couldn't have asked to be supported by a better group.

I am grateful to the many friends and colleagues who have supported this work at UCL. In particular, I would like to extend my sincere gratitude to my colleagues and mentors Aritz Irizar and Olga Giannakopoulou, who have taught me so much and without whom this work would not have been possible, and to Jasmine Harju-Seppänen who has made finishing a PhD during a pandemic significantly more enjoyable.

I am so lucky to have been supported by an incredible network of friends and family. Thank you so much to my little sisters, Lydia and Alexis, and to my step-mother, Ruth, my uncle, Nick, and my grandma, Natalie. I couldn't have made it through this degree without their love and encouragement. Thank you especially to my twin sister, Georgia, who is my best friend and greatest supporter. I am grateful to my husband, Josh, for making it possible for me to pursue this degree and for supporting me in everything that I do. Above all, I owe thanks to my parents, Fan and Keith, who provide the most loving and inspiring family life imaginable.

My sincere thanks to the Medical Research Council for funding this PhD, and to the participants of the UK Biobank, the Million Veteran Program, and the Pharmacogenetic Pilot Study for their vital contribution to science.

I dedicate this work to my late mother, Fan Austin, who would be so proud to see it complete.

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Introduction

Psychiatric diseases are estimated to affect over one billion people globally and are responsible for some 7% of global disease burden, as measured by disability adjusted life years (DALYs) [1, 2]. Psychiatric, or mental health, conditions account for five of the top 20 causes of global disease burden (with major depression ranking 5th, schizophrenia 7th, persistent depression/dysthymia 11th, and bipolar disorder 17th) [1]. Mental illness costs the UK economy £70–£100 billion per year (or 4.5% of gross domestic product)[1] [3, 4].

Historically, the classification of mental health conditions has been separated from more 'physical' neurological conditions such as epilepsy or Parkinson's disease. Though most agree this is counter to modern understanding of neuroscience, it has been demonstrated that the concept of 'dualism' has delayed progress in understanding the biological processes that underlie psychiatric disorders [1, 5]. In the absence of established biomarkers, psychiatric disorders are defined based on clinical observation, reported symptoms, and expert opinion [6]. Advances in the field of psychiatric genetics have identified hundreds of genetic risk variants that contribute to these mental diseases, and may help update disease classification, improve accuracy of diagnoses, and develop novel treatment strategies.

The World Health Organisation (WHO) International Classification of Diseases (ICD) 10[2] classification system separates psychiatric disorders into several groups, with codes ranging from F00 to F99. The focus of this thesis will be primarily focused on subcategories: F20-29 Schizophrenia, schizotypal and delusional disorders, and F30-39 Mood [affective] disorders [7]. This latter category includes depressive disorders, both with and without psychotic symptoms. In this section, I will give a brief introduction to the current

[1] Christensen *et al* provide a useful interactive breakdown of treatment costs by different types of mental disorder, with schizophrenia being second only to developmental disorders: `https://ncrr-au.shinyapps.io/mental-cost/#cost-measure-panel` [3]

[2] The updated ICD-11 classification was released and adopted by the WHO in May 2019. In keeping with the data available for use in this thesis, and current clinical practice in the UK, the previous version of ICD-10 will be referred to throughout this manuscript.

understanding of the symptoms, treatment, and genetics of depression and psychosis.

Depression and psychosis

As described above, major depressive disorder is the most common psychiatric disorder, and a leading cause of worldwide disability, with over 264 million people reportedly experiencing depression over their lifetime [1, 8–10]. The WHO defines depression as:

> *"characterised by persistent sadness and a lack of interest or pleasure in previously rewarding or enjoyable activities"[11].*

Diagnosis of depression is roughly twice as common in women as men; the lifetime prevalence is around 20-25% in women and around 7-12% in men [12]. Depression is often seen alongside other psychiatric disorders, such as bipolar disorder, generalised anxiety disorder and social anxiety disorder [13].

Symptoms of depression are wide-ranging and vary between patients. In the UK, a diagnosis of depression would typically be made following an assessment by a psychiatrist. The 'core symptoms' of depression are: depressed mood, loss of interest in everyday activities, and reduction in energy. Other officially listed symptoms are: loss of confidence or self-esteem, unreasonable feelings of self-reproach or inappropriate guilt, recurrent thoughts of death or suicide, diminished ability to think/concentrate or indecisiveness, change in psychomotor activity with agitation or retardation, sleep disturbance, change in appetite with weight change [14]. According to guidelines by the National Institute of Health and Care Excellence (NICE) in the UK, a diagnosis can be made when at least four of these symptoms are present at reasonable severity for at least two weeks. Specific wording varies between countries, but the broad criteria is similar across most of the world.

There are varying theories on the neurobiological basis of depression. Perhaps the most well known theory is the monoamine hypothesis[3], which states that depression is a result of a deficiency in the monoamine neurotransmitters; 5-hydroxytryptamine (5-HT), noradrenaline and dopamine. This is the theory upon which all available antidepressants are based [15–17]. However, it is by no means conclusive and there is evidence to suggest this is in fact a downstream or secondary effect of other primary causes [15]. Altered transmission of GABA (gamma-aminobutyric acid) [18, 19] and glutamate [18, 20] have also

[3]Monoamine in this context refers to monoamine neurotransmitters. These are neurotransmitters that contain one amino group attached by a two-carbon chain to an aromatic ring. Examples of monoamine neurotransmitters are 5-hydroxytryptamine (5-HT or serotonin) and the catecholamines adrenaline, dopamine and noradrenaline.

been associated with depression. There is reasonable evidence for the involvement of these systems, although drugs that target them have not been shown to have antidepressant effects [21]. Some studies have demonstrated that stimulation of specific brain regions (e.g., lateral frontal and temporal cortices, insula, and cerebellum) can have antidepressant effects [15, 22]. Further neuroimagining research is essential to determine if dysfunciton in these brain regions could give rise to depression. Stress hormones, inflammatory cytokines, and altered HPA (hypothalamic-pituitary-adrenal) axis activity have also been associated with depression pathogenesis [15]. Evidence suggests this may play a stronger role in women, which perhaps gives insight to the increased frequency of major depression in women [23]. Importantly, none of these theories are mutually exclusive. Depression is multi-faceted and complex, and it is likely that the neurobiology can vary between individuals and across the course of the illness.

There is a clear genetic component to depression, with heritablity estimates from twin studies as high as 37%, although the single nucleotide polymorphism (SNP)-based heritability is estimated at closer to 10%[4] [24]. Several candidate genes have been identified for major depression but, like other psychiatric traits, depression is a highly polygenic disease caused by complex interaction between environmental factors and multiple common genetic variants with small individual effect [13, 24, 25]. The most recent genome-wide association study (GWAS) of major depression identified 102 independent genomic risk loci and 269 genes associated with depression risk [24]. Several of the loci and genes highlighted in this study are involved in synaptic structure and neurotransmission. In addition, there is evidence of a shared genetic risk between depression and other neuropsychiatric traits [26, 27].

Psychosis is an umbrella term that describes a group of major psychiatric disorders in which a patients' perceptions, thoughts, mood and behaviour are altered significantly [28]. Psychosis is thought to affect approximately 3% of the global population [29]. Schizophrenia is the most common psychotic disorder, estimated to affect 0.3-0.7% of the global population [30]. Schizophrenia occurs across the world with a consistent frequency and seems more common in men (the incidence is approximately 15/100,000 in men versus 10/100,000 in women, though interestingly this sex difference is not observed in prevalence estimates) [31]. The WHO defines schizophrenia as:

[4] For more on the topic of the missing heritability problem see: Sandoval-Motta S *et al* The Human Microbiome and the Missing Heritability Problem. Front Genet. 2017;8:80

> *"characterised in general by fundamental and characteristic distortions of thinking and perception and affects that are inappropriate or blunted. Clear consciousness and intellectual capacity are usually maintained although certain cognitive deficits may evolve in the course of time. The most important psychopathological phenomena include thought echo; thought insertion or withdrawal; thought broadcasting; delusional perception and delusions of control; influence or passivity; hallucinatory voices commenting or discussing the patient in the third person; thought disorders and negative symptoms"[11].*

The three core symptoms of schizophrenia are hallucinations, delusions or thought disorder (disorganised speech). According to NICE guidelines and ICD-10 criteria, at least two of these three symptoms must be present and must be causing distress or impacting usual life in order for a diagnosis of schizophrenia to be made [28]. Importantly, psychotic symptoms can emerge in other psychiatric conditions such as major depression or bipolar disorder. A diagnosis of schizophrenia is made according to ICD-10 criteria and is typically reliant on duration of symptoms and/or number of episodes [7, 32]. Due to the syndromic nature of schizophrenia and the symptom overlap with other psychiatric diseases, diagnosis can be challenging. Many patients experience different symptoms to different degrees, and the course of disease and response to treatment is highly varied.

The major symptoms of schizophrenia are grouped into two categories, positive and negative. The positive symptoms are changes to thoughts and behaviour, often due to delusions and hallucinations. Negative symptoms include social withdrawal, lack of interest and motivation, affective flattening (reduction in emotional expression), alogia (difficulty or inability to speak), and cognitive impairment [28]. Co-morbid physical and psychiatric disease or disability is very common. The onset of disease is usually seen in between the ages of 16-30. Onset can be sudden but is often preceded by a prodromal phase where patients are considered to be in a high-risk mental state [33]. There is good evidence that early access to treatment improve prognosis and patient outcomes [31, 33]. The duration of untreated psychosis (DUP) is closely related to overall prognosis. A recent clinical trial found that DUP was associated with treatment response up to five years after the first treatment was provided [34]. In recent years, early intervention services have become more common in the UK, which is of great value to patients.

The societal burden of schizophrenia is very high. Patients with schizophrenia experi-

ence a 10-20 year reduction in life expectancy on average, a mortality gap that seems to be increasing despite ongoing research into the disease and its treatment [35–37]. People with schizophrenia are less likely to work, with unemployment rates reaching 80-90% [35, 38]. This, along with the high cost of health care provision for schizophrenic patients (especially for hospital admissions), results in an estimated annual cost of schizophrenia in England of £11.8 billion, £7.2 billion of which is a cost to the public sector [36].

There are many theories as to how and why schizophrenia develops. Perhaps the most prominent of which is the neurodevelopmental hypothesis, put forward by Weinberger, Murray and Lewis in the 1980s [38–40]. This hypothesis suggests that genetic and environmental factors can cause neurodevelopmental abnormalities as early as the first trimester of embryonic development, which can later result in the activation of pathological neural circuits that give rise to schizophrenia [40]. This theory is supported by neuroimaging evidence, which shows a reduction in grey matter and increased lateral ventricular volume in schizophrenic patients. Interestingly, the reduction in grey matter seems to be associated with antipsychotic treatment. Though it appears to progress throughout the course of the disease, even in untreated patients, the reductions are more pronounced in treated patients [41–43].

Another theory on the pathogenesis of schizophrenia is disturbed dopaminergic transmission. It is thought that this gives rise to the delusions and hallucinations seen in schizophrenia [43]. However, much like the neuroimaging abnormalities, this is not specific to schizophrenia and is seen in many other psychiatric conditions. Altered glutamatergic transmission has also been related to schizophrenia and is thought to be partly responsible for the cognitive dysfunction experienced by patients [44, 45]. Multiple brain regions (including prefrontal cortex, thalamus, caudate) have been associated with schizophrenia, but it is likely that other regions and circuits are also involved [46]. More recent theories point to an immune response, with increased oxidative stress and inflammatory mediators implicated [47].

Like depression, schizophrenia has a strong genetic component, with heritability estimates as high as 80% [48, 49]. It is a highly polygenic disease, with over 270 genetic risk loci identified in the most recent GWAS, including several risk loci in regions associated with glutamatergic and dopaminergic transmission [48–50]. Several of these SNPs were highly expressed in brain regions. In addition to these SNPs, several copy number

variants (CNVs: large sections of deleted or duplicated genome) have also been linked to increased risk of schizophrenia [48, 51–53]. There is evidence of substantial shared genetic risk between schizophrenia and other psychiatric traits, including bipolar disorder and autism spectrum disorder [27, 54–56].

Treatment

NICE recommends a combination of talking therapies and pharmacological interventions in the treatment of both depression and schizophrenia [28, 57]. However, There is a significant treatment gap in mental healthcare in England, and 75% of people with a psychiatric diagnosis receive no treatment at all [4]. The use of both antidepressant and antipsychotic medications has increased steadily in recent years. Antidepressant drugs were the third most commonly prescribed drug group in 2018, with 70.9 million prescriptions across the United Kingdom – an almost two-fold increase since 2008 [58, 59]. It is estimated that almost 20% of the British adult population has been prescribed an antidepressant at some stage [58–60]. A similar trend is seen in the prescription of antipsychotics, with an increase from eight to 12 million prescriptions between 2008 and 2018 [59].

Antidepressant drugs are used most frequently to treat depression, but are also used to treat various anxiety disorders, obsessive compulsive disorder and chronic pain. They are associated with common side effects including weight gain, lack of motivation, sleep disturbance, and dry mouth, as well as more severe but rarer effects, including increased suicidal thoughts and prolongation of QT interval with increased risk of arrhythmias. There are several types of antidepressants, categorised into four main groups: selective serotonin reuptake inhibitors (SSRIs), serotonin noradrenaline reuptake inhibitors (SNRIs), tricyclic antidepressants and monoamine oxidase inhibitors (MAOIs).

SSRIs, such as citalopram and fluoxetine, are the most widely prescribed antidepressants in most countries. They work by preventing 5-HT reabsorption to the presynapitic neuron, thus raising the extracellular concentration of 5-HT and increasing its binding to postsynaptic receptors. SNRIs work in a similar way but are less selective for 5-HT and also prevent the reuptake of noradrenaline. Examples include tramadol, venlafaxine and duloxetine. Tricyclic antidepressants work by blocking the 5-HT transporter (SERT) and the noradrenaline transporters (NET), which again results in an increase in the synaptic concentration and enhanced neurotransmission. Examples of tricyclic antidepressants include amitriptyline, clomipramine and doxepin. MOAIs work by inhibiting the activ-

ity of monoamine oxidase A and monoamine oxidase B. This prevents the breakdown of monoamine neurotransmitters and thus increases the synaptic availability. Early MAOIs, such as phenelzine and selegiline, bind irreversibly to monoamine oxidase enzymes. Newer drugs, such as moclobemide, bind reversibly.

Antipsychotic drugs are commonly used in the treatment of psychosis. Though effective at alleviating the symptoms of psychosis, antipsychotics are associated with common and severe side effects. These include weight gain, raised cholesterol, diabetes, sedation, movement disorders, raised prolactin, sexual dysfunction and osteoporosis. There is significant variation in clinical response to antipsychotics and approximately 30% of patients do not respond to treatment at all [61, 62]. The occurrence and severity of adverse events can also differ drastically between drugs and individuals [63]. Over 70 antipsychotics are licensed for use across the world. These can be categorised into two main groups: typical (first generation) and atypical (second generation). Initially it seemed that atypical drugs produced fewer or less severe side effects, but this may not be the case.

Antipsychotics work by reducing dopaminergic transmission by either dopamine receptor D2 antagonism or partial agonism. Typical antipsychotics work by blocking dopamine D2 receptors in the dopaminergic pathways of the brain. Atypical antipsychotics also block dopamine receptors, but have additional blocking effects on 5-HT receptors, particularly 5-HT2A and 5-HT2C. Examples of typical antipsychotics include benperidol, fluphenazine, haloperidol, chlorpromazine, perphenazine and zuclopenthixol. Examples of atypical antipsychotics are amisulpride, risperidone, aripiprazole, clozapine, olanzapine and quetiapine.

Both antidepressant and antipsychotic medication provide essential and often lifesaving treatment for many patients. However, they are also associated with a range of common and sometimes serious adverse drug reactions including sedation, weight gain, movement disorders, and an increased risk of developing diabetes mellitus [64, 65]. Although all available drugs are expected to meet high standards of safety and efficacy, there can be a significant degree of individual variability in response to a given drug. Adverse drug reactions are very common and can be severe. An estimated 5% of all hospital admissions are caused by adverse reactions to a medication [66]. An adverse drug reaction is defined by the WHO as:

"A harmful effect suspected to be caused by a drug. This term has been used

quite loosely to include all kinds of adverse events, many of which are not 'reactions' in the strict sense at all, and have not been subject to any assessment of causality. The term is properly reserved for late-stage analysis when the association between a medicine and an adverse effect has moved beyond 'unmeasurable' or 'uncertain'" [67].

Clinicians often rely on trial and error to identify the best treatment for a patient, and no prescription is devoid of risk of adverse reaction. These problems are not unique to psychiatry, but the ramifications in this field are considerable, as patients with certain psychiatric diseases, in particular psychosis, are already less compliant with their medication [68–71]. The risk of failed treatment, due to adverse drug reactions, non-compliance, non-response or partial response, is considerable and contributes to the poor quality of life and reduced life expectancy experienced by many patients [72–75].

Pharmacogenetics in psychiatry

A significant problem facing prescribers of psychotropic medication is the high amount of inter-individual variability in treatment response. Lack of efficacy and high rates of adverse events are frequently cited problems, which contribute to poor patient adherence and thus worsening outcomes. Multiple variables contribute to individual differences in therapeutic response, including environmental factors, lifestyle, disease severity, comorbidities or concomitant medication. These difference may, in part, be explained genetic variation in the enzymes, receptors and other proteins involved in the pharmacokinetic or pharmacodynamic processes. This thesis aims to elucidate some of the possible genetic reasons for these differences in treatment response.

The field of pharmacogenetics aims to understand and quantify the relationship between these genetic variants and drug response; both in terms of therapeutic success and risk of adverse drug reactions. It is possible that genotyping patients in advance of prescribing treatment could allow for these differences to be taken into account and lead to more successful treatment outcomes. Pharmacogenetic assessments are already in use in many areas of medicine, including oncology, and have resulted in significant improvements in treatment response [76, 77]. However, despite a growing body of evidence, genetic data is not yet routinely considered in psychiatric care [61, 78–84]. Pharmacogenetic testing has the potential to allow physicians and patients to make more informed decisions when pre-

scribing psychotropic treatment, which could result in significant clinical benefit to patients as well as the reduction of treatment costs [85].

There are a large number of alleles, haplotypes and genes already known to be of pharmacogenetic importance. There are currently 68 known pharmacogenes (i.e., genes of known pharmacogenetic importance), that have been defined as 'very important' by the PharmGKB consortium[5][86, 87]. Several of these 'very important pharmacogenes', predominantly members of the cytochrome P450 enzyme family, are catalogued by the Pharmacogene Variation Consortium[6] [88, 89]. The Clinical Pharmacogenetic Implementation Consortium (CPIC) has published clinical guidelines for 14 of these genes, suggesting dose alterations for several drugs based on genotype [79, 80]. Recent evidence suggests that 99.5% of individuals carry a risk variant in at least one of these genes, demonstrating the potential of pharmacogenetic testing to guide clinical practice [90].

There are several examples of pharmacogenetic interventions already in use in the NHS. Thiopurines (azathioprine, mercaptopurine, and thioguanine) are used to treat inflammatroy bowel disease, rheumatoid arthritis, and other immune-related diseases [91, 92]. These drugs are metabolised by thiopurine methyltransferase (encoded by the gene *TPMT*). Variants in this gene that decrease enzymatic activity greatly increase the risk of toxicity [92, 93]. Therefore, genetic testing is mandatory prior to prescription of this drug. Heterozygous individuals (carrying one decreased function and one wild-type copy of the gene) are started at a decreased dose. Individuals who are homozygous carriers of the decreased function gene cannot be treated with thiopurines [92, 93]. Another example is in HIV, where patients with the *HLA-B*57:01* mutation cannot be treated with abacavir [94]. The drug 5-fluorouracil, which is used to treat several types of cancer, works by inhibiting DNA and RNA synthesis. The metabolism of this drug is metabolised by dihydropyrimidine dehydrogenase (DPD), which is encoded by the gene *DYPD*. There are several known variants in *DYPD* that result in non-functional copies of the enzyme. Homozygous carriers of this mutation are at significantly greater risk of fatal toxicity following treatment with

[5]Pharmacogenetic Knowledge Base (PharmGKB) is a body funded by the United States National Institute for Health and based at Stanford University. It aims to collect, curate and disseminate information about clinically actionable gene-drug associations and genotype-phenotype relationships (`www.pharmgkb.org/`).

[6]The Pharmacogene Variation (PharmVar) Consortium is a centralised pharmacogene data repository, focusing on cataloging allelic variation of genes impacting drug pharmacokinetics, pharmacodymanics and response. It organises a unified nomenclature system for the pharmacogenetic/genomic community. It is central body working closely with PharmVar, the Pharmacogenomic KnowledgeBase (PharmGKB), and the Clinical Pharmacogenetic Implementation Consortium (CPIC), both of which will be referenced frequently throughout this thesis. For more detail see: `www.pharmvar.org` and `www.cpic.org`.

flurouracil, and thus pharmacogenetic testing is mandatory [95, 96]. The use of pharmacogenetic interventions is growing, and clinical guidelines for several additional drug/gene pairs are expected to be introduced to the NHS in the coming years (see chapter 5).

There have been some genome-wide association studies (GWAS) of antidepressant and antipsychotic drug response. In the case of antidepressants, the findings have been largely negative, which can be explained at least in part by the small sample sizes [97–99]. One study investigating drug response in a Korean sample did identify one genome-wide significant association in the *AUTS2* gene, which has been previously linked with both autism and schizophrenia [100]. A GWAS on side-effect risk in 2012 also found one genome-wide significant association in the *EMID2* gene [101]. Neither of these hits have been successfully replicated as yet. For antipsychotics there have been some more interesting findings. Several early GWAS investigating responses to single drugs (rather than grouped by class) yielded trend-level associations in *NPAS3*, *ATP2B2*, and *ANKS1B*, which was promising given the limited sample sizes [102]. In addition, a GWAS in European subjects taking olanzapine found a variant in the *DRD2* gene to be associated with improved working memory [103]. This gene encodes the dopamine D2 receptor, which is clearly relevant to antipsychotic pharmacodynamics. Several other GWAS have reported associations in genes involved in intracellular signal transduction (*PDE4D*), sodium/potassium transport (*ATP1A2*), and glutamatergic transmission (*GRM7* and *GRIA4*) [104–107]. Despite these promising early findings, most of these studies are relatively out of date, underpowered and lack replication.

In recent years we have seen the creation of several large-scale biobanks, which have opened up huge areas of potential for genomic and pharmacogenomic research. Several of these biobanks, such as UK Biobank, have designed their own genotyping assays to ensure, among other requirements, reasonable coverage of known pharmacogenes. This offers huge promise in overcoming the power issues faced by many smaller, prospective clinical studies, as well as allowing for more novel genome-wide approaches to pharmacogenetics.

Aims

The broad hypothesis of this thesis is that genetic variation in the proteins responsible for pharmacokinetic and pharmacodynamic processes contributes to varied clinical outcomes in response to psychotropic medication, and that prescription according to genotype could improve response, reduce side effect burden and increase patient adherence. I aim to address

this hypothesis in three key ways:

1. Conduct systematic reviews and meta-analyses to evaluate and quantify the current evidence for an association between *CYP2D6*, arguably the most important known pharmacogene, and two common adverse drug reactions to antipsychotics: hyperprolactinaemia and weight-gain.

2. Develop a computational method to assign large numbers of biobank participants into phenotypic groups based on genetic variation in CYP450 enzymes, and use this to test several gene-drug-adverse event relationships.

3. Design, plan and start a prospective clinical trial to test a new pharmacogenetic intervention for patients taking antipsychotics in the NHS.

Chapter 1

Systematic Review and meta-analyses of CYP2D6 variation and adverse drug reactions to antipsychotics

Some of the work contained in this chapter has been published in Calafato and Austin-Zimmerman *et al.*, (2019) [108] and Wannasuphoprasit *et al.*, (2022) [109] - see appendix A.1.

Abstract

Hyperprolactinemia is a known adverse drug reaction to antipsychotic treatment. Antipsychotic blood levels are influenced by cytochrome P450 enzymes, primarily CYP2D6. Variation in CYP450 genes may affect the risk of antipsychotic-induced hyperprolactinemia. I undertook a systematic review and meta-analysis to assess whether *CYP2D6* functional genetic variants are associated with antipsychotic-induced hyperprolactinemia. The systematic review identified sixteen relevant papers, seven of which were suitable for the meta-analysis (n=303 participants, including 134 extreme metabolisers). Participants were classified into four phenotype groups as poor, intermediate, normal (extensive) and ultra-rapid metabolisers. I calculated the standardised effect size for each primary study and, using a random effects meta-analysis, I obtained the pooled effect size (Cohen's d). I found no significant differences in prolactin levels between *CYP2D6* metabolic groups. In addition to this work, I supervised an undergraduate dissertation that followed the same methodology to investigate antipsychotic-induced weight gain. In this study, we found a suggestive association between *CYP2D6* poor metabolisers and increased BMI. These findings collectively suggest limited current evidence supports of using *CYP2D6* genotyping to reduce risk of

these common antipsychotic adverse drug reactions. However, statistical power is limited and there is high heterogeneity between the included studies. Thus far, very few prospective clinical trials have investigated CYP450 genotype status and antipsychotic-related adverse events. Future studies with larger, more diverse samples and comprehensive coverage of the CYP2D6 region will be necessary to understand these complex gene-environment interactions.

1.1 Introduction

Antipsychotics are the mainstay treatment for schizophrenia and are also licensed for use in bipolar disorder [110, 111]. Currently, the prescription of antipsychotics is largely empirical and patients may have several cycles of failed medications due to poor response and/or adverse events [112–114]. Antipsychotic adverse reactions are diverse and potentially serious, including metabolic as well as extrapyramidal side effects [115]. The emergence of adverse reactions is a contributing factor to poor adherence to antipsychotics [116]. Susceptibility to adverse reactions is likely to be dependent on multiple factors that influence drug metabolism and/or their action [102, 112].

Prolactin is a hormone produced in the anterior lobe of the pituitary gland. A rise in prolactin blood levels (hyperprolactinemia) is a common adverse reaction to several antipsychotics, having been reported in 47-52% of women and 26-28% of men [117–119]. A diagnosis of hyperprolactinemia is made when serum prolactin levels exceed the upper limit of well-established ranges for particular age/sex groups [120] (see appendix A.2 for normal ranges in men and women). Mild hyperprolactinemia can be asymptomatic, but when prolactin levels exceed twice the upper normal limits various symptoms may be observed. Women may report galactorrhoea and amenorrhea, and men may experience gynecomastia, decreased libido and erectile dysfunction [120, 121]. Long term hyperprolactinemia can cause osteoporosis in both sexes, with its associated increased risk of bone fractures [122]. Hyperprolactinaemia is therefore an adverse reaction that impacts on quality of life and has potentially serious consequences. Typical antipsychotics, such as haloperidol, are the most common cause of drug-induced hyperprolactinemia [123]. With the exception of risperidone and amisulpride, most atypical antipsychotics are less likely to cause hyperprolactinemia. The atypical antipsychotic aripiprazole can in fact be used independently or co-prescribed with other antipsychotics to correct or reduce antipsychotic-induced hyperprolactinaemia [123]. For prolactin raising antipsychotics, such as risperidone, prolactin

levels have been shown to be positively correlated with antipsychotic serum levels [124, 125].

The metabolic side effects of antipsychotics are an important concern in the successful treatment of patients with psychosis. Antipsychotics are known to cause weight gain, disrupt glucose metabolism, and to increase serum cholesterol and triglyceride levels. These factors combined can lead to the development of a metabolic syndrome, which has serious negative health implications, including increasing the risk of developing type 2 diabetes mellitus and cardiovascular diseases [126]. A meta-analysis in 2013 found that metabolic syndrome has an estimated prevalence of 32.5% in patients with schizophrenia [127]. The same study found that 49.9%, of all patients were clinically obese. The increase in obesity, metabolic syndrome and the related health risks are some of many factors contributing to reduced life expectancy for patients with schizophrenia; various estimates put the figure between 10 and 20 years below that of the general population [74, 128].

Antipsychotic-induced weight gain is most common in the atypical antipsychotics, and in particular clozapine and olanzapine [126, 129–132]. Several possible theories have been put forward as to the underlying cause, which all primarily agree that it is a result of increased appetite and thus increased food intake and reduced physical activity seen in patients taking antipsychotics. A 2007 study found that the orexigenic effects of antipsychotics appear to be mediated by activation of the hypothalamic AMP-activated protein kinase, which is linked to inhibition of histamine H1 receptors [133]. Further studies have identified alterations in neuropeptide levels, with increased leptin levels and decreased adiponectin levels seen in patients taking olanzapine [134, 135]. Altered ghrelin levels, a hormone known to increase appetite and stimulate the deposition of adipose tissue, have also been associated with antipsychotic use [135]. Importantly, these changes are believed to be a direct result of the antipsychotic drugs, rather than a secondary effect of the weight gain [126]. The weight gain and increased adipose tissue storage then increases lipid and glucose levels, which leads to insulin resistance and the release of triglycerides and very low density lipoproteins from adipocytes [126, 131]. These findings suggest that a combination of factors, including poor diet and sedentarism, come together to cause the weight gain seen in many patients with psychosis, which drastically increases the risk of developing metabolic syndrome.

A recent review paper summarised the evidence for a dose-dependent relationship between antipsychotics and adverse event risk, including hyperprolactinaemia, diabetes risk,

weight-gain and sedation [136]. They concluded that there was significant evidence that most antipsychotics elicit a dose-dependent increase in serum prolactin levels, although they note that quetiapine, aripiprazole and clozapine do not seem to increase prolactin levels and may even be associated with decreased risk of hyperprolactinaemia [136–138]. Almost all antipsychotics are associated with some degree of weight-gain, but literature on the dose-dependent nature of this relationship is mixed [136]. A meta-analysis of 14 studies and almost 6,000 patients found evidence of a dose-dependent increase in risk for extreme weight-gain (more than 7%) for patients taking olanzapine, quetiapine, and risperidone [139]. However, a literature review in 2009 demonstrated inconsistencies in the literature, and concluded that there is limited evidence of a dose-dependent relationship for amisulpride, aripiprazole, ziprasidone, and quetiapine [140].

Where an adverse drug reaction is dose-dependent, it follows that genetic or environmental factors that can influence serum level of the drug might in turn increase the risk of these adverse drug reactions. As discussed in the introduction, antipsychotic drugs are metabolised by the cytochrome P450 family of enzymes (CYP450), primarily by CYP2D6, CYP2C19, CYP3A4 and CYP1A2 [102, 107]. Given that antipsychotics serum levels are influenced by the functional status of CYP450 enzymes, genetic variation in these enzymes may explain some of the inter-individual difference in antipsychotic-induced hyperprolactinemia or weight gain [141].

CYP2D6 constitutes a major metabolic pathway for many antipsychotics, including haloperidol, risperidone, aripiprazole and zuclophenthixol [107, 142]. The pharmacogenetics of *CYP2D6* is covered in greater detail in chapter 2. Variation in the *CYP2D6* gene is known to influence enzyme activity and individuals can be classified as poor, intermediate, normal (extensive), or ultra-rapid metabolisers [143] (see table 2.3). Over 70 allelic variants of *CYP2D6* have been identified, including fully functional, reduced function, and non-functional alleles [142]. Individuals considered ‘poor metabolisers’ (PM) are those who carry two copies of any of the non-functional alleles (either homozygotes or heterozygotes). The intermediate metaboliser phenotype (IM) is typically a result of one non-functional allele and one reduced function allele. The most common phenotype, extensive or normal metabolisers (NM), results from one or two alleles with normal function. The ultra-rapid phenotype (UM) is less well defined, usually relating to copy number variations in *CYP2D6* [142].

Assigning individuals to these four phenotypic groups is not entirely straight-forward. The Human Cytochrome P450 Nomenclature Committee, established in 1999, was set-up in order to simplify and standardise the naming of the various polymorphisms discovered. This committee is now part of the Pharmacogene Variation (PharmVar) Consortium (`www.PharmVar.org`). Based on pre-existing guidelines, the standardised nomenclature system established is referred to as the star (*) allele system [88, 89]. This system assigns each haplotype a unique label made up of the gene names (e.g., *CYP2D6*), followed by the major star allele (e.g., *CYP2D6*2*) and then any sub-alleles (represented by a numerical string or a letter e.g., *CYP2D6*2.001/*2A*). The metabolic phenotype is assigned based on the diplotype combination of star alleles for each given CYP450 gene.

The wild-type allele, or consensus allele, is referred to as *1 (e.g. *CYP2D6*1*, *CYP2D6*1* etc.). This defines the reference sequence. The *1 alleles are typically described as the absence of any of the defining variants of another star allele. In most cases the *1 allele is the most common. A change in this reference sequence (a sequence variation) could be either a single nucleotide polymorphism (SNP) or a nucleotide insertion or deletion (indels). When a new sequence variation that results in a functional change to the protein is identified, such as a premature stop codon or splice defects, it is assigned a numeric label. Sub-alleles are defined as additional variants that don't result in a further change to the protein but may alter gene expression. These sub-alleles are allocated a numeric string and letter in addition to the number. The number-letter combination (e.g. *3B, as opposed to *3.002) is most frequently reported.

Generally, the most frequent version of the haplotype is discovered first, so naturally becomes the defining sequence for that numeric group (e.g. *CYP2D6*2A*). However in some cases, latterly-discovered sub-variants have been found to be more frequent. In these cases the genes are not typically re-ordered to reflect frequency [88, 89, 144].

If multiple effective polymorphisms are identified within the same haplotype, the star allele that results in the most sever functional consequence should be assigned. For example, if an individual has the defining variants for both *CYP2D6*2* and *CYP2D6*3* then the *3 allele would be assigned because that results in a non-functional enzyme, whereas *2 has normal function.

There is substantial evidence showing a relationship between *CYP2D6* genotypes and clinical outcomes to psychotropic medications, with poor metabolisers being more suscep-

tible to toxicity [145, 146] and ultra-rapid metabolisers being less likely to improve when treated with standard doses. Thus pharmacogenetic testing in the CYP450 region has the potential to benefit clinical practice [147–150].

In a systematic review and meta-analysis, Fleeman *et al* (2011) showed an association between *CYP2D6* genotype and extrapyramidal adverse effects, but, due to a lack of available studies at the time, they did not explore its influence on hyperprolactinaemia or weight gain [151]. In a more recent systematic review, Dodsworth *et al* (2018) looked at the impact of *CYP2D6* variation on side-effects caused by risperidone specifically in children and adolescents [152]. They concluded that clinical impact of the relationship between CYP2D6 metabolic phenotypes and risperidone levels on adverse side effects required further investigation. In this chapter, I have conducted a systematic review and meta-analysis to assess whether CYP2D6 functional genetic variants are associated with serum prolactin levels in individuals taking antipsychotics. In addition, I will discuss the findings of a similar study investigating antipsychotic-induced weight gain.

1.2 Methods

1.2.1 Search strategy and selection criteria

I searched the electronic databases PubMed, CINHAL, EMBASE, Medline and PsychINFO for literature published from January 1995 to July 2019 using the following search terms: (Cytochrome* or CYP* or P450*) and (antipsychotic* or neuroleptic* or risperidone or olanzapine or thioridazine or perphenazine or fluphenazine or zuclopenthixol or haloperidol or chlorpromazine or clozapine or quetiapine or ziprasidone or flupentixol or flupenthixol or benperidol or levomepromazine or methotrimeprazine or pericyazine or periciazine or pimozide or promazine or sulpiride or trifluoperazine or amisulpride or sertindole or zotepine) and (genot* or allel*) and (PRL or prolactin or hyperprolactinemia or hyperPRL or galactorrhoea or infertility or period* or amenorrhoea or hirsutism or gynecomastia or erectile dysfunction). I included a wide range of antipsychotics in our search to ensure that all studies investigating a link between hyperprolactinemia and CYP2D6 genotypes were identified. However, only studies involving antipsychotics that are known CYP2D6 substrates were included in the meta-analysis. I included only studies published in English. In addition, I searched review articles for any other relevant studies. I selected all suitable papers based on the inclusion/exclusion criteria outlined in table 1.1. This assessment was conducted independently by myself and a colleague (Stella Calafato) and any disagreement

was resolved by consensus or involvement of a third reviewer if required. The search process and results are described in figure 1.1. I reviewed all primary studies to ensure they had no sample overlap and contacted authors for clarification where necessary.

Table 1.1: Meta-analysis inclusion and exclusion criteria

Inclusion criteria	Randomised controlled or non-randomised trials, observational studies Samples genotyped for *CYP2D6* functional polymorphisms Blood/plasma prolactin levels measured and reported Patients treated with antipsychotics or healthy volunteers given a single oral dose of antipsychotic
Exclusion criteria	Single case report, narrative reviews, systematic reviews, opinions, editorials, conference abstracts, in vitro studies Participants not genotyped for *CYP2D6* gene Prolactin levels not measured/reported Studies in which aripiprazole was used, since despite being metabolised by CYP2D6, it tends to decrease prolactin levels

1.2.2 Data extraction

I extracted the data from the included studies, and SC reviewed the extracted data to ensure accuracy. The outcome of interest was the mean blood or plasma prolactin levels (ng/ml) defined as a quantitative variable. I also included studies with hyperprolactinemia defined as a categorical variable (present/absent). Genotypes for the *CYP2D6* gene were also extracted. I contacted the authors of 11 primary studies where data required for the meta-analysis was mentioned, but not actually reported in the paper. This enabled the inclusion of three additional primary studies.

1.2.3 Statistical analysis

I conducted a random effects meta-analysis to investigate the association between CYP2D6 genetic variation and prolactin levels. Study participants were classified into four groups reflecting their metabolic phenotype as described in table 2.3. The following comparisons were performed: (i) poor versus normal metabolisers, (ii) intermediate versus normal metabolisers, (iii) combined poor and intermediate against the combined normal and ultra-rapid metabolisers.

For each primary study, I calculated the effect size as the standardised difference in means between the two groups being compared (Cohen's d [153]). I used a random-effects meta-analysis with weighting by inverse variance due to the variability in methods across the primary studies, including diverse participants and a range of antipsychotic drugs used

for their treatment. I assessed heterogeneity between primary studies using the I^2 statistic [154]. Publication bias was assessed using Egger's test [155]. I used R version 3.4.3 with the package "meta" to conduct the meta-analysis (`https://CRAN.R-project.org/package=meta`).

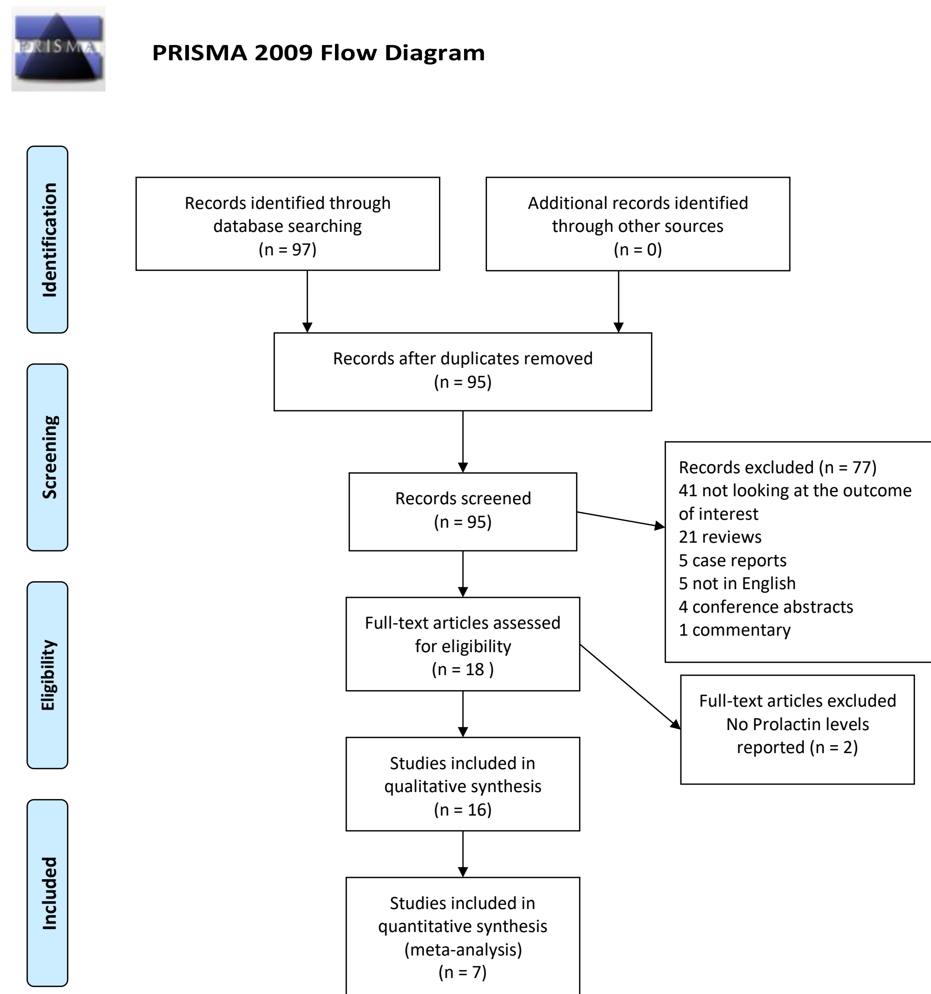
1.2.4 CYP2D6 and antipsychotic-induced weight gain

I worked on a closely related project that used the same methodology to investigate the relationship between polymorphisms in *CYP2D6* and antipsychotic-induced weight gain. A brief overview of this work is described here, and detail on the associated paper is included in appendix A.1. The search strategy and statistical analysis are the same as described above, with different search teams to capture the phenotype in question. The primary outcomes were weight (kg) and body mass index (BMI) (kg/m^2) after long-term use of antipsychotics. Due to the nature of this phenotype, healthy volunteer studies with only a single oral dose were not included.

1.3 Results

From 94 papers identified in the search, only 16 reported *CYP2D6* genotypes and either prolactin levels (13 studies: Pioro Rosenow 2006 [156], Ozdemir *et al* 2007 [157], Troost *et al* 2007 [158], Wang *et al* 2007 [159], Novalbos *et al* 2010 [160], Cabaleiro et al. 2013, 2014, 2015 [161–163], Roke *et al* 2013 [164], Youngster *et al* 2014 [165], Vandenberghe *et al* 2015 [166], Schoretsanitis *et al* 2018 [167]) or hyperprolactinemia (3 studies: dos Santos Júnior *et al* 2015 [168], Ivanova *et al* 2016 [169], Sukasem *et al* 2016 [170]) after antipsychotic administration. Of these 16 informative papers, seven provided the necessary data to undertake a meta-analysis.

Figure 1.1: PRISMA 2009 flow diagram outlining search process and results to identify studies for inclusion in the systematic review (qualitative synthesis) and meta-analysis (quantitative synthesis) [171]



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

1.3.1 Overview of the sixteen informative studies

The number of subjects genotyped in each study ranged from 22 to 150. Five of the 16 studies were conducted in children [158, 164, 165, 168, 170]. In five of the 11 studies

conducted in adults the participants were healthy volunteers receiving a single dose of antipsychotic medication [157, 160–163]. In the other five studies, the sample consisted of subjects with a psychotic disorder treated with antipsychotic medication for several weeks [159, 166–169, 172, 173]. Two of these studies used data from the same set of healthy volunteers [160, 162]. Only one of these studies was included in the meta-analysis for this reason [162]. Risperidone was the most commonly prescribed antipsychotic in the selected studies (12 out of 16 studies). The most commonly genotyped loss of function alleles were: *3 (11 studies) and *4 (12 studies). Followed by *5 (nine studies) and *6 (eight studies). The most commonly decreased function allele genotyped was *10 (eight studies). In regard to sex distribution, eight studies included 60% or more men and two studies included 60% or more female participants.

Table 1.2: Key characteristics of the sixteen informative studies included in this review

Study	Total n	Sex (% male)	No. PMs	Adults	Diagnosis	Antipsychotic	CYP2D6 Alleles Genotyped
Cabaleiro et al. 2013	61	52.4	2	Y	Healthy	Olanzapine (single dose)	Done but not stated
Cabaleiro et al. 2014	36	50	5	Y	Healthy	Risperidone (single dose)	*1, *2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *14A, *14B, *15, *17, *19, *20, *25, *26, *29, *30, *31, *35, *40, *41, *1xN, *2xN, *4xN, *10xN, *17xN, *35xN, and *41xN
Cabaleiro et al. 2015	26	38.5	5	Y	Healthy	Quetiapine (single dose)	Done but not stated
Choong et al. 2013	42	71.4	1	Y	Psychotic disorder	Risperidone long-acting injection	*3, *4, *5, *6
dos Santos Junior et al. 2015	120		$	N	Psychiatric Outpatients	Risperidone	*10
Ivanova et al. 2016	122	46.7	$	Y	Schizophrenia	Long-term neuroleptic therapy	*3, *4
Novalbos et al. 2010	36	50	6	Y	Healthy	Risperidone (single dose)	*3, *4, *5, *6, *7, *9
Ozdemir et al. 2007	22	100	0	Y	Healthy	Perphenazine (single dose)	*5, *10
Roke et al. 2012	46	100	2	N	Autism spectrum disorders and disruptive behavioural disorders	Risperidone	*3, *4, *5, *6
Schoretsanitis et al. 2018	110	55.5	3	Y	Schizophrenia, schizoaffective disorder, bipolar disorder, major depressive disorder	Risperidone	*2, *3, *4, *5, *6, *8, *9, *10, *11, *15, *17, *19, *20, *29, *35, *36, *40, *41, and a number of duplicated alleles
Sukasem et al. 2016	147	86.4	$	N	Autism spectrum disorders	Risperidone	*4, *10, *41 , *5
Troost et al. 2007	23	100	4	N	Pervasive Developmental Disorders	Risperidone	*3, *4, *5, *6, *7, gene duplication
Vandenberghe et al. 2015	150	54.7	10	Y	Psychiatric Cohort	Risperidone	*3, *4, *6
Wang et al. 2007	118	33.9		Y	Schizophrenia	Risperidone	*3, *4, *5, *10
Yasui-Furukori et al. 2001	76	36.8		Y	Schizophrenia	Haloperidol	*1, *3, *4, *5, *10
Youngster et al. 2014	35	82.8	2	N	Autism spectrum disorders	Risperidone	*2, *3, *4, *5, *6, *8, *9, *10, *11, *14, *15, *17, *18, *19, *20, *25, *26, *29, *30, *31, *35, *36, *37, *40, *41, *43, *52, and a number of duplicated alleles.

PM=Poor metabolisers; $=Number of PM not reported.

Table 1.3: Summary of the key characteristics and main conclusions of the 16 papers included in the systematic review and meta-analysis

Study	Reason for non-inclusion in meta-analysis	Sample	Summary of main conclusions
Cabaleiro *et al*. 2013	Included in meta-analysis and systematic review	61 (29 female, 32 male) healthy volunteers given a single oral dose of olanzapine.	Although this study looked at prolactin levels and *CYP2D6* genotype the authors made no comment on the association between the two. Unpublished data provided by authors. See forest plots, figures 1.2-1.4.
Cabaleiro *et al*. 2014		36 (18 female, 18 male) healthy volunteers given a single oral dose of risperidone.	Although this study looked at prolactin levels and *CYP2D6* genotype the authors made no comment on the association between the two. Unpublished data provided by authors. See forest plots, figures 1.2-1.4.
Cabaleiro *et al*. 2015		26 (16 female, 10 male) healthy volunteers given a single oral dose of quetiapine.	Although this study looked at prolactin levels and *CYP2D6* genotype the authors made no comment on the association between the two. Unpublished data provided by authors. See forest plots, figures 1.2-1.4.
Roke *et al*. 2012		46 patients (all male cohort) with autism spectrum disorders and disruptive behavioural disorders treated with risperidone.	Prolactin level was higher in normal versus reduced or no activity of CYP2D6. See forest plots, figures 1.2-1.4.

Table 1.3 continued from previous page

Troost *et al*. 2007		23 patients (all male paediatric cohort) with pervasive developmental disorder treated with risperidone.	Prolactin level was positively correlated with the number of functional *CYP2D6* alleles. See forest plots, figures 1.2-1.4.
Yasui-Furukori *et al*. 2001		76 (48 female, 28 male) patients with schizophrenia treated with haloperidol.	Males with one to two mutated alleles showed higher prolactin levels than those without. No difference was observed in females. See forest plots, figures 1.2-1.4.
Youngster *et al*. 2014		35 (6 female, 29 male) patients with autism spectrum disorders treated with risperidone.	The only two poor metaboliser patients included both had hyperprolactinemia. See forest plots, figures 1.2-1.4.
dos Santos Junior *et al*. 2015	These papers measured hyperprolactinemia. However, the samples in the dos Santos Junior *et al*. (2015) paper could not be sub-divided into the four CYP2D6 metabolic phenotypes and could not be meta-analysed.	120 (22 female, 98 male) children and adolescents treated with risperidone.	Only one *CYP2D6* polymorphism (*CYP2D6*10*) tested. No association between this polymorphism and hyperprolactinemia was observed.
Sukasem *et al*. 2016		147 (20 female, 127 male) children and adolescents treated with risperidone.	Investigated several *CYP2D6* polymorphisms (CYP2D6*4, *5, *10, *41). Found no association between these polymorphisms and hyperprolactinemia.
Ivanona *et al*. 2016		122 (65 female, 57 male) patients with schizophrenia treated long-term with neuroleptics.	Compared patients with hyperprolactinemia to patients with normal prolactin concentrations and found no significant difference in the genotype and allele distribution for the studied genes (*CYP2D6*3*, **4*, as well as *CYP1A2*1F*)

Table 1.3 continued from previous page

Choong *et al*. 2013	Prolactin levels reported in papers, but not possible to obtain information on prolactin levels by CYP2D6 metabolic phenotype.	42 (12 female, 30 male) psychiatric patients treated with risperidone	Prolactin levels and CYP450 alleles tested, but no comment made on the relationship between prolactin levels and CYP2D6 metabolic phenotype.
Novalbos *et al*. 2010		36 (18 female, 18 male) healthy volunteers given a single oral dose of risperidone	No significant influence of *CYP2D6* variant on prolactin levels identified.
Ozdemir *et al*. 2007		22 Chinese-Canadian healthy volunteers (all male cohort) given a single oral dose of perphenazine	No effect of CYP2D6 variant on prolactin levels identified following single oral dose of perphenazine.
Schoretsanitis *et al*. 2018		110 (49 female, 61 male) patients treated with risperidone	Grouped participants based on number of fully active CYP2D6 alleles. Found that each active *CYP2D6* allele was associated with a 30% decrease in plasma prolactin levels, but effect seen only in men.
Vandenberghe *et al*. 2015		150 (68 female, 82 male) psychiatric patients treated with risperidone	Prolactin levels found to be significantly higher in poor compared to extensive, but effect seen only in women.
Wang *et al*. 2007		118 (78 female, 40 male) Chinese schizophrenia patients treated with risperidone for 8 weeks	Prolactin levels and CYP450 alleles tested, but no comment made on the relationship between prolactin levels and CYP2D6 metabolic phenotype.

Table 1.4: Summary of outcomes as reported in each of the primary studies

Study	**Prolactin outcome measure**	**Unit**	**Drug**	**Males outcome as reported**	**Female outcome as reported**
Cabaleiro *et al*. 2013*	Mean±SD	ng/ml	Olanzapine (single dose)	PM (n=1): 9.03 IM (n=11): 7.98±4.52 EM (n=17): 11.22±5.29 UM (n=3): 14.36±9.02	PM (n=1): 25.75 IM (n=11): 15.19±6.62 EM (n=16): 14.58±6.2 UM (n=1): 15.87
Cabaleiro *et al*. 2014*	Mean±SD	ng/ml	Risperidone (single dose)	PM (n=2): 35.75±10.05 IM (n=5): 30.68±4.57 EM (n=7): 22.85±8.53 UM (n=4): 24.52±3.2	PM (n= 2): 55.53±6.97 IM (n=8): 77.57±33.93 EM (n=7): 62.38±15.48 UM (n=1): 42.21
Cabaleiro *et al*. 2015*	Mean±SD	ng/ml	Quetiapine (single dose)	PM (n=1): 7.98 IM (n=2): 6.14±0.88 EM (n=7): 7.5±2.83	IM (n=6): 8.07±1.79 EM (n=10): 11.19±4.02
Choong *et al*. 2013	Median ± IQR	ng/mL	Risperidone long-acting injection	Outcome not reported	Outcome not reported
dos Santos Junior *et al*. 2015	Hyperprolactinemia		Risperidone	Outcome not reported	Outcome not reported
Ivanova *et al*. 2016	Hyperprolactinemia		Long-term neuroleptic therapy	Outcome not reported	Outcome not reported

Table 1.4 continued from previous page

Novalbos *et al*. 2010	C_{max}	ng/ml	Risperidone (single dose)	Outcome not reported	Outcome not reported
Ozdemir *et al*. 2007	Net change from base-line over 6hrs	ng/ml	Perphenazine (single dose)	Outcome not reported	Outcome not reported
Roke *et al*. 2012	Mean±SD	ng/ml	Risperidone	PM (n=2): 49±0 IM (n=17): 18.4±17 EM (n=25): 19.8±17 UM (n=2): 6.8±6	
Schoretsanitis *et al*. 2018	Mean±SD	ng/ml	Risperidone	n=61: 31.9±13.1	n=49: 63.6±32.6
Sukasem *et al*. 2016	Hyperprolactinemia		Risperidone	Outcome not reported	Outcome not reported
Troost *et al*. 2007	Mean±SD	ng/ml	Risperidone	wt/wt (n=9): 31.7±12 wt/DUPwt (n=2): 58.5±27.6 m/wt (n=8): 32.4±6.4 m/m (n=4): 23.7±5.6	EM (n=2): 36.5±3.5
Vandenberghe *et al*. 2015	Median±IQR	µg/L	Risperidone	Outcome not reported	Outcome not reported
Wang *et al*. 2007	Mean±SD	MIU/L	Risperidone	Outcome not reported	Outcome not reported
Yasui-Furukori *et al*. 2001	Mean±SD	ng/ml	Haloperidol	wt/wt (n=11): 20.5±7.8	wt/wt (n=20): 71.2±46.5

Table 1.4 continued from previous page

				m/wt and m/m (n=17): 32.4±16.4	m/wt and m/m (n=28): 32.4±50.8
Youngster *et al*. 2014	Mean±SD	mcg/L	Risperidone	PM(n=2): 50.3±2.7 IM (n=4): 21.7±19.1 EM (n=21): 27±17.6 UM(n=2): 18.3±1.5	IM (n=2): 17±0 EM (n=4): 21.3±7.9

**Unpublished data provided by authors. Abbreviations: SD = standard deviation, IQR = interquartile range, C_{max} = Maximum serum concentration of a drug, PM = poor metaboliser, IM = intermediate metaboliser, EM = extensive metaboliser, UM = ultra-rapid metaboliser, wt = wild-type, DUPwt = duplication of normal function allele, m = mutated non-functional allele.*

1.3.2 Findings from the meta-analysis

After contacting authors where necessary, a total of seven primary studies provided the data required to undertake a meta-analysis (Table 1.5) (Yasui-Furukori *et al.* 2001 [172], Troost *et al.* 2007 [158], Cabaleiro *et al.* 2013, 2014, 2015 [161–163], Roke *et al.* 2013 [164], Youngster *et al.* 2014 [165]). This resulted in a total sample of 303 participants: 47 poor, 72 intermediate, 169 normal and 15 ultra-rapid metabolisers.

I found no significant differences in prolactin levels between CYP2D6 metabolic groups in any of the three comparisons with the following standardised mean differences: (i) poor versus normal metabolisers 0.19 (95% CI[-0.54, 0.92], p = 0.61), (ii) intermediate versus normal metabolisers -0.1 (95% CI[-0.44, 0.24], p = 0.58) and combined poor/intermediate versus combined normal/ultra-rapid metabolisers 0.06 (95% CI[-0.26, 0.38], p = 0.72). The results of these analyses are described in figures 1.2, 1.3, 1.4[1].

Although six of the seven studies included both male and female patients, there was a higher number of male patients than female patients overall. There is an established difference in prolactin levels in men and women, as well as in children compared to adults. Thus, I ran the meta-analysis twice, once combining male and female subjects and once including only male subjects. The conclusions were found to be the same. The forest plots showing the results of the analysis in only male subjects can be seen in figures 1.5, 1.6, and 1.7[1]. There were not enough studies to conduct a female-only analysis.

Three of the seven studies included in this meta-analysis were conducted in healthy volunteers following a single dose oral administration of antipsychotic medication (Cabaleiro *et al.* 2013, 2014, 2015 [161–163]). The remaining four studies were conducted in patients following long term treatment with antipsychotics. Given the limited literature available, I included all seven studies together in the primary meta-analysis. As hyperprolactinaemia is typically observed during ongoing treatment, I ran secondary meta-analyses excluding all the single-dose healthy volunteer primary studies. The conclusions from the results were found to be the same in both instances. Three of the seven studies included paediatric samples. To investigate the potential impact of age on prolactin levels I ran the analysis using data from the adult only studies. As with the previous analyses, I found no association between CYP2D6 metabolic status and prolactin levels. The forest plots showing the results of the analysis excluding paediatric data can be seen in the figures 1.8, 1.9, 1.10[1].

Table 1.5: Overview of data included in meta-analysis

Study	Sex	Poor			Intermediate			Extensive/Normal			Ultra-rapid		
		N	Mean PRL (ng/ml)	SD	N	Mean PRL (ng/ml)	SD	N	Mean PRL (ng/ml)	SD	N	Mean PRL (ng/ml)	SD
Cabaliro *et al*. 2013	M	1	9.03	NA	11	7.98	4.52	17	11.22	5.29	3	14.36	9.02
Cabaliro *et al*. 2013	F	1	25.75	NA	11	15.19	6.62	16	14.58	6.2	1	15.87	NA
Cabaliro *et al*. 2014	M	2	35.72	10.05	5	30.68	4.57	7	22.85	8.53	4	24.52	3.2
Cabaliro *et al*. 2014	F	2	55.53	6.97	8	77.57	33.93	7	62.38	15.48	1	42.21	NA
Cabaliro *et al*. 2015	M	1	7.98	NA	2	6.14	0.88	7	7.5	2.83		NA	
Cabaliro *et al*. 2015	F		NA		6	8.07	1.79	10	11.19	4.02		NA	
Roke *et al*. 2012	M	2	49	0.01	17	18.4	17	25	19.8	17	2	6.8	6
Troost *et al*. 2007	M	5	25	5.57	6	34.67	7.47	10	31.9	11.3	2	58.5	27.58
Youngster *et al*. 2014	M	2	50.3	2.69	4	21.68	19.14	21	26.98	17.63	2	18.31	1.54
Youngster *et al*. 2014	F		NA		2	17.01	0.01	4	21.27	7.94		NA	
Yasui-Furukori *et al*. 2001	M	11	20.5	7.8		NA		17	32.4	16.4		NA	
Yasui-Furukori *et al*. 2001	F	20	71.2	46.5		NA		28	72.1	50.8		NA	
Total (%)		**47 (15.5%)**			**72 (23.8%)**			**169 (55.8%)**			**15 (5.0%)**		

NA: No data for this group or standard deviation not applicable due to N of 1. Abbreviations: PRL = prolactin levels, SD = standard deviation, M = male, F = female.

Figure 1.2: Forest plot comparing prolactin levels between 44 poor (PMs) and 115 normal metabolisers (EMs)

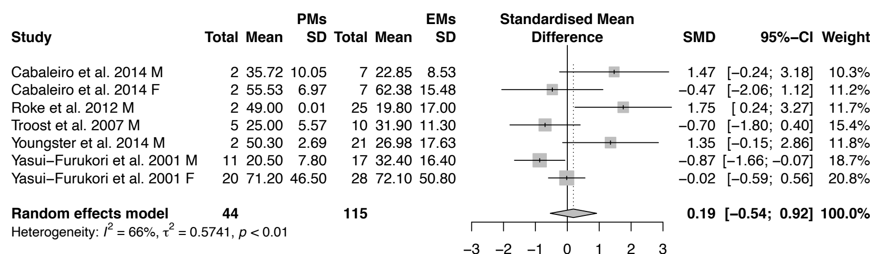
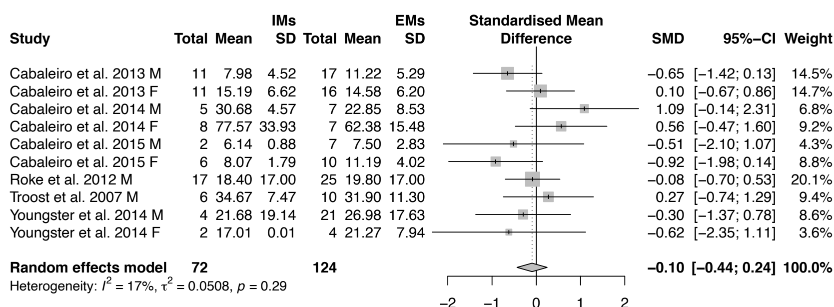


Figure 1.3: Forest plot comparing prolactin levels between 72 intermediate (IMs) and 124 normal metabolisers (EMs).



[1] At the time of conducting these analyses, normal metabolisers were called ‘extensive metabolisers’, thus the figures include the acronym EM. This nomenclature was updated by the PharmVar consortium following completion of this work, and as such the term ‘normal metabolisers’ is primarily used in this thesis.

Figure 1.4: Forest plot comparing prolactin levels between 133 poor and intermediate metabolisers combined (PMs + IMs) versus 170 normal and ultra-rapid metabolisers combined (EMs + UMs).

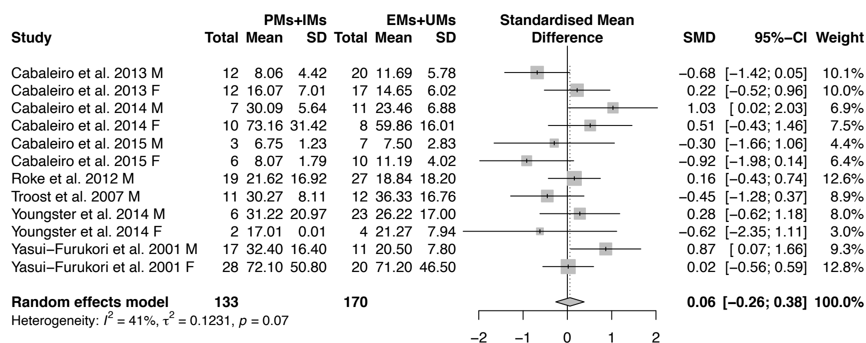


Figure 1.5: Forest plot comparing prolactin levels between 44 poor (PMs) and 115 normal metabolisers (EMs).

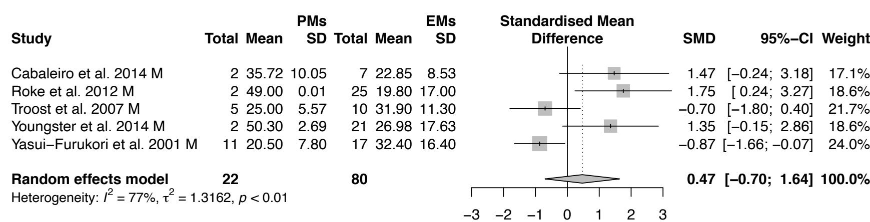


Figure 1.6: Forest plot comparing prolactin levels between 72 intermediate (IMs) and 124 normal metabolisers (EMs).

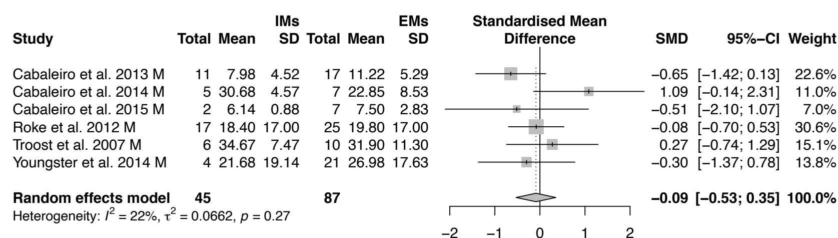


Figure 1.7: Forest plots comparing prolactin levels between 133 poor and intermediate metabolisers combined (PMs + IMs) versus 170 normal and ultra-rapid metabolisers combined (EMs + UMs).

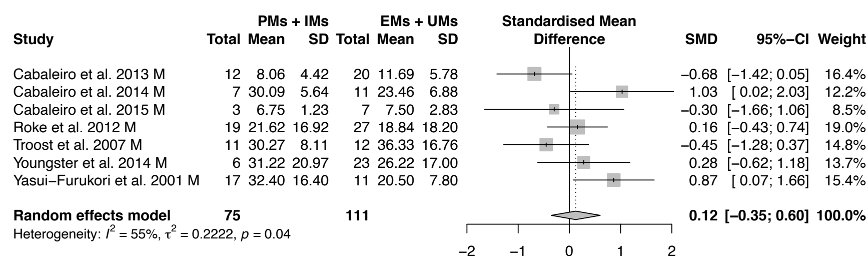


Figure 1.8: Forest plot comparing prolactin levels between 44 poor (PMs) and 115 normal metabolisers (EMs).

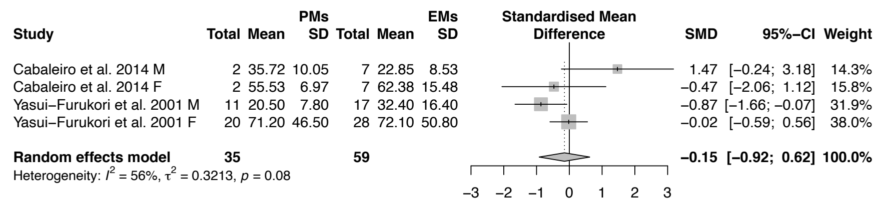


Figure 1.9: Forest plot comparing prolactin levels between 72 intermediate (IMs) and 124 normal metabolisers (EMs).

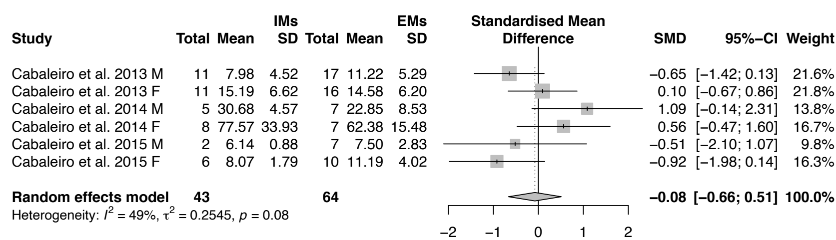
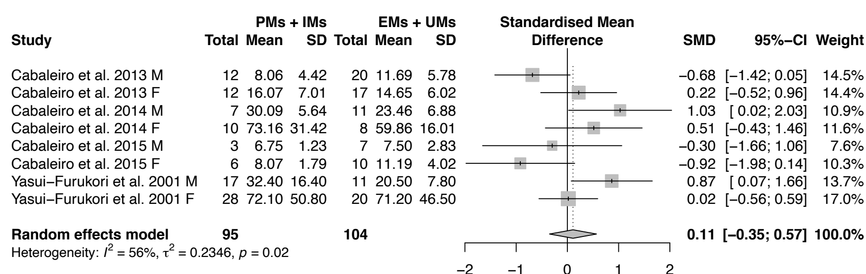


Figure 1.10: Forest plot comparing prolactin levels between 133 poor and intermediate metabolisers combined (PMs + IMs) versus 170 normal and ultra-rapid metabolisers combined (EMs + UMs).



1.3.3 Systematic review of a further nine informative studies

Of the 16 informative studies reviewed, nine could not be included in the meta-analysis. The reasons for their exclusion, as well as an overview of the studies and their conclusions, are summarised in table 1.3. Seven of these nine studies support the conclusions of the meta-analysis and do not show a significant relationship between *CYP2D6* genotype and hyperprolactinemia. One study (Vandenberghe *et al.* 2015 [166]) showed higher prolactin levels in patients with the poor metaboliser phenotype compared to normal metaboliser phenotype, but this result was only significant in female patients. A second study (Schoretsanitis *et al.* 2018 [167]) found that the number of active *CYP2D6* alleles was associated with a significant decrease in plasma prolactin levels, but this result was seen only in men.

1.3.4 CYP2D6 and antipsychotic-induced weight gain

We included a total of 13 studies in the systematic review and 16 in the meta-analysis (including several unpublished datasets provided by the authors when contacted). In the systematic review, four prospective studies did not find evidence that the presence of reduced-function or non-functional *CYP2D6* alleles was associated with greater weight. Four of the five cross-sectional studies included did not find any significant associations.

The 16 studies included in the meta-analysis covered a total of 1,424 patients, including 51 poor metabolisers, 418 intermediate metabolisers, 936 normal metabolisers and six ultra-rapid metabolisers. We found that poor metabolisers had higher BMI compared to normal metabolisers, though this failed to reach the threshold for significance, with the estimated pooled standardised difference of 0.66 (95%CI[-0.10, 1.42], p=0.09)). We found no difference in weight between poor and normal metabolisers with an estimated pooled difference of -0.02 (95% CI[-0.58, 0.55], p=0.96) and no difference in either weight or BMI between intermediate and normal metabolisers (estimated pooled differences were 0.14 (95% CI[-0.03, 0.31], p=0.11) and 0.20 (95%CI[-0.34, 0.74], p=0.47) respectively). The relatively small numbers of poor metabolisers included in the study mean this analysis is likely underpowered to identify significant associations. Additionally, statistical tests demonstrated high heterogeneity between studies in the analysis using BMI measurements ($I^2 \leq 75\%$ and $p < 0.05$ for Chi squared tests). See appendix A for further detail on this paper.

Table 1.6: Overview of data included in weight-gain meta-analysis

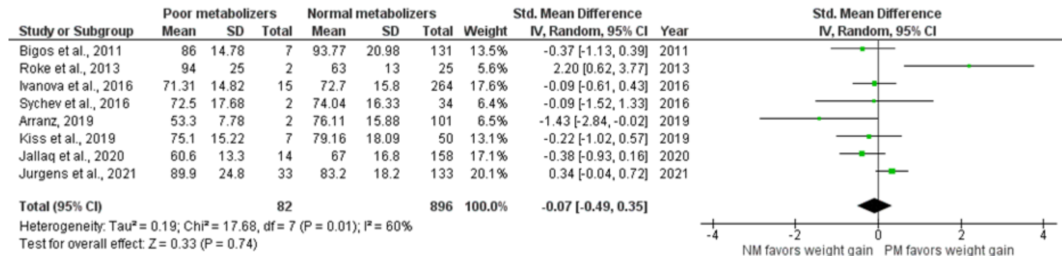
Study	N	Mean age±SD (range)	Gender, %Males	CYP2D6 Genotyping	Diagnosis	Antipsychotic drugs	Outcomes	Summary of findings
Jürgens *et al*. 2020	290	41.4 (30-53)	54.3%	*3, *4, *5, *6 and gene duplication.	Schizophrenic spectrum	Various antipsychotics Dose and treatment duration at baseline not reported.	BMI and weight	N/A
Jallaq e*et al*. 2021	277	14.3±12.5 (6.0-19.6)	34.3%	*2A, *3, *4, *5, *6, *7, *8, *9, *10, *11, *14, *15, *17, *18,*19, *20, *40, *41, *42, and *44.	Mood disorders (bipolar mania, major depressive disorder or disruptive mood dysregulation disorder)	Aripiprazole. 56.7% were on 5mg or more of Aripiprazole. Duration of treatment 367.8±464.7 days	Weight	BMI percentage change was associated with CYP2D6 phenotype groups.
Kloosterboer *et al*. 2021	40	Median age:9.7±5.3 (6-18).	76.2%	*3, *4, *5 and *41	Autism-spectrum disorder	Risperidone. Median dose:1.0±0.5 mg/day. Median duration of treatment:5.7±4.8 months	BMI and weight	CYP2D6 metabolism was not found to be a significant covariate that influenced antipsychotic-induced weight gain.
Ortega-Vázquez *et al*. 2021	48	38.7±10.5	58.3%	rs28371706, rs1065852, rs3892097, rs35742686 and gene duplication	Schizophreania, Schizoaffective disorder and bipolar disorder.	Clozapine. Mean dose:188.8±141.8 (10-700) mg/day for at least 6 months.	BMI and weight	N/A
Arranz *et al*. 2019	163	47.6±13.6	45.9%	*2, *3, *4, *5, *6, *9, *10, *35, *41 and gene duplication	Schizophrenia, Schizoaffective and delusional disorder.	Various antipsychotics Mean olanzapine dose-equivalent 11.0 ± 6.4 mg/day. Treatment duration at baseline not reported.	BMI and weight.	N/A
Kiss *et al*. (2019)	93	31 (18-65)	44.1%	*3, *4, *5, *6, *10 and *41	Schizophrenia, bipolar disorders	Aripiprazole. Mean dose 15mg/day (5–30 mg/day) for at least 4 weeks.	Weight	N/A
Akamine *et al*. 2017	41	36.4±12.5.	24.4%	*1, *2, *5 and *10	Schizophrenia	100-600 mg per day of Clozapine for at least 4 weeks	Weight	N/A
Sychev *et al*. 2017	54	43.6 ±13.5	49.4%	*4	Schizophrenia	Haloperidol monotherapy. Mean dose:12.6mg/day (SD:4.2mg/day).	Weight	N/A
Ivanova *et al*. 2016	475	40 (17-80)	Not reported	*3 and *4	Schizophrenia	Multiple drugs: Haloperidol, Chloroprotixene, Chloropromazin, Trifluoperazin, Clopiksol and Risperidone.	BMI and weight	N/A
Dos Santos-Junior *et al*. 2016	120	13.0±3.1 (8-20)	81.7%	*10	Mental and behavioural disorders	Risperidone. Mean dose:21.1mg/day (SD:1.3mg/day). Mean duration of treatment 25.9±27.2 months	BMI	The presence of *10 allele was associated with occurrence of obesity.
Nussbaum *et al*. 2014	81	15.7 (9-20)	46%	*4	Schizophrenia, Bipolar disorders	Either: Risperidone, Aripiprazole or Olanzapine. Dose and treatment duration not reported.	BMI was recorded at 0, 3, 6, 12 and 18 months.	No significant results at 0-3 months. Those with one *4 allele had significantly higher BMI than those with no * alleles at 6-18 months.
Suzuki *et al*. 2014	66	37.4±15.0	51.5%	*5 and *10	Schizophrenia	Risperidone. Mean dose 4.8±2.5mg/day for 4 weeks.	Weight	No statistically significant difference in weight between those with 2 mutant alleles, those with 1 mutant allele and those with wild type alleles.

Table 1.6 continued from previous page

Roke *et al*. 2013	46	14.7±2.1 (10-19).	100%.	*3, *4 *5, *6 and gene duplication.	Autism spectrum disorder or Disruptive behaviour disorder as well as any psychiatric disorder.	Risperidone. Mean dose 1.6mg/day or 0.026mg/kg. Mean duration of treatment 4.4±2.4 years.	BMI and weight	N/A
Bigos *et al*. 2011	178	Not reported	73%	*4	Schizophrenia	7.5-30 mg of Olanzapine per day. Treatment duration not reported.	BMI and weight	N/A
Mihara *et al*. 2003	85	44.6±14.4.	31.8%	*2, *3, *4, *5 and *10	Schizophrenia	No medication for at least 2 weeks followed by 3mg of Risperidone twice a day for at least 2 weeks.	Weight	N/A
Ellingrod *et al*. 2002	11	35.5±5.4	100%	*3 and *4	Schizophrenia	Olanzapine. Mean dose 14.2±3.3 (range:7.5-20) mg/day. Mean duration of treatment 13.8±12.9 months.	BMI	Those with *1/*3 or *1/*4 genotype had significant BMI increase compared to those with *1/*1.
Mihara *et al*. 2000	101	48±11.	30.7%	*3, *4, *5 and *10	Schizophrenia	12 mg/day of Haloperidol for at least 2 weeks.	Weight	N/A

N/A = outcome as related to weight/BMI and CYP2D6 metabolic phenotype not included in paper.

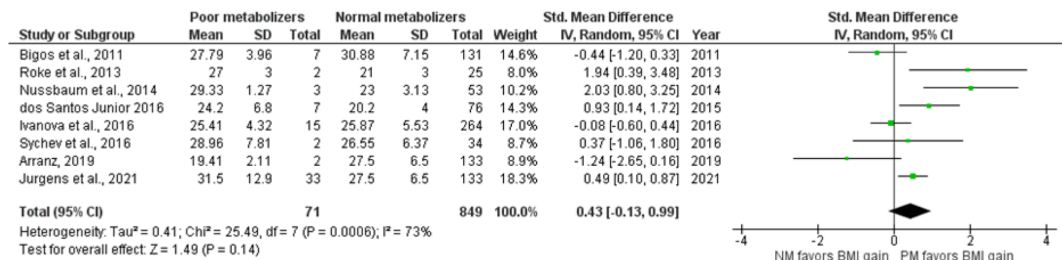
Figure 1.11: Forest plots comparing standardized mean weight differences between poor and normal metabolisers.



Study or Subgroup	Poor metabolizers Mean	SD	Total	Normal metabolizers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Bigos et al., 2011	86	14.78	7	93.77	20.98	131	13.5%	-0.37 [-1.13, 0.39]	2011
Roke et al., 2013	94	25	2	63	13	25	5.6%	2.20 [0.62, 3.77]	2013
Ivanova et al., 2016	71.31	14.82	15	72.7	15.8	264	17.6%	-0.09 [-0.61, 0.43]	2016
Sychev et al., 2016	72.5	17.68	2	74.04	16.33	34	6.4%	-0.09 [-1.52, 1.33]	2016
Arranz, 2019	53.3	7.78	2	76.11	15.88	101	6.5%	-1.43 [-2.84, -0.02]	2019
Kiss et al., 2019	75.1	15.22	7	79.16	18.09	50	13.1%	-0.22 [-1.02, 0.57]	2019
Jallaq et al., 2020	60.6	13.3	14	67	16.8	158	17.1%	-0.38 [-0.93, 0.16]	2020
Jurgens et al., 2021	89.9	24.8	33	83.2	18.2	133	20.1%	0.34 [-0.04, 0.72]	2021
Total (95% CI)			**82**			**896**	**100.0%**	**-0.07 [-0.49, 0.35]**	

Heterogeneity: Tau² = 0.19; Chi² = 17.68, df = 7 (P = 0.01); I² = 60%
Test for overall effect: Z = 0.33 (P = 0.74)

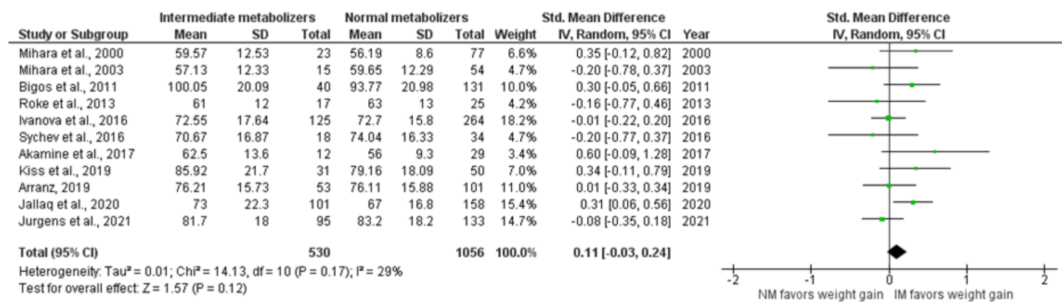
Figure 1.12: Forest plots comparing standardized mean BMI differences between poor and normal metabolisers.



Study or Subgroup	Poor metabolizers Mean	SD	Total	Normal metabolizers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Bigos et al., 2011	27.79	3.96	7	30.88	7.15	131	14.6%	-0.44 [-1.20, 0.33]	2011
Roke et al., 2013	27	3	2	21	3	25	8.0%	1.94 [0.39, 3.48]	2013
Nussbaum et al., 2014	29.33	1.27	3	23	3.13	53	10.2%	2.03 [0.80, 3.25]	2014
dos Santos Junior 2016	24.2	6.8	7	20.2	4	76	14.3%	0.93 [0.14, 1.72]	2015
Ivanova et al., 2016	25.41	4.32	15	25.87	5.53	264	17.0%	-0.08 [-0.60, 0.44]	2016
Sychev et al., 2016	28.96	7.81	2	26.55	6.37	34	8.7%	0.37 [-1.06, 1.80]	2016
Arranz, 2019	19.41	2.11	2	27.5	6.5	133	8.9%	-1.24 [-2.65, 0.16]	2019
Jurgens et al., 2021	31.5	12.9	33	27.5	6.5	133	18.3%	0.49 [0.10, 0.87]	2021
Total (95% CI)			**71**			**849**	**100.0%**	**0.43 [-0.13, 0.99]**	

Heterogeneity: Tau² = 0.41; Chi² = 25.49, df = 7 (P = 0.0006); I² = 73%
Test for overall effect: Z = 1.49 (P = 0.14)

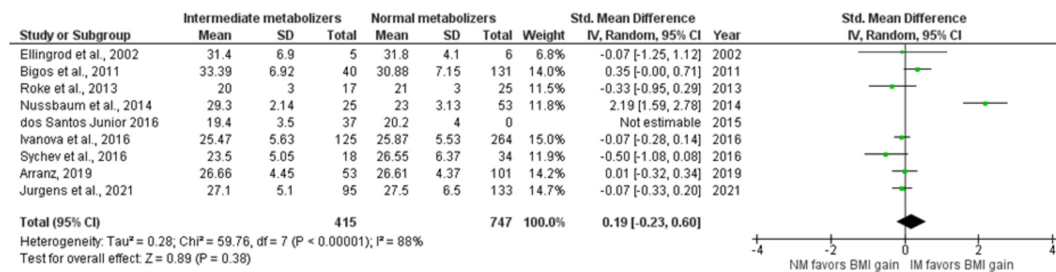
Figure 1.13: Forest plots comparing standardized mean weight differences between intermediate and normal metabolisers.



Study or Subgroup	Intermediate metabolizers Mean	SD	Total	Normal metabolizers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Mihara et al., 2000	59.57	12.53	23	56.19	8.6	77	6.6%	0.35 [-0.12, 0.82]	2000
Mihara et al., 2003	57.13	12.33	15	59.65	12.29	54	4.7%	-0.20 [-0.78, 0.37]	2003
Bigos et al., 2011	100.05	20.09	40	93.77	20.98	131	10.0%	0.30 [-0.05, 0.66]	2011
Roke et al., 2013	61	12	17	63	13	25	4.2%	-0.16 [-0.77, 0.46]	2013
Ivanova et al., 2016	72.55	17.64	125	72.7	15.8	264	18.2%	-0.01 [-0.22, 0.20]	2016
Sychev et al., 2016	70.67	16.87	18	74.04	16.33	34	4.7%	-0.20 [-0.77, 0.37]	2016
Akamine et al., 2017	62.5	13.6	12	56	9.3	29	3.4%	0.60 [-0.09, 1.28]	2017
Kiss et al., 2019	85.92	21.7	31	79.16	18.09	50	7.0%	0.34 [-0.11, 0.79]	2019
Arranz, 2019	76.21	15.73	53	76.11	15.88	101	11.0%	0.01 [-0.33, 0.34]	2019
Jallaq et al., 2020	73	22.3	101	67	16.8	158	15.4%	0.31 [0.06, 0.56]	2020
Jurgens et al., 2021	81.7	18	95	83.2	18.2	133	14.7%	-0.08 [-0.35, 0.18]	2021
Total (95% CI)			**530**			**1056**	**100.0%**	**0.11 [-0.03, 0.24]**	

Heterogeneity: Tau² = 0.01; Chi² = 14.13, df = 10 (P = 0.17); I² = 29%
Test for overall effect: Z = 1.57 (P = 0.12)

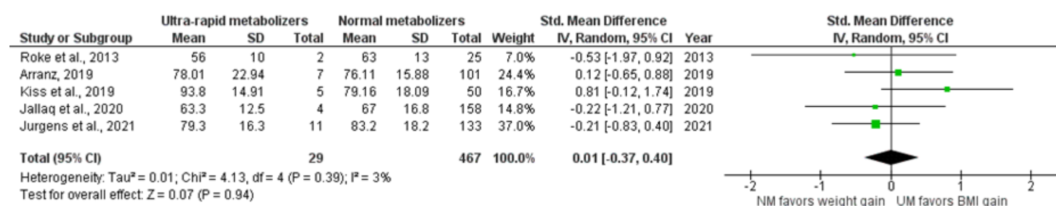
Figure 1.14: Forest plots comparing standardized mean BMI differences between intermediate and normal metabolisers.



Study or Subgroup	Intermediate metabolizers Mean	SD	Total	Normal metabolizers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Ellingrod et al., 2002	31.4	6.9	5	31.8	4.1	6	6.8%	-0.07 [-1.25, 1.12]	2002
Bigos et al., 2011	33.39	6.92	40	30.88	7.15	131	14.0%	0.35 [-0.00, 0.71]	2011
Roke et al., 2013	20	3	17	21	3	25	11.5%	-0.33 [-0.95, 0.29]	2013
Nussbaum et al., 2014	29.3	2.14	25	23	3.13	53	11.8%	2.19 [1.59, 2.78]	2014
dos Santos Junior 2016	19.4	3.5	37	20.2	4	0		Not estimable	2015
Ivanova et al., 2016	25.47	5.63	125	25.87	5.53	264	15.0%	-0.07 [-0.28, 0.14]	2016
Sychev et al., 2016	23.5	5.05	18	26.55	6.37	34	11.9%	-0.50 [-1.08, 0.08]	2016
Arranz, 2019	26.66	4.45	53	26.61	4.37	101	14.2%	0.01 [-0.32, 0.34]	2019
Jurgens et al., 2021	27.1	5.1	95	27.5	6.5	133	14.7%	-0.07 [-0.33, 0.20]	2021
Total (95% CI)			**415**			**747**	**100.0%**	**0.19 [-0.23, 0.60]**	

Heterogeneity: Tau² = 0.28; Chi² = 59.76, df = 7 (P < 0.00001); I² = 88%
Test for overall effect: Z = 0.89 (P = 0.38)

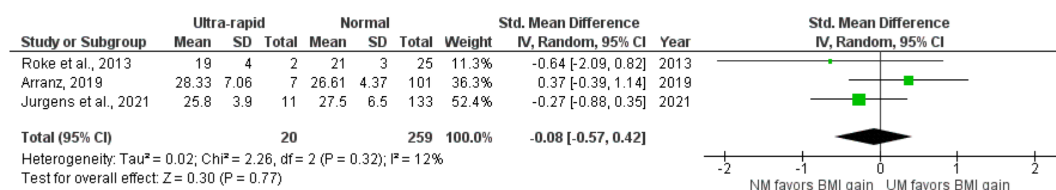
Figure 1.15: Forest plots comparing standardized mean weight differences between ultra-rapid and normal metabolisers.



Study or Subgroup	Ultra-rapid metabolizers Mean	SD	Total	Normal metabolizers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Roke et al., 2013	56	10	2	63	13	25	7.0%	-0.53 [-1.97, 0.92]	2013
Arranz, 2019	78.01	22.94	7	76.11	15.88	101	24.4%	0.12 [-0.65, 0.88]	2019
Kiss et al., 2019	93.8	14.91	5	79.16	18.09	50	16.7%	0.81 [-0.12, 1.74]	2019
Jallaq et al., 2020	63.3	12.5	4	67	16.8	158	14.8%	-0.22 [-1.21, 0.77]	2020
Jurgens et al., 2021	79.3	16.3	11	83.2	18.2	133	37.0%	-0.21 [-0.83, 0.40]	2021
Total (95% CI)			**29**			**467**	**100.0%**	**0.01 [-0.37, 0.40]**	

Heterogeneity: Tau² = 0.01; Chi² = 4.13, df = 4 (P = 0.39); I² = 3%
Test for overall effect: Z = 0.07 (P = 0.94)

Figure 1.16: Forest plots comparing standardized mean BMI differences between ultra-rapid and normal metabolisers.



Study or Subgroup	Ultra-rapid Mean	SD	Total	Normal Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Roke et al., 2013	19	4	2	21	3	25	11.3%	-0.64 [-2.09, 0.82]	2013
Arranz, 2019	28.33	7.06	7	26.61	4.37	101	36.3%	0.37 [-0.39, 1.14]	2019
Jurgens et al., 2021	25.8	3.9	11	27.5	6.5	133	52.4%	-0.27 [-0.88, 0.35]	2021
Total (95% CI)			**20**			**259**	**100.0%**	**-0.08 [-0.57, 0.42]**	

Heterogeneity: Tau² = 0.02; Chi² = 2.26, df = 2 (P = 0.32); I² = 12%
Test for overall effect: Z = 0.30 (P = 0.77)

Figure 1.17: Forest plots comparing standardized mean weight differences poor and intermediate metabolisers combined compared to normal and ultra-rapid metabolisers combined.

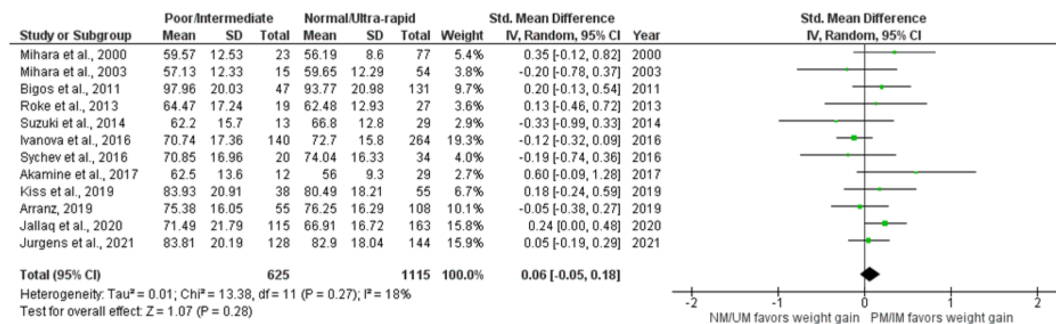
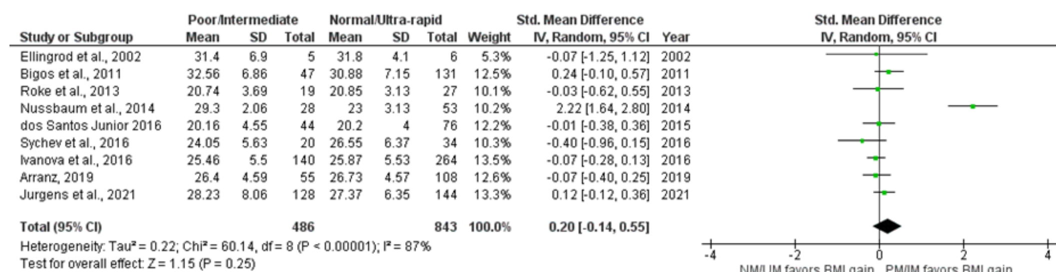


Figure 1.18: Forest plots comparing standardized mean BMI differences between poor and intermediate metabolisers combined compared to normal and ultra-rapid metabolisers combined.



1.4 Discussion

These systematic reviews and meta-analyses summarise and quantify the available evidence on the effect of *CYP2D6* variation on two important, but relatively under-researched adverse drug reactions: blood prolactin levels and weight gain after antipsychotic treatment. Both of these represent gene-environment interactions which have a major impact in clinical practice due to the potentially severe consequences of raised prolactin and/or significant weight-gain.

I did not find evidence of an association between *CYP2D6* genetic variation and prolactin blood levels during antipsychotic exposure. The pooled effect sizes from our meta-analyses were small (with Cohen's d of 0.2 or less) and none of them reached statistical significance. Though the effect sizes calculated for many of the primary studies were of a medium or large magnitude, they showed opposite directions and very few report statistically significant results. Only seven of the 16 studies identified provided the data necessary

to enable their inclusion in the meta-analysis. I also reviewed the findings of the remaining nine studies, which resulted in an overall sample of 867 participants and which mainly agreed in a lack of association. However, this finding cannot be considered conclusive since there are several limitations in the literature.

Only two of the 16 studies included in this review concluded that there was a relationship between *CYP2D6* genotype and prolactin levels [166, 167]. Vandenberghe *et al* observed that prolactin levels were more elevated in poor and intermediate metabolisers, or if the subjects were taking CYP2D6 inhibitors. This result was significant only in women. Schoretsanitis *et al* observed a significant decrease in plasma prolactin levels among subjects with more active *CYP2D6* alleles. This result was observed only in men, and was seen even after controlling for the serum concentration of risperidone and its metabolite. Notably, these studies are some of the largest included in this review, involving 150 (41 intermediate, 10 poor) and 110 (nine intermediate, three poor) patients respectively, treated with risperidone.

The 16 studies reviewed had similar caveats: Each had only between 22 and 150 participants and were individually underpowered to test this genetic association. There is extensive research showing that in the general population only 7% of white Europeans, 3% of Africans and 1% of Asians are *CYP2D6* poor metabolisers, and that ultra-rapid metabolisers are even rarer [174–176]. After pooling all the published data, the total number of participants included was 303 with only 44 poor and 72 intermediate metabolisers. Thus, even a meta-analysis would have limited power to detect a difference between metabolic groups. Therefore, further studies involving large samples and mega-analyses are necessary to guarantee inclusion of sufficient participants with these rarer phenotypes. This was a key recommendation from a Health Technology Assessment examining the potential benefits of *CYP2D6* testing for mental health drugs [147], but such studies have not yet been conducted.

The majority of studies included in this meta-analysis administered risperidone (four studies: [158, 162, 164, 165]) or haloperidol (one study: [172]). Both risperidone and haloperidol are known substrates of CYP2D6 and are primarily metabolised by this enzyme. However, two of the studies administered olanzapine [161] or quetiapine [163]. Although there is evidence that CYP2D6 is involved in their metabolism, it is not their only pathway. Olanzapine is primarily metabolised by CYP1A2 and quetiapine by CYP3A4 [177,

178]. This may explain why these two studies did not demonstrate any difference between CYP2D6 metabolic groups. In order to be confident that this was not skewing the results I ran the meta-analysis with only risperidone and haloperidol treated samples and found the results to be the same. Although this meta-analysis indicate that variation in *CYP2D6* does not influence antipsychotic-induced hyperprolactinemia, the statistical power of the current literature, with 303 participants and only 134 of them with impaired drug metabolism, is limited. Indeed, based on our meta-analysis the effect size for the association between *CYP2D6* and antipsychotic-induced hyperprolactinemia is estimated to be 0.2, which is a small yet clinically meaningful difference. Samples with at least 260 poor metabolisers would be required to reach 80% power to detect such a small group difference. The current literature does not have such large samples yet.

We did find some evidence suggesting that *CYP2D6* poor metabolisers taking antipsychotics have higher BMI than normal metabolisers, but with a p value of 0.09 this is a trend-level finding only. This finding is consistent with four prospective studies previously conducted, and discussed in greater detail the systematic review section of the associated paper. Of note, these four studies were all designed to control for variables affecting weight or BMI and represent one of the largest and most comprehensively genotyped samples included in this review. However, the rest of the meta-analysis and systematic review of the cross-sectional studies did not show evidence of a relationship between *CYP2D6* genetic variation and antipsychotic-induced weight gain or BMI increase.

The only randomised controlled clinical trial available looking at *CYP2D6* testing concluded that poor and ultra-rapid metaboliser patients incur higher treatment expenses and that *CYP2D6* testing can curtail these excess costs [85]; however, neither prolactin levels or weight gain were reported as outcome measures. This trial included over 200 participants and used screening methods to enrich the sample with sufficient numbers of extreme metabolisers (20% poor or ultra-rapid metabolisers in each treatment arm). There are very few trials investigating the effect of *CYP2D6* testing on specific adverse reactions to antipsychotics.

CYP2D6 is a crucial metabolic pathway for most antipsychotics [179, 180], but additional factors can mediate the effect of antipsychotics and risk of adverse drug reactions. For example, genetic variation in other proteins and enzymes involved in antipsychotic pharmacokinetics or pharmacodynamics. As with other complex traits, weight gain and prolactin

levels are influenced by several genetic and environmental factors as well as interactions between them. A genome-wide association study of prolactin identified 12 genome-wide significant loci associated with increased prolactin levels in the plasma and cerebrospinal fluid [181]. A recent meta-analysis of several clinical trials found that prolactin levels were significantly higher in dopamine D2 receptor gene (*DRD2*) Taq1A (rs1800497) carriers than in non-carriers, but only in the sub-population of patients with schizophrenia (five studies, n = 475, Hedges' g[2] = 0.250, 95%CI: 0.068 to 0.433, p = 0.007) [182].

There have been many candidate gene studies investigating putative associations between genes of interest and antipsychotic-induced weight gain, but they have largely yielded inconsistent findings, as is common with candidate gene approaches. A meta-analysis in 2016 attempted to overcome some of the inherent issues with the candidate gene approach by including only genetic variants that were reported in three or more independent samples [183]. They identified 13 SNPs across nine genes that were significantly associated with increased BMI in patients taking antipsychotics. Those with the largest effect sizes were adrenoceptor α2A (*ADRA2A* 1291C/G: Hedges' g = 0.20, 95% CI 0.06 to 0.33, p = 0.004), dopamine receptor D2 (*DRD2* 141C Ins/Del: Hedges g = 0.31, 95% CI 0.07 to 0.54, p = 0.01), 5-hydroxytryptamine receptor 2C (*HTR2C* 759C/T: Hedges' g = 0.23, 95% CI 0.04 to 0.42, p = 0.02) and melanocortin-4 receptor (*MC4R*: Hedges' g = 0.80, 95% CI 0.20 to 1.41, p = 0.009). Notably, seven of the nine genes identified are involved in pharmacodynamic processes. The *MC4R* gene has been associated with obesity in the general population, suggesting this link is not specific to antipsychotic use.

None of the 72 primary studied included in this review looked at CYP450 genes. In the future, investigating the influence of these genetic variants in combination with *CYP2D6* could improve the identification of patients at risk of experiencing adverse reactions to antipsychotic medication. Further research should also examine other pathways involved in the metabolism of antipsychotics and polygenic risk scores, capturing the effects of a wider range of putatively associated variants on the pharmacokinetics and pharmacodynamics of antipsychotics.

In addition to these candidate gene studies, there have been several recent GWAS investigating antipsychotic-induced weight gain. The first used date from the CATIE study,

[2] Hedges' g is another effect measure commonly used in meta-analyses, and is very similar to Cohen's d. The main difference is that Hedges' g takes each sample size into consideration when calculating the overall effect size.

but with a very small sample size identified no genome-wide significant associations [184]. A more recent paper in Chinese subjects found two SNPs in the *PTPRD* (protein tyrosine phosphatase, receptor type D) that reached genome-wide significance for predicting BMI change [185]. This is notable given the small sample size of just 546 patients. Interestingly, *PTPRD* was also associated with antipsychotic efficacy in a separate cross-population GWAS [186].

Several limitations are shared between both reviews reported in this chapter. In both cases, we are limited by small sample sizes in the primary studies, and a small number of extreme metabolisers (poor, intermediate, or ultra-rapid). Both phenotypes, hyperprolactinaemia and weight-gain, can be influenced by a wide variety of environmental and genetic factors, which cannot all be accounted for in the same way across the literature. There was high heterogeneity between the primary studies included in both meta-analyses, including in the SNPs genotyped and the definitions used to assign CYP2D6 metabolic status, as highlighted in table 1.2. Given the lack of consistency between the *CYP2D6* star allele genotyped, the definition of *1 (wild-type) will be varied across the primary studies. There is a need to standardise the definition of wild-type across the pharmacogenetic field, and doing so would likely improve the reliability of findings from meta-analyses such as these. Therefore, more standardised prospective studies with large sample sizes enriched for extreme metabolisers must be conducted to investigate these complex relationships further, and to establish if CYP2D6 testing will be clinically useful to mitigate adverse reactions to antipsychotic treatment.

Many of the primary studies included in both meta-analyses report non-significant associations with opposite directions of effect. This is unsurprising, given the high heterogeneity in the primary studies. There was a wide range of reported prolactin measurements for the studies (tables 1.4 amd 1.5. Prolactin measurement is a routine clinical assessment, and it is likely that these differences are largely explained by variability in age, sex, medication, treatment dose and duration. Ideally, the inclusion and exclusion criteria would have been more expansive, to ensure greater similarity between the included studies. The limited number of primary studies investigating these outcomes made it necessary to be more inclusive in our search. Where possible, secondary analyses were conducted in attempt to create more homogeneous groups; we found the results to be similarly inconclusive. If and when newer and larger studies become available, repeating these meta-analyses with more

restrictive criteria will likely clarify the true nature of these potential gene-environment interactions.

In summary, there is growing evidence from drug labels and clinical guidelines supporting the use of *CYP2D6* testing to guide personalised treatment with antidepressants and to a lesser extent antipsychotics [79, 80, 149, 187], but much of the current literature on this is inconclusive. The prescription of mental health medication remains primarily empirical and pharmacogenetics testing is not part of routine clinical practice in the UK. Larger studies and clinical trials designed to include sufficient extreme metabolisers are needed to investigate the potential cost-effectiveness of pharmacogenetics testing for antipsychotics [86, 107, 149, 188]. In the following chapters, I explore novel ways to address the limitations highlighted in these reviews, through use of large-scale biobank data, genome-wide approaches, and the set up of a prospective clinical trial.

Chapter 2

Investigating cytochrome P450 genetic variation among UK Biobank subjects taking psychotropic drugs

Abstract

This chapter uses data from the UK Biobank to assess the evidence that genetic variation in the cytochrome P450 genes impacts the likelihood and/or severity of adverse events in response to psychotropic medication. Although pharmacogenetic research has focused on these important genes for a long time, difficulties in defining the CYP450 risk alleles has prevented the wide-spread use of large biobank data for analysis. This chapter describes a novel pipeline, developed as part of this project, to assign individuals to metabolic phenotype groups, and to review the association between these phenotype groups and several proxy measures of psychotropic adverse drug reactions. I find that the frequencies of CYP450 metabolic phenotypes are within the range of expected frequencies compared to published estimates. I identify a total of 44,051 subjects who report taking at least one psychotropic drug of interest. I investigate the relationship between CYP450 metabolic phenotypes and risk of adverse drug reactions in patients taking antidepressants and antipsychotics. Overall, I find limited evidence of an association between the CYP450 phenotypic groups and the outcomes of interest. I suggest several potential explanations for this, and set out how I intend to probe them further in the following chapters, still using the data generated through the CYP450 calling pipeline, as described in this chapter.

2.1 Introduction

Through review of the literature as described in the introduction and chapter 1, it is clear that small sample size has been a consistent limitation in the investigation of putative relationship between genetic variation in cytochrome P450 (CYP450) enzymes and therapeutic response [84, 189]. I aimed to address that by making use of UK Biobank data, one of several efforts to capture genetic, medical and socioeconomic data for hundreds of thousands of participants. UK Biobank is a large, cross-sectional population study involving approximately 500,000 participants recruited from across the United Kingdom, with recruitment beginning in 2006. The aim was to provide extensive data to improve the prevention, diagnosis and treatment of a wide range of serious illnesses [190]. With all 500,00 participants providing blood samples for genotyping (and, later, whole exome and whole genome sequencing), this is one of the largest available samples for genetic research, with great potential for identifying novel markers of disease and genes of pharmacogenetic importance.

As mentioned previously, the CYP450 enzyme superfamily is perhaps the most pharmacogenetically important enzyme group. These enzymes are involved in the oxidative biotransformation and clearance of many endogenous and exogenous compounds [191]. CYP450 enzymes are responsible for 75-80% of all phase I metabolic reactions[1][115]. This makes them a hugely important target for understanding and improving treatment response. All CYP450 enzymes are encoded by highly polymorphic genes of the same names [179, 191]. There are 57 active CYP450 genes discovered so far in humans, which are commonly divided into 18 sub-families. The first three of these sub-families, CYP1-3, are those involved in the metabolism of exogenous compounds, whereas sub-families CYP4-18 are responsible for the metabolism of endogenous compounds [180]. As such, it is members of the first three sub-families that will be the focus of this thesis. The specific CYP450 enzymes most implicated in the metabolism of psychotropic drugs are CYP2C19 and CYP2D6, with minor pathways also mediated by CYP1A2, CYP2B6, CYP2C9, and CYP3A4 [195]. Tables 2.1 and 2.2 summarise which psychotropic drugs are substrates, inhibitors or inducers of the major CYP450 enzymes [115]. Genetic variation in these genes

[1]There are three major hepatic processes involved in the metabolism of xenobiotic compounds: phases I, II and III. Phase I metabolism makes the compounds more hydrophilic, to allow for their elimination through the renal system. This usually involves hydrolysis, oxidation or reduction mechanisms. Phase II metabolism is required where phase I has insufficiently cleared the compound from the circulation, or if phase I has resulted in a reactive compound. This step usually adds a large polar group to further increase solubility and thus improve removal through the renal system. Compounds are sometimes further metabolised through phase III reactions, which involves drug transporters to move the compounds across cellular barriers[192–194].

results in an altered catalytic activity of the enzymes, which ultimately can cause different people to metabolise their medication at different rates and may explain some of the inter-individual differences in plasma levels of drugs, and therefore differences in treatment response.

The nomenclautre system for CYP genes is described in greater detail in chapter 1. Typically, individuals are grouped into four to five phenotypic groups reflecting differing metabolic capabilities: poor, intermediate, normal (or extensive), rapid and ultra-rapid metabolisers [179, 195]. These phenotypes are defined in table 2.3. Poor metabolisers will have two mutated and non-functional copies of the CYP450 gene, resulting in a lack of functional enzyme. Intermediate metabolisers usually have one functional copy and one defective or deleted copy, causing reduced activity of the enzyme. Rapid and ultra-rapid metabolisers usually have multiple copies of a functional gene or possess variants that increase gene expression, resulting in increased enzymatic activity [196]. Normal metabolisers (previously described as 'extensive metabolisers'), or wild-type, are those with two fully functional copies of the gene and thus 'normal' enzymatic activity. The distribution of CYP2D6 and CYP2C19 phenotypes varies across populations, but the extreme metabolisers are typically the least commonly observed. Less than 10% of people are poor metabolisers, and less than 3% are ultra-rapid metabolisers, across all major populations and for both genes [197, 198].

Table 2.1: Showing primary metabolic pathways for common antipsychotic drugs [115]

Drug Name	Metabolic Pathways							
	CYP1A2	CYP2A6	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
amisulpride								
aripiprazole						s		s
asenapine	s					**i**		**d**
benperidol								
brexipiprazole						s		s
cariprazine						s		s
chlorpromazine	s					s/i		s
clozapine	s					s*/i		s*
flupentixol								
fluphenazine	s					s/i		
haloperidol						s/i		s
iloperidone						s		
levomepromazine						i		
lurasidone								s
olanzapine	s					s		
oxypertine								
pericyazine								
perphenazine	s/i					s/i		s/i
pimozide	s*					s*		s
pipotiazine								
prochlorperazine								
promazine								
quetiapine						s*		s
risperidone						s/i		s
sertindole								s
sulpiride								
thioridazine								
trifluoperazine								
ziprasidone								s
zuclopenthixol						s		

s = substrate; i = inhibitor; d = inducer; red = metabolic pathway unknown

Table 2.2: Showing primary metabolic pathways for common antidepressant [115]

Drug Name	Metabolic Pathways							
	CYP1A2	CYP2A6	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
agomelatine	s			s	s*			
amitriptyline	s			s	s	s/i		s
bupropion	s*	s*	s	s		i	s	s*
citalopram					s	s/i*		s
clomipramine	s*				s*	s/i		s*
dosulepin								s
doxepin								
duloxetine	s					s/i		
escitalopram					s/i*	s/i		s*
fluoxetine			i*	s/i*	s*	s/i		s*/i
fluvoxamine	s/i		i	i	i	s/i*		i
imipramine	s				s*	s		s
isocarboxazid								
lofepramine								
mianserin						s		
mirtazapine	s*					s*		s
moclobemide	i				s/i	s/i		
nortriptyline						s		
paroxetine			i*			s/i		i
phenelzine								
reboxetine						i*		s/i*
sertraline			s*/i*	s*	s*	s/i		s*
tranylcypromine		i						
trazodone						s*		s
trimipramine					s*	s		s*
venlafaxine						s/i*		s
vortioxetine						s		

s = substrate; i = inhibitor; d = inducer; red = metabolic pathway unknown; green = non-CYP450 mediated metabolic pathway

Table 2.3: Overview of cytochrome P450 variations and the resulting metabolic phenotypes

Phenotype	Genotype
Poor metaboliser (PM)	mt/mt
Intermediate metaboliser (IM)	mt/red
Normal/extensive metaboliser (NM)	wt/wt, wt/DUPred, red/DUPred, red/red, mt/wt, mut/DUPred
Ultra-rapid metaboliser (UM)	wt/wtDUP, red/wtDUP

wt = wild-type/normal function allele; mt = mutation/non-functional allele; red = reduced function allele; DUPwt = duplication of normal function allele; DUPred = duplication of reduced function allele [79, 180, 199]

Several studies have shown that CYP2C19 and CYP2D6 poor metabolisers taking psychotropic medication have higher serum drug concentration compared to normal metabolis-

ers [200–206]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published evidence-based clinical guidelines for SSRIs and tricyclic antidepressants, and recommend dose adjustments based on CYP2C19 or CYP2D6 metabolic status [79, 80]. There are currently no CPIC guidelines for antipsychotics. However, the Dutch Pharmacogenetics Working Group provides clinical guidelines recommending dose adjustments for people taking aripiprazole, haloperidol, pimozide and zuclopenthixol, based on CYP450 genotype [207]. Work to incorporate similar evidence based clinical guidelines to the UK National Health Service (NHS) is ongoing (see chapter 5, appendix E.3 and [84]).

To date, large biobanks such as UK Biobank have not been widely used in the investigation of CYP450 variation. A major reason for this is the difficulty in assinging individuals to the correct metabolic phenotype group. In order to do this accurately, a large number of variants in and around the gene in question must be reliably genotyped and/or imputed. It is then necessary to construct haplotype data, as several of the risk 'alleles' are in fact haplotypes defined by multiple variants. This is complex, and computationally demanding to apply to large numbers of people. A further challenge in using UK Biobank for this type of analysis is the cross-sectional, retrospective nature of the data. There is no measure of treatment response, or specific adverse drug reaction. In order to use this data to investigate these potential gene-drug relationships, it is necessary to define 'proxy' measures of adverse reactions based on the available data.

In the present study I sought to investigate the relationship between genetic polymorphisms in the CYP450 family of enzymes, specifically *CYP2C19* and *CYP2D6*, and adverse events in response to prescribed psychotropic medication. In this chapter I describe the development of a novel pipeline to assign individuals to CYP2D6 and CYP2C19 phenotypic groups based on genotype data, and the definition of proxy measures of adverse drug reactions. Finally, I will describe how I apply methods to test an association between CYP450 metabolic phenotype groups and proxy measures of several adverse drug reactions, including blood biomarker levels (cholesterol, HbA1c), weight and BMI, and sleep duration. Below, I will highlight the current literature on the two key CYP450 enzymes, CYP2C19 and CYP2D6, before describing the methods and results from my analyses.

CYP2C19: Cytochrome P450 family 2 subfamily C member 19

The CYP2C19 enzyme is found predominantly in the liver, and to a lesser extent in the small intestine. It is known to be of significant importance in the metabolism of a wide number

of drugs, including a number of psychotropic agents (see tables 2.1 and 2.2). *CYP2C19* is located on chromosome 10 q23.33. This gene has nine exons and is highly polymorphic, like many other CYP450 genes. Over 25 star alleles (again, see appendix **??**) have been identified by the Human Cytochrome P450 Allele Nomenclature Committee [195], of which eight loss-of-function and one gain-of-function alleles have been identified (Table 2.2).

The most common loss of function allele is *CYP2C19*2* (defined by risk allele A at rs4244285), with allele frequencies of 12% in white European ancestry populations, 15% in African ancestry populations and up to 35% in Asian ancestry populations [208, 209].

The SNP rs4986893 results in a prematurely truncated *CYP2C19* and defines *CYP2C19*3*, another loss-of-function allele. This variant is seen in less than 1% of most populations, though has been identified in up to 9% of samples with Asian ancestry [208, 209]. *CYP2C19*17* is the most common gain-of-function allele of this gene. This allele is identified in approximately 21% of white European populations, 16% of African samples and 3% in Asian populations [208, 209].

Individuals can be grouped into phenotypic groups based on the ability of their CYP2C19 enzyme to metabolise its substrates, as described above. The frequency of CYP2C19 poor metabolisers is estimated to be between 2 and 5% in European and African populations, but up to 15% in Asian populations [208, 209].

CYP2C19 is known to be very important in the metabolism of many psychotropic drugs, in particular antidepressants (see tables 2.1 and 2.2). Several studies have identified a relationship between CYP2C19 metabolic status and response to antidepressant drugs. Yin *et al* (2006) found that poor metabolisers experienced a 42% decrease in clearance rate of citalopram compared to normal metabolisers [210]. In 2010, Sim *et al* identified a link between individuals with homozygous *CYP2C19*2* and increased depressive symptoms, in groups both with and without antidepressant medication [211]. A 2011 study demonstrated significantly higher plasma concentrations of imipramine and desipramine in patients with poor metaboliser status compared to normal [212]. A further study in 2012 concluded that genetic polymorphisms in *CYP2C19* were associated with tolerance and remission rates in a large sample of white non-Hispanic patients taking citalopram [213]. The *CYP2C19*17* allele, resulting in the rapid and ultra-rapid metaboliser phenotype, has been associated with reduced serum concentration of several antidepressants compared to normal metabolisers [205, 214, 215].

There is some controversy on the clinical importance of these genetic polymorphisms, despite clear evidence that they can result in pharmacokinetic changes. A 2004 study showed that although plasma concentration of multiple antidepressant drugs were influenced by *CYP2C19* and *CYP2D6* polymorphisms, clinical response was not correlated to this [200]. The studies investigating *CYP2C19*17* also concluded that the clinical implication of their findings was unclear and warranted further study [205, 214, 215]. Further, a 2008 study involving the STAR*D sample (n=1,953) did not find any significant link between polymorphisms in *CYP2C19*, *CYP2D6* and *CYP3A4* and tolerance or response to antidepressants [216].

However, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has published guidelines on *CYP2C19* genotypes and antidepressants (SSRIs [79] and tricyclic antidepressants [80]). These guidelines summarise the existing evidence and conclude that CYP2C19 poor and ultra-rapid metabolisers should avoid drugs primarily metabolised through that enzyme. In the case of poor metabolisers, if an alternative drug is not a viable option the patients should start at a dose of 50% the recommended starting dose and titrate to response if necessary.

Table 2.4: Important CYP2C19 star alleles and their defining SNPs

Allele	Information
CYP2C19*1A	Wild-type
CYP2C19*2	Non-functional; defining variant rs4244285(A) (c.681G>A).
CYP2C19*2B	Non-functional; defining variant rs17878459(C)
CYP2C19*3	Decreased activity; defining variants rs4986893(A) and rs57081121(A)
CYP2C19*4	Non-functional; defining variant rs28399504(G)
CYP2C19*5	Decreased activity; defining variant rs56337013(T)
CYP2C19*6	Non-functional; defining variant rs72552267(A)
CYP2C19*7	Non-functional; defining variant rs72558186(A)
CYP2C19*8	Non-functional; defining variant rs41291556(C)
CYP2C19*17	Increased activity; defining variant rs12248560(T)

Information summarised from [79, 80, 209, 217]

CYP2D6: Cytochrome P450 family 2 subfamily D member 6

CYP2D6 is responsible for the metabolism of approximately 25% of all marketed drugs [196]. Uniquely for CYP450 enzymes, CYP2D6 is not susceptible to enzyme induction[2]

[2] An enzyme inducer is a substance that increases the metabolic activity of an enzyme. That can occur either when the inducing substrate binds to and activates the enzyme, or through increasing the expression of the enzyme-coding gene.

[196]. This means genetic variation is of even greater significance when considering interpersonal differences in enzyme activity, as genetic variation will account for nearly all ultra-rapid metabolism. Like all other members of the CYP450 family, *CYP2D6* is highly polymorphic. A summary of the star alleles, their defining variants and the resulting enzyme activity can be found in table 2.5.

Over 70 allelic variants of *CYP2D6* have been identified, including fully functional, reduced function and non-functional alleles [142]. Individuals considered poor metabolisers (PM) are those who carry two copies of any of the non-functional alleles (either homozygotes or heterozygotes). The intermediate metaboliser phenotype (IM) is typically a result of one non-functional allele and one reduced function allele. The most common phenotype, normal metabolisers (NM), results from one or two alleles with normal function. The ultra-rapid phenotype (UM) is less well defined in *CYP2D6*, usually relating to copy number variations in *CYP2D6* [142].

CYP2D6 constitutes a major metabolic pathway for many antipsychotics (including haloperidol, risperidone, aripiprazole and zuclophenthixol) and antidepressants (including citalopram, clomipramine and fluoxetine) [107, 142]. There is substantial evidence showing a relationship between *CYP2D6* genotypes and clinical outcomes to psychotropic medications, with poor metabolisers being more susceptible to toxicity [145, 146] and ultra-rapid metabolisers demonstrating significantly increased drug clearance rates, meaning they may be less likely respond positively when treated with standard doses [147–150].

A 2005 study identified a positive trend, suggesting that CYP2D6 poor metabolisers are more likely to experience tardive dyskinesia than intermediate, normal or ultra-rapid metabolisers [145]. A total of 43% of poor metabolisers experienced tardive dyskinesia versus 31% of non-poor metabolisers. This finding did not reach significance, perhaps due to small sample size (n=38). The authors concluded that a larger sample size should be investigated. A 2005 study concluded that pharmacogenetic testing prior to amitriptyline or nortriptyline prescription could identify patients at higher risk of adverse events [218].

A study investigating the influence of *CYP2D6* genotype on mirtazapine pharmacokinetics found a strong association between clearance of the S(+) enantiomer and *CYP2D6* genotype, with ultra-rapid metabolisers having significantly higher clearance rate compared to poor metabolisers [219]. This is significant as S(+)-mediated presynaptic antagonism is thought to be crucial for the efficacy of mirtazapine, meaning patients with ultra-rapid

metabolic status may be more likely to experience poor treatment response [220].

CPIC has published guidelines on *CYP2D6* genotypes and antidepressants (SSRIs [79] and tricyclic antidepressants [80]). These guidelines summarise the existing evidence and conclude that CYP2D6 poor and ultra-rapid metabolisers should avoid drugs primarily metabolised through that pathway. In the case of poor metabolisers, if an alternative drug is not a viable option the patients should start at a dose of 50% the recommended starting dose and titrate to response if necessary [79, 80].

A recent study by Walden et al. investigated treatment outcomes in psychiatric care when clinicians were informed of their subjects' CYP2C19 and CYP2D6 metabolic status [149]. In this study of 80 patients taking antidepressants or antipsychotics, the primary outcome measure was physician opinion on the impact of genetic testing on patient outcomes. They did also consider The Committee of Clinical Investigations (UKU) side effect rating scale[3] but the numbers were insufficient to draw meaningful conclusions from this. They reported that almost 25% of all physicians reported an improvement in patient outcomes, and none reported a negative impact. This type of study needs to be repeated with larger sample sizes and in a sample enriched with poor, intermediate and ultra-rapid metabolisers to be able to confirm the positive results here. The inclusion of more objective outcomes measures would also increase confidence in the findings.

In our meta-analysis and systematic review investigating *CYP2D6* and hyperprolactinaemia in subjects taking antipsychotics (see chapter 1), no significant association was observed. Only two of the 16 studies included in this review concluded that there was a relationship between *CYP2D6* genotype and prolactin levels [166, 167]. Both these studies report sex-specific differences. In our meta-analysis and systematic review investigating *CYP2D6* and weight gain, again in subjects taking antipsychotics, we observed inconsistent results in the 22 studies included. Overall, we found conflicting evidence that CYP2D6 poor metabolisers have a higher body mass index (BMI) compared to extensive. In both reviews, we found that the literature is limited by a lack of well-designed prospective clinical trials, and small sample sizes.

As with its fellow CYP450 genes, the data on the clinical significance of *CYP2D6* variation is not wholly conclusive. Many of the studies cited above were conducted in small

[3]UKU-SERS is a clinician-rated scale measured through semi-structured interview. It was developed in 1987 to provide a comprehensive side effect rating scale to systematically rate the side effects of psychotropic medications [221]

samples and lack statistical power to draw significant conclusions, especially as the frequency of CYP2D6 poor metabolisers is low, estimated to be between 1 and 7%, depending on the population [176, 197].

Table 2.5: Important CYP2D6 star alleles and their defining SNPs

Allele	Information
CYP2D6*1	Wild-type
CYP2D6*2	Normal activity; defining variants rs16947(A) and rs1135840(C)
CYP2D6*2XN	Increased activity; defining variants rs16947(A) and rs1135840(C) with multiple copies
CYP2D6*3A	Non-functional; defining variant rs35742686(-)
CYP2D6*3B	Non-functional; defining variants rs1135824(G) and rs35742686(-)
CYP2D6*4	Non-functional; defining variant rs3892097(A)
CYP2D6*6	Non-functional; defining variant rs5030655(-)
CYP2D6*7	Non-functional; defining variant rs5030867(C)
CYP2D6*8	Non-functional; defining variant rs5030865(A)
CYP2D6*9	Decreased activity; defining variant rs5030656(-)
CYP2D6*10	Decreased activity; defining variant rs1065852(T)
CYP2D6*12	Non-functional; defining variant rs5030862(A)
CYP2D6*14	Non-functional; defining variant rs5030865(T)
CYP2D6*17	Decreased activity; defining variants rs28371706(T) and rs16947(A)
CYP2D6*20	Non-functional; defining variant rs72549354(C)
CYP2D6*29	Decreased activity; defining variants rs61736512(T), rs1058164(C), rs16947(A), rs59421388(T) and rs1135840(C)
CYP2D6*39	Normal activity; defining variant rs1135840
CYP2D6*41	Decreased activity; defining variant rs28371725
CYP2D6*1XN	Increased activity; whole-gene duplication.
CYP2D6*4XN	Non-functional; whole-gene duplication (+1846G>A)
CYP2D6*10XN	Decreased activity; whole-gene duplication (+100C>T)
CYP2D6*41XN	Decreased activity; whole-gene duplication (+2988G>A)

Information summarised from [79, 80, 209, 217]

2.2 Methods

This research was conducted using UK Biobank data under application application ID 20737 (principal investigator: Andrew McQuillin, co-investigator: Elvira Bramon). The UK Biobank study was approved by the North-West Research Ethics Committee (ref 06/MREC08/65) in accordance with the Declaration of Helsinki. All participants provided written informed consent.

2.2.1 UK Biobank sample description

All participants attended one of 22 assessment centres across the country, and were expected to live within 10 miles of the assessment centre. Detailed baseline assessments were conducted, including comprehensive measures of physical and mental health as well as sociodemographic and lifestyle data. Samples for genetic analysis were collected for all participants. Detailed study protocols are available online (`www.ukbiobank.ac.uk/resources/` and `www.biobank.ctsu.ox.ac.uk/crystal/docs.cgi/`). All participants provided written informed consent, and those who withdrew consent after providing their sample for genetic analysis were excluded from the data extraction. Data for 502,527 UK Biobank participants were considered in this study. Of these, I included all participants who reported taking one or more psychotropic drugs (see below).

2.2.2 Identification of psychotropic drugs

The medication data recorded in the UK Biobank was collected through an interview with a trained nurse. Participants were asked during the interview if they were currently taking any *'regular prescription medication'*. If they answered yes, the nurse asked them to detail medication they were taking. Dose, formulation or duration of treatment were not recorded, and any short-term medications (i.e., one week course of antibiotics) were not included.

For the purposes of this thesis I was interested in patients taking psychotropic drugs. A psychotropic drug is defined in this context as any drug indicated, either licensed or through routine clinical practice, for the treatment of psychosis (schizophrenia) or depression. Anxiolytic drugs and mood stabilisers were not included in this study. The drugs of interest were identified through review of the British National Formulary (BNF) Drug Dictionary [222]. The list was expanded to include drugs licensed overseas and drugs no longer listed in the BNF. As the medication data in the UK Biobank is self-reported, it was necessary to identify all potential names (brand names and generic names in foreign languages) of the medications of interest. These alternate names were identified through review of published drug labels, the websites of the European Medicines Agency (EMA, `www.ema.europa.eu/en/medicines`), the Food and Drug Administration (FDA, `https://www.fda.gov/drugs`), The Mayo Clinic Drugs and Supplements database (`www.mayoclinic.org/drugs-supplements/`), the Drug Information Database from the independent website Drugs.com (`www.drugs.com`), and the National Library of Medicine MedlinePlus resource (`www.medlineplus.gov/druginfo/meds`) [223–

227]. In total, I searched for over 65 different antidepressants and antipsychotics, checking for the presence of over 4000 brand names. I the conducted a detailed review of this extracted data and removed items such as topical creams or branded items that contained a very low dose of the relevant drug.

2.2.3 Phenotype selection

The specific phenotypes of interest in this study were measures of well-established and relatively common adverse events to psychotropic medication. Adverse events were selected through review of the summaries of product characteristics (SmPCs) for the selected drugs. Any adverse events recorded as ‘very common’ or ‘common’ in two or more of the SmPCs were considered for inclusion. The variables available in the UK Biobank were then reviewed to find measures that could provide evidence that participants had experienced the adverse event. Table 2.6 lists the selected adverse events of interest. Detailed information on how this data was collected by UK Biobank, and how it was extracted and processed is provided in appendix B, table B.1.

Table 2.6: Common adverse drug reactions selected from review of the relevant SmPCs and other literature.

Reported adverse drug reaction
Increased weight
Raised cholesterol
Raised HbA1c or glucose levels (increased diabetes risk)
Glucose levels
Sedation; fatigue
Insomnia
Tardive dyskinesia
Raised prolactin levels
Prolonged QT interval

2.2.4 Genetic data and quality control

The UK Biobank core team conducted genome-wide genotyping for 488,377 participants. Genotyping was performed using the Affymetrix UK BiLEVE Axiom array on an initial sample of 50,000 and the Affymetrix UK Biobank Axiom array was used on all later participants [228]. These arrays are over 95% similar and include over 820,000 SNP and indel markers, including a good coverage of pharmacogenetics variants (`http://www.ukbiobank.ac.uk/`). Quality control and imputation of over 90 million variants was performed by a collaborative group led by the Wellcome Trust Centre for Human

Genetics [228]. The Haplotype Reference Consortium (HRC) data was chosen as the main imputation panel, due to it being the largest available dataset of European haplotypes [229]. In addition, the data was imputed using merged UK10K and 1000 Genome phase 3 reference panels [230], which optimises the imputation of low-frequency and rare variants. Where a SNP was present in both panels, the HRC panel was used [228]. The fully imputed genetic data used in this thesis was downloaded in March 2018.

A list of 407,500 participants of European ancestry was provided centrally by UK Biobank, based on a combination of principal component analysis (PCA) and self-reported ethnicity data [228]. In order to ensure individuals of all major ancestry groups were included, further local analysis was conducted to determine the genetic ancestry of the remaining participants: Two rounds of PCA were performed using the PC-AiR algorithm, and relatedness was estimated using PC-Relate [231–234]. This resulted in the identification of the following groups: East Asian 0.5% (N=2,464), South Asian 2% (N=8,964), African 2% (N=9,233) or admixed with predominantly European origin 2.5% (N=11,251). A further 6,686 subjects did not cluster with any main group and were excluded from the analyses. One of each pair of participants with a kinship score greater than 0.083 (approximately third-degree relatives) were excluded from the analysis. Individuals with greater than 10% missingness, excessive genetic relatedness (greater than 10 third-degree relatives based on kinship calculations as provided centrally by UK Biobank) or mismatch between reported and genetically inferred sex were removed. Across all five ancestry groups, this resulted in the exclusion of 40,129 participants.

The imputed genetic data were provided in the binary GEN (bgen) v1.2 format (.bgen, .bmi and .sample) with a file for each autosome. The positions are in GRCh37 coordinates. In order to extract the genetic data necessary to assign individuals to the correct CYP2C19 and CYP2D6 metabolic phenotype groups, I defined 'regions of interest': one megabase (Mb) either side of the CYP450 gene of interest (see table 2.7). This was selected to ensure that potential regulatory SNPs (i.e., SNPs that alter gene expression) were captured. I used QCTOOL version 2.0 (`https://github.com/gavinband/qctool`) to extract the regions of interest, conducting the extractions separately based on each CYP450 gene of interest. Start and stop coordinates were identified using the University of California Santa Cruz Human Genome Browser (`https://genome-euro.ucsc.edu`) [235]. Further post-imputation quality control was performed using PLINK version 2.0 [236] to remove

variants with minor allele frequency below 1% and/or Fisher information score (a measure of the imputation accuracy for each SNP) of less than 0.3.

Table 2.7: Start and stop positions for data extraction, one megabase either side of the start and end positions of the respective genes.

Gene (Chr:Position)	Start position	Stop position
CYP2C19 (CHR10: 96,447,882-96,612,671)	95,447,882	97,612,671
CYP2D6 (CHR 22: 42,522,501-42,526,883)	41,522,501	43,526,883

2.2.5 Assigning CYP450 metabolic phenotype

As this thesis focuses on subjects taking psychotropic medication, I limited this CYP450 calling to that subset. The developed approach could be readily upscaled for use in other sub-samples.

The imputed data made available to UCL was provided as unphased diploid genotypes, ordered by minor allele frequency (MAF). The phasing conducted centrally by the UK Biobank (described in detail here: `www.biobank.ctsu.ox.ac.uk`) was conducted on a large sample prior to imputation.

Several of the SNPs of interest in this study (i.e., those that define either *CYP2D6* or *CYP2C19* star alleles) are rare (MAF <0.1) and therefore fail standard quality control protocols. For rare SNPs of interest included on the genotype panel, I used Evoker version 2.4 to create intensity plots and performed visual checks to determine if the data for these SNPs was reliable enough to include [237]. Although quality control steps like genotyping call rate or Hardy-Weinberg equilibrium can be used to identify the majority of reliably-called common variants, a visual inspection of genotype cluster plots remains the most reliable method of verifying high quality genotype calls. Although this is not practical for all SNPs, it can be a valuable tool to recuse rare SNPs that might otherwise be excluded through standard quality control measures.

I reviewed a total of six genotyped SNPs for *CYP2C19* and five for *CYP2D6*. SNPs with distinct allelic clusters were included in this study. For the rare, imputed SNPs, I included only those that met a higher Fisher information score threshold of 0.6. I reviewed a total of seven imputed SNPs for *CYP2C19* and five for *CYP2D6*. These steps enabled the inclusion of an additional four relevant SNPs for *CYP2C19*, and three for *CYP2D6*. The extraction of data and identification of rare SNPs was conducted separately for each ancestry group, due to differences in linkage disequilibrium (LD) structure and allele frequency

across populations. Table 2.9 summarises the information on these additional SNPs. Figure 2.1 shows an example of a plot with three distinct clusters, that clearly defines the two homozygous groups (in red and blue) and the heterozygous group (in green). In addition, I highlight an example of a SNP that was rescued based on visual inspection of these plots, due to two clear clusters, and one example of a SNP that was not included, due to no distinct clustering of heterozygotes. A total of 95 plots per SNP were created and checked in total.

As detailed in table 2.8, some CYP450 star alleles are defined by a single genetic variant, but in other cases the star allele is defined based on a combination of variants across the gene, i.e., a haplotype. The phenotype is then dependent on the haplotypes present on both chromosomes, i.e., the diplotype. Accurately inferring the maternal and paternal contribution is essential to this study in order to define the CYP450 genes according to the star allele nomenclature. Therefore, it was necessary to re-phase the imputed data.

Haplotypes for this sample were constructed based on the imputed genetic data using Beagle version 5.0 [238, 239]. Each job was parallelised on 10 threads. An input map and reference panel from the 1,000 genome project was used [240]. The phased data was used to construct haplotypes for all participants according to the star allele nomenclature system [88, 89, 195]. A summary of the defining SNPs for each star allele is provided in Table 2.8. This table was used to identify which SNPs were available within our sample and therefore which star alleles could be accurately assigned.

I created tables to assign each star allele based on the defining SNPs, as per data provided by PharmGKB. Some individuals carry the defining variants for multiple star alleles. Where this was the case, both star alleles were listed in the output, separated by a semicolon (e.g., *CYP2C19*2;CYP2C19*17*). I then reviewed all examples over overlapping alleles, and determined which had the most functionally significant outcome. Where one star allele resulted in a more significant change than the other, the individual was assigned that star allele. In the example given above, an individual with the defining variants for both *CYP2C19*2* and *CYP2C19*17* would be assigned *CYP2C19*17*, because that results in increased enzymatic activity whereas *CYP2C19*2* is normal function. Where an individual possesses the defining variants for two alleles with the same functional consequence, I did not assign a single allele but rather kept the information on both variants.

Once each individual was assigned their two star alleles, I reviewed all the potential diplotype combinations for the two genes. I grouped individuals into CYP2C19 metabolic

phenotype groups based on the activity of the individual haplotypes and resulting diplotypes [88, 89]. We grouped individuals into CYP2D6 metabolic phenotype groups according to the Gaedigk activity score method [144, 241]. The fully annotated script for this process is provided on my GitHub page: `https://github.com/isabelleazimm/UKBiobank/tree/main/DATA`.

Not all SNPs known to define certain star alleles were available in the UK Biobank data, even after reviewing the intensity plots for some of the rare missing variants. Where no defining SNP for a known star allele was observed, individuals were classified as 'wild-type' (*1) for the respective genes. We did not have data on *CYP2D6* copy number variants (CNVs) and as such were not able to define CYP2D6 ultra-rapid metabolisers. I reviewed the minor allele frequency of the SNPs that are known to define certain star alleles, but were not available in the UK Biobank data. Based on this, I estimated the number of individuals within my sample that might be carriers of the risk allele and therefore might be incorrectly assigned as wild-type. This is summarised within table 2.10.

Table 2.8: Translation table to assign CYP2C19 and CYP2D6 star alleles based on the defining SNPs, including nucleotide change.

Star allele	Defining SNP1	Defining SNP2	Defining SNP3	Defining SNP4
CYP2C19*2	rs4244285 (G>A)	rs3758581 (A>G)		
CYP2C19*3	rs4986893 (G>A)	rs3758581 (A>G)		
CYP2C19*4	rs28399504 (A>G)	rs3758581 (A>G)		
CYP2C19*5	rs17885098 (C>T)	rs3758581 (A>G)	rs56337013 (C>T)	
CYP2C19*6	rs17885098 (C>T)	rs3758581 (A>G)	rs72552267 (A>A)	
CYP2C19*7	rs72558186 (T>A)			
CYP2C19*8	rs41291556 (T>C)	rs3758581 (A>G)		
CYP2C19*9	rs17884712 (G>A)	rs3758581 (A>G)		
CYP2C19*10	rs6413438 (C>T)	rs3758581 (A>G)		
CYP2C19*11	rs58973490 (G>A)	rs3758581 (A>G)		
CYP2C19*12	rs17885098 (C>T)	rs3758581 (A>G)	rs55640102 (A>C)	
CYP2C19*13	rs17879685 (C>T)	rs3758581 (A>G)		
CYP2C19*14	rs55752064 (T>C)	rs3758581 (A>G)	rs17885098 (C>T)	
CYP2C19*15	rs17882687 (A>C)	rs3758581 (A>G)		
CYP2C19*17	rs12248560 (C>T)	rs3758581 (A>G)		
CYP2D6*2	rs1135840 (G>C)	rs16947 (C>T)		
CYP2D6*3	rs35742686 (delT)			

CYP2D6*4	rs3892097 (G>A)			
CYP2D6*5	Whole gene deletion			
CYP2D6*6	rs5030655 (delA)			
CYP2D6*7	rs5030867 (A>C)			
CYP2D6*8	rs5030865 (G>A/T)	rs16947 (C>T)	rs1135840 (G>C)	
CYP2D6*9	rs5030656 (delCTT)			
CYP2D6*10	rs1065852 (C>T)	rs1135840 (G>C)		
CYP2D6*11	rs201377835 (G>C)	rs16947 (C>T)	rs1135840 (G>C)	
CYP2D6*12	rs5030862 (G>A)	rs16947 (C>T)	rs1135840 (G>C)	
CYP2D6*14	rs5030865 (G>A/T)	rs16947 (C>T)	rs1135840 (G>C)	
CYP2D6*15	rs774671100 (insT)			
CYP2D6*17	rs28371706 (C>T/A)	rs16947 (C>T)	rs1135840 (G>C)	
CYP2D6*20	rs72549354 (insG)	rs199535154 (T>C)	rs16947 (C>T)	rs1135840 (G>C)
CYP2D6*29	rs61736512 (G>A)	rs16947 (C>T)	rs59421388 (G>A)	rs1135840 (G>C)
CYP2D6*39	rs1135840 (G>C)			
CYP2D6*41	rs16947 (C>T)	rs28371725 (G>A)	rs1135840 (G>C)	
CYP2D6*52	rs1065852 (C>T)	rs28371733 (G>A)	rs1135840 (G>C)	

Table 2.9: Summary of SNPs that define CYP450 star alleles but are missing from UK Biobank data

Gene	SNP	Defines star allele	Typed/ imputed (y/n)	Passed visual checks (y/n) (N batches)	Info score	MAF
CYP2C19	rs4986893	*3	y/y	y (95)	n/a	n/a
CYP2C19	rs28399504	*4	y/y	n/a	0.74	0.0022
CYP2C19	rs41291556	*8	y/y	y (95)	n/a	n/a
CYP2C19	rs17884712	*9	y/n	n (95)	n/a	n/a
CYP2C19	rs6413438	*10	n/y	n/a	0.79	0.000019
CYP2C19	rs58973490	*11	y/y	n/a	0.94	0.0064
CYP2C19	rs17879685	*13	n/y	y (95)	n/a	n/a
CYP2C19	rs17882687	*15	y/y	n/a	0.87	0.0007
CYP2D6	rs5030867	*7	n/y	n/a	0.68	0.0004
CYP2D6	rs5030865	*8	y/y	n/a	0.68	0.0004
CYP2D6	rs28371706	*17	y/y	y (95)	n/a	n/a
CYP2D6	rs59421388	*29	y/y	y (95)	n/a	n/a
CYP2D6	rs61736512	*29	y/y	n (95)	n/a	n/a

Figure 2.1: Examples of A) 'gold standard' intensity plot with three distinct clusters, B) an example of a plot for a SNP that was deemed high enough quality to enable inclusion, and C) and example of a plot that was not accepted for inclusion.

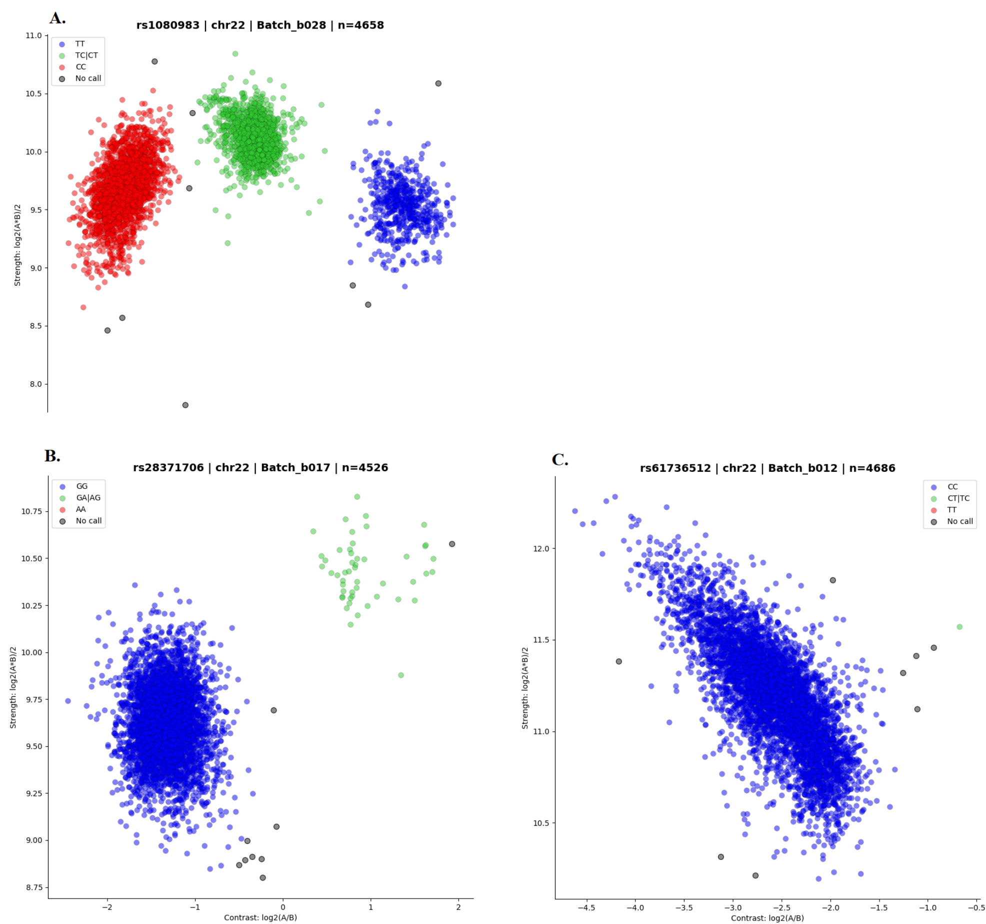


Table 2.10: Summary of uncalled star alleles, and the expected frequency of risk alleles within sample

Star allele	Missing SNP	Risk allele: MAF (European populations)	Predicted no. alleles within sample
CYP2C19*4	rs28399504	G: 0.001	73
CYP2C19*5	rs56337013	T: <0.000001	0
CYP2C19*6	rs72552267	A: 0.0001	7
CYP2C19*7	rs72558186	C: <0.000001	0
CYP2C19*8	rs41291556	C: 0.003	219
CYP2C19*9	rs17884712	A: <0.000001	0
CYP2C19*10	rs6413438	T: <0.000001	0
CYP2C19*11	rs58973490	A: 0.004	292
CYP2C19*12	rs55640102	C: <0.000001	0
CYP2C19*14	rs55752064	C: <0.000001	0
CYP2D6*5	Whole gene deletion	del: 0.028	2041
CYP2D6*6	rs5030655	delA: 0.02	1,458
CYP2D6*11	rs201377835	C: <0.000001	0
CYP2D6*12	rs5030862	A: <0.000001	0
CYP2D6*20	rs72549354	<0.000001	0
CYP2D6*52	rs28371733	T: <0.000001	0

2.2.6 Statistical analysis

I grouped the drugs of interest into three main group for analysis: tricyclic antidepressants, SSRI antidepressants and antipsychotic drugs. Tricyclic antidepressants that are known CYP2C19 substrates are: amitriptyline, clomipramine, doxepin, imipramine and trimipramine. SSRIs that are known CYP2C19 substrates are: citalopram, escitalopram, and sertraline. Tricyclic antidepressants that are known substrates for CYP2D6 include: amitriptyline, clomipramine, duloxetine, and doxepin. SSRIs that are known substrates for CYP2D6 are: fluoxetine, fluvoxamine, paroxetine, sertraline, as well as the SNRIs mirtazapine and venlafaxine [115, 194]. Antipsychotic drugs known to be metabolised at least in part by CYP2D6 are: aripiprazole, clozapine, fluphenazine, haloperidol, olanzapine, perphenazine, pimozide, risperidone, zuclopenthixol, thioridazine. CYP2C19 does not play a significant role in the metabolism of antipsychotics [115].

For each drug group, I ran regression models with each of the selected phenotypes as the outcome of interest and CYP450 metabolic phenotype and diabetes status as the main explanatory variables. All statistical models were adjusted for age, sex, and genetically determined ancestry group.

I ran a total of 18 models (six outcomes across three drug subgroups, tricycilics, SSRIs,

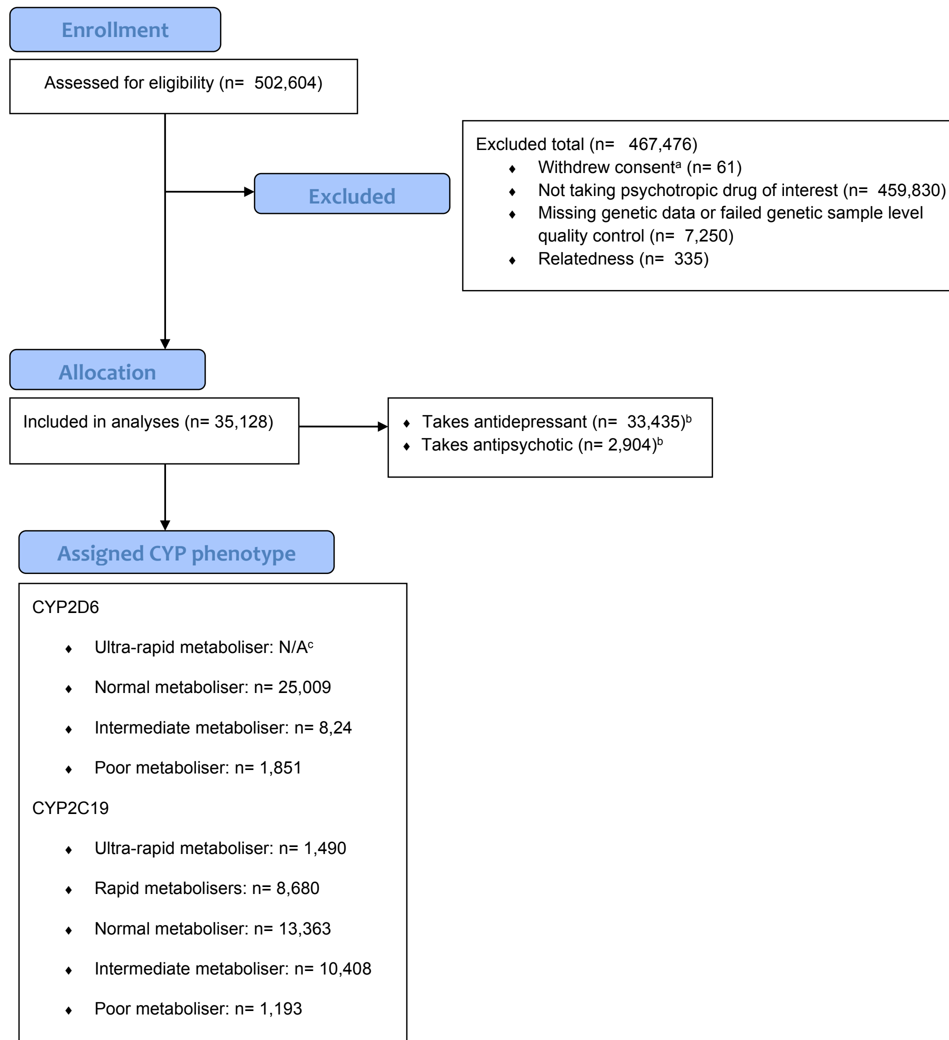
and antipsychotics), however since this is a hypothesis driven study with candidate genes, applying a Bonferroni correction is considered too stringent [242, 243]. Therefore, I applied a multiple testing correction for the five independent outcomes I tested (although I test six outcomes, weight and BMI are highly correlated, given BMI is calculated from weight). The resulting adjusted significance threshold is $p < 0.05/5 = 0.01$ (threshold for a trend level association $p < 0.1/5 = 0.02$). Uncorrected p values are reported in all text and tables, but this adjusted threshold is used to interpret the level of evidence. All statistical analyses were performed using R version 3.6.0 [244–246].

2.3 Results

2.3.1 Study sample description

I identified a sub-sample of 44,051 UK Biobank participants who were taking one or more psychotropic medication at the time of their baseline visit. After conducting all sample and variant level quality control procedures as described in the methods, I was left with a sample of 35,128 participants taking antidepressant and/or antipsychotic medication that could be included in these analyses. Figure 2.2 shows an adapted CONSORT diagram detailing how this final sample was arrived at. The sex, age and ethnicity of these samples is summarised in table 2.11 below. The sample sizes included in each specific phenotype analysis is included in the relevant tables included in appendix B.

Figure 2.2: Adapted CONSORT statement detailing steps taken to arrive at the final included in these analyses.



[a]Number of participants who withdrew consent after the initial download of all phenotype data and before the analysis was conducted. Participants who withdrew consent prior to initial download of data were already removed; [b]Note that several subjects report taking both antidepressant(s) and antipsychotic(s) and are included in both analyses; [c]See main body of text for detail on why CYP2D6 ultra-rapid metabolisers were not defined in this analysis.

2.3.2 Psychotropic drugs in the UK Biobank

There were 28 different antidepressants identified in our sample (figure 2.3). Amitriptyline was the most common drug in our cohort (taken by 25.9% of all antidepressant drug users, N=8,667). We identified 24 different antipsychotic drugs (figure 2.3), with the most frequent antipsychotics being prochlorperazine (taken by 32% of all antipsychotic drug users, N=928), followed by olanzapine (18.5%, N=538). The number of participants taking each drug is shown in figure 2.2[4].

[4]Note that some participants reported taking more than one of the included medications, therefore the total number of samples in this table is greater than the number of individual participants included in this study.

Figure 2.3: Frequency of identified antipsychotics (top, darker green) and antidepressants (bottom, lighter green) in UK Biobank.

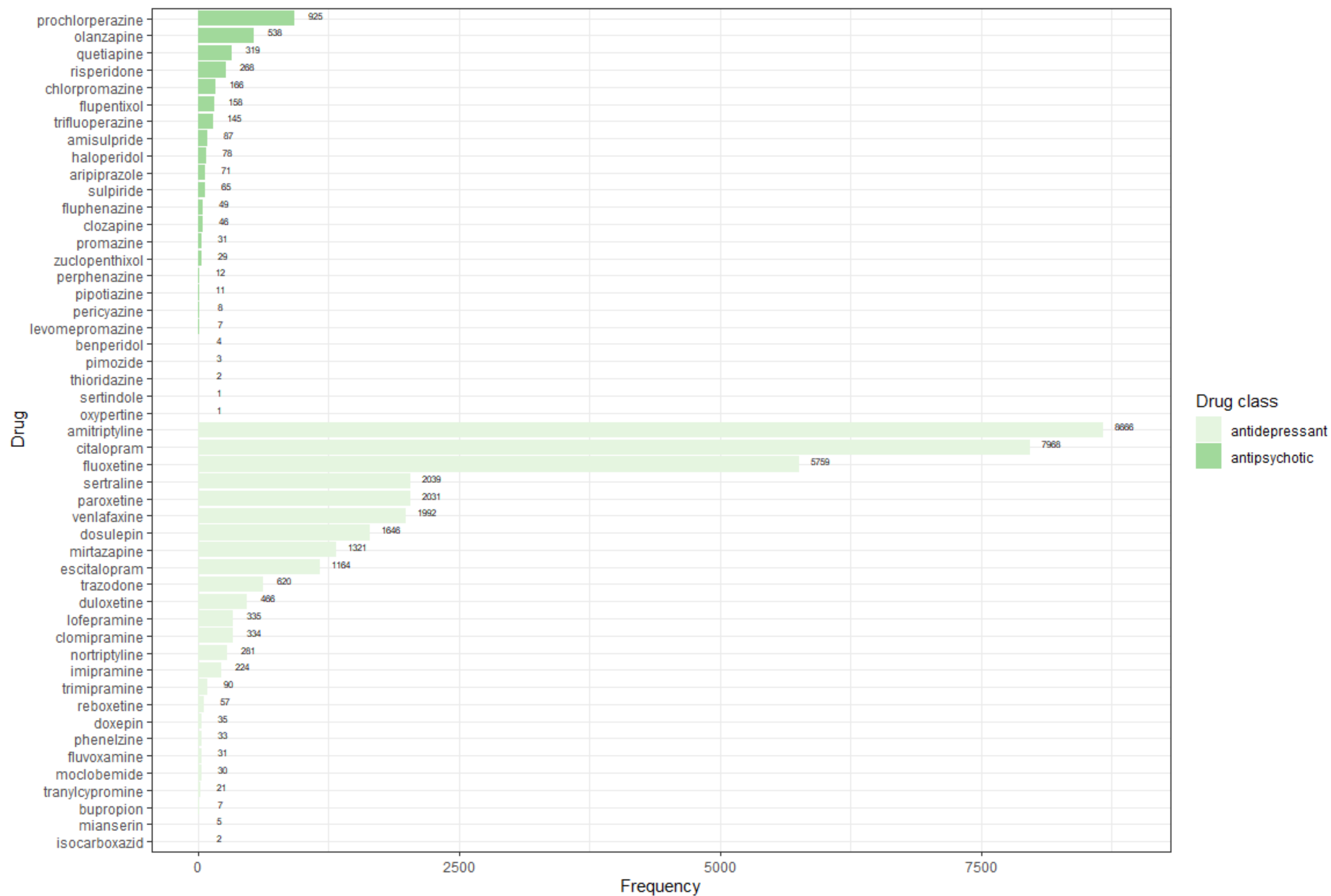


Table 2.11: Sample demographics

	Antidepressants (N=33,435)	**Antipsychotics (N=2,904)**
Sex		
Female	22,967 (68.69%)	1,669 (57.49%)
Male	10,465 (31.30%)	1,234 (42.50%)
Age		
Mean (SD)(years)	56.54 (7.79)	56.32 (8.10)
Range (median)(years)	40-71 (58)	40-70 (57)
Ethnicity		
White European	31,276 (93.55%)	2,559 (88.1%)
Admixed European	849 (2.53%)	77 (2.65%)
African	372 (1.11%)	118 (4.06%)
East Asian	44 (0.13%)	13 (0.45%)
Admixed	483 (1.44%)	64 (2.20%)
South Asian	408 (1.22%)	72 (2.48%)
CYP2D6 metabolic phenotype		
Normal metabolisers	23,818 (71.24%)	2,052 (70.69%)
Intermediate metabolisers	7,851 (23.48%)	705 (24.1%29
Poor metabolisers	1,764 (5.27%)	146 (5.03%)
CYP2C19 metabolic phenotype		
Normal metabolisers	12,770 (38.05%)	1,088 (37.47%)
Intermediate metabolisers	9,909 (29.63%)	846 (29.14%)
Poor metabolisers	1,122 (3.36%)	111 (3.82%)
Rapid metabolisers	8,266 (24.72%)	730 (25.15%)
Ultra-rapid metabolisers	1,416 (4.24%)	128 (4.41%)
Takes CYP2D6 inhibitors[a]		
No	31,441 (94.04%)	2,738 (94.32%)
Yes	1,992 (5.96%)	165 (5.68%)
Takes CYP2C19 inhibitors[a]		
Yes	8,480 (25.36%)	656 (22.60%)
No	24,953 (74.64%)	2,247 (77.40%)

[a] *CYP2C19 and CYP2D6 inhibitors identified through review of literature, including British National Formulary*

2.3.3 Haplotype estimation and CYP450 metabolic phenotype

I extracted the genetic data for the UK Biobank sub-sample of 44,051 participants taking a psychotropic drug of interest. After applying the quality control measures described above, I was left with a sample of 35,128 individuals with high quality genetic data. I phased this data and used the resulting phased VCF (variant call format) file as the input for the CYP450 metabolic phenotype R script to assign CYP450 star alleles and translate this to the appropriate metabolic phenotype group. I compared the frequency of the resulting star alleles and phenotype groups to published frequencies [88]. Table 2.12 summarises the observed fre-

quencies of the different metabolic phenotype groups in this sample compared to published values. Across both genes, the observed frequencies are broadly consistent with published frequencies for the white European populations. There is a greater discrepancy between observed and published frequencies of CYP2D6 metabolic phenotypes, which was expected due to the relatively frequent structural variants that were not identified in this study. There are larger differences between the observed and published frequencies in the non-European populations, however the sample sizes are underpowered to detect the statistical significance of these differences.

Table 2.12: Observed frequency of metabolic phenotype per population group, with expected frequency based on published data where available[176]

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Phenotype	European		Admixed Eur.		African		East Asian		South Asian		Admixed	
	Obs. N (%)	Exp. %	Obs. N (%)	Exp. %	Obs. N (%)	Exp. %	Obs. N (%)	Exp. %	Obs. N (%)	Exp. %	Obs. N (%)	Exp. %
CYP2C19:												
Poor	1,231 (3.43)	2.5	31 (3.1)	N/A	16 (3.1)	4.3	1 (1.52)	14.5	13 (2.5)	12.4	21 (3.8)	N/A
Intermediate	10,648 (27.7)	26.8	266 (27.0)	N/A	158 (30.6)	32.6	17 (25.8)	47.0	156 (30.5)	45.6	171 (30.8)	N/A
Normal	13,635 (38.0)	39.2	399 (40.4)	N/A	186 (36.0)	35.7	32 (48.5)	36.6	188 (36.7)	22.6	214 (38.5)	N/A
Rapid	8,856 (24.7)	26.9	236 (23.9)	N/A	134 (25.9)	23.6	14 (21.2)	1.7	133 (26.0)	16.4	124 (22.3)	N/A
Ultra-rapid	1,496 (4.2)	4.6	55 (5.6)	N/A	23 (4.5)	3.4	2 (3.0)	0.2	22 (4.3)	3.0	26 (4.7)	N/A
CYP2D6:												
Poor	1,1895 (5.3)	6.0	38 (3.9)	N/A	30 (5.8)	2.9	6 (9.1)	0.8	23 (4.5)	0.2	29 (5.2)	N/A
Intermediate	8,427 (23.5)	37.9	215 (21.8)	N/A	114 (22.1)	41.7	13 (19.7)	42.3	130 (25.4)	28.7	126 (22.7)	N/A
Normal	25,544 (71.2)	52.5	734 (74.4)	N/A	373 (71.2)	50.8	47 (71.2)	56.4	359 (70.1)	68.8	401 (72.1)	N/A
Ultra-rapid	N/A	3.6	N/A	N/A	N/A	4.6	N/A	0.6	N/A	2.4	N/A	N/A

Obs. N = Observed number of participants in sample per metabolic phenotype; Exp. % = Published frequency of metabolic phenotypes for that population, where available; N/A Data not available.

2.3.4 Selected phenotypes

The common adverse events and the related variables identified in the UK Biobank data are summarised in table 2.13 below. These were the ‘proxy’ variables I selected as the best available measurement of the reported adverse drug reactions. However, not all traits were included in the final analysis. The number of subjects identified who reported taking a drug indicated for tardive dyskinesia was too low for analysis (N=107, with no CYP2D6 poor metabolisers and seven CYP2C19 poor metabolisers). In order to simplify the analyses in this chapter, I excluded binary traits such as ’reported trouble falling or staying asleep’. For more detailed sleep analyses see chapter 4. Additional detail on all phenotypes extracted from UK Biobank or derived or use in these analyses is provided in appendix B, table B.1.

Table 2.13: Variables selected from the UK Biobank to derive psychotropic-induced adverse events.

Reported adverse drug reaction	UK Biobank variable(s) selected
Increased weight	BMI (km/m^2); weight (kg)
Raised cholesterol	Cholesterol (HDL, LDL, overall cholesterol) measure (mmol/L)
Raised HbA1c; diabetes risk	HbA1c measure (mmol/mol)
Sedation; fatigue; sleep disturbance	Sleep duration (hrs)
Insomnia	Reported trouble falling or staying asleep
Tardive dyskinesia	Taking antipsychotic and anticholinergic drugs indicated for tardive dyskinesia
Prolonged QT interval	QT reading from electrocardiogram (ms)

2.3.5 CYP2C19 and CYP2D6 metabolic phenotypes and adverse drug reactions

Overall, I observed limited evidence of a relationship between CYP450 metabolic phenotype and the selected measures of adverse drug reactions. In subjects taking SSRIs (table 2.15), there was a suggestive association between longer sleep and CYP2C19 ultrarapid metabolisers (0.14hours, 95% CI [0.02,0.25]; p = 0.018). Among subjects taking tricyclic antidepressants (table 2.14) there was evidence of a significant association between CYP2C19 intermediate and poor metabolisers and decreased BMI compared to normal metabolisers (IM: -0.38kg/m^2, 95% CI [-0.63–0.13], p = 0.003; PM: -0.8kg/m^2, 95% CI [-1.39–0.2], p = 0.008). This association is in the opposite direction to my initial hypothesis. There is evidence of trend level associations between CYP2C19 metabolic phenotype and weight, sleep duration and QT interval (see table 2.14). There was no evidence of a significant association between CYP2D6 metabolic status and any of the selected outcomes among

subjects taking antipsychotics (table 2.16), potentially due to limited statistical power (see appendix B table B.5).

Table 2.14: Association between CYP450 metabolic phenotype and measures of adverse drug reactions among participants taking tricyclic antidepressants.

		Phenotype											
		BMI (kg/m^2)		Weight (kg)		HbA1c (mmol/mol)		Cholesterol (mmol/L)		Sleep duration (hours)		QT (ms)	
Gene	Metabolic phenotype	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P
CYP2C19	Poor	-0.79 (0.30)	0.008	-2.12 (0.86)	0.014	0.17 (0.43)	0.705	-0.04 (0.07)	0.569	0.08 (0.08)	0.318	1.96 (8.89)	0.826
	Intermediate	-0.39 (0.12)	0.003	-0.66 (0.36)	0.069	-0.40 (0.18)	0.031	-0.01 (0.03)	0.621	-0.009 (0.03)	0.775	-8.29 (3.58)	0.021
	Rapid	-0.17 (0.14)	0.224	-0.21 (0.39)	0.591	0.02 (0.20)	0.905	-0.02 (0.03)	0.505	-0.08 (0.04)	0.023	-4.70 (3.69)	0.203
	Ultra-rapid	-0.23 (0.26)	0.388	-0.56 (0.74)	0.452	-0.0002 (0.37)	0.999	0.03 (0.06)	0.662	-0.09 (0.07)	0.188	-14.17 (7.71)	0.067
CYP2D6	Poor	0.09 (0.24)	0.705	-0.20 (0.69)	0.777	-0.27 (0.35)	0.433	-0.02 (0.05)	0.713	0.15 (0.07)	0.025	15.31 (8.50)	0.072
	Intermediate	0.13 (0.12)	0.313	0.38 (0.35)	0.281	0.13 (0.18)	0.476	-0.01 (0.03)	0.650	0.02 (0.03)	0.630	2.97 (3.58)	0.407

Table 2.15: Association between CYP450 metabolic phenotype and measures of adverse drug reactions among participants taking SSRI antidepressants.

		Phenotype											
		BMI (kg/m^2)		Weight (kg)		HbA1c (mmol/mol)		Cholesterol (mmol/L)		Sleep duration (hours)		QT (ms)	
Gene	Metabolic phenotype	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P
CYP2C19	Poor	0.04 (0.24)	0.868	0.03 (0.68)	0.960	-0.24 (0.31)	0.428	0.009 (0.05)	0.861	-0.00002 (0.008)	0.854	6.96 (6.09)	0.253
	Intermediate	-0.14 (0.10)	0.165	-0.53 (0.29)	0.071	-0.15 (0.13)	0.259	0.007 (0.02)	0.732	-0.01 (0.06)	0.999	-2.65 (2.32)	0.254
	Rapid	-0.13 (0.11)	0.226	-0.09 (0.31)	0.759	-0.09 (0.14)	0.548	-0.02 (0.02)	0.442	0.02 (0.03)	0.401	-2.14 (2.45)	0.382
	Ultra-rapid	-0.21 (0.22)	0.330	-0.78 (0.63)	0.217	-0.21 (0.29)	0.475	-0.07 (0.05)	0.144	0.14 (0.05)	0.018	-7.90 (5.01)	0.116
CYP2D6	Poor	0.17 (0.19)	0.353	0.37 (0.53)	0.488	0.13 (0.25)	0.593	-0.06 (0.04)	0.103	-0.02 (0.05)	0.671	4.41 (4.34)	0.310
	Intermediate	0.06 (0.10)	0.567	0.14 (0.29)	0.630	-0.24 (0.13)	0.064	-0.03 (0.02)	0.162	0.03 (0.03)	0.320	-3.10 (2.23)	0.165

Table 2.16: Association between CYP450 metabolic phenotype and measures of adverse drug reactions among participants taking antipsychotics.

		Phenotype											
		BMI (kg/m^2)		Weight (kg)		HbA1c (mmol/mol)		Cholesterol (mmol/L)		Sleep duration (hours)		QT (ms)	
Gene	Metabolic phenotype	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P	Effect (SD)	P
CYP2D6	Poor	0.40 (0.51)	0.433	-0.11 (1.47)	0.939	-0.92 (0.73)	0.207	-0.20 (0.11)	0.057	0.29 (0.18)	0.118	-3.30 (12.26)	0.789
	Intermediate	0.08 (0.26)	0.748	0.24 (0.74)	0.745	0.35 (0.37)	0.349	0.07 (0.05)	0.189	0.11 (0.09)	0.256	7.39 (9.22)	0.426

2.4 Discussion

Previous studies have reported that variation in *CYP2D6* and *CYP2C19* genes is associated with QT prolongation [247, 248], weight gain [170, 247–250], and hormonal changes among patients taking psychotropic medication, as well as increased risk of extrapyramidal adverse reactions to antipsychotics [151]. This has resulted in the publication of several clinical guidelines, recommending dose adjustments for certain tricyclic and SSRI antidepressants (from CPIC: [79, 80]), as well as a small number of antipsychotics (from DWPG: [207]). However, these guidelines are a rather cautious interpretation of the available evidence, and are not in widespread clinical use. Indeed, recent studies and meta-analyses, including those described in this thesis (see chapter 1), have yielded inconclusive or negative findings, and the clinical significance of CYP450 metabolic phenotypes in terms of treatment response and adverse reactions is still in question [108, 206]. As described in the introduction to this chapter, many studies of pharmacogenetics are limited by small sample sizes, low representation of poor and ultra-rapid metabolisers, and a lack of reproducible results.

With many now aiming to recruit hundreds of thousands, or even millions, of subjects, large scale biobanks have great potential in the study of drug-gene interactions. In this chapter, I present the largest pharmcogenetic analysis of psychotropic drug users to date, with a sample of over 30,000 individuals, including a much higher number of extreme CYP450 metabolisers than seen in previous publications (N=10,511 non-wild-type CYP2D6 metabolisers and N=21,771 non-wild-type CYP2C19 metabolisers). I present a reliable and scalable method for assigning CYP450 metabolic phenotypes based on SNP-based array data, and demonstrate how this data can be used to probe for evidence of a relationship between metabolic phenotypes and adverse drug reactions. I demonstrate that, while it is possible to use SNP-array data to assign individuals to CYP450 metabolic phenotype groups, I find limited evidence of a relationship between CYP450 metabolic status and risk of adverse drug reactions using these methods. There are several clear limitations to my methods, which may explain in part why I was not able to confirm my initial hypotheses. I will discuss these limitations in detail throughout this discussion, and describe how subsequent work can address them.

The final sample included in this analysis was 35,128 subjects taking at least one of 28 antidepressants (N = 33,345) and/or 24 antipsychotics (N = 2,904) (figures 2.2 and 2.3).

Overall, I do observe some evidence of a relationship between CYP450 metabolic phenotype and the selected proxy measures of adverse drug reactions. There is evidence of an association between shorter sleep duration and CYP2C19 rapid metabolisers taking tricyclic antidepressants (-0.08 hours, 95% CI [-0.16,-0.01]; p = 0.023) and longer sleep duration among CYP2C19 ultra-rapid metabolisers taking SSRIs (0.14 hours, 95% CI [0.02,0.25]; p = 0.018). After multiple testing correction, these observations are suggestive only. Effect sizes of 0.08 and 0.14 hours are equivalent to a difference of less than 15 minutes in reported sleep duration, so these findings are not necessarily clinically significant on their own. That said, small effect sizes are expected when studying common genetic variants and complex traits, so this finding is still potentially of scientific interest. The fact that the effect sizes are in opposite directions to each other is unexpected, although this could point to a non-linear relationship between sleep and the included explanatory variables. Chapter 4 discusses the relationship between CYP450 metabolic status and sleep duration in greater depth.

Among subjects taking tricyclic antidepressants there is evidence of a suggestive association between CYP2C19 metabolic phenotype with both weight and QT interval. In addition, I observe a significant association between CYP2C19 intermediate and poor metabolisers and decreased BMI (IM: -0.38kg/m^2, 95% CI [-0.63–0.13], p = 0.003; PM: -0.8kg/m^2, 95% CI [-1.39–0.2], p = 0.008). Interestingly, all of these associations are in the opposite direction to my initial hypotheses; which would have expected poor or intermediate metabolisers to have higher BMI due to increased serum drug concentration. One potential explanation for this is the complex metabolic pathway of tricyclic antidepressants, particularly tertiary amine tricyclics. It involves two steps, with the first step catalysed by CYP2C19 and producing an active metabolite. The second step is the metabolism of this active metabolite to an inactive metabolite, and is catalysed by CYP2D6 [251, 252]. In order to account for this, metabolic phenotypes for both CYP2C19 and CYP2D6 were included together in the analysis of tricyclic antidepressants. However, further studies investigating the synergistic action of CYP2D6 and CYP2C19 on tricyclic metabolism are required, that take into account the potentially complex pharmacokinetic activity in patients with altered metabolism in both enzymes. With a sample size of over 8,000 subjects, amitriptyline accounts for more than two thirds of all subjects taking a tricyclic antidepressant. Chapters 3 and 4 therefore include single drug analyses of amitriptyline, and other highly prescribed drugs, in an effort to elucidate some of these complexities still further.

I did not observe any significant associations between CYP2D6 metabolic status and any of the selected outcomes among subjects taking antipsychotics. Although a sample of just under three thousand participants represents one of the largest available samples of antipsychotic drug users, these analyses were likely still underpowered given the heterogeneity of the sample. Indeed, a post-hoc power calculation demonstrated that this study had just 12% power, and that a sample with at least 500 poor metabolisers would be necessary to detect a statistically significant change in any of the phenotypes considered in this chapter (see appendix B). No single antipsychotic had a high enough frequency to allow for single drug analysis. The UK Biobank is a population study of healthy volunteers, and thus the number of subjects who report taking an antipsychotic is low. In addition, it is reasonable to assume that many of the subjects who do report taking antipsychotics are relatively well, and thus may be taking a low dose that is less likely to cause side effects.

Of the 2,904 subjects who report taking an antipsychotic, prochlorperazine (N=928 individuals) is the most common, followed by olanzapine (N=538). Prochlorperazine is rarely prescribed for the treatment of psychosis in the UK, but rather is often used as an antiemetic (anti-sickness drug). Amitriptyline was the most common antidepressant in this sample, but again that does not line up with reported prescribing rates for depression in the UK. Therefore it is also likely that this drug is being used for other indications, such as sleep problems or pain, rather than depression. I decided to include these in my analysis regardless, because the risk of adverse event is theoretically unchanged by the indication. Though this can be considered a strength of these analyses, it is worth noting that several of the proxy measures of adverse drug reaction included are, independently of treatment, more common among patients with both psychosis and depression. Therefore this analysis would likely be improved with a more clearly defined patient sample. The UK Biobank does have data on diagnoses (e.g., ICD-10 codes) for some participants, but the number of subjects with a psychosis/schizophrenia diagnosis is small, and an analysis using this data would be even more underpowered. I determined that in this analysis I was primarily interested in the relationship between CYP450 metabolic phenotype and risk of adverse drug reactions, and that a simplified model with the largest possible sample was the best first step here. Repeating this analysis in another cohort with a larger proportion of psychiatric patients, such as the Million Veteran Program, will be valuable [253].

A related limitation is the reliance on self-reported data, and the lack of data on treat-

ment dose and duration. Most adverse drug reactions are dose-dependent, and thus further analysis including this data is warranted. Recently, the UK Biobank has released primary care records for some 200,000 participants, and this data is expected to be available for all subjects in due course[5]. Analysis of this data may provide valuable detail to strengthen this analysis, especially if this can be conducted without sacrificing the large sample size.

Not all adverse events described as ‘very common’ or ‘common’ for the drugs of interest are captured in the UK Biobank. For example, raised prolactin levels (hyperprolactinemia, see chapter 1) is common among patients taking antipsychotics, but prolactin levels were not measured by the UK Biobank. Tardive dyskinesia is a known adverse reaction to certain antipsychotics, but the only potential indicator of tardive dyskinesia in this data would be the co-prescription of anticholinergic and antipsychotic medication, which would be indicative of extrapyramidal symptoms. Although I investigated this, the cases were too few to include in this analysis. Psychiatric symptoms, (e.g suicidal thoughts, anxiety, low mood), were collected by the UK Biobank, but were not useful in this context as this data would be particularly impacted by the lack of temporal data, since they are more likely to be the reason for the patient seeking treatment in the first place than as a side effect of the drugs.

A prospective clinical trial (as described in chapter 5) will enable the collection of more detailed and less heterogeneous data, with information on treatment dose, duration, and indication, as well as the timing and severity of any adverse drug reactions. While the trial data may be from a much smaller sample, it will be of a higher quality. It will be necessary to pair analysis of highly granular trial data with these larger scale computational analyses in order to probe all angles of these complex drug-gene relationships. In addition, the cross-sectional nature of the UK Biobank data naturally suits some phenotypes better than others. For example, BMI and weight can vary significantly, for many different reasons. Without having information on baseline (pre-treatment) weight, it is difficult confirm if higher BMI or weight is the result of a adverse-drug reaction, or if those subjects were of higher BMI/weight to begin with. Prospectively designed clinical trials are essential to address this.

Among UK Biobank participants taking antidepressants, 5.2% report taking more than one different antidepressant concurrently (of these, 2% report taking three or four). Of those

[5]This data is actually currently available for all UK Biobank participants where the focus of the research is Covid-19, and will shortly be available for researchers in other fields.

taking antipsychotics, 4.5% report taking more than one different antipsychotic medication concurrently (of these, 7.4% report taking three or four). The co-prescription of an antidepressant with antipsychotics is very common, with 41.4% of subjects taking antipsychotics also taking at least one antidepressant. In addition, many subjects report taking concomitant medication that is known to inhibit CYP2C19 (23.8%) and/or CYP2D6 (5.75%). In order to simplify the statistical models, I did not include this information as covariates in these analyses. In chapters 3 and 4, I take a deeper look into two specific outcome (HbA1c levels and sleep duration) to allow me to investigate more complex statistical models that account for this polypharmacy.

All of the outcomes included in these analyses are complex traits, with many genetic and environmental risk factors. The SNP-based heritability of these traits ranges from as little as 7% for sleep duration, to over 40% for QT interval [254–257]. The inclusion of polygenic risk scores for the outcome traits may improve analyses of pharmacogenetic associations by capturing background genetic disease risk. A genome-wide gene-environment interaction study may also highlight other genes of potential interest.

The first section of this chapter describes the assigning individuals to their correct CYP450 metabolic phenotype groups. Compared to published frequencies of the resulting phenotypes, this method appears to be reliable in the European sample with the calculated frequencies being similar to the published data. With limited numbers of non-European subjects in my sub-sample, it is difficult to confirm the accuracy of this method for all populations, but it does appear to be less consistent. This draws attention to another major limitation of this work, and of the field of pharmacogenetics at large. The full UK Biobank sample of almost half a million individuals is, like the UK population at large, predominantly white European. Some 93.1% of my sub-sample is defined as white European according based on their genetic data (this is in line with UK census data for the UK Biobank recruitment period, which reported the population to be 91.3% white European when accounting for the recruited age range[6]) [258].

There is a great deal of variation in the frequency of functional variants within the CYP450 genes across different populations [176, 197]. Many of the SNPs of interest in this study are more common in non-European populations, which means a more diverse sample could in fact elucidate much more about these gene-drug relationships than a predominantly

[6]Note that the UK Census data is self-reported, whereas the definitions here are based on principal component analysis of genotype data. Recruitment for the UK Biobank was conducted between 2006 an 2010.

European sample. Many of the variants that are common in non-European populations are also very rare among Europeans, which means they are less likely to be included on a SNP array or imputation panel, and are more likely to be removed in the quality control process when they are included. A review in 2017 found that only one third of studies on CYP450 genes conducted ‘minimal genotyping’ that included all risk variants needed to accurately assign CYP450 metabolic phenotype status [197]. This has a greater impact in studies of non-European populations, as often the risk variants with higher frequency among European populations will be prioritised. To account for this, I conducted the quality control of the genetic data and reviewed the rare SNPs separately for each major population group. However, the methods described here are undoubtedly less robust when applied to non-European populations, and are naturally limited by the UK Biobank genotype arrays and imputation panels. Given we assume that the known risk variants will have the same functional outcome on the resulting proteins, data from all populations groups can be analysed together (following quality control conducted separately per population group given differences in LD structure and allele frequency). That said, multiple risk variants give rise to the same metabolic phenotype (from poor to ultra-rapid), and these have different frequencies among different populations. The field of pharmacogenetics as a whole will be greatly benefited by further study in more diverse cohorts.

Future work could be improved with regard to ancestral diversity through the use of microarrays that specifically target rare variants [259], and improved imputation panels, such as TopMed [260]. In addition, the availability of whole genome sequencing data will significantly improve pharmacogenetic research opportunities, and will be especially beneficial to the study on non-European populations.

Although both arrays used by UK Biobank have relatively good coverage of *CYP2C19* and *CYP2D6*, several SNPs that define known star alleles were neither genotyped nor imputed or did not otherwise meet the criteria for inclusion as described in the methods. The wild-type allele (*1) is defined by the absence of any defining variants of the other star alleles. Therefore, I expect a number of individuals to be misclassified as normal metabolisers. I anticipate this number to be small given the low minor allele frequency of the missing variants, although the impact of this is likely higher among non-European populations as discussed above. Any individuals in this study that have been incorrectly defined as normal metabolisers will result in ‘false negatives’ that weaken the significance of any findings,

rather than 'false positives' that lead us to incorrect conclusions. Not all published studies will include the same variants on the SNP array and/or imputation panel, and therefore the precise definition of the *1 allele will vary across samples. This makes it challenging to compare results across studies and samples (as discussed in greater detail in chapter 1). Again, the use of large-scale biobank data should help define the frequency of these risk variants in different populations, which would enable researchers to more accurately quantify the impact of any excluded variants.

I was not able to include CYP2D6 ultra-rapid metabolisers in this study, as copy number and other structural variants were not defined. CYP2D6 ultra-rapid metabolisers are the least common phenotypic group across all populations, with a frequency of less than 2% in European, South Asian, East Asian and Admixed European groups, and approximately 3-6% in African ancestry groups [176, 197]. CYP2D6 ultra-rapid metabolisers therefore represent a very small minority in this sample, and they have been combined with the normal metaboliser group by default. I estimate this to have a small effect on our results as we would expect ultra-rapid metabolisers to be less susceptible to adverse drug reactions, though it will be important to consider this group in future studies of treatment failure or treatment resistance. The availability of whole genome sequencing data will improve the accuracy with which highly polymorphic pharmacogenes like *CYP2D6* can be characterised, whilst still capturing the important splicing or non-coding variants that may be missed with exome sequencing data [90].

Overall, the UK Biobank is very exciting and valuable resource for the study of pharmacogenetic variation in psychiatry. However, this analysis makes clear that the data may be too heterogenous and varied to allow for simplified analyses.

Chapter 3

The influence of CYP2D6 and CYP2C19 genetic variation on diabetes mellitus risk in people taking antidepressants and antipsychotics

Some of the information presented in this chapter has formed the basis of a publication: Austin-Zimmerman et al. (2021) [261] - see appendix C.

Abstract

CYP2D6 and CYP2C19 enzymes are essential in the metabolism of antidepressants and antipsychotics. Genetic variation in these genes may increase risk of adverse drug reactions. After investigating the putative relationship between CYP540 metabolic phenotypes and several adverse drug reactions, I decided to investigate one adverse drug reaction, raised HbA1c, in greater detail. Antidepressants and antipsychotics have previously been associated with risk of developing diabetes. I examined whether individual genetic differences in *CYP2D6* and *CYP2C19* contribute to these effects.

As detailed in chapter 2, I identified all UK Biobank participants who reported taking at least one antidepressant or antipsychotic. These participants were classified as poor, intermediate, or normal metabolisers of CYP2D6, and as poor, intermediate, normal, rapid and ultra-rapid metabolisers of CYP2C19. Risk of diabetes mellitus represented by HbA1c level was examined in relation to the metabolic phenotypes. In this chapter, I include 31,579 individuals taking antidepressants and 2,699 taking antipsychotics, for whom I was also able to extract data on HbA1c levels and diabetes diagnosis. I have analysed drugs either

individually (where sample size permitted) or grouped by class.

CYP2D6 poor metabolisers taking paroxetine, regardless of diabetes status, had higher Hb1Ac than normal metabolisers (mean difference: 2.29mmol/mol; p <0.001). Among participants with diabetes who were taking venlafaxine, CYP2D6 poor metabolisers had higher HbA1c levels compared to normal metabolisers (mean differences: 10.15 mmol/mol; p <0.001. Among participants with diabetes who were taking fluoxetine, I observe that CYP2D6 intermediate metabolisers had decreased HbA1c, compared to normal metabolisers (mean difference -7.74mmol/mol; p = 0.017). I do not observe any relationship between CYP2D6 or CYP2C19 metabolic status and HbA1c levels in participants taking antipsychotic medication.

These results indicate that the impact of genetic variation in *CYP2D6* differs depending on diabetes status. Although these findings support existing clinical guidelines, further research is essential to inform pharmacogenetic testing for people taking antidepressants and antipsychotics.

3.1 Introduction

Most first-generation antipsychotics, as well as olanzapine and clozapine, have been shown to impair glucose regulation. Other second generation (or atypical) antipsychotics such as amisulpride, ziprasidone, and aripiprazole seem less associated with this risk [65, 129, 262–264]. Several studies have linked tricyclic antidepressants to increased diabetes risk (4,11–13). The evidence for selective serotonin reuptake inhibitors (SSRIs) is inconsistent, with some studies showing improved diabetic control and others showing the opposite [64, 265]. Research into serotonin-noradrenaline reuptake inhibitors (SNRIs), such as venlafaxine, has reported both a lack of influence on glycaemic control and diabetes risk [115, 266–268]. Some research suggests that the risk of antidepressant-induced diabetes varies substantially between similar drugs of the same class, and thus may not be a mechanism-based adverse effect, but rather an off-target effect of a single drug [269].

As described in previous chapters, CYP450 pharmacogenetics may help explain inter-individual differences in drug response and adverse drug reactions. Several studies have shown that poor metabolisers of CYP2D6 or CYP2C19 have higher serum levels of antidepressants and antipsychotics, compared to normal metabolisers and several clinical guidelines exist based on this evidence [79, 80, 84, 200–207].

Thus far, research on the putative association between CYP450 metabolic phenotype

and adverse drug reactions in response to antidepressants and antipsychotics has been limited by small sample sizes [84, 189]. My previous work (see chapter 2) demonstrated that even when large samples are available, these gene-drug relationships are complex and the effect sizes of the individual risk variants may be quite small and thus difficult to detect in 'noisy' datasets. Little is known about diabetes risk associated with pharmacogenetics of these drugs. This chapter aims to examine the association between CYP2C19 and CYP2D6 metabolic phenotypes and the risk of diabetes mellitus in UK Biobank participants taking antidepressants and antipsychotics. By focusing on just one adverse drug reaction, I am able to investigate more complex statistical models, account for a wider range of covariates, and consider the potential interactions between CYP450 metabolic status and other variables.

3.2 Methods

The methods to define the subsample included in this analysis, and to assign them to the appropriate CYP450 metabolic phenotype groups are described in chapter 2. We identified a sample of 44,051 participants taking a drug of interest for this study. Data on HbA1c levels, diabetes diagnosis (self-reported and confirmed by ICD-10 diagnosis when available), antidiabetic medications, enzyme inhibitors and body mass index (BMI) were also downloaded.

3.2.1 HbA1c measure and diabetes status

The UK Biobank measured a variety of biochemical markers in blood samples collected at the baseline visit. Glycated haemoglobin (HbA1c) was measured with the High Performance Liquid Chromatography (HPLC) method on a Bio-Rad VARIANT II Turbo analyser. The HbA1c analytical range was 15-184 mmol/mol and this measurement was recorded for over 92% of the UK Biobank cohort. Table 3.1 defines HbA1c levels for non-diabetic, pre-diabetic and diabetic subjects, as per NHS guidelines.

Table 3.1: Normal, prediabetic and diabetic ranges of HbA1c (mmol/mol).

Status	HbA1c (mmol/mol)
Normal	<42 mmol/mol
Pre-diabetes	42 to 47 mmol/mol
Diabetes	>47 mmol/mol

There were several items in the UK Biobank which could act as a source of information for whether the patient had diabetes or not, namely ICD-10 diagnosis, self-reported

diagnosis of diabetes, or self-reported use of antidiabetic medications. Though ICD-10 data would be the most reliable item to use for the purpose of our research, this data was incomplete, covering 410,316 participants and recording 3,399 (0.82%) cases of diabetes mellitus. Based on self-reported data, the prevalence of diabetes mellitus in the UK Biobank was 5.28%. This is consistent with UK epidemiological studies which report the prevalence of diabetes mellitus at 7%. I thus concluded that the self-reported data was reliable in this instance. Where possible, diabetes status was confirmed by ICD-10 code. Based on the available data it was not possible to differentiate between type 1 and type 2 diabetes, but given the prevalence, and age range of this patient population, I would expect the majority of cases to be type 2.

I identified 49 individuals who reported taking antidiabetic medication but stated they do not have diabetes. They were excluded from the analysis due to uncertainty about their diagnosis. A total of 40,783 participants taking a psychotropic drug of interest also had HbA1c measurements available. After applying all genetic quality control procedures described in chapter 2, a total of 33,149 participants taking antidepressant and/or antipsychotic medication were included in this analysis (see figure 3.1).

3.2.2 Covariates

I identified participants taking insulin, metformin, thiazolidinediones, sulfonylureas, meglitinides, alpha-glucosidase inhibitors. There were no participants within our dataset who reported taking glucagon-like peptide-1 or gastric inhibitory peptide and gliflozins. Using the same method described in chapter 2 to exctract psychotropic drugs, the brand names of anti-diabetic medications were reviewed and compiled to identify all potential self-report cases. These were converted to their generic equivalents and a dichotomous variable which reflected the use of the antidiabetic medications was created.

A high BMI is an independent risk factor for diabetes; hence it was included in our analyses. BMI (kg/m^2) at baseline was downloaded directly from the UK Biobank data showcase.

I identified all participants taking drugs that inhibit CYP2D6 and CYP2C19 activity, using the same method applied to the extraction of all other drugs (see chapter 2). In the sample, 1,969 participants were taking a CYP2D6 inhibitor drug including: ranitidine, celecoxib, metoclopramide, chlorphenamine, terbinafine, hydroxyzine or promethazine. I also identified 8,340 participants taking drugs that are known CYP2C19 inhibitors including

omeprazole, esomeprazole, lansoprazole, pantoprazole, oestrogen, cimetidine, modafinil, topiramate, indomethacin or oxcarbazepine.

Further detail on the how this data was collected by UK Biobank, and how it was extracted and processed is provided in appendix B, table B.1.

3.2.3 Statistical analysis

I conducted a grouped analysis of all tricyclic antidepressants, as previous evidence suggests that they all cause an increase in HbA1c to some extent [270]. I did not analyse SSRIs as a group due to variable evidence on their influence on HbA1c in the literature [267, 269, 270].

We calculated that a minimum of 97 CYP2D6/CYP2C19 poor metabolisers were necessary in order to achieve 80% to detect a unit change in HbA1c level. Given the frequency of CYP2D6 poor metabolisers was approximately 5%, any drug taken by at least 1,940 subjects should have a sufficient number of poor metabolisers to allow for independent analysis. Therefore, any drug with at least 1,800 subjects was considered, and was analysed independently if there were at least 97 CYP2D6 poor metabolisers.

Medications were grouped according to whether their primary metabolic pathway was catalysed by CYP2D6 or CYP2C19, based on the Maudsley Prescribing Guidelines and CPIC guidelines [79, 80, 115]. Tricyclic antidepressants that are known CYP2C19 substrates are: amitriptyline, clomipramine, doxepin, imipramine and trimipramine. SSRIs that are known CYP2C19 substrates are: citalopram, escitalopram, and sertraline. Tricyclic antidepressants that are known substrates for CYP2D6 include: amitriptyline, clomipramine, duloxetine, and doxepin. SSRIs that are known substrates for CYP2D6 are: fluoxetine, fluvoxamine, paroxetine, sertraline, as well as mirtazapine and venlafaxine [58, 194].

As described previously, no single antipsychotic drug had sufficient sample size to allow for individual analysis. Therefore, we included all antipsychotic drugs known to be metabolised at least in part by CYP2D6: aripiprazole, clozapine, fluphenazine, haloperidol, olanzapine, perphenazine, pimozide, risperidone, zuclopenthixol, thioridazine. CYP2C19 does not play a significant role in the metabolism of antipsychotics [115].

For each drug or drug group, I ran linear regression models with HbA1c as the outcome of interest and CYP450 metabolic phenotype and diabetes status as the main explanatory variables. All statistical models were adjusted to account for any participant taking antidiabetic treatment or taking drugs, psychotropic or otherwise, that are known inhibitors of the

enzymes of interest. Additional covariates included were BMI, sex, age, and genetically determined ancestry group. I investigated the interaction of diabetes status and CYP metabolic phenotype. Where this interaction was significant ($p < 0.05$) I conducted a stratified analysis separating participants into two groups based on their diabetes status.

Some of these analyses are nested (individual drug analyses overlap with drug group analyses), and as such, I concluded that a Bonferroni correction for multiple testing would be excessively stringent [242]. Therefore, we report uncorrected p values in all text and tables, but as recommended by Li *et al* (2012) [243], I have an adjusted significance threshold of $p < 0.05/2 = 0.025$ (threshold for a suggestive association $p < 0.1/2 = 0.05$) for the two grouped analyses, and $p < 0.05/6 = 0.0083$ (threshold for a suggestive association $p < 0.1/6 = 0.017$) for the individual drug analyses examining six specific drugs. All statistical analyses were performed using R version 3.6.0 [244–246].

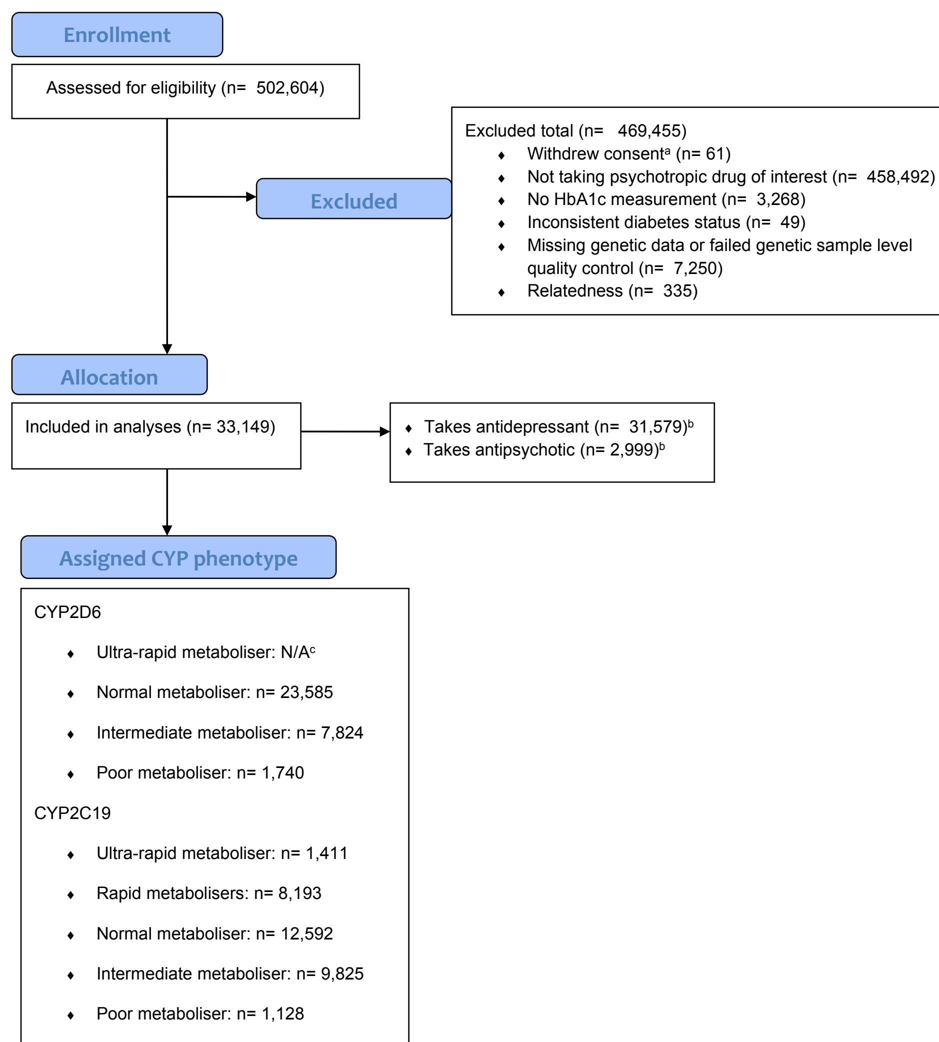
3.3 Results

3.3.1 Dataset

I identified 33,149 UK Biobank participants who reported taking at least one antidepressant or antipsychotic and had HbA1c and genetic data passing quality control (antidepressants N=31,579, antipsychotics N=2,699). This sample includes 22,632 (68.3%) females and 10,517 (31.7%) males. The mean age was 56.6 ± 7.8 years, range 40 to 70 years. Full demographic data and summary statistics of our sample are shown in table 3.2. A more detailed breakdown of demographics and CYP450 metabolic status is included in appendix C.2.

There were 28 different antidepressants identified in our sample. Amitriptyline was the most common drug in our cohort (N=8,191). We identified 24 different antipsychotic drugs, with the most frequent antipsychotics being prochlorperazine (870 individuals, 30.9%), followed by olanzapine (499 individuals, 17.7%) (figure 3.2; appendix table C.3). Among UK Biobank participants taking antidepressants, 5.2% report taking more than one different antidepressant concurrently (of these, 2% report taking three or four). Of those taking antipsychotics, 4.5% report taking more than one different antipsychotic medication concurrently (of these, 7.4% report taking three or four). The co-prescription of an antidepressant with antipsychotics is very common, with 41.4% of subjects taking antipsychotics also taking at least one antidepressant.

Figure 3.1: Adapted CONSORT statement detailing steps taken to arrive at the final included in these analyses.



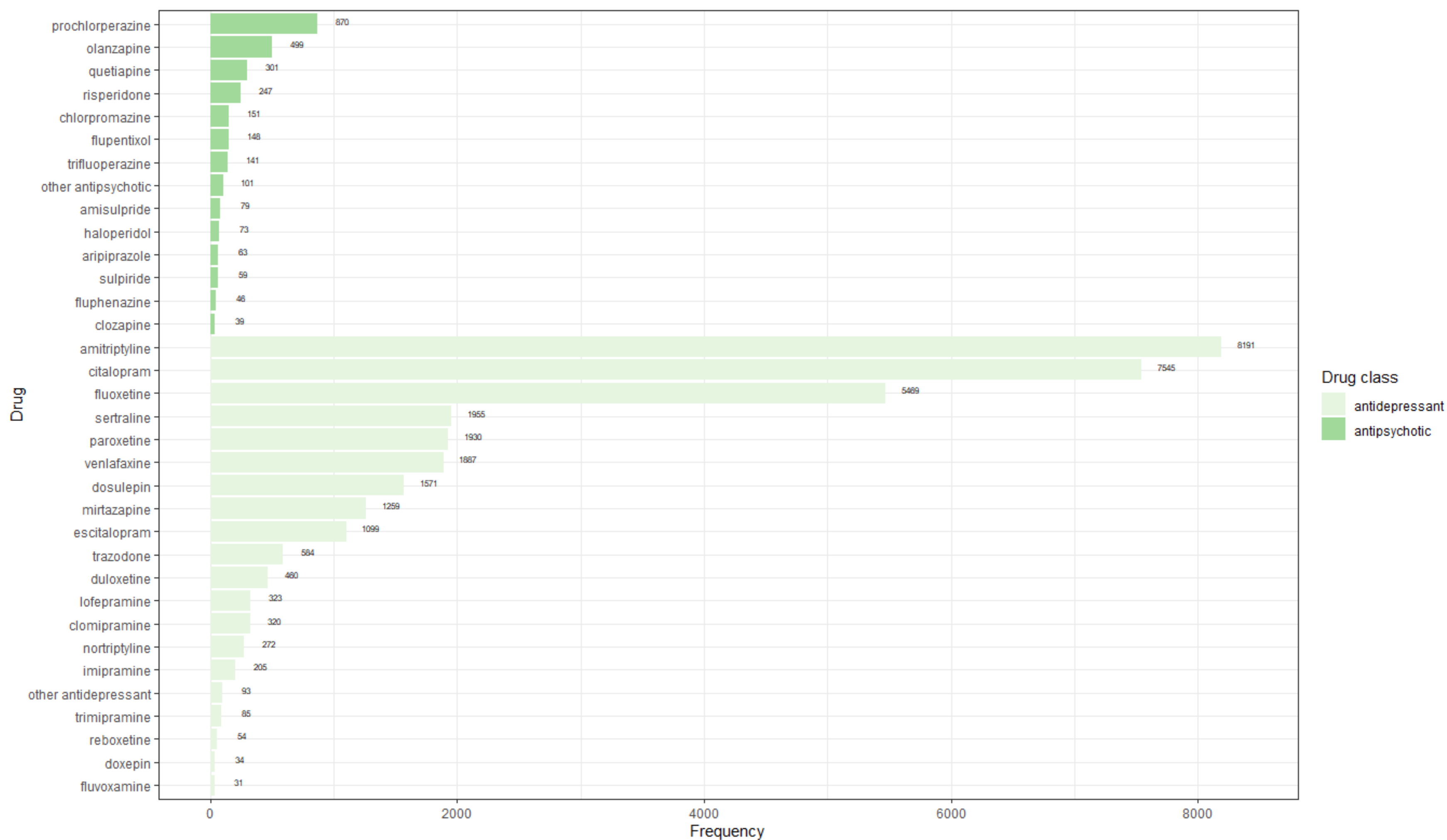
[a]Number of participants who withdrew consent after the initial download of all phenotype data and before the analysis was conducted. Participants who withdrew consent prior to initial download of data were already removed; [b]Note that several subjects report taking both antidepressant(s) and antipsychotic(s) and are included in both analyses; [c]See chapter 2 for detail on why CYP2D6 ultra-rapid metabolisers were not defined in this analysis.

Table 3.2: Sample demographics

	Antidepressants (N=31,579)	**Antipsychotics (N=2,699)**
Sex		
Female	21,754 (68.9%)	1,553 (57.5%)
Male	9,827 (31.1%)	1,146 (42.5%)
Age		
Mean (SD)(years)	56.6 (7.78)	56.4 (8.12)
Range (median)(years)	40-70 (58)	40-70 (57)
Ethnicity		
White European	29,628 (93.8%)	2,403 (89.0%)
Admixed European	795 (2.5%)	72 (2.7%)
African	289 (0.9%)	90 (3.3%)
East Asian	43 (0.1%)	12 (0.4%)
South Asian	374 (1.2%)	65 (2.4%)
Admixed	450 (1.4%)	57 (2.1%)
HbA1c		
Mean (SD) (mmol/mol)	37.1 (7.75)	37.5 (8.31)
BMI		
Mean (SD) (kg/m^2)	28.8 (5.66)	29.1 (5.94)
CYP2D6 metabolic phenotype		
Normal metabolisers	22,486 (71.2%)	1,914 (70.9%)
Intermediate metabolisers	7,433 (23.5%)	650 (24.1%29
Poor metabolisers	1,660 (5.3%)	135 (5.0%)
CYP2C19 metabolic phenotype		
Normal metabolisers	12,001 (38.0%)	1,004 (37.2%)
Intermediate metabolisers	9,367 (29.7%)	789 (29.2%)
Poor metabolisers	1,065 (3.4%)	100 (3.7%)
Rapid metabolisers	7,805 (24.7%)	686 (25.4%)
Ultra-rapid metabolisers	1,341 (4.2%)	120 (4.4%)
Takes CYP2D6 inhibitors[a]		
No	29,713 (94.1%)	2,548 (94.32%)
Yes	1,866 (5.9%)	151 (5.6%)
Takes CYP2C19 inhibitors[a]		
No	23,608 (74.8%)	2,091 (77.5%)
Yes	7,971 (25.4%)	608 (22.5%)
Diabetes status		
No	28,776 (91.1%)	2,415 (89.5%)
Yes	2,803 (8.9%)	284 (10.5%)
Takes antidiabetic medications[b]		
No	29,573 (93.6%)	2,491 (92.3%)
Yes	2,006 (6.4%)	208 (7.7%)

[a] CYP2C19 and CYP2D6 inhibitors identified through review of literature, including British National Formulary; [b] as defined by British National Formulary [222]; SD = standard deviation

Figure 3.2: Frequency of identified antipsychotics (top, darker green) and antidepressants (bottom, lighter green) in UK Biobank.

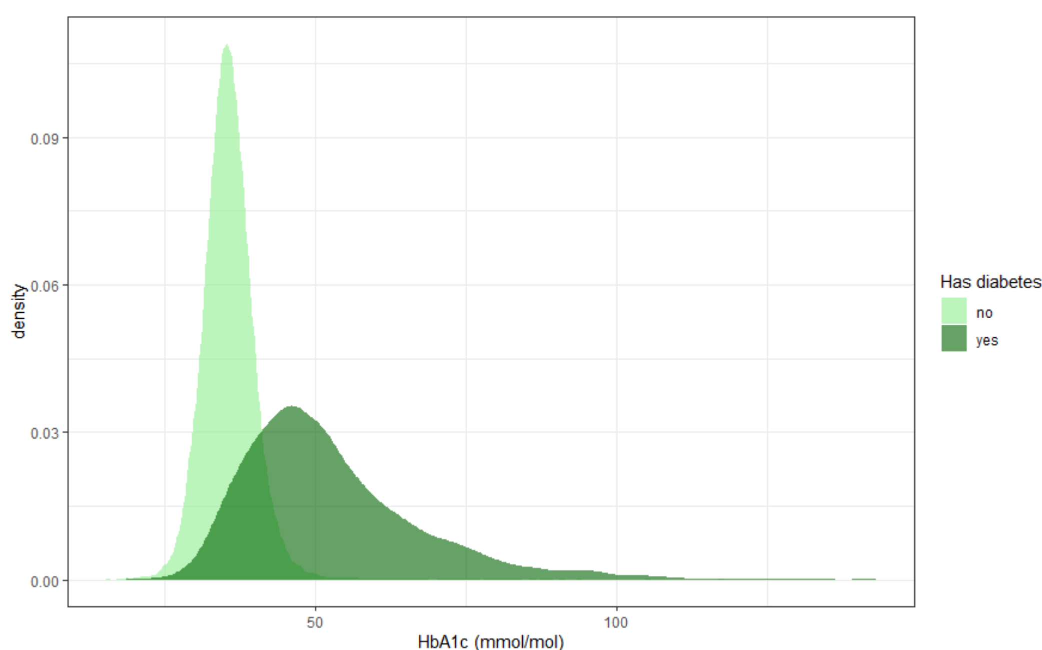


Other antipsychotic (N): *promazine (30), zuclopenthixol (25), perphenazine (12), pipotiazine (10), pericyazine (8), levopromazine (6), benperidol (4), pimozide (3), thioridazine (2), sertindole (1);* ***Other antidepressants (N):*** *moclobemide (30), phenelzine (30), tranylcypromine (21), bupropion (6), mianserin (4), isocarboxazid (2).*

3.3.2 HbA1c and diabetes status within sample

Of the 33,149 participants included in this data, 2,936 (8.9%) were defined as diabetic, based on a combination of self-reported information and ICD-10 codes. As anticipated, the diabetic group had higher average levels of HbA1c (figure 3.3). Among the non-diabetic group, the mean HbA1c was 35.6 (SD 14.5) mmol/mol. Among the diabetic group, the mean HbA1c was 52.1 (SD 4.5) mmol/mol.

Figure 3.3: Density plot showing HbA1c (mmol/mol) distribution among diabetic and non-diabetic subjects.



3.3.3 Antidepressants and CYP450 metabolic status

For several antidepressants, I identified a statistically significant interaction between diabetes status and CYP2D6 and CYP2C19 metabolic phenotype (figure 3.4). Where this was the case, the analyses was stratified by whether participants had diabetes or not.

Stratified analyses of citalopram, sertraline, and amitriptyline did not reveal any significant association between the relevant CYP450 metabolic status and HbA1c levels (see additional detail in appendix C, tables C.4, C.5, and C.6).

Several tricyclic antidepressants were reported too infrequently to allow for singe-drug analysis. Therefore, we grouped the remaining drugs of this class, excluding amitriptyline as its higher frequency would have heavily driven the findings. I again stratified the

group based on diabetes status and found no significant associations between CYP2D6 or CYP2C19 derived metabolic groups and HbA1c (see additional detail in appendix C, table C.7).

In addition, I find that participants taking drugs that act as CYP2C19 inhibitors, regardless of CYP2C19 metabolic status, experience higher levels of HbA1c. Citalopram: mean difference: 0.36mmol/mol, 95% CI [0.07,0.65]; p = 0.016); Amitriptyline: mean difference: 0.37mmol/mol; 95% CI [0.09,0.64]; p = 0.009; Tricyclics: mean difference = 0.39mmol/mol; 95% CI [0.13,0.66]; p = 0.004). I did not see this relationship with sertraline (see additional detail in appendix C, tables C.4 to C.7).

As expected, in every antidepressants' model, having diabetes, taking antidiabetic medications, raising BMI and increasing age was associated with higher HbA1c (all p <0.001). In all models apart from venlafaxine, South Asian ethnicity was associated with higher HbA1c level (p range <0.001 - 0.050). African ethnicity was associated with higher HbA1c in following models: citalopram, paroxetine, venlafaxine (p range <0.001 – 0.005). admixed European ethnicity was associated with higher HbA1c levels in fluoxetine models (p = 0.006 and p = 0.027 accordingly) and admixed European ethnicity in tricyclic and amitriptyline models (p <0.001, p = 0.002). Male sex was associated with higher HbA1c in citalopram and fluoxetine models. See additional tables in appendix C.

3.3.3.1 Paroxetine

Among all participants (regardless of diabetes status) taking paroxetine (SSRI), I observe significantly higher HbA1c levels among CPY2D6 poor metabolisers (mean difference: 2.43mmol/mol; 95% CI [1.23,3.63]; p = 7.77x10-5) (see table 3.3).

Table 3.3: Association between CYP2D6 metabolic phenotype and HbA1c levels among participants taking paroxetine.

Predictors	Estimates	CI	p
(Intercept)	22.92	20.37,25.48	$<2x10^{-11}$
Has diabetes (n=174)	6.85	5.11,8.59	$1.7x10^{-14}$
CYP2D6 IM (n=457)	0.23	-0.42,0.87	0.489
CYP2D6 PM (n=106)	2.43	1.23,3.63	$7.8x10^{-5}$
Takes CYP2D6 inhibitor (n=103)	-0.34	-1.55,0.87	0.577
Takes anti-diabetic (n=125)	12.89	10.88,14.89	$<2x10^{-16}$
BMI	0.14	0.09,0.19	$3.8x10^{-8}$
Sex (n male=680)	0.41	-0.16,0.98	0.158
Age at recruitment	0.15	0.11,0.19	$9.5x10^{-16}$
Ethnicity: Admix Eur (n=62)	0.14	-1.39,1.68	0.856
Ethnicity: African (n=12)	6.41	2.96,9.86	0.0003
Ethnicity: East Asian (n=1)	-0.90	-12.83,11.03	0.882
Ethnicity: South Asian (n=18)	6.76	3.92,9.59	$3.2x10^{-6}$
Ethnicity: Other (n=23)	0.56	-1.94,3.06	0.661
Observations	1930		
R^2/R^2 adjusted	0.454 / 0.450		

Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, diabetes status, taking antidiabetics and BMI; Normal metabolisers of CYP2D6 taking paroxetine: 1,367

3.3.3.2 Fluoxetine

A stratified analysis of diabetic participants taking fluoxetine (SSRI) reveals a suggestive association between CYP2D6 intermediate metabolisers and lower HbA1c levels compared to normal metabolisers (mean difference = -3.74mmol/mol; 95% CI [-6.82,-0.67]; p = 0.017) (see table 3.4).

Table 3.4: A) Association between CYP2D6 metabolic phenotype and HbA1c levels among participants taking fluoxetine[a]; B) Stratified analysis of diabetes status among participants taking fluoxetine[b].

A) Predictors	Estimates	CI	p
(Intercept)	22.58	21.34,23.81	$<2x10^{-11}$
Has diabetes (n=426)	7.22	6.20,8.23	$<2x10^{-16}$
CYP2D6 IM (n=1,282)	0.06	-0.29,0.41	0.728
CYP2D6 PM (n=299)	0.04	-0.62,0.69	0.916
Diabetes: CYP2D6 IM	-3.78	-5.03,-2.53	$3.1x10^{-9}$
Diabetes: CYP2D6 PM	-1.81	-4.11,0.49	0.124
Takes CYP2D6 inhibitor (n=304)	0.01	-0.62,0.64	0.970
Takes anti-diabetic (n=292)	12.50	11.39,13.62	$<2x10^{-16}$
BMI	0.16	0.13,0.18	$<2x10^{-16}$
Sex (n male=1,541)	0.36	0.04,0.67	0.027
Age at recruitment	0.15	0.13,0.17	$<2x10^{-16}$
Ethnicity: Admix Eur (n=135)	1.27	0.36,2.17	0.006
Ethnicity: African (n=46)	0.80	-0.75,2.34	0.314
Ethnicity: East Asian (n=9)	1.84	-1.63,531	0.299
Ethnicity: South Asian (n=40)	3.68	2.03,5.34	$1.3x10^{-5}$
Ethnicity: Other (n=78)	0.28	-0.91,1.47	0.640
Observations	1930		
R^2/R^2 adjusted	0.454 / 0.450		

	Diabetes				No diabetes			
B) Predictors	N	Est.	CI	p	N	Est.	CI	p
CYP2D6 IM	100	-3.74	-6.82,-0.67	0.017	1182	0.05	-0.21,0.31	0.696
CYP2D6 PM	24	-0.94	-6.61,4.73	0.745	275	0.04	-0.43,0.52	0.859
Observations			426		5043			
R2 / R2 adjusted			0.196 / 0.175		0.130 / 0.128			

[a]Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, diabetes status, taking antidiabetics and BMI; Normal metabolisers of CYP2D6: 3,888; [b]Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, taking antidiabetics and BMI; Normal metabolisers of CYP2D6: diabetes = 302

3.3.3.3 Venlafaxine

In participants taking venlafaxine (SNRI), I found that poor metabolisers for CYP2D6 with diabetes had higher HbA1c than normal metabolisers with diabetes (mean difference: 10.15mmol/mol; 95% CI [2.63,17.67]; p = 0.008) (see table 3.5).

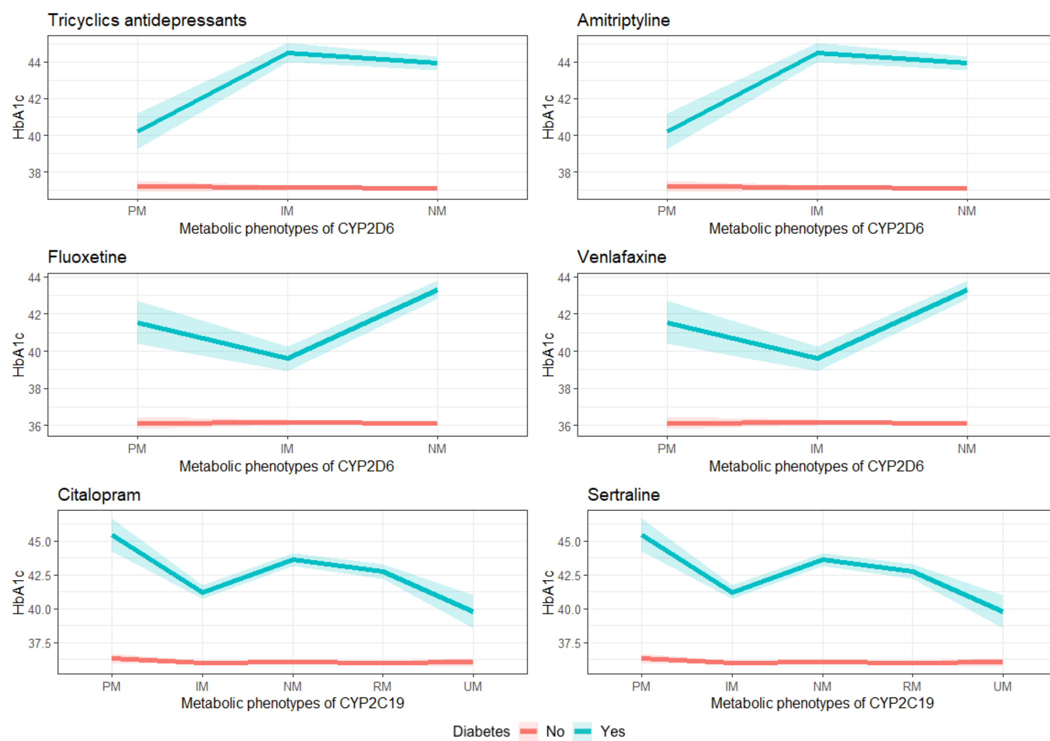
Table 3.5: A) Association between CYP2D6 metabolic phenotype and HbA1c levels among participants taking venlafaxine [a]; B) Stratified analysis of diabetes status among participants taking venlafaxine[b].

A) Predictors	Estimates	CI	p
(Intercept)	23.36	20.83,25.89	$<2x10^{-11}$
Has diabetes (n=182)	5.68	4.04,7.33	$1.77x10^{-11}$
CYP2D6 IM (n=430)	-0.23	-0.89,0.43	0.495
CYP2D6 PM (n=103)	-0.46	-1.73,0.80	0.473
Diabetes: CYP2D6 IM	3.62	1.27,5.98	0.003
Diabetes: CYP2D6 PM	11.44	8.05,14.84	4.79^{-11}
Takes CYP2D6 inhibitor (n=410)	-0.37	-1.50,0.77	0.525
Takes anti-diabetic (n=127)	14.82	12.97,16.67	$<2x10^{-11}$
BMI	0.15	0.10,0.20	$3.5x10^{-10}$
Sex (n male=668)	0.41	-0.14,0.97	0.146
Age at recruitment	0.14	0.11,0.18	$7.0x10^{-15}$
Ethnicity: Admix Eur (n=53)	0.42	-1.17,2.02	0.602
Ethnicity: African (n=13)	4.59	1.37,7.80	0.005
Ethnicity: East Asian (n=1)	-0.96	-12.42,10.51	0.870
Ethnicity: South Asian (n=18)	2.63	-0.09,5.35	0.058
Ethnicity: Other (n=20)	1.97	-0.62,4.56	0.136
Observations	1887		
R^2/R^2 adjusted	0.528 / 0.524		

			Diabetes				No diabetes	
B) Predictors	N	Est.	CI	p	N	Est.	CI	p
CYP2D6 IM	32	3.55	-1.75,8.85	0.188	398	-0.22	-0.71,0.26	0.367
CYP2D6 PM	15	10.15	2.63,17.67	0.008	88	-0.44	-1.36,0.49	0.356
Observations			182				1703	
R2 / R2 adjusted			0.28 / 0.233				0.122 / 0.116	

[a]*Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, diabetes status, taking antidiabetics and BMI; Normal metabolisers of CYP2D6: 1352;* [b]*Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, taking antidiabetics and BMI; Normal metabolisers of CYP2D6: diabetes = 135*

Figure 3.4: Interaction between diabetes status and metabolic phenotypes among subjects taking, from left to right, (a) tricyclic antidepressants; (b) Amitriptyline; (c) Fluoxetine; (d) Venlafaxine; (e) Citalopram; (f) Sertraline.



3.3.4 Antipsychotics and CYP450 metabolic status

I find no evidence that the metabolic phenotypes of CYP2D6 influenced HbA1c levels amongst 2,699 people taking antipsychotic medications. Similarly, taking a CYP2D6 inhibitor drug was not significantly associated with HbA1c levels amongst people taking antipsychotic medication (see table 3.6, figure **??**.

Table 3.6: Association between CYP2D6 metabolic phenotype and HbA1c levels in participants taking antipsychotics.

Predictors	Estimates	CI	p
(Intercept)	24.8	22.6,27.0	<0.001
Has diabetes (n=284)	4.55	3.13,5.97	<0.001
CYP2D6 IM (n=650)	-0.02	-0.58,0.53	0.93
CYP2D6 PM (n=135)	-0.93	-2.01,0.16	0.093
Takes CYP2D6 inhibitor (n=151)	-0.59	-0.43,1.61	0.26
Takes anti-diabetic (n=208)	14.18	12.55,15.81	<0.001
BMI	0.19	0.15,0.23	<0.001
Sex (n male=1,146)	0.40	-0.07,0.99	0.097
Age at recruitment	0.09	0.06,0.12	<0.001
Ethnicity: Admix Eur (n=72)	0.78	-0.67,2.23	0.291
Ethnicity: African (n=90)	3.81	2.49,5.13	<0.001
Ethnicity: East Asian (n=12)	2.31	-1.21,5.13	0.198
Ethnicity: South Asian (n=65)	3.72	2.19,5.26	<0.001
Ethnicity: Other (n=57)	0.94	-0.70,2.58	0.263
Observations	2699		
R^2/R^2adjusted	0.449 / 0.446		

Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, diabetes status, taking antidiabetics and BMI Normal metabolisers of CYP2D6 = 1,914.

3.4 Discussion

This study is the first to explore if variation in the *CYP2D6* and *CYP2C19* genes influences HbA1c levels in individuals taking antidepressants and antipsychotics. Several studies agree that long-term antidepressant treatment increases risk of developing diabetes [64, 271–273], but the extent to which this specific adverse drug reaction is impacted by genetics is unknown. As described previously in this thesis, many studies of CYP450 metabolic status and adverse drug reactions are limited by small sample sizes and low representation of the less common poor or ultra-rapid metabolisers. This study represents one of the largest available samples of individuals taking antidepressants and antipsychotics and includes a much higher number of extreme CYP450 metabolisers than seen in previous publications (N=9,878 non-wild-type CYP2D6 metabolisers and N=21,273 non-wild-type CYP2C19 metabolisers).

In this chapter I demonstrate a significant association between CYP2D6 poor metabolisers and higher levels of HbA1c among all participants taking paroxetine with an average increase of 2.3mmol/mol, which is a clinically relevant change in HbA1c levels. The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines recommend using lower doses of paroxetine for poor metabolisers of CYP2D6 [79]. Thus, these find-

ings are consistent with existing pharmacokinetic evidence and provide further support for the CPIC guidelines. Of interest, some research found that prolonged use of paroxetine was associated with phenocopying, an environmentally induced conversion of normal metabolisers to poor metabolisers [274–276].

I observe a significant interaction between diabetic status and non-wild-type CYP450 status for participants taking amitriptyline, fluoxetine, citalopram, sertraline, and venlafaxine. I conducted stratified analyses of these drugs and found suggestive evidence that, in diabetic participants taking venlafaxine, CYP2D6 poor and intermediate metabolisers have higher HbA1c levels. Like paroxetine, venlafaxine has been previously associated with an increased risk of diabetes [64, 267, 277]. This analysis finds that diabetic CYP2D6 poor metabolisers treated with venlafaxine have on average 10.15 mmol/mol higher HbA1c levels than diabetic normal metabolisers. Though this is a suggestive association only with a comparatively small sample size, it is consistent with the guidelines published by the Dutch Pharmacogenetics Working Group which suggest that CYP2D6 poor metabolisers should have their venlafaxine dose reduced or consider an alternative treatment option [207]. In addition, a stratified analysis reveals suggestive evidence that diabetic CYP2D6 intermediate metabolisers taking fluoxetine have lower HbA1c levels compared to diabetic CYP2D6 normal metabolisers. Although this is contrary to my initial hypothesis, there is some evidence to suggest that fluoxetine can lower HbA1c levels in diabetic patients, despite increasing risk of type 2 diabetes in non-diabetic patients [278–280]. These findings add support to this theory, suggesting that decreased CYP2D6 metabolism may in fact be somewhat beneficial for patients with diabetes who take fluoxetine.

Overall, I find that diabetes status seems to be relevant to the importance of the CYP450 genetic variants. This highlights a potential gene-environment interaction and demonstrates that CYP450 genotype may be of greater or lesser significance among patients with specific characteristics. Again, this points to the general complexity of these analyses and provides another explanation for the lack of significant associations observed in chapter 2.

Contrary to my hypotheses, we did not find evidence of associations between CYP2D6 or CYP2C19 metabolic status and HbA1c in people treated with amitriptyline and other tricyclics. This was somewhat surprising, given tricyclic antidepressants are associated with weight gain and hyperglycaemia [64, 265]. Although CPIC guidelines exist for CYP2C19 and CYP2D6 poor metabolisers taking tricyclic antidepressants, they state that suggested

dose alterations or treatment changes are optional based on the limited strength of existing evidence [80]. The analyses of tricyclics antidepressants and amitriptyline alone was adequately powered with over 400 poor metabolisers of each gene, making it one of the largest samples of abnormal CYP metabolisers available. However, as described in chapter 2, the metabolic pathway of amitriptyline (and other tertiary amine tricyclic antidepressants) involves two steps: the first step is catalysed by CYP2C19 and produces an active metabolite (nortriptyline). The second step is the metabolism of nortriptyline to an inactive metabolite, via CYP2D6 [251, 252]. For this reason, I included both CYP2C19 and CYP2D6 metabolic phenotype in the analyses of tricyclic antidepressants. Despite this, it is likely that pairing these analyses with dose data, or ideally serum drug level data, would be necessary to fully elucidate the extent of the synergistic action of CYP2C19 and CYP2D6 data on tricyclic metabolism. A larger sample may reveal more in a study of the interaction of CYP2C19 and CYP2D6 metabolic status.

In addition, I did not find associations between *CYP2D6* variation and HbA1c amongst people taking antipsychotics. Given the total sample size of 2,699, I undertook a combined analysis including all antipsychotics, which have various levels of influence on glucose regulation and diabetes risk. Although this sample is the largest available with 135 CYP2D6 poor metabolisers overall, statistical power remains limited given the heterogeneity of the sample. Analysis in a larger sample would allow for the separate analysis of individual drugs and should yield more conclusive results. This limitation also applies to the less common antidepressants in this sample, which were included in grouped analyses only. Given that UK Biobank is a population study, utilizing existing data from large patient-based biobanks such as the Million Veteran Program could be a valuable continuation of this work [253]. Biobanks from countries with more historically isolated populations, such as Finngen, may contain a higher proportion of some rare SNPs that are necessary to define additional CYP450 star alleles.

As well as being impacted by genetic variation, CYP2D6 and CYP2C19 enzyme activity is susceptible to inhibition by other compounds. I observed that taking CYP2C19 inhibitors (of which proton pump inhibitors were the most common in our sample) led to higher HbA1c levels in people taking tricyclic antidepressants, amitriptyline, and citalopram. Thus, based on this data, there is substantial potential for drug interactions and drug-drug-CYP2C19 interactions. These should be investigated further and considered for

inclusion in future clinical guidelines. I did not find evidence that taking CYP2D6 inhibitors affected HbA1c levels in people taking antipsychotics or antidepressants. This enzyme inhibition could, however, still be important for other psychotropic adverse effects such as QT prolongation. It is also worth noting that CYP2C19 inhibitor drugs were taken by almost a quarter of subjects in this study. This may have decreased our ability to detect a difference in HbA1c levels between the genetically-determined CYP2C19 metabolic phenotype groups, because if the inhibitory actions of the concomitant drugs were strong enough it would reduce or eliminate the relative importance of the genetic variants. For example, a CYP2C19 normal metaboliser taking an inhibitor drug may have the same enzymatic activity as a CYP2C19 poor metaboliser. This is an example of phenocopying and future studies should investigate this interaction further.

As discussed in chapter 2, a clear limitation of this study is the reliance on certain self-reported data (including diabetes diagnosis). In addition, I have used only the baseline, cross-sectional UK Biobank data and therefore lack detail on treatment dose and duration. Most adverse drug reactions to antidepressants and antipsychotics are dose-dependent, and thus further analysis including this data is warranted. Besides, diabetes is a complex disease with many genetic and environmental risk factors. Although the SNP-based heritability of diabetes is estimated to be less than 20%, the inclusion of polygenic risk scores for diabetes may improve analyses of pharmacogenetic associations by capturing background genetic disease risk [255]. A genome-wide gene-environment interaction study may also highlight other genes of potential interest. Finally, although I included participants of all population groups in this analysis, UK Biobank is predominantly European. There is a great deal of variation in the frequency of functional variants within the CYP450 genes across different populations [176, 197], as well as in the risk of diabetes. Future study in more ancestrally diverse populations is essential.

This analysis includes a some subjects who will be misclassified as normal metabolisers, and CYP2D6 ultra-rapid metabolisers as described in chapter 2. CYP2D6 ultra-rapid metabolisers are the least common phenotypic group across all populations, and therefore represent a small minority in our sample. They have been combined with the normal metabolisers group by default and I estimate this to have a small effect on our results.

Overall, these findings are broadly consistent with existing guidelines for antidepressants and point towards the necessity to include more antidepressants and antipsychotics

in pharmacogenetic clinical trials and experimental medicine studies. These results also suggest that there is a need for randomised double-blinded clinical trials to further explore genetic testing as a guide to antidepressant/antipsychotic treatment. Indeed, studies show that pharmacogenetic testing is practical [281], accurately predicts the outcomes of antidepressant treatments [282], and improves outcomes [283, 284]. It has also been demonstrated that it can reduce the total cost of antipsychotic treatment by 28% [85]. Findings from this study need to be followed up with further longitudinal testing, with a focus on singular antidepressants and antipsychotics, more adverse drug reactions, and in more diverse populations.

Chapter 4

The influence of CYP2D6 and CYP2C19 genetic variation on self-reported sleep duration in people taking antidepressants and antipsychotics

Abstract

Disordered sleeping has been linked to a wide range of negative health outcomes, including major depressive disorder, anxiety, and psychosis, as well as overall decreased life expectancy. In addition to being a well-documented symptom of depression, sleep disturbance is a frequently reported side effect of antidepressants, with 78% of antidepressants drug labels listing sleep disturbance as a common side effect. In this chapter, I discuss the results of a hypothesis lead investigation into the impact of *CYP2C19* and *CYP2D6* genetic variation on changes in sleep duration among subjects taking antidepressants and antipsychotics, using the methods described in chapters 2.

I observe some evidence that CYP2C19 and/or CYP2D6 metabolic activity increases or decreases the risk of sleep-related adverse drug reactions, especially among participants taking tricyclic antidepressants. CYP2C19 rapid and ultra-rapid metabolisers both report decreased sleep duration compared to normal metabolisers. In addition, taking a CYP2D6 inhibitor drug alongside a tricyclic antidepressant was associated with increased sleep duration. By contrast, taking a CYP2C19 inhibitor drug alongside a tricyclic antidepressant lead to decreased sleep duration. In the same group of participants taking tricyclic antidepressants, CYP2C19 ultra-rapid metabolisers were more likely to experience sleeplessness

or insomnia, to report a change in their sleep patterns due to depression, and to describe that change as *‘trouble falling asleep’*, compared to normal metabolisers. The co-prescription of CYP2C19 inhibitory drugs increased risk of insomnia and experiencing trouble falling asleep, but was also associated with increased daytime drowsiness. Subjects who are co-prescribed an SSRI and CYP2D6 inhibitory drug are more likely to experience increased sleeplessness/insomnia and to report changes in their sleep pattern during their most recent bout of depression. Interestingly, these same subjects also experience more daytime drowsiness. In participants taking fluoxetine, the co-prescription of a CYP2D6 inhibitory drug is significantly associated with short sleep duration.

These findings offer support to existing clinical guidelines that patients with non-wild-type CYP2C19 and CYP2D6 metabolic status may be at increased risk of sleep-related adverse drug reactions to antidepressants, and that care should be taken when co-prescribing certain antidepressants with other drugs that inhibit CYP2C19 or CYP2D6. Well-designed. prospective clinical trials will help establish how clinically significant these changes are.

4.1 Introduction

Sleep can be broadly defined as a state of decreased arousal and responsiveness. It occurs in repeating cycles that, in humans, are controlled by the internal circadian clock. It is one of the most highly conserved traits across the animal kingdom, indicating a strong evolutionary advantage. Sleep is an essential and fundamental property of many networks across the brain [285, 286]. It occurs in any organism with even a very simple neuronal/glial network (e.g. *C. elegans*), and is preserved in subjects surviving lesions in any brain region [285, 287–289]. However, many of the molecular processes underlying how and why we sleep remain unclear.

Sleep health has been defined as

> *“a multidimensional pattern of sleep-wakefulness, adapted to individual, social, and environmental demands, that promotes physical and mental well-being. Good sleep health is characterized by subjective satisfaction, appropriate timing, adequate duration, high efficiency, and sustained alertness during waking hours [290].”*

Disordered sleeping has been linked to a wide range of negative health outcomes, as well as overall decreased life expectancy [291–297]. Both unusually long and unusually short

sleep duration have been associated with multiple psychiatric conditions, including major depressive disorder, anxiety, and psychosis, though the causal relationship between sleep and these diseases is not well established [254, 298–300].

In addition to being a well-documented symptom of depression, sleep disturbance is a commonly reported side effect of antidepressants [301–303]. Different drugs are known to have either stimulatory or sedative effects [303]. A 2018 study reviewed the summary of product characteristics (SmPCs) for 64 commonly prescribed antidepressants and found that 78% of them listed at least one sleep-related adverse reaction [302]. A systematic review by Mayers and Baldwin (2005) found that many tricyclic antidepressants, such as amitriptyline and trimipramine, were associated with sedation and oversleeping [304]. By contrast, SSRIs, including sertraline and fluoxetine, were associated with increased incidence of insomnia. However, only 21% of clinical trials employed structured methods to evaluate antidepressant-induced adverse drug reactions, and more research is needed to explore which patient groups are at higher risk [305]. Some antidepressants, such as amitriptyline and mirtazapine are used (off-label) to treat insomnia [303]. Using the methods described in chapter 2 and 3, I assess the relationship between *CYP2C19* and *CYP2D6* and sleep duration among subjects taking antidepressants.

4.2 Methods

4.2.1 Genotyping, imputation and quality control

The methods describing genotyping, imputation and central and local quality control metrics for the UK Biobank data are described in chapter 2.

4.2.2 Phenotypic assessment

I selected participants who had provided self-reported data on sleep duration. Participants were asked *“About how many hours sleep do you get in every 24h? (Please include naps)”* as part of the baseline assessment. Responses were given in hour increments and participants who claimed to sleep less than three hours or more than 12 were prompted to confirm their answer. In addition, subjects were asked several additional sleep related questions as part of the baseline assessment as via an online follow-up questionnaire focusing on mental health. At the baseline assessment, participants were asked:

- *“Do you have trouble falling asleep at night or do you wake up in the middle of the night?”*.

- *"How likely are you to doze off or fall asleep during the daytime when you don't mean to? (e.g. when working, reading or driving)"*

As part of the mental health online follow up questionnaire, participants were asked if their sleep changed as a result of their depression. If they selected yes, they were asked *"Was that: Sleeping too much"* or *"Was that: Trouble falling asleep"*.

4.2.3 CYP450 pharmacogenetics

This analysis is based on UK Biobank data only, due to the restricted access of MVP data. The methods to define the subsample included in this analysis, and to assign them to the appropriate CYP450 metabolic phenotype groups are described in chapter 2. I identified a sample of 37,752 participants taking at least one drug of interest for this analysis. After applying all genetic quality control procedures described in chapter 2, a total of 33,433 participants taking antidepressant medication were included in this part of the analysis (see figure 4.1). Where possible, drugs were analysed independently given the reported differences in their impact on sleep. Where the sample size did not permit this, drugs were grouped by class as described in previous chapters. This chapter focuses on depression, and therefore antipsychotic drugs were not included.

For each drug or drug group, I ran linear regression models with sleep as the outcome of interest and CYP450 metabolic phenotype as the main explanatory variables. In addition, I ran logistic regression models to consider the impact of CYP450 metabolic status on the risk of reporting frequent insomnia or fatigue. All statistical models were adjusted to account for any participant taking drugs, psychotropic or otherwise, that are known inhibitors of the enzymes of interest. Additional covariates included were sex, age, and genetically determined ancestry group.

Some of these analyses are nested (individual drug analyses overlap with drug group analyses), and as such, I concluded that a Bonferroni correction for multiple testing would be excessively stringent [242]. Therefore, I report uncorrected p values in all text and tables, but as recommended by Li *et al* (2012) [243], I have an adjusted significance threshold of $p < 0.05/2 = 0.025$ (threshold for a suggestive association $p < 0.1/2 = 0.05$) for the two grouped analyses, and $p < 0.05/6 = 0.0083$ (threshold for a suggestive association $p < 0.1/6 = 0.017$) for the individual drug analyses examining six specific drugs. I did not perform multiple testing correction for the separate outcome measures, as they are all related to usual patterns of sleep and thus highly correlated. All statistical analyses were performed using

R version 3.6.0 [244–246].

4.3 Results

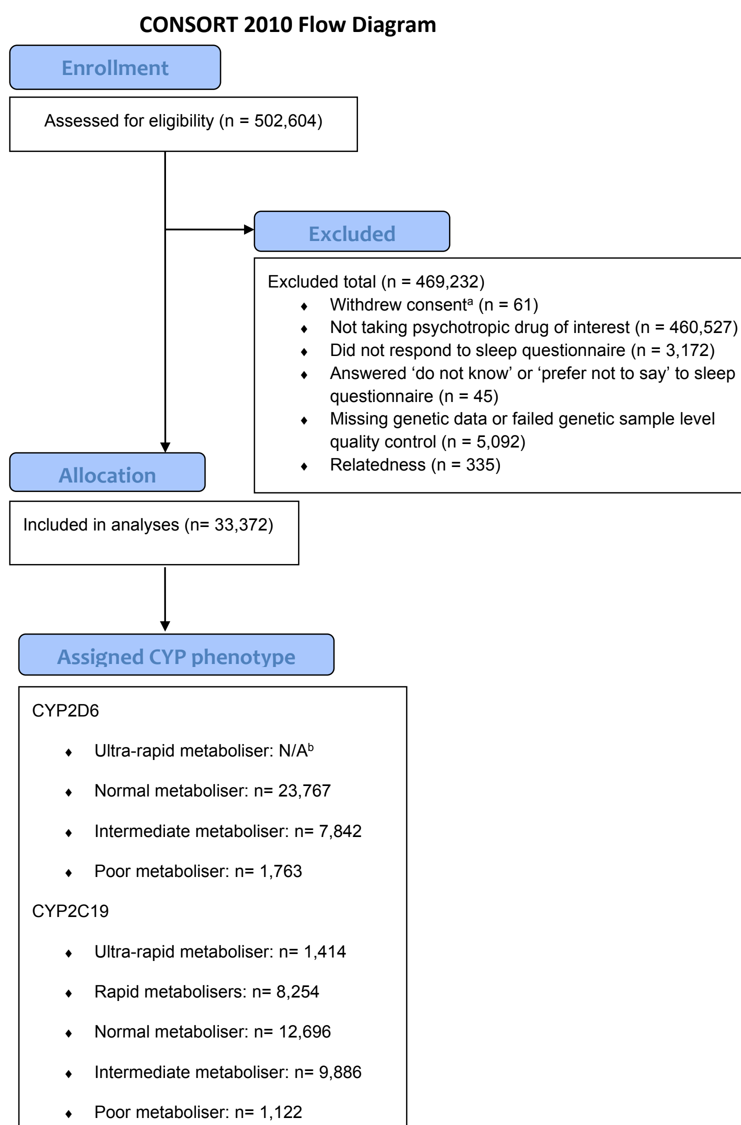
4.3.1 Sample

I identified a sample of 42,347 UK Biobank participants taking at least one drug of interest for this analysis. After applying all genetic quality control procedures described in chapter 2, a total of 33,372 participants taking antidepressant medication were included in this part of the analysis. This sample includes 22,934 (68.7%) females and 10,437 (31.3%) males. The mean age was 56.6±7.8 years, range 40 to 71 years. Full demographic data and summary statistics of this sample are shown in table 4.1.

As described in chapters 2 and 3, there were 28 different antidepressants identified in our sample. Amitriptyline was the most common drug in this cohort (24.3%, N=8,120). See also figure 3.2; appendix table C.3. A total of 8,480 participants (25.4%) report taking a CYP2C19 inhibitor drug and 1,992 participants (6.0%) reported taking drugs that are known CYP2D6 inhibitors; these participants were identified using the same method applied to the extraction of all other drugs (see chapter 2).

The distribution of CYP450 metabolic phenotypes, age, sex and ethnicity is the same as described in previous chapters. Sleep duration was normally distributed across the sample, with an average reported sleep duration of 7.35±1.53 hours. The most common response was eight hours sleep (27.9%, n=9,301), closely followed by seven hours (27.8%, n=9,292)(figure 4.2). A small number of people reported sleeping less than three or greater than 12 hours per day. These individuals were asked for a second time if they were sure this was an accurate response. Any response of less than one hour or greater than 23 hours was excluded. Among the full UK Biobank sample, excluding the subjects included in this analysis, reported sleep duration was slightly lower, with a mean sleep duration of 7.01±1.06 hours. There were also more ‘normal’ sleepers (those sleeping 7-8 hours); the most common response was seven hours sleep (35.8%, n=180,129), followed by eight hours (26.3%, n=132,024) (see appendix D).

Figure 4.1: Adapted CONSORT statement detailing steps taken to arrive at the final included in these analyses.



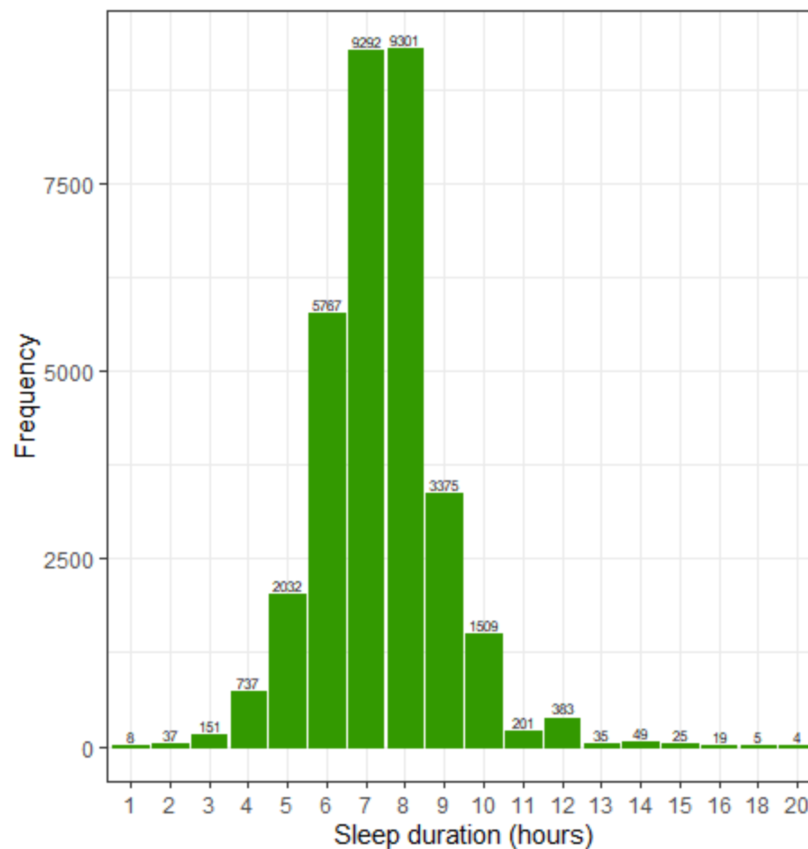
[a]Number of participants who withdrew consent after the initial download of all phenotype data and before the analysis was conducted. Participants who withdrew consent prior to initial download of data were already removed; [b]See chapter 2 for detail on why CYP2D6 ultra-rapid metabolisers were not defined in this analysis.

Table 4.1: Sample demographics

	Participants taking antidepressants (N=33,372)
Sex	
Female	22,935 (68.7%)
Male	10,437 (31.3%)
Age	
Mean (SD)(years)	56.55 (7.79)
Range (median)(years)	40-71 (58)
Ethnicity	
White European	31,249 (93.5%)
Admixed European	845 (2.5%)
African	364 (1.1%)
East Asian	43 (0.1%)
South Asian	395 (1.2%)
Admixed	447 (1.4%)
Sleep duration	
Mean (SD)(hours)	7.35 (1.53)
Range (median)(hours)	1-20[c] (7)
Reports sleeping too much	
No	31,381 (94.0%)
Yes	1,990 (6.0%)
Reports frequent sleeplessness/insomnia[c]	
No	31,381 (94.0%)
Yes	1,990 (6.0%)
CYP2D6 metabolic phenotype	
Normal metabolisers	23,767 (71.2%)
Intermediate metabolisers	7,842 (23.5%)
Poor metabolisers	1,763 (5.3%)
CYP2C19 metabolic phenotype	
Normal metabolisers	12,696 (38.0%)
Intermediate metabolisers	9,886 (29.6%)
Poor metabolisers	1,122 (3.4%)
Rapid metabolisers	8,254 (24.7%)
Ultra-rapid metabolisers	1,414 (4.2%)
Takes CYP2D6 inhibitors[a]	
No	31,381 (94.0%)
Yes	1,990 (6.0%)
Takes CYP2C19 inhibitors[a]	
No	24,911 (74.6%)
Yes	8,461 (25.4%)

[a] CYP2C19 and CYP2D6 inhibitors identified through review of literature, including British National Formulary [222]; [b] Participants who reported sleeping fewer than three or greater than 12 hours per 24-hour period were automatically asked to confirm this was accurate; [c] Participants responded either 'Usually' assigned as cases, those that respond 'Sometimes' or 'Never/rarely' considered controls.

Figure 4.2: Frequency of reported hours of sleep duration in this UK Biobank sub-sample



4.3.2 Self-reported sleep duration and CYP450 metabolic status

Overall, I observe some significant findings indicating that either CYP2D6 or CYP2C19 metabolic status is associated with self-reported sleep duration in subjects taking antidepressants. Among all subjects taking tricyclic antidepressants, I observe a trend level association with CYP2D6 poor metabolisers reporting increased sleep duration (mean difference: -0.14 hours; 95% CI [0.01,0.27]; p = 0.041). CYP2C19 rapid metabolisers and ultra-rapid metabolisers both report decreased sleep duration compared to normal metabolisers (RM mean difference: -0.14 hours; 95% CI [-0.23,-0.05]; p = 0.001; UM mean difference: -0.17 hours; 95% CI [-0.34,-0.01]; p = 0.043), although the latter was not significant after multiple testing correction. In addition, I observe a significant association between increased sleep duration and taking a CYP2C19 inhibitory drugs (mean difference: -0.14 hours; 95% CI [-0.23,-0.04], p = 0.006. There is an increase in sleep duration among participants taking CYP2D6 inhibitory drugs (mean difference: 0.13 hours; 95% CI [0.004,0.26]; p = 0.043) (table 4.2), but again this was not significant after correction. Of all the tricyclic antidepres-

sants reported in the UK Biobank sample, only amitriptyline and dosulepin had sufficient sample sizes to be analysed individually. None of these observations remain significant after multiple testing correction (table 4.2).

I did not observe any association between CYP2D6 metabolic status and reported sleep duration in a grouped analysis of all subjects taking SSRIs. Subjects taking fluoxetine alongside a CYP2D6 inhibitory drug report significantly decreased sleep duration (mean difference: -0.3 hours; 95% CI [-0.44,-0.08]; p = 0.004), but there is no association between genetically-determined CYP2D6 metabolic groups (table 4.3). There is also no observed significant associations among subjects taking venlafaxine (an SNRI, serotonin-noradrenaline reuptake inhibitor) (table 4.4). See appendix D.2 for further detail on these results.

Table 4.2: Association between CYP450 metabolic phenotype and sleep duration among participants taking tricyclic antidepressants

Sleep Duration	Tricyclic antidepressants			Amitriptyline			Dosulepin		
	Estimates	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
Intercept	6.68	6.45 – 6.92	**<0.001**	6.77	6.50 – 7.04	**<0.001**	6.47	5.88 – 7.07	**<0.001**
CYP2C19 intermediate metabolisers	-0.06	-0.14 – 0.03	0.175	-0.06	-0.15 – 0.03	0.214	0.02	-0.15 – 0.19	0.839
CYP2C19 poor metabolisers	0.02	-0.18 – 0.21	0.878	-0.02	-0.25 – 0.21	0.853	0.35	-0.01 – 0.71	0.06
CYP2C19 rapid metabolisers	-0.14	-0.23 – -0.05	**0.001**	-0.12	-0.22 – -0.02	***0.019***	-0.16	-0.34 – 0.01	0.072
CYP2C19 ultra-rapid metabolisers	-0.17	-0.34 – -0.01	***0.043***	-0.17	-0.36 – 0.03	0.089	0.05	-0.28 – 0.37	0.784
CYP2D6 intermediate metabolisers	0.04	-0.03 – 0.11	0.267	0.05	-0.03 – 0.13	0.185	0.04	-0.13 – 0.20	0.675
CYP2D6 poor metabolisers	0.14	0.01 – 0.27	***0.04***	0.14	-0.01 – 0.29	0.075	0.19	-0.15 – 0.53	0.28
Takes CYP2C19 inhibitor	-0.14	-0.23 – -0.04	**0.006**	-0.12	-0.24 – -0.01	0.027	0	-0.16 – 0.15	0.957
Takes CYP2D6 inhibitor	0.13	0.00 – 0.26	***0.043***	0.1	-0.05 – 0.24	0.186	0.18	-0.09 – 0.45	0.182
Observations	11,383			8,519			1,616		
R^2 / R^2 adjusted	0.008 / 0.006			0.008 / 0.005			0.014 / 0.006		

Table 4.3: Association between CYP450 metabolic phenotype and sleep duration among participants taking SSRIs

Sleep Duration	SSRIs			Citalopram			Fluoxetine			Paroxetine			Sertraline		
	Estimates	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
Intercept	6.97	6.81 – 7.13	**<0.001**	7.01	6.77 – 7.26	**<0.001**	7.05	6.74 – 7.35	**<0.001**	6.77	6.23 – 7.30	**<0.001**	7.18	6.68 – 7.68	**<0.001**
CYP2D6 intermediate metabolisers	0.03	-0.03 – 0.08	0.329	-0.01	-0.09 – 0.07	0.833	0.06	-0.04 – 0.15	0.253	0.06	-0.10 – 0.22	0.485	0.06	-0.10 – 0.22	0.449
CYP2D6 poor metabolisers	-0.02	-0.12 – 0.08	0.705	-0.09	-0.24 – 0.06	0.226	-0.02	-0.20 – 0.15	0.786	0.2	-0.09 – 0.49	0.184	-0.04	-0.33 – 0.25	0.785
Takes CYP2D6 inhibitor	-0.07	-0.17 – 0.03	0.182	0.08	-0.07 – 0.23	0.322	-0.26	-0.44 – -0.08	**0.004**	-0.14	-0.44 – 0.15	0.342	0	-0.30 – 0.29	0.987
Observations	18608			7,882			5,663			2,002			2,007		
R^2 / R^2 adjusted	0.003 / 0.003			0.002 / 0.001			0.005 / 0.003			0.012 / 0.007			0.004 / -0.000		

Table 4.4: Association between CYP450 metabolic phenotype and sleep duration among participants taking venlafaxine

Sleep Duration	Venlafaxine		
	Estimates	*CI*	*p*
Intercept	6.89	6.31 – 7.47	**<0.001**
CYP2D6 intermediate metabolisers	-0.01	-0.19 – 0.17	0.925
CYP2D6 poor metabolisers	-0.09	-0.42 – 0.23	0.579
Takes CYP2D6 inhibitor	0.27	-0.06 – 0.59	0.111
Observations	1959		
R^2 / R^2 adjusted	0.013 / 0.008		

4.3.3 Measures of sleep quality and CYP450 metabolic status

In addition to considering self-reported sleep duration as a quantitative trait, I investigated the influence of CYP450 metabolic phenotype on three questions on sleep quality. In a grouped analysis of all subjects taking tricyclic antidepressants, CYP2C19 rapid and ultra-rapid metabolisers and those taking a CYP2C19 or CYP2D6 inhibitor drug were at significantly greater risk of experiencing regular insomnia or sleeplessness (see table 4.5). In addition, both taking CYP2C19 or CYP2D6 inhibitor drugs significantly increased the risk of reporting daytime sleeping and/or narcolepsy in the same group ((CYP2C19 inhibitor: β= 0.03±0.004, p=5x10^{-14}, CYP2D6 inhibitor: β= 0.02±0.008, p = 0.004).

A grouped analysis of all participants taking SSRIs demonstrated a significant association between taking a CYP2D6 inhibitory drug and increased likelihood of reporting insomnia (β= 0.1±0.02, p = 2.1x10^{-9}), but no difference between CYP2D6 metabolic groups. As seen in the grouped analysis of tricyclic antidepressants, taking a CYP2D6 inhibitor drugs increased the risk of reporting daytime sleeping and/or narcolepsy in subjects taking SSRIs (β= 0.03±0.008, p = 4.25x10^{-6}). In addition, there is a significant association between CYP2D6 intermediate metabolisers and decreased risk of experiencing daytime drowsiness and/or narcolepsy (β= -0.01±0.004, p = 0.017).

Of those who responded, 77% of participants taking tricyclic antidepressants and 80% of participants taking SSRIs reported a change in their sleep patterns due to depression. As summarised in table 4.6, the impact of genetically-determined CYP450 metabolic activity is more relevant to sleep phenotypes among participants taking tricyclic antidepressants compared to SSRI users. Table 4.6 and appendix D.2 summarise the findings from these analyses and the individual drug level analyses.

Table 4.5: Association between CYP2C19 and CYP2D6 metabolic phenotype and measures of sleep quality among participants taking tricyclic antidepressants

Tricyclic antidepressants	Sleeplessness/insomnia			Daytime dozing/narcolepsy			Change in sleep			Sleeping too much			Trouble falling asleep		
	Estimates	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
Intercept	0.61	0.53 – 0.68	**<0.001**	0.1	0.07 – 0.13	**<0.001**	1.08	0.93 – 1.22	**<0.001**	0.82	0.64 – 1.00	**<0.001**	0.74	0.58 – 0.90	**<0.001**
CYP2C19 intermediate metabolisers	0	-0.02 – 0.02	0.838	0	-0.01 – 0.01	0.351	-0.01	-0.05 – 0.04	0.801	0	-0.06 – 0.05	0.867	0.05	0.00 – 0.10	***0.043***
CYP2C19 poor metabolisers	0.05	0.00 – 0.11	***0.046***	-0.01	-0.04 – 0.01	0.288	0.04	-0.06 – 0.14	0.486	-0.01	-0.13 – 0.11	0.844	0.1	-0.01 – 0.21	0.072
CYP2C19 rapid metabolisers	0.03	0.01 – 0.06	**0.007**	0	-0.01 – 0.01	0.651	0.03	-0.02 – 0.07	0.240	-0.02	-0.07 – 0.04	0.535	0.07	0.02 – 0.12	**0.006**
CYP2C19 ultra-rapid metabolisers	0.04	-0.01 – 0.08	0.129	0.01	-0.01 – 0.03	0.175	0.1	0.01 – 0.18	**0.022**	-0.01	-0.11 – 0.09	0.816	0.09	0.00 – 0.18	***0.039***
CYP2D6 intermediate metabolisers	0.02	-0.00 – 0.04	0.118	0	-0.01 – 0.01	0.769	0.01	-0.03 – 0.05	0.604	-0.01	-0.06 – 0.04	0.742	-0.02	-0.06 – 0.02	0.387
CYP2D6 poor metabolisers	0	-0.04 – 0.04	0.997	0.01	-0.01 – 0.03	0.166	-0.03	-0.12 – 0.06	0.474	0.05	-0.06 – 0.16	0.399	-0.04	-0.14 – 0.07	0.490
Takes CYP2C19 inhibitor	0.09	0.07 – 0.11	**<0.001**	0.03	0.02 – 0.04	**<0.001**	0	-0.04 – 0.04	0.888	0	-0.04 – 0.05	0.870	0.08	0.03 – 0.12	**<0.001**
Takes CYP2D6 inhibitor	0.04	0.00 – 0.07	***0.042***	0.02	0.01 – 0.04	**0.004**	0	-0.07 – 0.06	0.930	0.03	-0.05 – 0.11	0.476	-0.05	-0.12 – 0.02	0.166
Observations	11,383			11,383			2,254			1,742			1,742		
R^2 / R^2 adjusted	0.011 / 0.010			0.012 / 0.011			0.016 / 0.010			0.026 / 0.018			0.021 / 0.012		

Table 4.6: Association between CYP2D6 metabolic phenotype and measures of sleep quality among participants taking SSRIs

SSRIs	Sleeplessness/insomnia			Daytime dozing/narcolepsy			Change in sleep			Sleeping too much			Trouble falling asleep		
	Estimates	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
Intercept	0.33	0.28 – 0.39	**<0.001**	0.06	0.04 – 0.09	**<0.001**	1.17	1.08 – 1.25	**<0.001**	0.72	0.60 – 0.84	**<0.001**	0.65	0.54 – 0.76	**<0.001**
CYP2D6 intermediate metabolisers	-0.01	-0.03 – 0.01	0.306	-0.01	-0.02 – -0.00	**0.017**	0	-0.02 – 0.03	0.840	0.01	-0.03 – 0.04	0.680	-0.02	-0.05 – 0.02	0.362
CYP2D6 poor metabolisers	-0.02	-0.05 – 0.01	0.188	0	-0.02 – 0.01	0.688	0	-0.05 – 0.05	0.945	-0.05	-0.12 – 0.02	0.191	0	-0.06 – 0.07	0.934
Takes CYP2D6 inhibitor	0.1	0.07 – 0.13	**<0.001**	0.04	0.02 – 0.05	**<0.001**	0.07	0.02 – 0.12	**0.007**	-0.01	-0.08 – 0.06	0.865	0.04	-0.02 – 0.10	0.208
Observations	18,608			18,608			4,925			3,951			3,951		
R^2 / R^2 adjusted	0.003 / 0.003			0.006 / 0.006			0.021			0.010 / 0.007			0.012 / 0.006		

4.4 Discussion

Sleep is a central part of all animal life, and disrupted sleep or a decrease in sleep quality can have significant ramifications for health, both short and long term. The question of how to improve sleep quality is of constant interest, with the global market for sleep aids and technologies exceeding 80 billion US Dollars per year. As well as an established genetic component, a wide variety of environmental factors can impact sleep duration and quality, including hours of daylight exposure, caffeine intake, shift work, alcohol consumption, medication, mental and physical health conditions. Indeed, sleep disturbance can be considered both a cause and symptom of many health conditions, and the relationship between sleep and mental health in particular is an area of increasing scientific focus. As a complex trait with so many environmental (for an example, see appendix D.3) and genetic risk factors, it is a challenging outcome to study. However, given the high correlation between sleep disturbance and depression, it is a vital area of research. As well as being a common cause and symptom of depression, sleep related adverse drug reactions to antidepressants are common.

This chapter investigates the impact of CYP450 metabolic phenotype on self-reported sleep duration and measures of sleep quality in subjects taking antidepressants. Overall, I do observe some evidence that CYP2C19 and/or CYP2D6 metabolic activity increases or decreases the risk of sleep-related adverse drug reactions. This seems particularly apparent in the group that use tricyclic antidepressants. Tricyclic antidepressants are known to have sedative effects, due in part to their blockage of histamine H1 receptors [187, 306]. Therefore, my hypothesis was that decreased CYP2C19 or CYP2D6 metabolic activity (either due to genetic variation or the co-prescription of inhibitory drugs) would increase the duration of histamine H1 blockage and increase sedation. In contrast, more efficient CYP2C19 metabolism (or CYP2D6, which could not be assessed in this analysis) would reduce the duration of H1 blockage compared to normal metabolisers, and thus decrease sedation. In line with this hypothesis, CYP2C19 rapid and ultra-rapid metabolisers both reported decreased sleep duration compared to normal metabolisers. Also, the co-prescription of taking tricyclic antidepressants with a CYP2D6 inhibitor drug was associated with increased sleep duration. Interestingly, taking a CYP2C19 inhibitory drug was also associated with decreased sleep, which is a somewhat contradictory finding. This may be a result of some confounding through co-morbidity. For example, omeprazole was one of the most com-

monly prescribed CYP2C19 inhibitor, which is prescribed for reflux and alongside long-term use of non-steroidal anti-inflammatory drugs for chronic pain patients. Both reflux and chronic pain are common causes of sleep disruption [307, 308].

In addition to investigating the impact of variation in CYP450 metabolism on sleep duration as a quantitative variable, I investigated several measures of sleep quality, considered in this study as binary traits. Again, subjects taking tricyclic antidepressants appear to be more likely to experience changes in their sleep quality as a result of CYP2C19 and/or CYP2D6 metabolic activity. CYP2C19 ultra-rapid metabolisers are more likely to experience sleeplessness or insomnia, to report a change in their sleep patterns due to depression, and to describe that change as *'trouble falling asleep'*, compared to normal metabolisers. The co-prescription of CYP2C19 inhibitory drugs increases risk of insomnia and experiencing trouble falling asleep, but is also associated with increased daytime drowsiness.

I have discussed the complexity of the two-stage metabolic pathway of tricyclic drugs in chapter 3; in brief CYP2C19 catalyses the first step of the metabolic pathway which gives rise to an active metabolite, which is then metabolised via CYP2D6. This analysis may not adequately account for the potential interaction of altered CYP2C19 and CYP2D6 metabolism. In addition, CYP2C19 inhibitor drugs were taken by approximately a quarter of the subjects included in these analyses, which may have decreased the power to detect a significant association between the (genetically-determined) CYP2C19 metabolic status and sleep duration.

As a group, SSRIs are known to have less sedative effects. Subjects who are co-prescribed a CYP2D6 inhibitory drug were more likely to experience increased sleeplessness/insomnia and to report changes in their sleep pattern during their most recent bout of depression. By contrast, these same subjects also appear to experience more daytime drowsiness. In participants taking fluoxetine, it appears that the co-prescription of a CYP2D6 inhibitory drug is significantly associated with short sleep duration. Though is the opposite direction of effect to my general hypothesis, further investigation into the side effect profile of fluoxetine reveals it can have stimulatory effects [309, 310]. Therefore, the observed decrease in sleep duration reported by these subjects supports the idea that impaired CYP2D6 metabolism increases the risk of adverse drug reactions. These findings highlight the importance of conducting drug-specific analyses, as the impact of these drugs on many adverse reactions can be very different even among drugs of the same class. It is

also worth noting that the pharmacokinetic profile of many SSRIs in non-linear, which adds an additional layer of complexity to the interpretation of these results. Large, randomised, controlled clinical trials that measure serum concentrations of these drugs over time will be necessary to understand the extent of the impact of impaired CYP2D6 metabolism.

The limitations of these analyses on CYP450 genotype and proxy measures of adverse drug reactions has been discussed in detail in chapters 2 and 3. In brief, this study is limited by lack of dose, treatment duration, and temporal data, and the method to assign CYP450 genotype varies in accuracy across the two genes and across population groups. This specific analysis is subject to some additional limitations. Firstly, the specific impact of antidepressants on sleep is complex. Though some have known sedative effects, many have been associated with both increased and decreased sleep. This potentially non-linear relationship is clear from the results of this analysis, where the effect estimates are often not in the expected directions. As concluded in previous chapters, larger analyses at the individual drug level are necessary to draw any firm conclusions. The inclusion of dose, treatment duration, co-prescribed drugs, and specific diagnosis is likely to be a significant benefit.

Secondly, sleep is impacted by a wide range of environmental outcomes. The UK Biobank did collect data on many of these (e.g., shift patterns, caffeine, alcohol consumption) but as not all subjects responded to all questions, including these variables would reduce the available sample size and limit power[1]. Finally, where I do report significant associations, the effect sizes are very small and amount to a difference of minutes. Unlike the changes observed in HbA1c levels, for example, this would not be considered a clinically meaningful change in sleep pattern on an individual level.

A final clear limitation of this work is the reliance on self-reported data. However, a previous study in Europeans in the UK Biobank confirmed a high level of consistency between the top variants identified in a GWAS of self-report data and those seen using accelerometer-derived data [254]. Their findings offer confidence in the reliability of self-reported data in this context, but future work using accelerometer or otherwise impartially collected biological data in larger samples and including non-European populations is recommended. A further limitation comes from the age range in the UK Biobank study, which

[1] It is worth noting that an analysis of two of these potential environmental risk factors, caffeine intake and hours of solar irradiation at the time of recruitment, demonstrated that both are indeed significantly associated with changes in sleep duration (see appendix D.3 for detail on these analyses).

recruited adults between 40 and 70 years of age. Sleep disturbance (particularly short sleep) is more common as we get older.

Overall, this chapter highlights that patients with impaired CYP450 metabolism may be at greater risk of sleep-related adverse reactions to their antidepressants, particularly those taking tricyclic antidepressants. Given the high genetic correlation between sleep disturbance and depression, this is clearly an important symptom for further study.

Chapter 5

Pharmacogenetic interventions in a clinical psychiatric setting

Abstract

In the final chapter of my thesis, I described the work conducted to design, set up and start a pilot study investigating a pharmacogenetic intervention in patients taking antipsychotics. This study aims to recruit 420 participants in total, with the first 50 being recruited in a pilot phase to be analysed as part of this thesis. In the interest of the safety of myself, colleagues and potential participants, recruitment was paused as a result of the Covid-19 pandemic. Therefore, limited patient data is available for analysis. The work formed the basis of a successful grant application and recruitment restarted in August 2021. This pilot will inform on the design and feasibility of a future fully-powered randomised controlled trial. We will recruit up to 420 patients for the study. The primary outcome will be the number of days under acute service care. Patients who consent to take part in the study will provide a blood or saliva sample for pharmacogenetic testing. Their clinician will be provided with a detailed report listing the test results as well as other relevant information from the patient's medical records. The report will use a simple method to explain if a patient carries a genetic variant that could alter drug metabolism and, if so, help the clinician to choose the most suitable drug and dose. This report will be informed by evidence-based clinical guidelines. These guidelines will be informed in part by the Clinical Pharmacogenetics Implementation Consortium [79, 80, 311, 312]. In addition this chapter describes the development of new clinical implementation guidelines for the NHS for four antidepressants, that I developed as part of the NHS-Genomics England pharmacogenetics working group.

5.1 Introduction

A large number of drugs have a marketing authorisation in the UK for the treatment of psychosis, but the evidence guiding choice for an individual patient is limited. In clinical practice, the selection of drug is effectively made by a trial and error approach. This can lead to several failed cycles of medications until an effective treatment is identified often weeks or months later. In addition, standard doses are offered to all patients and doses are changed only in response to observed symptom changes and tolerability. Furthermore, many patients fail to show sufficient clinical improvement from antipsychotic medication, and the side effect burden of these drugs is substantial. This is a key reason for the low levels of medication adherence seen in psychiatric conditions (e.g. depression, schizophrenia) and is likely to be a contributing factor to the severe reduction in life expectancy among such patients.

As described in previous chapters, characterising the metabolic status of patients using genetic testing could improve the prescribing of commonly used psychotropic medicines by helping clinicians to adjust the dose in an individualised, biologically-informed way. Such pharmacogenetic interventions have been successful in oncology and haematology, and testing is already in use for the management of some drugs such as tamoxifen and warfarin [313, 314]. However, although there has been extensive research on the pharmacogenetics of psychotropic drugs, there is little evidence from clinical trials on this subject.

In psychiatry, two recent studies have shown very promising results. One of these is the study by Walden *et al* (described in chapter 2), which reported that 25% of physicians using pharmacogenetic testing reported an improvement in their patient outcomes, and no clinicians reported negative findings [315]. A second study by Perez *et al* recently reported the results of a 12-week, double-blind randomised controlled trial investigating the benefit of pharmacogenetic testing in 316 adults with major depressive disorder [316]. They found that pharmacogenetic testing resulted in a significant improvement in treatment response at 12 weeks and that the difference between the two arms was strengthened when patients whose clinicians had not followed the pharmacogenetic recommendations were removed from the analysis. The difference was most pronounced in patients who had experienced one to three failed drug trials. A significant reduction in adverse event burden was also observed.

Previous chapters in this thesis have discussed approaches to using large, existing

biobank data to investigate evidence of increased side effect burden in people taking psychotropic medication. However, there are several key limitations to these approaches. Firstly, the retrospective, cross-sectional natures of these analyses mean that it is difficult to confirm that a measured effect is a result of an adverse drug reaction. Secondly, the available data often lacks information on treatment dose and duration, which is highly relevant to the study of adverse drug reactions. Finally, many genotype and imputation panels do not have good coverage of known pharmacogenes, meaning the reliability of assigning participants to different phenotypic groups is limited. The 'gold standard' of pharmacogenetic research is a large, well-designed clinical trial that captures detailed medical history and includes measures of treatment response and adverse reactions. In addition, primary data collection will allow a choice of the best genotyping array and/or targeted sequencing methods for the specific pharmacogenes of interest.

This project aims to build on some promising but limited clinical research by conducting a study to investigate the use of a pharmacogenetic testing to optimise the management of antipsychotic drugs. I will examine feasibility, acceptability and gather data to test whether personalised prescribing of antipsychotic agents results in higher efficacy and reduced side effects, compared to treatment as usual.

5.2 Study aims

The aim of this study is to establish the feasibility of conducting a large, randomised controlled trial investigating the use of pharmacogenetic tools in the prescription of antipsychotic medications. The outcome measures are as follows:

- Confirm appropriate end points
- Assess recruitment rate
- Confirm variant frequency within our sample population
- Establish acceptability of process from the perspective of patients and clinicians
- Confirm the most appropriate genetic panel
- To identify the most appropriate and cost-effective laboratory for genetic analysis

5.3 Trial design

This study will be conducted according to Good Clinical Practice and International Conference on Harmonisation guidelines. Up to 40 clinician-participants will be recruited, through whom 420 participants will be recruited to the study. All inpatient and community mental health services can be considered as trial sites. All of these participants will provide a blood and/or saliva sample for pharmacogenetic analysis. Participants will be stratified according to diagnosis, baseline assessments and genetic metabolic status. Participants will have the results of the pharmacogenetic test made available to their clinicians as soon as possible following sample collection. The clinician will then be able to review this and consider using the information to guide their treatment decisions. All clinicians will be asked to consent to discuss the results of their patients' genetic test with them and to explain how, if at all, they will use the results to guide treatment decisions. If a clinician does not consent to this, we will not recruit their patients to the study. All participants will be asked to consent to the storage of the pharmacogenetic data for future research.

If participants consent, the results of their genetic testing will be uploaded to their electronic health record to be used in fully anonymised research undertaken in the Clinical Record Interactive Search (CRIS) database (see below). This will be optional and participants will still be able to join the study if they do not consent to this.

The 420 participants will be compared to an equivalent group, receiving treatment as usual, within the CRIS database. This is a large anonymised collection of health records available within the UCL and KCL mental health associated trusts. Natural language processing has identified medication data for approximately 50,000 individuals, of whom at least 7,000 are taking an antipsychotic. Participants will be matched based on age, sex and treatment. Genetic data are not available within CRIS yet, so it will not be possible to match according to metabolic phenotype. If genetic data become available prior to final analysis, participants will be matched according to this too.

Patients will be stratified according to their metabolic profile, which will be identified through their genetic test. Approximately half of the population are expected to be normal metabolisers and these patients do not require any modification to their treatment in response to their genetic test result. Knowing that the metabolic status is normal may be reassuring and enhance medication adherence.

The clinician-participants will be asked to consent to answer a short questionnaire

at the end of their involvement in the study. The questionnaire collects both quantitative and qualitative data on their opinion on the intervention and will be analysed using mixed-methods.

5.4 Participants and recruitment

Up to 40 consultant adult psychiatrist from the trial sites (NHS trusts) will be approached to discuss the study. If they consent to take part, they will recommend potential participants from their case load. Clinician participants will seek verbal consent from their patients before sharing their contact details with the research team. Up to 420 participants (the first 50 recruited in the pilot stage) will undergo screening assessments, including genotyping. Any patient that clinical staff believes to meet inclusion criteria will first be approached by a staff member to make a preliminary enquiry. If they are interested, the researchers will then fully explain the process of the study and gain informed consent. This will include an assessment of capacity where relevant. All participants will be paid for their time. Inclusion criteria:

- Aged 18 years or over.
- With ICD-10 diagnosis of schizophrenia or schizoaffective disorder, or bipolar disorder.

Exclusion criteria:

- Patients with current high risk of self-harm or harm to others.
- Patients who lack capacity to consent to taking part in the research.
- Patients who are pregnant.

We estimate we can recruit three to six patients per week (thus 150 to 300 patients in a year). Thus recruiting a total of 420 patients, can be achieved in two to three years.

5.5 Study set up

Although ethical approval for recruiting and genotyping patients is already in place, it was necessary to prepare a new ethical application in order to provide the genetic results to the patients clinician so that they can be used to guide treatment decisions. In order to obtain ethical approval, I prepared the Integrated Research Application Service (IRAS) form and

related study documents (protocol, patient information sheet, patient consent form, HRA statement of activities, HRA schedule of events).

5.6 Interventions

All participants will be treated within British National Formulary licensed limits (Joint Formulary Committee 2012). All participants will have their plasma levels of medication monitored. This is to confirm they are compliant and can be used to confirm appropriate dosage. Non-compliance and partial compliance will be accounted for in all statistical analyses.

Participants will be aware of what medication they have been prescribed and at what dose. The clinical teams will be directed not to discuss participant allocation or medication with the researcher carrying out follow-up assessments.

5.7 Trial procedures

Prior to registration, participants will be given study information and asked to sign an informed consent form. Patients will be assessed for capacity to consent at this time and their medical notes will be reviewed to confirm their diagnosis meets the criterion for inclusion.

Participant and clinician-participant registration will be undertaken centrally by the trial manager or delegate at UCL. All participants will be registered prior to providing a sample for genotyping. Patients will be registered to the trial following participant consent and confirmation of eligibility. Patients will be allocated a unique trial number. The prescribing clinicians and participants cannot be blind as the purpose of the trial is to guide prescription using the intervention (genetic test) to make an informed choice.

Baseline assessments will take place on the hospital wards or in clinical outpatient settings. At the baseline visit, all participants will provide a DNA sample for genotyping of known pharmacogenes. In addition, they will also be invited to consent to genome-wide genotyping and exome sequencing, to contribute to novel discovery (this is optional). The initial assessment will take place on the hospital ward. The follow-up assessment can take place on the ward or in the community if the patient has been discharged. The final follow-up will be undertaken based on clinical records.

I have undertaken phlebotomy training to enable me to recruit patients directly. We initially planned for DNA samples to be collected predominantly from blood samples, with saliva samples as an additional or alternative option for patients who prefer it. However, this plan was updated in light of the Covid-19 pandemic, and subsequent patients will be asked

to post a saliva sample to the UCL laboratory where the DNA extraction will be conducted. A DNA aliquot from all participants will be frozen and stored for further study.

During these baseline assessments, participants will be interviewed and asked to complete a set of validated questionnaires widely used in clinical trials in psychiatry. Participants will also complete quality of life questionnaires.

Follow-up visits can take place either on the ward or in the community for participants being treated as outpatients. The final follow up assessment (6 months) will be undertaken based on clinical records and interview with the participant's primary nurse or care coordinator.

Table 5.1: Schedule of trial assessments and procedures

Intervention	**Baseline**	**3 month**	**6 month**
Medical history	x		
Physical examination including height and weight	x	x	
Questionnaires	x	x	
Blood and/or saliva sample for DNA analysis[a]	x		
Measurement of medication plasma levels	x	x	
Serum biochemistry	x	x	
Medical record review[b]			x

[a]Both blood and saliva samples collected where possible; [b]Conducted by research team

5.8 Outcome measures

Primary outcome

The primary outcome measure is the number of days under acute service care.

Secondary outcomes

- Recruitment rate
- Clinical global impression score
- Movement disorder scale
- Glasgow antipsychotic side effect scale
- Clinical Assessment of Treatment Score (CATS)
- Plasma medication levels

- Positive and Negative Symptoms Scale (PANSS)
- Liverpool University Neuroleptic Side Effect Rating Scale (LUNSERS)
- Quality of life with EQ-5D
- service usage and costs with the client services receipt inventory (CSRI)
- HbA1c, cholesterol levels, prolactin levels

5.9 Statistical analysis

A total of up to 420 participants will be recruited to the study. They will be compared to a group of 6,000 participants in the CRIS database, with the groups being matched on age, sex and treatment distribution. The primary outcome measure selected after much consideration is the number of days under acute service care. This was selected as it is a very reliably recorded metric on electronic health records. In addition, it has been previously demonstrated that pharmacogenetic interventions can improve treatment cost, which is a highly valuable measure for the NHS [36, 147, 317, 318]. Other potential endpoints, such as biomedical markers of adverse drug reactions (e.g., HbA1c), may take longer to demonstrate change and are harder to capture from review of medical notes.

Recently published data from the CRIS system in a sample of 17,666 patients treated by the crisis resolution teams, shows that the average duration of crisis team care was 20.2 days per patient with a standard deviation of 20.8 days[319]. An intervention achieving a 3.5-day average change in crisis team care gives an effect size of 0.17 (Cohen's d)[153]. This is a small yet clinically meaningful change in the duration of crisis team care. Therefore, the sample we propose to collect (420 intervention and 6,000 treatment as usual) gives 92% power to detect an effect size as small as 0.17 (two-sided test, 5% significance).

Linear models, including CYP450 metabolic status, diagnosis, medication, baseline value of the outcome and experimental group as main effects, will be used to compare the two treatment groups (trial group and CRIS sample) on the outcome measures.

The principal aim of the study is to undertake a combined analysis including all participants. In addition, we will undertake supporting analyses to examine the effect of further covariates that are known to influence medication response including duration of illness, concurrent medication, smoking status and illegal substance use. We will explore the effects of experimental group within registration strata, including exploratory tests for interaction

between stratum and experimental group. We will conduct linear models, controlling for age and sex as main effect, to compare the four metabolic groups on the outcome measures. The questionnaires returned by the clinician-participants will be analysed to assess the opinion of the recruited clinicians on the value of pharmacogenetic interventions for their patients.

We will undertake sensitivity analyses to account for missing data. We will be aware of compliance and non-compliance with medication as we will monitor blood levels of medication during the trial. A full statistical analysis plan will be drafted after the pilot stage is complete (first 50 participants recruited). This plan will cover the primary and secondary analyses and will be updated as a result of the blind review of the initial data and will be finalised before breaking the blind. We will keep records of analyses conducted and note when blind is broken.

5.10 Progress to September 2021

The IRAS application and related documents were submitted to the London Central Research Ethics Committee on 18th March 2019. After several revisions requested by the ethics committee, the study was approval in October 2019, and recruitment planned to start in January 2020. A copy of the approved trial protocol is provided in appendix E.1, and the patient information documents can be found in appendix E.2.

A total of 12 participants were recruited before it was necessary to pause recruitment due to the Covid-19 pandemic. Recruitment was paused from March 2020 until July 2021. During this time, we opened two new sites and submitted a substantial amendment to allow us to conduct some trial procedures virtually. This was approved in May 2021. This amendment allows us to contact potential participants by phone, post a consent form and saliva sample kit, and conduct baseline/follow-up interviews over the phone. Those who consent will be asked to return their consent form and saliva sample to UCL via a prepaid envelope.

This study was adopted to the Clinical Research Network (CRN) in December 2019. Through this, we were contacted by several interested sites. As of September 2021, one site is fully open to recruitment (Camden and Islington NHS Foundation Trust) and we are in the process of opening five more: Soth West London and St Georges, Kent, Sussex, Essex, and South London and Maudsley.

5.10.1 Participants

The characteristics of the 12 patients recruited to the study so far are reported in table 5.2.

Table 5.2: Sample demographics

	Summary (N=12)
Age (mean years ± SD)	43.2 ± 14.8
Sex (N(%))	
Female	4 (66.7%)
Male	8 (33.3%)
Ethnicity (self-reported, N(%)))	
White British	7 (58.3%)
Asian or Asian British	2 (16.6%)
Mixed - White and South Asian	2 (16.6%)
White - other, unspecified	1 (8.3%)
CYP2D6 metabolic phenotype[a]	
Normal metabolisers	10 (83.3%)
Intermediate metabolisers	0
Poor metabolisers	2 (16.7%)
Diagnosis (N(%))	
Paranoid schizophrenia	9 (75.5%)
Bipolar disorder	2 (16.7%)
Acute polymorphic disorder with symptoms of schizophrenia	1 (8.3%)
Primary medication (N(%))	
Clozapine	3 (25.0%)
Risperidone	3 (25.0%)
Olanzapine	2 (16.7%)
Aripiprazole	1 (8.3%)
Paliperidone	1 (8.3%)
Not currently taking any psychotropic medication[b]	2 (16.7%)
BMI (mean kg/m^2 ± SD)	27.2 ± 8.1
Weight (mean kg ± SD)	79.3 ± 28.1

[a]Data on *CYP2C19* and other pharmacogenes not returned yet; [b]Two participants not currently prescribed treatment but are likely to be offered medical intervention soon.

5.10.2 Implementation to the UK National Health Service

Alongside the set up of this clinical trial, I was invited to join the NHS-Genomics England Pharmacogenetics working group, to help draft the first evidence based guidelines for the implementation of pharmacogenetic testing in the NHS. I was tasked with drafting guidelines for four antidepressants: citalopram, escitalopram, nortriptyline and paroxetine.

This involved researching existing clinical guidelines (e.g., CPIC, Dutch Pharmacogenetics Working Group), as well as recent scientific literature to make an assessment of the appropriate level of interventions. The final versions of these documents are provided in appendix E.3.

SSRIs, as the name suggests, are a class of antidepressant that selectively increase 5-hydroxytriptamine (5-HT, serotonin) activity by inhibiting presynaptic 5-HT reuptake. Both citalopram and its pharmacologically active S-enantiomer escitalopram are extensively metabolised by CYP2C19. The metabolites produced are less active but do inhibit 5-HT reuptake to some extent [79]. CYP2C19 ultra-rapid and rapid metabolisers have increased metabolism of SSRIs to less active compounds as compared with normal metabolisers. Lower plasma concentrations of active drug will increase the probability of pharmacotherapy failure [79, 320]. Intermediate metabolisers have reduced metabolism of SSRIs to less active compounds as compared to normal metabolisers. Higher plasma concentrations of active drug will increase the probability of adverse reactions. Poor metabolisers have greatly reduced metabolism of SSRIs to less active compounds as compared with normal metabolisers. Higher plasma concentrations of active drug will increase the probability of adverse reactions [79].

Paroxetine is an SSRI extensively metabolised by CYP2D6. Its metabolites have minimal pharmacological activity [79, 321]. Because of the importance of CYP2D6 in the metabolism of paroxetine, genetic polymorphisms in *CYP2D6* may increase or decrease the exposure to the drug and subsequently impact clinical response.

Tricyclic antidepressants are combined 5- hydroxytriptamine (5-HT) and noradrenaline reuptake inhibitors. Tricyclic antidepressants can be sub-divided into two similar but distinct categories: secondary and tertiary amines. Most tertiary amines (e.g. amitriptyline) are metabolised through CYP2C19 and result in demethylated active metabolites – the secondary amines. Nortriptyline is a secondary amine and an active metabolite of amitriptyline. Secondary amines are primarily metabolised through CYP2D6 to form less active hydroxy-metabolites [322]. Generally, tertiary amines are more potent blockers of the reuptake of 5-HT, whereas the secondary amines (e.g. nortriptyline) are more potent blockers of noradrenaline reuptake. Therefore, genetic polymorphisms in *CYP2C19* and *CYP2D6* are relevant to the clinical response to tricyclic antidepressants.

I concluded, along with the working group, that patients who meet the following crite-

ria should be tested for decreased or increased function alleles in *CYP2C19* and/or *CYP2D6*:

- Patients who failed to respond to two or more antidepressants, despite evidence of adherence.
- Patients who are unable to tolerate treatment with one or more antidepressant/s due to serious adverse reactions.
- Patients with a history of hospital admissions for mental health reasons.

In addition, care should be taken when prescribing these drugs alongside known CYP2C19 inhibitors (e.g. fluvoxamine or omeprazole) and/or CYP2D6 inhibitors (e.g. fluoxetine).

5.11 Discussion

The design of this study was informed, in part, by the limitations identified in the previous chapters of this thesis. Much of the available literature on CYP450 pharmacogenetics cites low sample size and poor statistical power as the main limitations. My work using UK Biobank data represents one of the largest studies of psychiatric pharmacogenetics to date, and although I observed some interesting findings, I found that large sample size alone was not sufficient to answer outstanding questions on these complex gene-drug-environment interactions. This prospectively designed clinical trial will collect high-quality data on treatment dose, duration, comorbidities, concomitant medication, and measures of adverse drug reactions over time.

Due to the necessary pause in recruitment during the Covid-19 pandemic, it has not been possible to conduct any statistical analysis of this clinical work as part of this thesis. Nevertheless, this chapter described the successful set up and opening of what will become a large clinical trial. This study and all the associated study documents have been approved by the Central London Research Ethics Committee, who have also subsequently approved a substantial amendment to allow virtual recruitment and interviews. The study was successfully adopted to the Clinical Research Network, which lead to the identification of six appropriate study sites, one of which is now open to recruitment. A total of 12 subjects have been recruited thus far, which, given the time frame, is in line with our estimates on recruitment rate. The work described in this chapter was essential for a grant application, and Professor Elvira Bramon was awarded an NIHR grant to continue this trial. This grant

has allowed the recruitment of a full time clinical trial manager, Dr Eirini Zartaloudi, with whom I have been working closely to hand over trial responsibility.

The clinical guidelines described in this chapter have been approved by the working group and are in the process of being approved by The National Institute for Health and Care Excellence (NICE). This too was paused due to the Covid-19 pandemic, but it is expected that they will be introduced to the NHS in early 2022. These are the first example of such pharmacogenetic guidelines in psychiatric medicine.

Although these are not the milestones I hoped and planned to reach by the end of this PhD, a successful grant application, the interest of sites, the swift recruitment of the first 12 participants, and the outcomes of the pharmacogenetic working group demonstrate that the psychiatric clinical community understands the potential value of pharmacogenetic research and interventions. The work described in this chapter will be essential for the successful running of a large clinical trial, which is now underway, and which I anticipate will address many of the questions left unanswered in this thesis.

Conclusions

Although both public and scientific interest in pharmacogenetics, or personalised medicine, has been growing for several years, there remain very few examples of clinical implementation in the UK. The most notable examples currently in use in the NHS are, as mentioned in the introduction to this thesis, abacavir/*HLA-B*, thiopurines/*TPMT*, and 5-fluoruracil/*DYPD* [222]. In addition, testing for warfarin/*CYP2C9*/*VKORC-1* and carbamazepine/*HLA-A* is expected to be introduced imminently for certain patients [222, 323].

As described in this chapter 5, I used the knowledge gained from this PhD research to assist in developing new clinical guidelines for CYP450 genetic testing for patients taking certain antidepressants. One potential reason for the relatively slow uptake of pharmacogenetic testing is related to the centralised, single-payer nature of the UK healthcare system. The National Institute for Health and Care Excellence (NICE) requires a high standard of evidence in order to make a favourable cost-benefit assessment of a new intervention or product. This sets the UK apart from countries like the United States, where pharmacogenetic testing is much more widespread, albeit far from nationwide (indeed these services are often available only where the healthcare provider is attached to centres of academic excellence) [84].

Although there are many studies investigating putative pharmacogenetic associations with psychotropic drugs, until very recently the available data was not conclusive enough to be considered for NHS implementation, due in large part to small sample sizes in the available clinical studies. The primary goal of this thesis, therefore, was to consider novel approaches to conducting psychiatric pharmacogenetic research, using biobank data to greatly improve the sample size and discovery potential, and to design the UK's largest psychiatric pharmacogenetic clinical study to date. In doing so, I have found evidence to support existing and emerging clinical guidelines on CYP450 genetic variation. At the same time, I have highlighted many current and future challenges in this field.

Much pharmacogenetic research, especially in psychiatry, focuses on the CYP450 enzyme family. *CYP2D6* and *CYP2C19* remain the most studied candidate genes for pharmacogenetic intervention and are the focus of the first three chapters of this thesis. In chapter 1, I describe two complimentary systematic reviews and meta-analyses investigating the available literature on *CYP2D6* variation and antipsychotic-induced hyperprolactinaemia or weight gain. Both are common and, as I conclude from my research, relatively under-researched adverse drug reactions. These are examples of gene-environment interactions that could lead to significant clinical outcomes. In both analyses, I found a lack of compelling evidence through the meta-analyses linking CYP2D6 phenotype to increased or decreased risk of the adverse drug reaction. However, a systematic review of the available literature revealed that many of the cohort or prospective clinical studies did identify some association between CYP2D6 metabolic phenotype and the respective adverse reaction, especially weight gain. This inconsistency speaks to challenges identified throughout this thesis in analysing CYP450 genetic variation — the study design and quality of data collected can make a significant difference in the ability to confirm an association. In both reviews, I found that many of the primary studies did not publish the data necessary to allow for their inclusion in the meta-analyses. Contacting the authors did allow the inclusion of several additional studies, but had it been possible to include a wider range of the potentially relevant studies in the meta-analysis, the results might have proved more conclusive.

One major challenge in both meta-analyses and reviews was the high level of heterogeneity across the studies, in terms of the demographic background of the subjects, their treatment (drug type, duration), and the method of assigning CYP2D6 phenotype. Differences in demographic background are a common fact of meta-analyses, and can be overcome through use of a random effects model and/or assessing the I^2 statistic. Differences in treatment type, dose, and duration likely present a larger issue to these analyses. Although we included only those antipsychotics known to be metabolised at least partially through CYP2D6, the metabolic pathways of many of these drugs is complex and restricting analyses to individual drugs would certainly yield clearer results (as I discovered through my UK Biobank analyses described in chapter 2). Additionally, both hyperprolactinaemia and weight-gain are dose dependent adverse drug reactions, and some knowledge of the dose would guide more accurate interpretation of these findings. While prolactin levels can spike after just one dose of antipsychotic, weight-gain is likely to be seen only in those subjects

who have taken the drug for an extended period [324]. Therefore, only primary studies conducted in a patients receiving treatment (excluding single dose experimental studies) could be included in the weight-gain meta-analysis and review.

A lack of standard approach to assigning *CYP2D6* genotype was arguably the most significant problem to overcome in these analyses. Some studies genotyped only those SNPs necessary to call one or two star alleles, and many of the papers lacked detail on how the CYP2D6 phenotype calling was done, and why. This lack of standardisation makes it difficult to aggregate the findings from these studies and presents a significant challenge when considering the arguments for implementation of pharmacogenetic testing in the NHS. This is an important oversight. As highlighted in a recent review by Bousman *et al* (2019)[1], there exist many regulations around the accuracy and standard expected of genetic laboratories, and consortium efforts (e.g. CPIC) to describe how clinicians can interpret the results of pharmacogenetic testing, but that middle step — defining which gene and alleles should be included on genetic assays, remains highly varied [325]. These discrepancies have been reported previously and were confirmed through the reviews described in chapter 1 [326]. It should be noted that this lack of standardisation is likely to have a more significant impact on non-European populations. Many of the primary papers included in these reviews conducted selective sequencing or genotyping of only a small number of SNPs that define one or two risk alleles. In almost all cases, the chosen SNPs were more common in Europeans, and the risk alleles highly common in non-European populations were overlooked. Although my findings in this first chapter did not provide evidence of association between CYP2D6 variation and hyperprolactinaemia/weight gain and did not support existing clinical guidelines, the limitations identified in the primary literature are too extensive to confirm the null hypothesis. In the subsequent chapters of this thesis, I attempt to highlight multiple methods that might clarify the impact of pharmacogenetic variation on psychotropic drug response.

In the second chapter of this thesis, I describe using data from the UK Biobank study to investigate further evidence that genetic variation in CYP450 enzymes increases or decreases risks of adverse drug reactions in participants taking antidepressants and antipsychotics. This represents the largest pharmcogenetic analysis of psychotropic drug users to date, with over 30,000 individuals and including a much higher number of ex-

[1]This paper focuses on US regulations, but the same can be said of the UK.

treme CYP450 metabolisers than seen in previous publications (N=10,511 non-wild-type CYP2D6 metabolisers and N=21,771 non-wild-type CYP2C19 metabolisers).

When embarking on this project, I was surprised to find that very few pharmacogenetic researchers had taken advantage of this large data resource to probe the question of CYP450 genetic variation. It became clear that this was, in part, due to the complexity in assigning CYP450 metabolic phenotype groups. Therefore, this chapter describes the method I developed to do so. This involved extracting the imputed genetic data for the relevant regions upstream and downstream of *CYP2C19* and *CYP2D6*, phasing the data and performing sample and individual level quality control for all major population groups, establishing which star alleles I could accurately define based on the available SNPs, aggregating and translating the haplotype data to the star alleles, and assigning a metabolic phenotype based on the diplotype combination. At the time of conducting this analysis, this was to my knowledge the first example of assigning CYP450 metabolic phenotype groups across a large biobank population. Recently, McInnes and Lavertu *et al* (2020) released a new software, PGxPOP (`https://github.com/PharmGKB/PGxPOP`), that allows the quick calling of 14 known pharmacogenes [90]. A comparison of the observed frequencies of the CYP450 metabolic phenotype groups based on my method and PGxPOP reveals similar results, which adds confidence to my method. The availability of this software will undoubtedly allow more researchers to utilise biobank scale data to address pharmacogenetic questions, which opens the door for many novel discoveries.

As described in chapter 2, I expect some degree of inaccuracy in assigning the CYP450 metabolic phenotypes for all populations. As table 2.12 shows, the frequencies of CYP2C19 metabolic phenotype groups in the white European population are highly concordant with published data, with differences of just a few percent for each group [176]. For CYP2D6, the method is less reliable, owing to the relatively common CNVs in that gene. I did not define CNVs in this project. Although methods do exist to call CNVs from genotype data, it is difficult and the results not highly reliable [90]. It is likely that whole exome sequencing data would also improve the accuracy of the assigned phenotypic groups, especially for *CYP2D6* which is highly polymorphic (thus reducing the reliability of imputation) and where structural variation is common. However, for genes like *CYP2C19*, with important splicing and non-coding variants, whole genome sequencing would be the gold-standard. This data will be released in the coming months for most UK Biobank participants, and will

be of great value for pharmacogenetic, and other, research.

Among the non-European population the differences in the accuracy of the CYP450 calling method described here are more extreme, although the numbers in the UK Biobank sample are too low to confirm the accuracy of the method with a high degree of certainty. That said, it is not surprising that the ability to reliably assign phenotypic groups based on the data from the UK Biobank array(s) would be lower for non-European populations. There are significant differences in the frequencies of risk alleles among population groups, and several variants that are common in a non-European population were not included on either the genotype assay or imputation panel (or, they were included but failed to pass quality control steps). For example, *CYP2C19*27*, a reduced function allele, is observed in 21% of people of African ancestry, and *CYP2C19*35*, a non-functional allele, is seen in 9% of people of African ancestry [176, 197, 327, 328]. Neither of these alleles are seen in the European population at all, and the defining SNPs were neither typed nor imputed in the UK Biobank sample. Over 1% of African individuals carry the risk alleles for *CYP2D6*40*, and 1% of East Asian individuals carry the risk allele for *CYP2D6*69*. Both are non-functional alleles, and neither are observed in the European population. Again, the defining SNPs for these star alleles were not included on the genotype array or imputation panel. In addition, *CYP2D6*5*, a whole gene deletion, is almost twice as common in African and East Asian and South Asian populations compared to European. Although this deletion was not characterised at all in this sample, this exclusion will be of greater consequence in the non-European populations. Where possible, I did make efforts to rescue rare variants that were initially excluded due to low frequency. For example, the defining variants for *CYP2C19*9* were initially removed but were added in following a visual check of the Evoker intensity plots (as described in the methods section of chapter 2) [237]. This is a reduced function allele that is common in African populations (around 4%), but very rare in European (<0.025%). Similarly, *CYP2C19*15* is more common in both African (5%) and East Asian (1%) populations compared to European (0.2%). *CYP2D6*29* was also rescued following a visual check of the intensity plots. This allele is seen in around 10% of African individuals and 6% of South Asian individuals, but only 0.09% of European individuals [176, 197, 327, 328]. Future work should consider an improved genotype panel, with more complete coverage of common variants in non-European populations. The use of whole genome sequencing data will also be highly valuable in accurately assigning individuals of

all populations to the correct phenotypic groups.

Here I have set out some clear limitations of using genotype data to investigate pharmacogenetic variation. Undoubtedly, whole genome sequencing data would be more reliable and should be chosen where possible. However, whole genome sequencing is much more expensive - often prohibitively so - and not yet available for many of the largest biobank cohorts. Whole exome sequencing is a lower cost alternative, but this has it's own shortcomings. Many known pharmacogenes, including *CYP2C19*, have relatively common splicing and non-coding variants that would not be captured through exome sequencing. Therefore, it remains important to elucidate the potential of genotype data for these types of analyses. Although all findings should be considered carefully in light of the known limitations, I demonstrate successfully that it is possible to assign biobank subjects to the complex phenotypic groups with a reasonably high degree of accuracy, and a preliminary assessment of six proxy measures of adverse drug reaction (BMI, weight, HbA1c, cholesterol levels, sleep duration and QT interval) revealed some evidence that CYP450 metabolic activity was associated with increased or decreased risk.

The results of the preliminary analyses described in this chapter led me to the conclusion that for certain adverse events, it would be valuable to consider the impact CYP450 metabolic status on individual drugs, rather than drug groups. This is because some drugs of the same class can still cause very different adverse events. The risk of antidepressant-induced diabetes is known to vary between drugs of the same class. For example, paroxetine increases HbA1c levels, while fluoxetine, also an SSRI, seems to lower it [269, 278–280]. I chose to focus the third chapter of this thesis on a more in-depth analysis of this specific adverse drug reaction, which allowed me to consider individual drugs, as well as to account for additional variables likely to impact HbA1c levels (such as diabetes status and concomitant medication).

The work presented in chapter 3 represents the first study to explore if variation in the *CYP2D6* and *CYP2C19* genes influences HbA1c levels in individuals taking antidepressants and antipsychotics. I observed a significant (both statistically and clinically) association between CYP2D6 poor metabolisers and higher levels of HbA1c among all participants taking paroxetine. This offers support to existing CPIC guidelines that recommend reducing paroxetine dose for CYP2D6 poor metabolisers [79].

I found that taking a CYP2C19 inhibitor drug was also associated with changes in

HbA1c levels. Enzyme inhibition and induction are important factors to consider in these analyses, as the co-prescription of such agents could effectively change the metabolic status of the individual (i.e., a participant grouped as a normal metaboliser, who takes an inhibitory agent, is likely to have their enzymatic activity reduced). The challenge here is quantifying the extent of the inhibition and/or induction. Not all inhibitory agents will completely prevent enzymatic activity. The fact that some inhibitory agents are very commonly prescribed in this sample (25% of participants took a CYP2C19 inhibitor drug) adds an additional layer of complexity, because the importance of that inhibitory agent will vary depending on the (genetically determined) metabolic status. If someone is a CYP2C19 poor metaboliser, and they take a drug that inhibits CYP2C19, the impact of that inhibitory drug will be much less compared to a normal metaboliser. It is difficult to account for such complexities when conducting a retrospective analysis using large data sets, but future clinical trials may include serum drug monitoring methods to more clearly define the extent to which inhibitory agents (and inducers) impacts CYP450 metabolic activity, and how this interacts with the genetic variation.

I demonstrate a significant interaction between diabetic status and non-wild-type CYP450 status for participants taking amitriptyline, fluoxetine, citalopram, sertraline, and venlafaxine. Stratifying these analyses provides some evidence that the impact of *CYP2D6* genetic variation is somewhat contingent on diabetes status. Among diabetic participants taking venlafaxine, CYP2D6 poor and intermediate metabolisers have higher HbA1c levels. Although this is a suggestive association only, this adds support to existing clinical guidelines published by the Dutch Pharmacogenetics Working Group which state that CYP2D6 poor metabolisers should be treated with reduced venlafaxine doses or seek an alternative treatment. In contrast, I observed a decreased HbA1c levels among diabetic CYP2D6 intermediate metabolisers taking fluoxetine. As discussed in chapter 3, this initially appeared to contradict my hypothesis, but further research into the literature surrounding fluoxetine and diabetes risk revealed that fluoxetine can have a protective effect in diabetic patients by lowering HbA1c levels. Thus, the slower metabolism experienced by the intermediate metabolisers would lead to increased serum fluoxetine levels and an increased protective effect [278–280].

In the fourth chapter of this thesis, I describe the impact of CYP2C19 and CYP2D6 metabolic phenotype on self-reported sleep duration and measures of sleep quality. I

demonstrate that CYP450 metabolic activity does seem to be associated with sleep (both duration and quality), particularly among people taking tricyclic antidepressants. This makes sense, given how common sleep-related side effects (particularly sedation) are for this drug class due to their blockage of the histamine H1 receptors [187, 306]. Although the findings among SSRI users were less strong, I do observe some evidence that the co-prescription of a CYP2D6 inhibitor drug alongside fluoxetine decreases self-reported sleep duration, suggesting decreased CYP2D6 activity may result in increased sensitivity to the stimulatory effects of fluoxetine. Again, my findings in this chapter highlight the importance of conducting single drug analyses where possible, as many adverse reactions cannot be generalised across drug class.

Chapters 2, 3, and 4 describe various limitations to these methods. Firstly, these analyses rely on self-report data and the derivation of proxy phenotypes. The UK Biobank did not include information on medication dose or treatment duration. Towards the end of my work on this project, the UK Biobank released primary care data for a subset of the UK Biobank sample. This data would likely include much more detail on dose, treatment duration and response. Follow up research by our group will endeavour to make use of this data, especially once it is available for the full sample. A second limitation is the low number of antipsychotic drug users in the UK Biobank sample, where we know that people with severe psychiatric disorders are under-represented. Alternative cohorts, such as the Million Veteran Program, which is a large patient-based biobank, would be a valuable extension of this work [253].

A third, important, limitation lies in the complexity of CYP450-mediated metabolism. For many drugs, this is non-linear, and may involve several steps and secondary pathways that may be mediated by different enzymes. This is difficult to account for in statistical models. The best, and perhaps only, solution to this is to include serum drug monitoring, which is not practical in a biobank cohort. This is something that can be incorporated into clinical trials, and future pharmacogenetic research should take account of any new and emerging knowledge on the pharmacokinetic profiles of these drugs. Adding to this complexity, some CYP2C19 and CYP2D6 inhibitory agents were commonly co-prescribed alongside the psychotropic drugs. This will have likely impacted my analyses because the inhibitory action may reduce or eliminate the impact of the genetic variation. Future analyses with larger sample sizes on individual drugs will be better suited to investigate this

interaction in greater detail.

Overall, these analyses provide support to existing and emerging clinical guidelines that suggest altered dosing or the choice of a different drug for CYP2C19 and CYP2D6 poor, intermediate or ultra-rapid metabolisers [79, 80, 84, 207]. Beyond that, this work is the first in-depth consideration of how the ever-growing availability of large datasets may be valuable in the assessing the extent of CYP450 genetic variation on adverse drug reactions. The UK Biobank is an extremely valuable resource, and its open-access policy allows a wide range of researchers across the world to conduct innovative analyses. That said, the limitations I highlight here demonstrate that it may not be the best available data to probe the question of CYP450 genetic variation among psychotropic drug users, particularly among non-European populations. These findings will help future researchers carefully consider the most appropriate data for their analyses.

Some of the most important findings of my research on CYP450 pharmacogenetics, both the meta-analyses described in chapter 1 and the UK Biobank analyses described in chapters 2 to 4, are the limitations. Many previous studies on CYP450 genetic variation have concluded that small sample size and the resulting lack of statistical power is the primary limitation. Though this is undoubtedly an essential consideration, my results highlight many additional complexities in understanding CYP450 pharmacogenetics, that cannot be overcome by large samples alone.

The genetic variants that give rise to altered CYP450 metabolism represent a tiny number of genetic variants that may contribute to sleep disturbance in depression - both in the presence of antidepressants and otherwise. Gaining a broader understanding of this background genetic variation will help place the pharmacogenetic differences into context and may present novel loci of pharmacogenetic importance. As part of a paper in preparation, but not included in this thesis, I demonstrate that the SNP-based heritability of both long and short sleep duration was relatively low. This confirms that environmental factors are a highly important consideration alongside genetic factors. In terms of adding value to pharmacogenetic analyses, it would be possible to use the GWAS summary statistics to calculate a polygenic risk score, and then include as a covariate in the models described in chapters 2, 3, and 4. This would perhaps be most valuable where the outcome trait has a high genetic component; most of the traits considered in this thesis have heritability estimates under 20%.

In the aforementioned paper, a GWAS of sleep duration, one of my most interesting observations was low positive genetic correlation between short and long sleep. Intuitively, one might anticipate a high degree of negative correlation between these traits. This finding indicates that short and long sleep duration are two distinct traits, rather than being opposite ends of a continuum. I also demonstrate that these traits share similar profiles of genetic correlation to other neuropsychiatric traits; both are, for example, positively genetically correlated with depression. Taken together, these findings indicate a non-linear relationship between sleep duration and related traits. This adds an additional layer of complexity when interpreting the sleep pharmacogenetics findings described above and in chapter 4 and is something that should be considered in future analyses. Logistic regression models, which assume a linear relationship between the predictors and the outcome, may not be the most appropriate option.

As I discuss in the introduction to this thesis, there has been a shift in recent years away from candidate gene studies towards genome-wide approaches. Though there has been some uptake of these genome-wide approaches in pharmacogenetic, or pharmacogenomic, research, it has not been adopted as widely. Much research has remained focused on known pharmacogenes, or genetic variants in proteins with an established role in drug transport or metabolism. These types of studies play an important role; many genotyping arrays do not offer full coverage of regions of pharmacogenetic importance, and it is difficult to reliably identify structural variation based on genotyped data. The work described in chapters 2, 3, and chapter 4 demonstrate that it is possible to conduct candidate gene type analyses based on genotype data, albeit with clear limitations. There have been several GWAS on psychotropic drug response and toxicity, but few have yielded clear findings [97–100]. The negative findings from these studies are largely due to power issues, as it is difficult to gather large enough samples of users of a specific drug or drug class. Patient based biobanks like the Million Veteran Program, the 100,000 and 5 million Genomes Projects, and Psychiatric Genomics and other consortia efforts will be essential to overcome this.

The final chapter of this thesis describes the work undertaken to design and set up a clinical trial investigating pharmacogenetic interventions in psychiatry. A total of 40 clinician-participants and 420 patient-participants are to be recruited, with the first 50 being recruited to a pilot stage to be analysed in this thesis. Unfortunately, recruitment had to be paused due to the Covid-19 pandemic, in the interest of safety. Although this means there

is not enough data to conduct a meaningful statistical analysis, I successfully opened the first study site and recruited of the first 12 patients, which helps to confirm the feasibility of the trial. The response from sites and clinicians has been overwhelmingly positive, with over 15 NHS trusts proactively reaching out to be involved. Recruitment is now underway, following an amendment to the protocol to allow virtual recruitment and assessments.

All chapters of this thesis were limited by the poor availability of data from non-European subjects. This is a significant problem when considering the clinical relevance of these findings. Any future studies must include data from a wider range of worldwide population groups, in order to ensure the equity, generalisability and relevance of the findings and to increase discovery potential.

The limitations identified in this thesis can be broadly grouped as issues with 1) phenotyping and 2) genotyping. The major phenotyping issues (cross-sectional data, lack of information on dose or treatment duration, reliance of self-report measures) can be addressed through inclusion of the increasingly available primary care data for UK Biobank participants. By the end of 2020, UK Biobank released data from linked electronic patient records. This was initially limited to researchers studying Covid-19, but has since been expanded. Data from a variety of medical records, including Covid-19 tests, death records, GP records, and hospital episodes are now available for over 400,000 UK Biobank participants. Updating some of the analyses described in this thesis with this data will be of huge value improving our understanding of pharmacogenetics within psychiatry.

The genotype data is missing some of the SNPs necessary to define star alleles. As described previously, this will have a disproportionate impact on non-European populations. In addition, missing data on CNVs means I could not define CYP2D6 ultra-rapid metabolisers. This missingness results in a certain degree of known error in my ability to assign CYP450 metabolic phenotypes. These issues can be neatly addressed through use of whole genome sequencing data, which UK Biobank will soon make available for all participants (data for the first 200,000 participants was released in November 2021). This will represent, by some margin, the largest release of whole genome sequencing data in the world. The use of this data will ensure all relevant SNPs can be identified, allow for the study of structural variants such as CNVs, and ensure the inclusion of important splicing and non-coding variants that might be missed with whole exome sequencing data. In addition, it will eliminate the reliance on reference and imputation panels, which disadvantage the

study of non-European populations. It also opens the door for the discovery of potentially novel areas of genetic variation that impact CYP450 function.

The overarching conclusion of this thesis is that no single methodological approach will be sufficient to fully elucidate the role of pharmacogenetic variation in psychiatry. The combination of well-designed, prospective clinical trials with large, biobank based studies is essential to consider this question from several directions. The data collected through these methods can be considered complimentary, and each type of study should be analysed with the other(s) in mind. In the future, larger samples of patient data (including, perhaps, health record data) will allow for further GWAS of drug response to be conducted. This may lead to the identification of novel genes and variants of pharmacogenetic importance, including non-coding variants that are typically excluded from candidate-gene approaches. Where possible, exome as well as whole-genome sequencing data should also be utilised, to learn more about the role of structural variation in drug response.

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Appendix A

Appendices for chapter 1

A.1 Publications associated with this chapter

The work described in chapter 1 has formed the basis of two peer reviewed, published articles, both included below. The first, ”The effect of CYP2D6 variation on antipsychotic-induced hyperprolactinaemia: a systematic review and meta-analysis” was accepted for publication in The Pharmacogenomics Journal in December 2019, and published online in February 2020 (https://www.nature.com/articles/s41397-019-0142-9). The second, ”CYP2D6 and antipsychotic-induced weight gain: a meta-analysis and systematic review”, was accepted for publication in a special issue of Frontiers in Psychology, focusing on pharmacogenetics, and published online in January 2022 (https://www.frontiersin.org/articles/10.3389/fpsyg.2021.768748/full).

A.2 Normal prolactin ranges

Table taken from NHS laboratory guidelines:

Age	Male reference range (mU/L)	Female reference range (mU/L)
Up to 30 days	900-6751	900-6751
31 – 60 days	689-4208	689-4208
61 – 90 days	151-2820	151-2820
91 days – 5 months	113-2813	113-2813
5 – 8 months	121-2213	121-2213
8 – 1 year	148-1105	148-1105
1 – 2 years	93-1063	96-1165
2 – 4 years	82-967	81-864
4 – 8 years	69-593	66-630
8 – 18 years	60-324	65-496
> 18 years	86-324	102-496

Table A.1: Normal prolactin ranges for all ages

Appendix B

Appendices for chapter 2

B.1 UK Biobank phenotype derivation

Detailed information on all phenotypes extracted and/or derived from UK Biobank data can be found in the following table.

Table B.1: Description of all UK Biobank phenotypes included in this thesis and how they were captured, recorded, coded, and derived.

Phenotype	UKB Data Field	Description	Question asked	Valid responses	Data type	Processing measure
Age at recruitment (years)	21022	Age of participant.	Derived variable	37-73 years	Integer	This is a derived variable based on date of birth and date of attending an initial assessment centre and refers to the age of the participant on the day they attended an Initial Assessment Centre, truncated to whole year.
Sex	31	Sex of participant.	Derived variable	Male, female	Categorical	Acquired from central registry at recruitment, but in some cases updated by the participant. Checked against genetic sex (22001) for quality control. Participants with discrepancy between reported and genetic sex excluded from analyses.
Ethnicity	21000	Self-reported ethnicity of participant	"What is your ethnic group?"	White, Mixed, Asian or Asian British, Black or Black British, Chinese, Other ethnic group, Do not know, Prefer not to answer	Categorical	Self-report.
Genetic ethnicity	22006	Indicates samples who self-identified as 'White British' according to Field 21000 and have very similar genetic ancestry based on a principal components analysis of the genotypes.	Derived variable	Caucasian	Categorical	Principal component analysis
	-	Derived for all populations using PC-AiR (see chapter 2, methods)	Derived variable	European, African, East Asian, South Asian, Admixed, other	Categorical	Principal component analysis
Medication	2492	Self-reported medications status	"Do you regularly take any other PRESCRIPTION medications? (Do not forget medications such as puffers or patches)"	Yes, no, do not know, prefer not to answer	Categorical	Participants who answer YES informed they will be asked about this later by an interviewer.
	20003	Self-reported medication history	Asked to list all prescribed medication duruing verbal interview.	Free text entry	Categorical	Coded post-interview.
Body mass index (BMI, kg/m2)	21001	BMI of participant	Derived variable	12 to 75	Continuous	BMI value here is constructed from height and weight measured during the initial Assessment Centre visit.
Weight (kg)	21002	Weight of participant	Measured	30 to 198	Continuous	Weight was measured by a variety of means during the initial Assessment Centre visit. This variable is an amalgamation of these results.

Table B.1 continued from previous page

HbA1c (mmol/mol)	30750	Glycated haemoglobin	Blood biochemistry	15 to 516	Continuous	Measured by HPLC analysis on a Bio-Rad VARIANT II Turbo
Cholesterol (mmol/L)	30690	Total cholesterol	Blood biochemistry	0.5 to 15.5	Continuous	Measured by CHO-POD analysis on a Beckman Coulter AU5800
Sleep duration (hours)	1160	Self-reported sleep duration	"About how many hours sleep do you get in every 24 hours? (please include naps)"	1 to 23 hours, do not know, prefer not to answer	Integer	If answer <1 then rejected, if answer >23 then rejected, Participant asked to confirm if <3 or >12.
QT interval (ms)	22331	QT interval is measured from an electrocardiogram (ECG) - calculated as the time from the start of the Q wave to the end of the T wave. This represents the time from start of cardiac ventricles contracting to full relaxation.	Measured	114 to 752	Integer	QT interval during ECG
Diabetes status	2443	Self-reported diabetes diagnosis	"Has a doctor ever told you that you have diabetes?"	Yes, no, do not know, prefer not to answer	Categorical	Participant were advised to select 'do not know' if they were unsure, and were then asked about this during a verbal interview.
ICD-10 diagnoses	41270	Diagnoses - ICD10: A summary of the distinct primary or secondary diagnosis codes a participant has had recorded across all their hospital inpatient records.	Information taken from hospital records	Diagnoses are coded according to the International Classification of Disease version 10 (ICD-10).	Categorical	Corresponding date of diagnosis can be found in data field 41280.
	41202	Diagnoses - main ICD10: A summary of the distinct primary/main diagnosis codes a participant has had recorded across all their hospital inpatient records.				Corresponding date of diagnosis can be found in data field 41262.
	41204	Diagnoses - secondary ICD10: A summary of the distinct secondary diagnoses codes a participant has had recorded across all their hospital inpatient records.				No corresponding date field available.

B.2 Additional detail for tables in chapter 2

The complete results from the regression models described in chapter 2 are provided in the following tables. Please also see tables 2.14, 2.15, and 2.16. The included covariates (age, sex, ethnicity), largely impact the outcomes as anticipated, which serves as a valuable control. In most cases, the variance explained (R^2) is low, demonstrating that these simplified models explain only a small portion of the overall variance. This is discussed in greater detail in the discussion of chapter 2, and in chapters 3 and 4.

Table B.2: Association between CYP450 metabolic phenotype and measures of adverse drug reactions among participants taking tricyclic antidepressants.

	body_mass_index		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	29.88	29.03 – 30.74	**<0.001**
cyp2c19_metapheno [IM]	-0.38	-0.63 – -0.13	**0.003**
cyp2c19_metapheno [PM]	-0.80	-1.39 – -0.20	**0.008**
cyp2c19_metapheno [RM]	-0.17	-0.43 – 0.10	0.224
cyp2c19_metapheno [UM]	-0.23	-0.74 – 0.29	0.388
cyp2d6_metapheno [IM]	0.13	-0.12 – 0.37	0.313
cyp2d6_metapheno [PM]	0.09	-0.38 – 0.57	0.705
sex [Female]	-0.30	-0.53 – -0.07	**0.009**
age	-0.01	-0.03 – 0.00	0.072
genetic_eth [Admix]	-0.13	-1.04 – 0.78	0.777
genetic_eth [Admix European]	-0.90	-1.62 – -0.17	**0.015**
genetic_eth [African]	1.86	0.94 – 2.77	**<0.001**
genetic_eth [East Asian]	-4.01	-6.94 – -1.09	**0.007**
genetic_eth [South Asian]	0.06	-0.84 – 0.96	0.893
Observations	11274		
R^2 / R^2 adjusted	0.005 / 0.004		

	weight		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	98.60	96.17 – 101.03	**<0.001**
cyp2c19_metapheno [IM]	-0.66	-1.38 – 0.05	0.069
cyp2c19_metapheno [PM]	-2.12	-3.81 – -0.44	**0.014**
cyp2c19_metapheno [RM]	-0.21	-0.97 – 0.55	0.591
cyp2c19_metapheno [UM]	-0.56	-2.01 – 0.89	0.452
cyp2d6_metapheno [IM]	0.38	-0.31 – 1.08	0.281
cyp2d6_metapheno [PM]	-0.20	-1.55 – 1.16	0.777
sex [Female]	-13.65	-14.30 – -13.01	**<0.001**

Table B.2: cont.

age	-0.16	-0.20 – -0.12	**<0.001**
genetic_eth [Admix]	-3.02	-5.61 – -0.43	**0.023**
genetic_eth [Admix European]	-3.47	-5.53 – -1.42	**0.001**
genetic_eth [African]	4.19	1.59 – 6.78	**0.002**
genetic_eth [East Asian]	-17.34	-25.65 – -9.03	**<0.001**
genetic_eth [South Asian]	-5.30	-7.84 – -2.76	**<0.001**
Observations	11290		
R^2 / R^2 adjusted	0.139 / 0.138		

	glycated_haemoglobin_hba1c		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	29.17	27.94 – 30.40	**<0.001**
cyp2c19_metapheno [IM]	-0.40	-0.76 – -0.04	**0.031**
cyp2c19_metapheno [PM]	0.16	-0.69 – 1.02	0.705
cyp2c19_metapheno [RM]	0.02	-0.36 – 0.41	0.905
cyp2c19_metapheno [UM]	-0.00	-0.73 – 0.73	0.999
cyp2d6_metapheno [IM]	0.13	-0.22 – 0.48	0.476
cyp2d6_metapheno [PM]	-0.27	-0.96 – 0.41	0.433
sex [Female]	-1.50	-1.83 – -1.18	**<0.001**
age	0.16	0.14 – 0.18	**<0.001**
genetic_eth [Admix]	2.90	1.59 – 4.20	**<0.001**
genetic_eth [Admix European]	0.63	-0.42 – 1.68	0.238
genetic_eth [African]	5.26	3.84 – 6.68	**<0.001**
genetic_eth [East Asian]	2.21	-1.90 – 6.33	0.292
genetic_eth [South Asian]	6.55	5.26 – 7.83	**<0.001**
Observations	10860		
R^2 / R^2 adjusted	0.043 / 0.042		

Table B.2: cont.

	cholesterol		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	5.46	5.27 – 5.65	**<0.001**
cyp2c19_metapheno [IM]	-0.01	-0.07 – 0.04	0.620
cyp2c19_metapheno [PM]	-0.04	-0.17 – 0.09	0.569
cyp2c19_metapheno [RM]	-0.02	-0.08 – 0.04	0.505
cyp2c19_metapheno [UM]	0.03	-0.09 – 0.15	0.622
cyp2d6_metapheno [IM]	-0.01	-0.07 – 0.04	0.650
cyp2d6_metapheno [PM]	-0.02	-0.13 – 0.09	0.714
sex [Female]	0.60	0.55 – 0.65	**<0.001**
age	-0.00	-0.01 – 0.00	0.083
genetic_eth [Admix]	-0.14	-0.34 – 0.07	0.195
genetic_eth [Admix European]	0.03	-0.13 – 0.19	0.709
genetic_eth [African]	-0.42	-0.62 – -0.21	**<0.001**
genetic_eth [East Asian]	0.18	-0.47 – 0.82	0.594
genetic_eth [South Asian]	-0.52	-0.72 – -0.32	**<0.001**
Observations	10843		
R^2 / R^2 adjusted	0.051 / 0.050		

	sleep_duration		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.66	6.43 – 6.89	**<0.001**
cyp2c19_metapheno [IM]	-0.01	-0.08 – 0.06	0.776
cyp2c19_metapheno [PM]	0.08	-0.08 – 0.24	0.318
cyp2c19_metapheno [RM]	-0.08	-0.16 – -0.01	**0.023**
cyp2c19_metapheno [UM]	-0.09	-0.23 – 0.05	0.188
cyp2d6_metapheno [IM]	0.02	-0.05 – 0.08	0.630
cyp2d6_metapheno [PM]	0.15	0.02 – 0.28	**0.025**
sex [Female]	0.02	-0.04 – 0.08	0.451

Table B.2: cont.

age	0.01	0.01 – 0.01	**<0.001**
genetic_eth [Admix]	-0.15	-0.40 – 0.10	0.230
genetic_eth [Admix European]	-0.27	-0.47 – -0.08	**0.006**
genetic_eth [African]	-0.44	-0.68 – -0.19	**<0.001**
genetic_eth [East Asian]	0.42	-0.37 – 1.21	0.296
genetic_eth [South Asian]	-0.22	-0.46 – 0.02	0.069
Observations	11383		
R^2 / R^2 adjusted	0.006 / 0.005		

		qt_interval	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	405.14	380.94 – 429.33	**<0.001**
cyp2c19_metapheno [IM]	-8.29	-15.32 – -1.25	**0.021**
cyp2c19_metapheno [PM]	1.96	-15.52 – 19.43	0.826
cyp2c19_metapheno [RM]	-4.70	-11.94 – 2.54	0.203
cyp2c19_metapheno [UM]	-14.17	-29.31 – 0.98	0.067
cyp2d6_metapheno [IM]	2.97	-4.06 – 10.01	0.407
cyp2d6_metapheno [PM]	15.31	-1.40 – 32.02	0.072
sex [Female]	1.82	-4.78 – 8.42	0.588
age	0.10	-0.29 – 0.50	0.606
genetic_eth [Admix]	-1.61	-38.15 – 34.93	0.931
genetic_eth [Admix European]	2.22	-21.71 – 26.15	0.856
genetic_eth [African]	2.02	-23.91 – 27.94	0.879
genetic_eth [South Asian]	-27.25	-63.94 – 9.44	0.145
Observations	494		
R^2 / R^2 adjusted	0.028 / 0.004		

Table B.3: Association between CYP450 metabolic phenotype and measures of adverse drug reactions among participants taking SSRI antidepressants.

	body_mass_index		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	28.53	27.91 – 29.16	**<0.001**
cyp2c19_metapheno [IM]	-0.14	-0.34 – 0.06	0.165
cyp2c19_metapheno [PM]	0.04	-0.43 – 0.51	0.868
cyp2c19_metapheno [RM]	-0.13	-0.34 – 0.08	0.226
cyp2c19_metapheno [UM]	-0.21	-0.65 – 0.22	0.330
cyp2d6_metapheno [IM]	0.06	-0.14 – 0.25	0.567
cyp2d6_metapheno [PM]	0.17	-0.19 – 0.54	0.353
sex [Female]	-0.45	-0.63 – -0.27	**<0.001**
age	0.01	0.00 – 0.02	**0.049**
genetic_eth [Admix]	0.20	-0.50 – 0.89	0.578
genetic_eth [Admix European]	-0.29	-0.80 – 0.21	0.257
genetic_eth [African]	2.24	1.34 – 3.15	**<0.001**
genetic_eth [East Asian]	-5.48	-7.72 – -3.23	**<0.001**
genetic_eth [South Asian]	-0.42	-1.22 – 0.39	0.307
Observations	18497		
R^2 / R^2 adjusted	0.004 / 0.004		

	weight		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	96.54	94.76 – 98.33	**<0.001**
cyp2c19_metapheno [IM]	-0.53	-1.10 – 0.05	0.071
cyp2c19_metapheno [PM]	0.03	-1.29 – 1.36	0.960
cyp2c19_metapheno [RM]	-0.09	-0.70 – 0.51	0.759
cyp2c19_metapheno [UM]	-0.78	-2.02 – 0.45	0.213
cyp2d6_metapheno [IM]	0.14	-0.42 – 0.70	0.630
cyp2d6_metapheno [PM]	0.37	-0.68 – 1.42	0.488

Table B.3: cont.

sex [Female]	-14.28	-14.79 – -13.77	**<0.001**
age	-0.12	-0.15 – -0.09	**<0.001**
genetic_eth [Admix]	-2.60	-4.58 – -0.61	**0.010**
genetic_eth [Admix European]	-2.49	-3.94 – -1.03	**0.001**
genetic_eth [African]	5.88	3.31 – 8.45	**<0.001**
genetic_eth [East Asian]	-20.10	-26.49 – -13.70	**<0.001**
genetic_eth [South Asian]	-7.11	-9.39 – -4.82	**<0.001**
Observations	18515		
R^2 / R^2 adjusted	0.145 / 0.144		

	glycated_haemoglobin_hba1c		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	27.97	27.15 – 28.79	**<0.001**
cyp2c19_metapheno [IM]	-0.15	-0.41 – 0.11	0.259
cyp2c19_metapheno [PM]	-0.24	-0.85 – 0.36	0.428
cyp2c19_metapheno [RM]	-0.09	-0.36 – 0.19	0.548
cyp2c19_metapheno [UM]	-0.21	-0.77 – 0.36	0.474
cyp2d6_metapheno [IM]	-0.24	-0.50 – 0.01	0.064
cyp2d6_metapheno [PM]	0.13	-0.35 – 0.61	0.593
sex [Female]	-1.47	-1.70 – -1.24	**<0.001**
age	0.18	0.16 – 0.19	**<0.001**
genetic_eth [Admix]	1.18	0.27 – 2.09	**0.011**
genetic_eth [Admix European]	0.31	-0.35 – 0.98	0.359
genetic_eth [African]	3.19	1.91 – 4.47	**<0.001**
genetic_eth [East Asian]	0.29	-2.64 – 3.21	0.848
genetic_eth [South Asian]	5.56	4.51 – 6.61	**<0.001**
Observations	17697		
R^2 / R^2 adjusted	0.050 / 0.049		

Table B.3: cont.

	cholesterol		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	5.36	5.23 – 5.50	**<0.001**
cyp2c19_metapheno [IM]	0.01	-0.04 – 0.05	0.732
cyp2c19_metapheno [PM]	0.01	-0.09 – 0.11	0.861
cyp2c19_metapheno [RM]	-0.02	-0.06 – 0.03	0.442
cyp2c19_metapheno [UM]	-0.07	-0.16 – 0.02	0.144
cyp2d6_metapheno [IM]	-0.03	-0.07 – 0.01	0.162
cyp2d6_metapheno [PM]	-0.06	-0.14 – 0.01	0.103
sex [Female]	0.43	0.39 – 0.47	**<0.001**
age	0.00	-0.00 – 0.00	0.061
genetic_eth [Admix]	-0.07	-0.22 – 0.07	0.317
genetic_eth [Admix European]	-0.03	-0.14 – 0.08	0.563
genetic_eth [African]	-0.32	-0.51 – -0.13	**0.001**
genetic_eth [East Asian]	0.22	-0.25 – 0.69	0.367
genetic_eth [South Asian]	-0.38	-0.55 – -0.21	**<0.001**
Observations	17719		
R^2 / R^2 adjusted	0.030 / 0.029		

	sleep_duration		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.96	6.79 – 7.12	**<0.001**
cyp2c19_metapheno [IM]	-0.00	-0.05 – 0.05	0.999
cyp2c19_metapheno [PM]	-0.01	-0.14 – 0.11	0.854
cyp2c19_metapheno [RM]	0.02	-0.03 – 0.08	0.401
cyp2c19_metapheno [UM]	0.14	0.02 – 0.25	**0.018**
cyp2d6_metapheno [IM]	0.03	-0.03 – 0.08	0.320
cyp2d6_metapheno [PM]	-0.02	-0.12 – 0.08	0.671

Table B.3: cont.

sex [Female]	0.03	-0.02 – 0.08	0.194
age	0.01	0.01 – 0.01	**<0.001**
genetic_eth [Admix]	-0.17	-0.36 – 0.01	0.065
genetic_eth [Admix European]	-0.09	-0.23 – 0.04	0.175
genetic_eth [African]	-0.18	-0.42 – 0.06	0.131
genetic_eth [East Asian]	0.06	-0.54 – 0.65	0.851
genetic_eth [South Asian]	-0.37	-0.59 – -0.16	**0.001**
Observations	18608		
R^2 / R^2 adjusted	0.003 / 0.003		

		qt_interval	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	396.33	381.77 – 410.89	**<0.001**
cyp2c19_metapheno [IM]	-2.65	-7.21 – 1.91	0.254
cyp2c19_metapheno [PM]	6.96	-4.98 – 18.90	0.253
cyp2c19_metapheno [RM]	-2.14	-6.94 – 2.66	0.382
cyp2c19_metapheno [UM]	-7.90	-17.74 – 1.94	0.116
cyp2d6_metapheno [IM]	-3.10	-7.48 – 1.28	0.165
cyp2d6_metapheno [PM]	4.41	-4.11 – 12.93	0.310
sex [Female]	6.87	2.75 – 10.98	**0.001**
age	0.38	0.12 – 0.64	**0.004**
genetic_eth [Admix]	3.45	-16.98 – 23.87	0.741
genetic_eth [Admix European]	-4.30	-18.09 – 9.49	0.541
genetic_eth [African]	-19.69	-55.02 – 15.64	0.274
genetic_eth [East Asian]	-32.89	-76.01 – 10.22	0.135
genetic_eth [South Asian]	183.18	139.86 – 226.50	**<0.001**
Observations	1067		
R^2 / R^2 adjusted	0.084 / 0.072		

Table B.4: Association between CYP450 metabolic phenotype and measures of adverse drug reactions among participants taking antipsychotics.

	body_mass_index		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	32.09	30.52 – 33.66	**<0.001**
cyp2d6_metapheno [IM]	0.08	-0.43 – 0.59	0.748
cyp2d6_metapheno [PM]	0.40	-0.60 – 1.39	0.433
sex [Female]	0.51	0.07 – 0.95	**0.024**
age	-0.06	-0.09 – -0.03	**<0.001**
genetic_eth [Admix]	-0.30	-1.81 – 1.21	0.701
genetic_eth [Admix European]	0.20	-1.15 – 1.54	0.774
genetic_eth [African]	1.69	0.59 – 2.79	**0.003**
genetic_eth [East Asian]	-5.19	-8.41 – -1.97	**0.002**
genetic_eth [South Asian]	-0.75	-2.16 – 0.65	0.292
Observations	2878		
R^2 / R^2 adjusted	0.016 / 0.012		

	weight		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	104.14	99.61 – 108.67	**<0.001**
cyp2d6_metapheno [IM]	0.24	-1.22 – 1.71	0.745
cyp2d6_metapheno [PM]	-0.11	-2.99 – 2.76	0.939
sex [Female]	-11.66	-12.93 – -10.40	**<0.001**
age	-0.28	-0.36 – -0.20	**<0.001**
genetic_eth [Admix]	-3.40	-7.76 – 0.96	0.127
genetic_eth [Admix European]	-0.26	-4.15 – 3.64	0.897
genetic_eth [African]	4.17	0.99 – 7.34	**0.010**
genetic_eth [East Asian]	-19.51	-28.82 – -10.21	**<0.001**
genetic_eth [South Asian]	-7.86	-11.88 – -3.83	**<0.001**
Observations	2880		
R^2 / R^2 adjusted	0.133 / 0.130		

Table B.4: cont.

	glycated_haemoglobin_hba1c		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	31.59	29.36 – 33.82	**<0.001**
cyp2d6_metapheno [IM]	0.35	-0.38 – 1.07	0.349
cyp2d6_metapheno [PM]	-0.92	-2.34 – 0.51	0.207
sex [Female]	-0.93	-1.55 – -0.30	**0.004**
age	0.11	0.07 – 0.15	**<0.001**
genetic_eth [Admix]	2.37	0.26 – 4.48	**0.028**
genetic_eth [Admix European]	0.11	-1.80 – 2.01	0.912
genetic_eth [African]	6.02	4.29 – 7.74	**<0.001**
genetic_eth [East Asian]	0.15	-4.49 – 4.80	0.948
genetic_eth [South Asian]	5.66	3.70 – 7.63	**<0.001**
Observations	2719		
R^2 / R^2 adjusted	0.037 / 0.034		

	cholesterol		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	5.85	5.51 – 6.18	**<0.001**
cyp2d6_metapheno [IM]	0.07	-0.04 – 0.18	0.189
cyp2d6_metapheno [PM]	-0.20	-0.41 – 0.01	0.057
sex [Female]	0.53	0.44 – 0.62	**<0.001**
age	-0.01	-0.02 – -0.00	**<0.001**
genetic_eth [Admix]	0.09	-0.22 – 0.40	0.576
genetic_eth [Admix European]	0.11	-0.18 – 0.40	0.454
genetic_eth [African]	-0.56	-0.79 – -0.32	**<0.001**
genetic_eth [East Asian]	0.62	-0.10 – 1.34	0.093
genetic_eth [South Asian]	-0.48	-0.78 – -0.19	**0.001**
Observations	2750		
R^2 / R^2 adjusted	0.061 / 0.058		

Table B.4: cont.

	sleep_duration		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	7.98	7.42 – 8.55	**<0.001**
cyp2d6_metapheno [IM]	0.11	-0.08 – 0.29	0.256
cyp2d6_metapheno [PM]	0.29	-0.07 – 0.65	0.118
sex [Female]	-0.15	-0.31 – 0.01	0.059
age	-0.01	-0.02 – 0.00	0.133
genetic_eth [Admix]	0.20	-0.33 – 0.74	0.456
genetic_eth [Admix European]	-0.50	-0.98 – -0.01	**0.045**
genetic_eth [African]	-0.67	-1.07 – -0.27	**0.001**
genetic_eth [East Asian]	-0.94	-2.16 – 0.27	0.129
genetic_eth [South Asian]	-1.21	-1.71 – -0.70	**<0.001**
Observations	2901		
R^2 / R^2 adjusted	0.016 / 0.013		

	qt_interval		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	335.67	275.41 – 395.93	**<0.001**
cyp2d6_metapheno [IM]	7.39	-11.00 – 25.78	0.426
cyp2d6_metapheno [PM]	-3.30	-27.75 – 21.15	0.789
sex [Female]	4.99	-11.16 – 21.14	0.540
age	1.36	0.34 – 2.38	**0.010**
genetic_eth [Admix]	-9.35	-77.41 – 58.71	0.785
genetic_eth [Admix European]	11.66	-36.77 – 60.09	0.633
genetic_eth [African]	-17.64	-86.95 – 51.67	0.613
genetic_eth [South Asian]	43.46	-25.28 – 112.19	0.212
Observations	81		
R^2 / R^2 adjusted	0.127 / 0.030		

B.3 Power calculations

In order to consider the reliability of the findings for the antipsychotic analyses, which I predicted to be underpowered, I conducted a post-hoc power analysis using the 'pwr' package in R (`https://github.com/heliosdrm/pwr`). This takes into account the observed R^2 of the linear models used to estimate statistical power, and can also be used to derive a minimum sample size required for 80% power. The following table summarises the observed R^2, estimated power, and minimum sample required for 80% power for each phenotype included in this analysis:

Table B.5: Post-hoc power analyses

Phenotype (No. PMs)	R^2	Estimated power of model	Estimated min. sample for 80% power
BMI (145)	0.016	0.193	733
Weight (145)	0.133	0.678	191
HbA1c (136)	0.037	0.407	313
Cholesterol (139)	0.061	0.643	187
Sleep duration (146)	0.016	0.193	733
QT interval (9)	0.127	0.114	54

Appendix C

Appendices for chapter 3

C.1 Publications associated with this chapter

The work described in chapters 2 and 3 has lead to one submitted article so far. This paper was accepted for publication by Genes in October 2021, and published online in November 2021: `https://www.mdpi.com/2073-4425/12/11/1758`

C.2 Additional detail for tables in chapter 3

The following tables provide more detail to accompany the tables provided in 3, including a more detailed demographic breakdown (tables C.1 and C.2).

Table C.1: Sample demographics by CYP450 metabolic phenotypes.

Characteristics of CYP2C19 metabolic phenotype in our sample

	NM (N=12592)	**IM** (N=9825)	**PM** (N=1128)	**RM** (N=8193)	**UM** (N=1411)	**Overall** (N=33149)
Age (years)						
Mean (SD)	56.6 (7.79)	56.6 (7.85)	56.2 (8.01)	56.7 (7.72)	56.6 (7.80)	56.6 (7.80)
Sex						
Female	8623 (68.5%)	6751 (68.7%)	749 (66.4%)	5547 (67.7%)	962 (68.2%)	22632 (68.3%)
Male	3969 (31.5%)	3074 (31.3%)	379 (33.6%)	2646 (32.3%)	449 (31.8%)	10517 (31.7%)
Ethnicity						
European	11762 (93.4%)	9205 (93.7%)	1062 (94.1%)	7670 (93.6%)	1307 (92.6%)	31006 (93.5%)
Admix European	342 (2.7%)	223 (2.3%)	27 (2.4%)	198 (2.4%)	46 (3.3%)	836 (2.5%)
African	127 (1.0%)	110 (1.1%)	10 (0.9%)	95 (1.2%)	16 (1.1%)	358 (1.1%)
East Asian	25 (0.2%)	13 (0.1%)	1 (0.1%)	12 (0.1%)	0 (0%)	51 (0.2%)
Other	178 (1.4%)	149 (1.5%)	19 (1.7%)	109 (1.3%)	24 (1.7%)	479 (1.4%)
South Asian	158 (1.3%)	125 (1.3%)	9 (0.8%)	109 (1.3%)	18 (1.3%)	419 (1.3%)
HbA1c (mmol/mol)						
Mean (SD)	37.2 (7.89)	37.0 (7.44)	37.0 (7.02)	37.2 (7.90)	37.1 (7.84)	37.1 (7.73)
Diabetes						
Yes	1117 (8.9%)	827 (8.4%)	105 (9.3%)	762 (9.3%)	125 (8.9%)	2936 (8.9%)
No	11475 (91.1%)	8998 (91.6%)	1023 (90.7%)	7431 (90.7%)	1286 (91.1%)	30213 (91.1%)
Taking antidiabetic medication						

Table C.1: cont.

Yes	819 (6.5%)	580 (5.9%)	67 (5.9%)	532 (6.5%)	100 (7.1%)	2098 (6.3%)
No	11773 (93.5%)	9245 (94.1%)	1061 (94.1%)	7661 (93.5%)	1311 (92.9%)	31051 (93.7%)
BMI						
Mean (SD)	28.8 (5.67)	28.7 (5.69)	28.6 (5.34)	28.7 (5.66)	28.6 (5.73)	28.7 (5.67)
Taking CYP2C19 inhibitor						
Yes	3184 (25.3%)	2364 (24.1%)	283 (25.1%)	2068 (25.2%)	360 (25.5%)	8259 (24.9%)
No	9408 (74.7%)	7461 (75.9%)	845 (74.9%)	6125 (74.8%)	1051 (74.5%)	24890 (75.1%)

Characteristics of CYP2D6 metabolic phenotype in our sample

	NM (N=23585)	**IM (N=7824)**	**PM (N=1740)**	**Overall (N=33149)**
Age (years)				
Mean (SD)	56.6 (7.80)	56.6 (7.82)	56.6 (7.71)	56.6 (7.80)
Median [Min, Max]	58.0 [40.0, 71.0]	58.0 [40.0, 70.0]	58.0 [40.0, 70.0]	58.0 [40.0, 71.0]
Sex				
Female	16086 (68.2%)	5355 (68.4%)	1191 (68.4%)	22632 (68.3%)
Male	7499 (31.8%)	2469 (31.6%)	549 (31.6%)	10517 (31.7%)
Ethnicity				
Caucasian	22027 (93.4%)	7342 (93.8%)	1637 (94.1%)	31006 (93.5%)
Admix Caucasian	620 (2.6%)	181 (2.3%)	35 (2.0%)	836 (2.5%)
African	262 (1.1%)	78 (1.0%)	18 (1.0%)	358 (1.1%)
East Asian	36 (0.2%)	10 (0.1%)	5 (0.3%)	51 (0.2%)

Table C.1: cont.

Other	346 (1.5%)	108 (1.4%)	25 (1.4%)	479 (1.4%)
South Asian	294 (1.2%)	105 (1.3%)	20 (1.1%)	419 (1.3%)
HbA1c (mmol/mol)				
Mean (SD)	37.1 (7.74)	37.0 (7.57)	37.3 (8.34)	37.1 (7.73)
Diabetes				
Yes	2106 (8.9%)	670 (8.6%)	160 (9.2%)	2936 (8.9%)
No	21479 (91.1%)	7154 (91.4%)	1580 (90.8%)	30213 (91.1%)
Taking antidiabetic medication				
Yes	1488 (6.3%)	487 (6.2%)	123 (7.1%)	2098 (6.3%)
No	22097 (93.7%)	7337 (93.8%)	1617 (92.9%)	31051 (93.7%)
BMI (kg/m²)				
Mean (SD)	28.7 (5.65)	28.8 (5.67)	28.8 (5.80)	28.7 (5.67)
Taking CYP2D6 inhibitor				
Yes	1401 (5.9%)	440 (5.6%)	102 (5.9%)	1943 (5.9%)
No	22184 (94.1%)	7384 (94.4%)	1638 (94.1%)	31206 (94.1%)

Table C.2: HbA1c levels and CYP phenotypes across individual and groups of medications.

	CYP2D6			**CYP2C19**				
	PM	IM	NM	PM	IM	NM	RM	UM
Models	HbA1c *[mmol/mol]**, (SD)							
Antipsychotics	36.78 (7.26)	37.60 (8.54)	37.53 (8.30)	-	-	-	-	-
Tricyclics	37.60 (7.79)	37.79 (8.38)	37.86 (8.21)	37.75 (7.80)	37.6 (7.73)	37.88 (8.30)	38.00 (8.81)	38.13 (7.88)
Amitriptyline	37.52 (7.86)	37.93 (8.60)	37.85 (8.18)	37.61 (7.28)	37.64 (7.84)	37.92 (8.31)	38.00 (8.86)	38.08 (8.06)
Fluoxetine	36.50 (6.90)	36.47 (6.50)	36.65 (7.50)	-	-	-	-	-
Paroxetine	40.46 (15.05)	37.50 (8.27)	37.38 (7.27)	-	-	-	-	-
Citalopram	-	-	-	36.89 (7.54)	36.48 (6.90)	36.67 (7.56)	36.51 (7.23)	36.01 (6.16)
Sertraline	-	-	-	35.30 (4.35)	36.99 (7.24)	37.15 (7.43)	37.13 (7.37)	37.04 (8.50)
Venlafaxine	39.49 (12.38)	37.13 (8.56)	37.58 (8.06)	-	-	-	-	-

* HbA1c levels diagnostic for impaired glucose regulation: normal < 42 mmol/mol, prediabetes 42 - 47 mmol/mol, diabetes ≥48 mmol/mol

Table C.3: Number of subjects taking each psychotropic drug by CYP450 metabolic phenotype group.

CYP2C19 metabolic phenotypes of people taking antidepressants

	NM (N=12689)	**IM** (N=9889)	**PM** (N=1122)	**RM** (N=8241)	**UM** (N=1426)	**Overall** (N=33367)
Antidepressant	12689 (100%)	9889 (100%)	1122 (100%)	8241 (100%)	1426 (100%)	33367 (100%)
Tricyclic						
amitriptyline	3116 (24.6%)	2483 (25.1%)	263 (23.4%)	1961 (23.8%)	368 (25.8%)	8191 (24.5%)
dosulepin	603 (4.8%)	439 (4.4%)	60 (5.3%)	388 (4.7%)	81 (5.7%)	1571 (4.7%)
lofepramine	127 (1.0%)	95 (1.0%)	4 (0.4%)	82 (1.0%)	15 (1.1%)	323 (1.0%)
clomipramine	126 (1.0%)	96 (1.0%)	12 (1.1%)	75 (0.9%)	11 (0.8%)	320 (1.0%)
nortriptyline	98 (0.8%)	73 (0.7%)	7 (0.6%)	85 (1.0%)	9 (0.6%)	272 (0.8%)
imipramine	74 (0.6%)	59 (0.6%)	5 (0.4%)	59 (0.7%)	8 (0.6%)	205 (0.6%)
trimipramine	38 (0.3%)	26 (0.3%)	2 (0.2%)	13 (0.2%)	6 (0.4%)	85 (0.3%)
SSRI						
citalopram	2923 (23.0%)	2205 (22.3%)	232 (20.7%)	1882 (22.8%)	303 (21.2%)	7545 (22.6%)
fluoxetine	2065 (16.3%)	1642 (16.6%)	195 (17.4%)	1338 (16.2%)	229 (16.1%)	5469 (16.4%)
sertraline	760 (6.0%)	587 (5.9%)	67 (6.0%)	465 (5.6%)	76 (5.3%)	1955 (5.9%)
paroxetine	731 (5.8%)	580 (5.9%)	79 (7.0%)	478 (5.8%)	62 (4.3%)	1930 (5.8%)
escitalopram	396 (3.1%)	345 (3.5%)	39 (3.5%)	263 (3.2%)	56 (3.9%)	1099 (3.3%)
SNRI						
venlafaxine	717 (5.7%)	536 (5.4%)	71 (6.3%)	483 (5.9%)	80 (5.6%)	1887 (5.7%)
duloxetine	167 (1.3%)	143 (1.4%)	20 (1.8%)	105 (1.3%)	25 (1.8%)	460 (1.4%)

Table C.3: cont.

Tetracyclic						
mirtazapine	439 (3.5%)	359 (3.6%)	40 (3.6%)	356 (4.3%)	65 (4.6%)	1259 (3.8%)
SARI						
trazodone	220 (1.7%)	169 (1.7%)	18 (1.6%)	155 (1.9%)	22 (1.5%)	584 (1.8%)
NRI						
reboxetine	21 (0.2%)	15 (0.2%)	2 (0.2%)	13 (0.2%)	3 (0.2%)	54 (0.2%)
OTHER						
other	68 (0.5%)	37 (0.4%)	6 (0.5%)	40 (0.5%)	7 (0.5%)	158 (0.5%)

NM - normal metaboliser, PM - poor metaboliser, IM – intermediate metaboliser, RM – rapid metaboliser, UM – ultra-rapid metaboliser, SSRI – Selective Serotonin Reuptake Inhibitor, SNRI – Selective Noradrenaline Reuptake Inhibitor, MOI – Monoamine Oxidase Inhibitor, NRI – Noradrenaline Reuptake Inhibitor, NDRI – Noradrenaline Dopamine Reuptake Inhibitor, SARI – Serotonin Antagonist and Reuptake Inhibitor, Other - doxepin, fluvoxamine, phenelzine, moclobemide, tranylcypromine, bupropion, mianserin, isocarboxazid

CYP2D6 metabolic phenotypes of people taking antidepressants

	NM (N=23749)	**IM (N=7816)**	**PM (N=1757)**	**Overall (N=33367)**
Antidepressant	23794 (100%)	7816 (100%)	1757 (100%)	33367 (100%)
Tricyclic				
amitriptyline	5840 (24.5%)	1929 (24.7%)	422 (24.0%)	8191 (24.5%)
dosulepin	1158 (4.9%)	349 (4.5%)	64 (3.6%)	1571 (4.7%)
lofepramine	231 (1.0%)	71 (0.9%)	21 (1.2%)	323 (1.0%)
clomipramine	221 (0.9%)	91 (1.2%)	8 (0.5%)	320 (1.0%)
nortriptyline	197 (0.8%)	63 (0.8%)	12 (0.7%)	272 (0.8%)
imipramine	139 (0.6%)	51 (0.7%)	15 (0.9%)	205 (0.6%)

Table C.3: cont.

trimipramine	62 (0.3%)	19 (0.2%)	4 (0.2%)	85 (0.3%)
SSRI				
citalopram	5381 (22.6%)	1753 (22.4%)	411 (23.4%)	7545 (22.6%)
fluoxetine	3888 (16.3%)	1282 (16.4%)	299 (17.0%)	5469 (16.4%)
sertraline	1394 (5.9%)	456 (5.8%)	105 (6.0%)	1955 (5.9%)
paroxetine	1367 (5.7%)	457 (5.8%)	106 (6.0%)	1930 (5.8%)
escitalopram	795 (3.3%)	249 (3.2%)	55 (3.1%)	1099 (3.3%)
SNRI				
venlafaxine	1354 (5.7%)	430 (5.5%)	103 (5.9%)	1887 (5.7%)
duloxetine	325 (1.4%)	119 (1.5%)	16 (0.9%)	460 (1.4%)
Tetracyclic				
mirtazapine	869 (3.7%)	316 (4.0%)	74 (4.2%)	1259 (3.8%)
SARI				
trazodone	412 (1.7%)	139 (1.8%)	33 (1.9%)	584 (1.8%)
NRI				
reboxetine	38 (0.2%)	10 (0.1%)	6 (0.3%)	54 (0.2%)
OTHER				
other	123 (0.5%)	32 (0.4%)	3 (0.2%)	158 (0.5%)

NM - normal metaboliser, PM - poor metaboliser, IM – intermediate metaboliser, SSRI – Selective Serotonin Reuptake Inhibitor, SNRI – Selective Noradrenaline Reuptake Inhibitor, MOI – Monoamine Oxidase Inhibitor, NRI – Noradrenaline Reuptake Inhibitor, NDRI – Noradrenaline Dopamine Reuptake Inhibitor, SARI – Serotonin Antagonist and Reuptake Inhibitor

Other - doxepin, fluvoxamine, phenelzine, moclobemide, tranylcypromine, bupropion, mianserin, isocarboxazid

Table C.3: cont.

CYP2D6 metabolic phenotype of individuals taking antipsychotics

	NM (N=2004)	**IM (N=671)**	**PM (N=142)**	**Overall (N=2817)**
Antipsychotic	2004 (100%)	671 (100%)	142 (100%)	2817 (100%)
Medication				
prochlorperazine	607 (30.3%)	221 (32.9%)	42 (29.6%)	870 (30.9%)
olanzapine	352 (17.6%)	114 (17.0%)	33 (23.2%)	499 (17.7%)
quetiapine	215 (10.7%)	74 (11.0%)	12 (8.5%)	301 (10.7%)
risperidone	181 (9.0%)	56 (8.3%)	10 (7.0%)	247 (8.8%)
chlorpromazine	105 (5.2%)	40 (6.0%)	6 (4.2%)	151 (5.4%)
flupentixol	107 (5.3%)	36 (5.4%)	5 (3.5%)	148 (5.3%)
trifluoperazine	110 (5.5%)	25 (3.7%)	6 (4.2%)	141 (5.0%)
amisulpride	57 (2.8%)	17 (2.5%)	5 (3.5%)	79 (2.8%)
haloperidol	51 (2.5%)	17 (2.5%)	5 (3.5%)	73 (2.6%)
aripiprazole	43 (2.1%)	16 (2.4%)	4 (2.8%)	63 (2.2%)
sulpiride	41 (2.0%)	14 (2.1%)	4 (2.8%)	59 (2.1%)
other	135 (6.7%)	41 (6.1%)	10 (7.0%)	186 (6.6%)

NM - normal metaboliser, PM - poor metaboliser, IM – intermediate metaboliser. Other - fluphenazine, clozapine, promazine, zuclopenthxol, perphenazine, pipotiazine, periciazine, levomepromazine, benperidol, pimozide, thioridazine, sertindole

Table C.4: A) Association between CYP2C19 metabolic phenotype and HbA1c within individuals taking citalopram; B) Stratified analysis of people taking citalopram

A.

		Citalopram	
Predictors	*Estimates*	*CI*	*p*
CYP2C19 IM	-0.06	-0.36,0.24	0.701
CYP2C19 PM	0.25	-0.48,0.99	0.500
CYP2C19 RM	-0.07	-0.39,0.24	0.650
CYP2C19 UM	-0.04	-0.68,0.61	0.913
CYP2C19 IM: Diabetes	-2.33	-3.41,-1.25	**<0.001**
CYP2C19 PM: Diabetes	1.62	-0.97,4.21	0.221
CYP2C19 RM: Diabetes	-0.76	-1.92,0.40	0.198
CYP2C19 UM: Diabetes	-3.78	-6.28,-1.28	**0.003**
Takes CYP2C19 inhibitor	0.36	0.07,0.65	**0.016**
Sex: Male	0.29	0.02,0.55	**0.032**
Age at recruitment	0.13	0.12,0.15	**<0.001**
Ethnicity: Admix Caucasian	0.02	-0.75,0.80	0.958
Ethnicity: African	1.90	0.58,3.22	**0.005**
Ethnicity: East Asian	0.45	-2.83,3.73	0.788
Ethnicity: Other	0.81	-0.16,1.78	0.100
Ethnicity: South Asian	3.80	2.78,4.81	**<0.001**
Diabetes	7.53	6.55,8.51	**<0.001**
BMI	0.16	0.14,0.19	**<0.001**
Antidiabetics	12.59	11.65,13.52	**<0.001**
Observations		7545	
R^2 / R^2 adjusted		0.470 / 0.468	

Table C.4: cont.

B.

			Citalopram					
			Diabetes				**No diabetes**	
Predictors	*N*	*Est.*	*CI*	*p*	*N*	*Est.*	*CI*	*p*
CYP2C19 IM	181	-2.42	-4.99, 0.16	0.066	2024	-2.42	-0.29, 0.18	0.635
CYP2C19 PM	19	1.37	-4.81, 7.54	0.664	213	1.37	-0.31, 0.80	0.392
CYP2C19 RM	140	-1.03	-3.82, 1.76	0.470	1742	-1.03	-0.31, 0.17	0.557
CYP2C19 UM	20	-4.07	-10.09, 1.94	0.184	283	-4.07	-0.52, 0.46	0.894
Observations			583			6962		
R^2 / R^2adjusted			0.189 / 0.170			0.127 / 0.125		

Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, taking antidiabetics and BMI

Normal metabolisers of CYP2C19: citalopram diabetes = 223, sertraline diabetes = 74

Table C.5: A) Association between CYP2C19 metabolic phenotype and HbA1c within individuals taking sertraline; B) Stratified analysis of people taking sertraline

A.

		Sertraline	
Predictors	*Estimates*	*CI*	*p*
CYP2C19 IM	0.13	-0.49,0.76	0.679
CYP2C19 PM	-0.58	-2.02,0.86	0.429
CYP2C19 RM	-0.17	-0.84,0.50	0.618
CYP2C19 UM	-0.47	-1.82,0.89	0.500
CYP2C19 IM: Diabetes	-0.64	-2.68,1.40	0.539
CYP2C19 PM: Diabetes	-5.84	-11.12,-0.56	**0.030**
CYP2C19 RM: Diabetes	0.17	-1.96,2.30	0.876
CYP2C19 UM: Diabetes	8.52	3.31,13.73	**0.001**
Takes CYP2C19 inhibitor	-0.05	-0.62,0.52	0.860
Sex: Male	0.04	-0.49,0.58	0.876
Age at recruitment	0.13	0.10,0.16	**<0.001**
Ethnicity: Admix Caucasian	-0.37	-1.71,0.97	0.588
Ethnicity: African	2.31	-0.51,5.13	0.108
Ethnicity: East Asian	2.91	-2.51,8.32	0.293
Ethnicity: Other	-0.46	-2.61,1.68	0.671
Ethnicity: South Asian	2.29	0.00,4.57	**0.050**
Diabetes	5.80	4.01,7.60	**<0.001**
BMI	0.18	0.13,0.22	**<0.001**
Antidiabetics	11.57	9.84,13.30	**<0.001**
Observations		1955	
R^2 / R^2 adjusted		0.438 / 0.433	

Table C.5: cont.

B.

	Sertraline							
	Diabetes				No diabetes			
Predictors	*N*	*Est.*	*CI*	*p*	*N*	*Est.*	*CI*	*p*
CYP2C19 IM	54	-2.42	-5.07, 3.85	0.787	533	-2.42	-0.35, 0.59	0.621
CYP2C19 PM	5	1.37	-20.28, 3.49	0.165	62	1.37	-1.66, 0.52	0.306
CYP2C19 RM	47	-1.03	-4.28, 5.10	0.863	418	-1.03	-0.69, 0.33	0.494
CYP2C19 UM	71	-4.07	-3.85, 19.04	0.192	71	-4.07	-1.53, 0.52	0.330
Observations	185				1770			
R^2 / R^2adjusted	0.232 / 0.174				0.133 / 0.127			

Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, taking antidiabetics and BMI

Normal metabolisers of CYP2C19: citalopram diabetes = 223, sertraline diabetes = 74

Table C.6: A) Association between CYP2D6 and CYP2C19 metabolic phenotype and HbA1c within amitriptyline; B) Stratified analysis of people taking amitriptyline

A.

	Amitriptyline		
Predictors	*Estimates*	*CI*	*p*
CYP2D6 IM	0.04	-0.28,0.37	0.789
CYP2D6 PM	0.10	-0.51,0.72	0.740
CYP2C19 IM	-0.10	-0.43,0.23	0.545
CYP2C19 PM	0.01	-0.79,0.81	0.978
CYP2C19 RM	-0.13	-0.49,0.23	0.476
CYP2C19 UM	0.02	-0.66,0.70	0.951
Diabetes: CYP2D6 IM	1.03	0.02,2.03	**0.046**
Diabetes: CYP2D6 PM	-3.41	-5.44,-1.38	**0.001**
CYP2C19 IM: Diabetes	-0.07	-1.12,0.97	0.889
CYP2C19 PM: Diabetes	-1.67	-4.01,0.67	0.163
CYP2C19 RM: Diabetes	0.46	-0.61,1.53	0.401
CYP2C19 UM: Diabetes	0.06	-2.06,2.18	0.955
Takes CYP2D6 inhibitor	-0.28	-0.78,0.23	0.280
Takes CYP2C19 inhibitor	0.37	0.09,0.65	**0.009**
Sex: Male	0.09	-0.20,0.38	0.532
Age at recruitment	0.12	0.10,0.14	**<0.001**
Ethnicity: Admix Caucasian	0.07	-0.84,0.98	0.886
Ethnicity: African	2.57	1.42,3.72	**<0.001**
Ethnicity: East Asian	-1.41	-5.83,3.00	0.531
Ethnicity: Other	1.74	0.66,2.82	**0.002**
Ethnicity: South Asian	2.90	1.81,4.00	**<0.001**

Table C.6: cont.

Diabetes	6.68	5.64,7.73	**<0.001**
Antidiabetics	12.36	11.43,13.29	**<0.001**
BMI	0.16	0.13,0.18	**<0.001**
Observations	8191		

B.

Amitriptyline								
		Diabetes			**No diabetes**			
Predictors	*N*	*Est.*	*CI*	*p*	*N*	*Est.*	*CI*	*p*
CYP2D6 IM	197	1.18	-0.93, 3.30	0.273	1732	0.04	-0.19, 0.28	0.733
CYP2D6 PM	39	-2.92	-7.23, 1.39	0.184	383	0.10	-0.35, 0.55	0.671
CYP2C19 IM	248	-0.26	-2.46, 1.95	0.819	2235	-0.09	-0.33, 0.15	0.473
CYP2C19 PM	31	-1.43	-6.33, 3.48	0.568	232	0.04	-0.54, 0.62	0.902
CYP2C19 RM	235	0.07	-2.19, 2.32	0.952	1726	-0.12	-0.38, 0.14	0.366
CYP2C19 UM	38	-0.09	-4.55, 4.37	0.969	330	0.05	-0.45, 0.54	0.851
Observations			874		7317			
R^2 / R^2 adjusted			0.158 / 0.142		0.100 / 0.098			

Table C.7: A) Association between CYP2D6 and CYP2C19 metabolic phenotype and HbA1c within individuals taking tricyclic antidepressants; B) Stratified analysis of people taking tricyclic antidepressants

A.

	Tricyclic antidepressants		
Predictors	*Estimates*	*CI*	*p*
CYP2D6 IM	0.04	-0.26,0.34	0.793
CYP2D6 PM	0.13	-0.46,0.72	0.660
CYP2C19 IM	-0.11	-0.42,0.20	0.495
CYP2C19 PM	-0.07	-0.83,0.69	0.857
CYP2C19 RM	-0.09	-0.43,0.24	0.594
CYP2C19 UM	0.12	-0.52,0.77	0.709
Diabetes: CYP2D6 IM	0.53	-0.43,1.50	0.279
Diabetes: CYP2D6 PM	-3.85	-5.76,-1.95	**<0.001**
CYP2C19 IM: Diabetes	-0.49	-1.48,0.51	0.338
CYP2C19 PM: Diabetes	-1.34	-3.50,0.81	0.222
CYP2C19 RM: Diabetes	0.27	-0.75,1.29	0.604
CYP2C19 UM: Diabetes	-0.52	-2.52,1.49	0.613
Takes CYP2D6 inhibitor	-0.26	-0.73,0.21	0.279
Takes CYP2C19 inhibitor	0.39	0.13,0.66	**0.004**
Sex: Male	0.11	-0.16,0.38	0.428
Age at recruitment	0.12	0.11,0.14	**<0.001**
Ethnicity: Admix Caucasian	0.18	-0.67,1.03	0.686
Ethnicity: African	2.47	1.36,3.57	**<0.001**
Ethnicity: East Asian	0.81	-2.55,4.16	0.638
Ethnicity: Other	2.19	1.16,3.22	**<0.001**
Ethnicity: South Asian	2.55	1.52,3.59	**<0.001**

Table C.7: cont.

Diabetes	6.98	5.99,7.97	**<0.001**
BMI	0.16	0.13,0.18	**<0.001**
Antidiabetics	12.45	11.58,13.33	**<0.001**
Observations	9095		
R^2 / R^2 adjusted	0.484 / 0.482		

B.

	Tricyclics							
	Diabetes				No diabetes			
Predictors	*N*	*Est.*	*CI*	*p*	*N*	*Est.*	*CI*	*p*
CYP2D6 IM	1949	0.73	-1.33, 2.78	0.488	208	0.04	-0.18, 0.26	0.740
CYP2D6 PM	419	-3.30	-7.36, 0.76	0.111	44	0.12	-0.31, 0.54	0.596
CYP2C19 IM	2475	-0.61	-2.73, 1.50	0.570	274	-0.09	-0.32, 0.13	0.414
CYP2C19 PM	252	-1.26	-5.77, 3.25	0.584	37	-0.04	-0.59, 0.52	0.899
CYP2C19 RM	1940	-0.08	-2.24, 2.09	0.945	257	-0.08	-0.33, 0.16	0.501
CYP2C19 UM	362	-0.59	-4.84, 3.66	0.786	42	0.15	-0.32, 0.62	0.526
Observations			955			8140		
R^2 / R^2 adjusted			0.162 / 0.147			0.101 / 0.099		

Model adjusted by age, ethnicity, sex, taking inhibitors of CYP2D6, taking antidiabetics and BMI, Normal metabolisers of CYP2D6: tricyclics diabetes 703, amitriptyline diabetes = 638, Normal metabolisers of CYP2C19: tricyclics diabetes = 345, amitriptyline = 322

Appendix D

Appendices for chapter 4

D.1 Publications associated with this chapter

Building on the work described in this thesis, I have led a study entitled ”Genome-wide association study investigating genetic loci for self-reported sleep duration: a meta-analysis with data from UK Biobank and the Million Veteran Program”. This project is the result of a research trip I undertook as part of the UCL-Yale Collaborative Exchange Programme. The paper is in the final stages of preparation and will be submitted to Nature Communications in the coming weeks.

D.2 CYP450 pharmacogenetics and sleep: Additional figures and tables

The following figure and tables provide additional detail on the CYP450 pharmacogenetic investigation of sleep disturbance as an adverse reaction to antidepressants, to be read alongside 4.

Figure D.1: Frequency reported hours of sleep duration across the full UK Biobank sample, excluding the subjects taking antidepressants

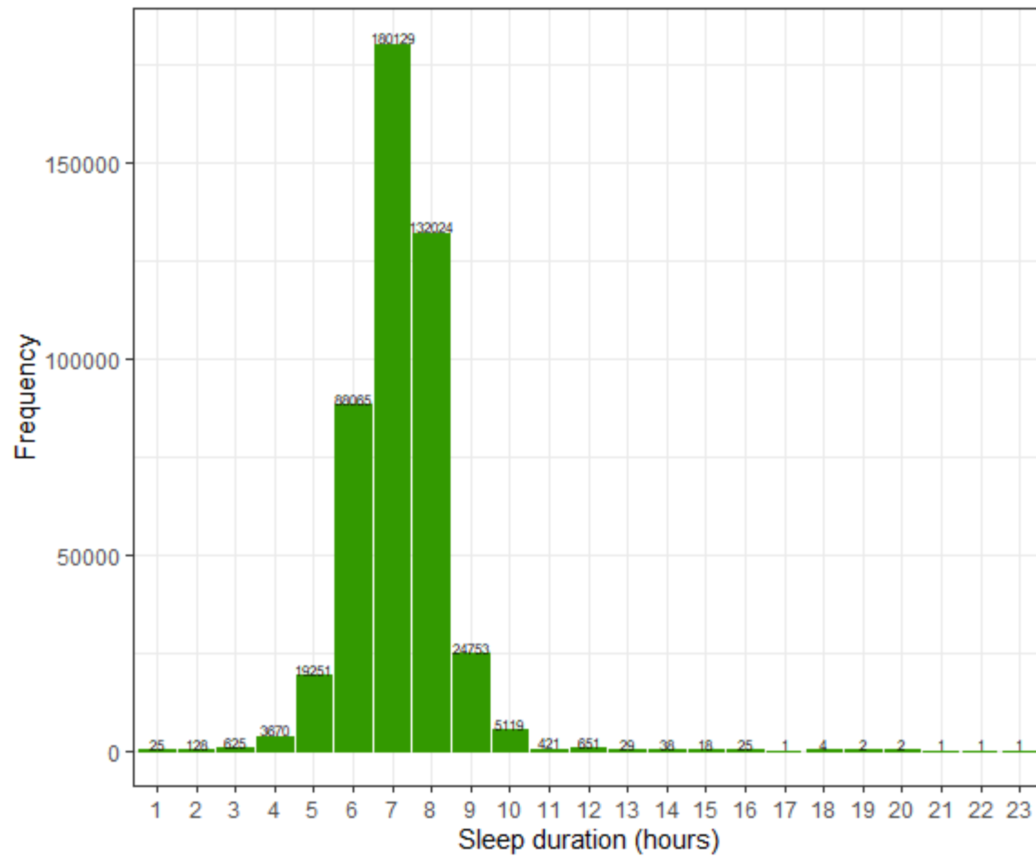

Table D.1: Additional detail on the association between CYP450 metabolic phenotype and measures of sleep duration and quality among participants taking antidepressants.

Tricyclic antidepressants	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.68	6.45 – 6.92	**<0.001**
CYP2C19 intermediate metabolisers	-0.06	-0.14 – 0.03	0.175
CYP2C19 poor metabolisers	0.02	-0.18 – 0.21	0.878
CYP2C19 rapid metabolisers	-0.14	-0.23 – -0.05	**0.001**
CYP2C19 ultra-rapid metabolisers	-0.17	-0.34 – -0.01	**0.043**
CYP2D6 intermediate metabolisers	0.04	-0.03 – 0.11	0.267
CYP2D6 poor metabolisers	0.14	0.01 – 0.27	**0.040**
Female	0.02	-0.04 – 0.09	0.433
Age	0.01	0.01 – 0.01	**<0.001**
Admix	-0.15	-0.40 – 0.10	0.233
Admix European	-0.28	-0.47 – -0.08	**0.005**
African	-0.45	-0.69 – -0.20	**<0.001**
East Asian	0.40	-0.39 – 1.19	0.323
South Asian	-0.21	-0.45 – 0.03	0.080
Takes CYP2C19 inhibitor	-0.14	-0.23 – -0.04	**0.006**
Takes CYP2D6 inhibitor	0.13	0.00 – 0.26	**0.043**
Observations	11,383		
R^2 / R^2 adjusted	0.008 / 0.006		

Amitriptyline	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.77	6.50 – 7.04	**<0.001**
CYP2C19 intermediate metabolisers	-0.06	-0.15 – 0.03	0.214
CYP2C19 poor metabolisers	-0.02	-0.25 – 0.21	0.853
CYP2C19 rapid metabolisers	-0.12	-0.22 – -0.02	**0.019**
CYP2C19 ultra-rapid metabolisers	-0.17	-0.36 – 0.03	0.089

Table D.1: cont.

CYP2D6 intermediate metabolisers	0.05	-0.03 – 0.13	0.185
CYP2D6 poor metabolisers	0.14	-0.01 – 0.29	0.075
Female	0.05	-0.02 – 0.13	0.131
Age	0.01	0.00 – 0.01	**<0.001**
Admix	-0.23	-0.51 – 0.04	0.098
Admix European	-0.28	-0.50 – -0.05	**0.015**
African	-0.56	-0.83 – -0.29	**<0.001**
East Asian	0.30	-0.81 – 1.42	0.595
South Asian	-0.25	-0.52 – 0.01	0.062
Takes CYP2C19 inhibitor	-0.12	-0.24 – -0.01	**0.027**
Takes CYP2D6 inhibitor	0.10	-0.05 – 0.24	0.186
Observations	8,519		
R^2 / R^2 adjusted	0.008 / 0.005		

Dosulepin	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.47	5.88 – 7.07	**<0.001**
CYP2C19 intermediate metabolisers	0.02	-0.15 – 0.19	0.839
CYP2C19 poor metabolisers	0.35	-0.01 – 0.71	0.060
CYP2C19 rapid metabolisers	-0.16	-0.34 – 0.01	0.072
CYP2C19 ultra-rapid metabolisers	0.05	-0.28 – 0.37	0.784
CYP2D6 intermediate metabolisers	0.04	-0.13 – 0.20	0.675
CYP2D6 poor metabolisers	0.19	-0.15 – 0.53	0.280
Female	-0.13	-0.28 – 0.03	0.103
Age	0.01	0.00 – 0.02	**0.006**
Admix	-0.06	-0.86 – 0.74	0.877
Admix European	-0.21	-0.72 – 0.29	0.408
African	-0.11	-0.83 – 0.61	0.760
South Asian	-0.05	-0.73 – 0.62	0.878

Table D.1: cont.

Takes CYP2C19 inhibitor	-0.00	-0.16 – 0.15	0.957
Takes CYP2D6 inhibitor	0.18	-0.09 – 0.45	0.182
Observations	1,616		
R^2 / R^2 adjusted	0.014 / 0.006		

SSRIs	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.97	6.81 – 7.13	**<0.001**
CYP2D6 intermediate metabolisers	0.03	-0.03 – 0.08	0.329
CYP2D6 poor metabolisers	-0.02	-0.12 – 0.08	0.705
Takes CYP2D6 inhibitor	-0.07	-0.17 – 0.03	0.182
Female	0.03	-0.02 – 0.08	0.218
Age	0.01	0.01 – 0.01	**<0.001**
Admix	-0.17	-0.35 – 0.01	0.068
Admix European	-0.09	-0.23 – 0.04	0.175
African	-0.18	-0.42 – 0.06	0.132
East Asian	0.05	-0.54 – 0.65	0.859
South Asian	-0.37	-0.58 – -0.16	**0.001**
Observations	18608		
R^2 / R^2 adjusted	0.003 / 0.003		

Citalopram	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	7.01	6.77 – 7.26	**<0.001**
CYP2D6 intermediate metabolisers	-0.01	-0.09 – 0.07	0.833
CYP2D6 poor metabolisers	-0.09	-0.24 – 0.06	0.226
Takes CYP2D6 inhibitor	0.08	-0.07 – 0.23	0.322
Female	-0.02	-0.09 – 0.05	0.543

Table D.1: cont.

Age	0.01	0.00 – 0.01	**0.003**
Admix	-0.11	-0.37 – 0.15	0.412
Admix European	-0.06	-0.27 – 0.16	0.605
African	-0.15	-0.49 – 0.20	0.403
East Asian	0.23	-0.66 – 1.11	0.615
South Asian	-0.30	-0.58 – -0.02	**0.037**
Observations	7,882		
R^2 / R^2 adjusted	0.002 / 0.001		

Fluoxetine	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	7.05	6.74 – 7.35	**<0.001**
CYP2D6 intermediate metabolisers	0.06	-0.04 – 0.15	0.253
CYP2D6 poor metabolisers	-0.02	-0.20 – 0.15	0.786
Takes CYP2D6 inhibitor	-0.26	-0.44 – -0.08	**0.004**
Female	0.05	-0.04 – 0.14	0.246
Age	0.01	0.00 – 0.01	**0.006**
Admix	0.04	-0.30 – 0.38	0.816
Admix European	-0.08	-0.34 – 0.18	0.553
African	-0.29	-0.73 – 0.14	0.184
East Asian	-0.25	-1.33 – 0.82	0.643
South Asian	-0.61	-1.09 – -0.13	**0.013**
Observations	5,663		
R^2 / R^2 adjusted	0.005 / 0.003		

Paroxetine	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.77	6.23 – 7.30	**<0.001**
CYP2D6 intermediate metabolisers	0.06	-0.10 – 0.22	0.485

Table D.1: cont.

CYP2D6 poor metabolisers	0.20	-0.09 – 0.49	0.184
Takes CYP2D6 inhibitor	-0.14	-0.44 – 0.15	0.342
Female	0.22	0.07 – 0.36	**0.003**
Age	0.01	0.00 – 0.02	**0.004**
Admix	-0.48	-1.12 – 0.17	0.147
Admix European	-0.20	-0.59 – 0.19	0.312
African	0.49	-0.42 – 1.40	0.291
East Asian	-0.77	-3.78 – 2.24	0.615
South Asian	-0.06	-0.84 – 0.72	0.884
Observations	2,002		
R^2 / R^2 adjusted	0.012 / 0.007		

Sertraline	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	7.18	6.68 – 7.68	**<0.001**
CYP2D6 intermediate metabolisers	0.06	-0.10 – 0.22	0.449
CYP2D6 poor metabolisers	-0.04	-0.33 – 0.25	0.785
Takes CYP2D6 inhibitor	-0.00	-0.30 – 0.29	0.987
Female	-0.04	-0.18 – 0.11	0.609
Age	0.00	-0.00 – 0.01	0.304
Admix	-0.62	-1.20 – -0.04	**0.037**
Admix European	-0.12	-0.49 – 0.25	0.536
African	-0.33	-1.11 – 0.45	0.406
East Asian	0.60	-0.90 – 2.10	0.433
South Asian	-0.21	-0.80 – 0.38	0.484
Observations	2,007		
R^2 / R^2 adjusted	0.004 / -0.000		

Table D.1: cont.

Venlafaxine	**Sleep duration**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.89	6.31 – 7.47	**<0.001**
CYP2D6 intermediate metabolisers	-0.01	-0.19 – 0.17	0.925
CYP2D6 poor metabolisers	-0.09	-0.42 – 0.23	0.579
Takes CYP2D6 inhibitor	0.27	-0.06 – 0.59	0.111
Female	0.03	-0.13 – 0.18	0.730
Age	0.01	-0.00 – 0.02	0.083
Admix	1.43	0.69 – 2.16	**<0.001**
Admix European	0.04	-0.41 – 0.49	0.862
African	-0.64	-1.43 – 0.15	0.113
East Asian	2.73	-0.59 – 6.06	0.107
South Asian	0.09	-0.72 – 0.90	0.822
Observations	1959		
R^2 / R^2 adjusted	0.013 / 0.008		

Measures of sleep quality

Tricyclic antidepressants	**Sleeplessness/insomnia**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.61	0.53 – 0.68	**<0.001**
CYP2C19 intermediate metabolisers	0.00	-0.02 – 0.02	0.838
CYP2C19 poor metabolisers	0.05	0.00 – 0.11	**0.046**
CYP2C19 rapid metabolisers	0.03	0.01 – 0.06	**0.007**
CYP2C19 ultra-rapid metabolisers	0.04	-0.01 – 0.08	0.129
CYP2D6 intermediate metabolisers	0.02	-0.00 – 0.04	0.118
CYP2D6 poor metabolisers	0.00	-0.04 – 0.04	0.997
Takes CYP2C19 inhibitor	0.09	0.07 – 0.11	**<0.001**
Takes CYP2D6_inhibitor	0.04	0.00 – 0.07	**0.042**

Table D.1: cont.

Female	0.01	-0.01 – 0.03	0.156
Age	-0.00	-0.00 – -0.00	**<0.001**
Admix	0.04	-0.05 – 0.12	0.387
Admix European	-0.01	-0.08 – 0.05	0.729
African	-0.03	-0.11 – 0.05	0.457
East Asian	-0.30	-0.56 – -0.05	**0.021**
South Asian	-0.01	-0.09 – 0.07	0.759
Observations	11,383		
R^2 / R^2 adjusted	0.011 / 0.010		

Tricyclic antidepressants	**Daytime dozing/narcolepsy**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.10	0.07 – 0.13	**<0.001**
CYP2C19 intermediate metabolisers	-0.00	-0.01 – 0.01	0.351
CYP2C19 poor metabolisers	-0.01	-0.04 – 0.01	0.288
CYP2C19 rapid metabolisers	-0.00	-0.01 – 0.01	0.651
CYP2C19 ultra-rapid metabolisers	0.01	-0.01 – 0.03	0.175
CYP2D6 intermediate metabolisers	-0.00	-0.01 – 0.01	0.769
CYP2D6 poor metabolisers	0.01	-0.01 – 0.03	0.166
Takes CYP2C19 inhibitor	0.03	0.02 – 0.04	**<0.001**
Takes CYP2D6_inhibitor	0.02	0.01 – 0.04	**0.004**
Female	-0.03	-0.03 – -0.02	**<0.001**
Age	-0.00	-0.00 – -0.00	**0.007**
Admix	0.06	0.02 – 0.10	**0.001**
Admix European	-0.04	-0.06 – -0.01	**0.014**
African	0.05	0.01 – 0.08	**0.012**
East Asian	-0.05	-0.16 – 0.07	0.432
South Asian	0.01	-0.03 – 0.04	0.673

Table D.1: cont.

Observations	11,383
R^2 / R^2 adjusted	0.012 / 0.011

Tricyclic antidepressants	**Change in sleep**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.08	0.93 – 1.22	**<0.001**
CYP2C19 intermediate metabolisers	-0.01	-0.05 – 0.04	0.801
CYP2C19 poor metabolisers	0.04	-0.06 – 0.14	0.486
CYP2C19 rapid metabolisers	0.03	-0.02 – 0.07	0.240
CYP2C19 ultra-rapid metabolisers	0.10	0.01 – 0.18	**0.022**
CYP2D6 intermediate metabolisers	0.01	-0.03 – 0.05	0.604
CYP2D6 poor metabolisers	-0.03	-0.12 – 0.06	0.474
Takes CYP2C19 inhibitor	0.00	-0.04 – 0.04	0.888
Takes CYP2D6_inhibitor	-0.00	-0.07 – 0.06	0.930
Female	0.03	-0.01 – 0.07	0.164
Age	-0.01	-0.01 – -0.00	**<0.001**
Admix	0.06	-0.12 – 0.25	0.505
Admix European	0.03	-0.08 – 0.14	0.582
African	0.12	-0.11 – 0.35	0.313
East Asian	-0.32	-0.90 – 0.26	0.278
South Asian	-0.06	-0.31 – 0.18	0.615
Observations	2,254		
R^2 / R^2 adjusted	0.016 / 0.010		

Tricyclic antidepressants	**Sleeping too much**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.82	0.64 – 1.00	**<0.001**
CYP2C19 intermediate metabolisers	-0.00	-0.06 – 0.05	0.867
CYP2C19 poor metabolisers	-0.01	-0.13 – 0.11	0.844

Table D.1: cont.

CYP2C19 rapid metabolisers	-0.02	-0.07 – 0.04	0.535
CYP2C19 ultra-rapid metabolisers	-0.01	-0.11 – 0.09	0.816
CYP2D6 intermediate metabolisers	-0.01	-0.06 – 0.04	0.742
CYP2D6 poor metabolisers	0.05	-0.06 – 0.16	0.399
Takes CYP2C19 inhibitor	0.00	-0.04 – 0.05	0.870
Takes CYP2D6_inhibitor	0.03	-0.05 – 0.11	0.476
Female	-0.00	-0.05 – 0.05	0.998
Age	-0.01	-0.01 – -0.01	**<0.001**
Admix	-0.00	-0.22 – 0.21	0.979
Admix European	-0.00	-0.14 – 0.13	0.943
African	0.17	-0.08 – 0.43	0.186
East Asian	0.58	-0.31 – 1.47	0.198
South Asian	-0.10	-0.41 – 0.21	0.528
Observations	1,742		
R^2 / R^2 adjusted	0.026 / 0.018		

Tricyclic antidepressants	**Trouble falling asleep**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.74	0.58 – 0.90	**<0.001**
CYP2C19 intermediate metabolisers	0.05	0.00 – 0.10	**0.043**
CYP2C19 poor metabolisers	0.10	-0.01 – 0.21	0.072
CYP2C19 rapid metabolisers	0.07	0.02 – 0.12	**0.006**
CYP2C19 ultra-rapid metabolisers	0.09	0.00 – 0.18	**0.039**
CYP2D6 intermediate metabolisers	-0.02	-0.06 – 0.02	0.387
CYP2D6 poor metabolisers	-0.04	-0.14 – 0.07	0.490
Takes CYP2C19 inhibitor	0.08	0.03 – 0.12	**<0.001**
Takes CYP2D6_inhibitor	-0.05	-0.12 – 0.02	0.166
Female	0.05	0.01 – 0.10	**0.020**

Table D.1: cont.

Age	-0.00	-0.00 – 0.00	0.557
Admix	0.07	-0.12 – 0.27	0.469
Admix European	-0.01	-0.14 – 0.11	0.828
African	-0.14	-0.37 – 0.09	0.241
East Asian	0.26	-0.54 – 1.06	0.524
South Asian	0.19	-0.09 – 0.47	0.187
Observations	1,742		
R^2 / R^2 adjusted	0.021 / 0.012		

SSRIs	**Sleeplessness/insomnia**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.33	0.28 – 0.39	**<0.001**
CYP2D6 intermediate metabolisers	-0.01	-0.03 – 0.01	0.306
CYP2D6 poor metabolisers	-0.02	-0.05 – 0.01	0.188
Takes CYP2D6_inhibitor	0.10	0.07 – 0.13	**<0.001**
Female	0.01	-0.00 – 0.03	0.131
Age	0.00	0.00 – 0.00	**0.012**
Admix	0.05	-0.01 – 0.10	0.129
Admix European	-0.01	-0.05 – 0.03	0.621
African	0.02	-0.06 – 0.10	0.623
East Asian	0.08	-0.11 – 0.27	0.407
South Asian	0.11	0.04 – 0.18	**0.001**
Observations	18,608		
R^2 / R^2 adjusted	0.003 / 0.003		

SSRIs	**Daytime dozing/narcolepsy**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.06	0.04 – 0.09	**<0.001**

Table D.1: cont.

CYP2D6 intermediate metabolisers	-0.01	-0.02 – -0.00	**0.017**
CYP2D6 poor metabolisers	-0.00	-0.02 – 0.01	0.688
Takes CYP2D6_inhibitor	0.04	0.02 – 0.05	**<0.001**
Female	-0.03	-0.04 – -0.02	**<0.001**
Age	0.00	-0.00 – 0.00	0.108
Admix	0.06	0.03 – 0.09	**<0.001**
Admix European	-0.00	-0.03 – 0.02	0.670
African	0.05	0.01 – 0.08	**0.018**
East Asian	0.02	-0.07 – 0.12	0.614
South Asian	0.02	-0.02 – 0.05	0.313
Observations	18,608		
R^2 / R^2 adjusted	0.006 / 0.006		

SSRIs	**Change in sleep**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1.17	1.08 – 1.25	**<0.001**
CYP2D6 intermediate metabolisers	0.00	-0.02 – 0.03	0.840
CYP2D6 poor metabolisers	-0.00	-0.05 – 0.05	0.945
Takes CYP2D6_inhibitor	0.07	0.02 – 0.12	**0.007**
Female	0.03	0.01 – 0.06	**0.015**
Age	-0.01	-0.01 – -0.01	**<0.001**
Admix	-0.00	-0.11 – 0.11	0.934
Admix European	-0.01	-0.08 – 0.05	0.643
African	-0.04	-0.19 – 0.11	0.601
East Asian	0.15	-0.20 – 0.50	0.396
South Asian	0.08	-0.09 – 0.26	0.342
Observations	4,925		
R^2 / R^2 adjusted	0.021 / 0.019		

Table D.1: cont.

SSRIs	**Sleeping too much**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.72	0.60 – 0.84	**<0.001**
CYP2D6 intermediate metabolisers	0.01	-0.03 – 0.04	0.680
CYP2D6 poor metabolisers	-0.05	-0.12 – 0.02	0.191
Takes CYP2D6_inhibitor	-0.01	-0.08 – 0.06	0.865
Female	0.01	-0.03 – 0.04	0.725
Age	-0.01	-0.01 – -0.00	**<0.001**
Admix	0.01	-0.14 – 0.16	0.869
Admix European	0.04	-0.05 – 0.12	0.405
African	-0.12	-0.32 – 0.09	0.254
East Asian	0.17	-0.26 – 0.60	0.432
South Asian	0.09	-0.13 – 0.32	0.429
Observations	3,951		
R^2 / R^2 adjusted	0.010 / 0.007		

SSRIs	**Trouble falling asleep**		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.65	0.54 – 0.76	**<0.001**
CYP2D6 intermediate metabolisers	-0.02	-0.05 – 0.02	0.362
CYP2D6 poor metabolisers	0.00	-0.06 – 0.07	0.934
Takes CYP2D6_inhibitor	0.04	-0.02 – 0.10	0.208
Female	0.01	-0.02 – 0.04	0.548
Age	0.00	-0.00 – 0.00	0.109
Admix	0.09	-0.04 – 0.23	0.188
Admix European	-0.03	-0.11 – 0.04	0.411
African	-0.00	-0.18 – 0.18	0.999
East Asian	-0.14	-0.52 – 0.25	0.491

Table D.1: cont.

South Asian	0.05	-0.16 – 0.25	0.663
Observations	3,951		
R^2 / R^2 adjusted	0.002 / -0.000		

D.3 Environmental risk factors for sleep disturbance

There are many environmental factors that are likely to impact significantly on sleep duration. I identified two potential environmental risk factors, hours of daylight exposure and caffeine intake, to investigate as a comparison to the genetic influences on sleep.

To evaluate possible effects of hours of daylight on sleep hours, as this is known to have a significant impact on reported sleep duration[1,2,3,4] I calculated solar irradiance for each participant based on the location of their recruitment site and the month of their recruitment. I downloaded monthly total normal solar irradiation data from the European Commission Photovoltaic Geographical Information System[5]. To evaluate the impact of caffeine intake, I downloaded data on tea and coffee intake from the UK Biobank. I considered these separately, and created a joint variable summing the number of cups of tea and coffee together. For both analyses, I conducted linear regression analyses, including age and sex as covariates.

As hypothesised, both hours of daylight and coffee, but not tea, intake were significantly associated with decreased sleep duration, with effect estimates on a similarly small scale to those observed for the genetic variants. Higher levels of solar irradiation were significantly associated with shorter reported sleep duration (estimate = $-4.8 \times 10^{-4} \pm 5 \times 10^{-5}$ hours, $p < 2 \times 10^{-16}$). Increased coffee intake was also associated with shorter sleep duration (estimate = -0.01 ± 0.003 hours, $p < 2 \times 10^{-16}$).

[1] **(1)** Czeisler CA *et al.* Exposure to Bright Light and Darkness to Treat Physiologic Maladaptation to Night Work. N Engl J Med. 1990;322(18):1253–9.

[2] Czeisler CA *et al.* Sleep and circadian rhythms in humans. In: Cold Spring Harbor Symposia on Quantitative Biology. 2007. p. 579–97.

[3] Sivertsen B *et al.* Seasonal Variations in Sleep Problems at Latitude 63 -65 in Norway: The Nord-Trondelag Health Study, 1995-1997. Am J Epidemiol. 2011;174(2):147–53.

[4] **(4)** Leger D *et al.* Underexposure to light at work and its association to insomnia and sleepiness. A cross-sectional study of 13296 workers of one transportation company. J Psychosom Res. 2011;70(1):29–36.

[5] JRC Photovoltaic Geographical Information System (PVGIS) - European Commission [Internet]. [cited 2021 May 13]. Available from: `https://re.jrc.ec.europa.eu/pvg_tools/en/#PVP`.

Appendix E

Appendices for chapter 5

E.1 Protocol

This protocol was drafted based on the UCL interventional trial protocol version 1. It sets out the scientific rationale, background and objectives of the trial, and details the trial design and procedures. This document is provided to all site staff, along with detailed training, to serve as a manual for running the study. The protocol was approved by the London Central Research Ethics Committee, and any subsequent changes to it, however minor, must be documented and approved by the same group.

Pharmacogenetics in Mental Health

Full title of trial	Personalised medicine for psychotropic drugs (antipsychotics, antidepressants, antimanic drugs and other psychotropic agents)
Short title	Pharmacogenetics in Mental Health
Version and date of protocol	Version 1.2 , 27th September 2019
Sponsor:	University College London (UCL)
Sponsor protocol number	18/0557
Funder (s):	Medical Research Council British Medical Association Biomedical Research Council
ISRCTN / Clinicaltrials.gov no:	[Insert ISRCTN or Clinicaltrials.gov reference no]
Intervention:	Pharmacogenetic testing
Single site/multi-site:	Multi-site

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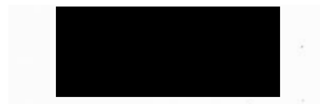
Protocol Version History

Version Number	Date	Protocol Update Finalised By (insert name of person):	Reasons for Update
1.0	5th June 2019	Elvira Bramon	NA
1.1	20th July 2019	Elvira Bramon	REC response
1.2	27th September 2019	Elvira Bramon	REC response

Signatures

The Chief Investigator and the JRO have discussed this protocol. The investigator agrees to perform the investigations and to abide by this protocol.

The investigator agrees to conduct the trial in compliance with the approved protocol, the General Data Protection Regulation (2016/679), the Trust Information Governance Policy (or other local equivalent), the current Research Governance Framework, the Sponsor's SOPs, and other regulatory requirements as amended.



27/09/2019

Chief investigator

Professor Elvira Bramon
UCL

Signature Date

Sponsor

Suzanne Emerton
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Signature Date

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List of abbreviations

AE	Adverse Event
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
GAfREC	Governance Arrangements for NHS Research Ethics
GCP	Good Clinical Practice
HTA	Human Tissue Authority
IB	Investigator's Brochure
ICF	Informed Consent Form
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
NHS R&D	National Health Service Research & Development
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
TMG	Trial Management Group
TSC	Trial Steering Committee

Trial personnel

See protocol cover page for Chief Investigator and Sponsor contact details.

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NHS Laboratory Services will also be used in this study.

1 Summary

Objectives:	To investigate the use of pharmacogenetic tools in guiding the prescribing and dosing of psychotropic drugs. To prospectively investigate the efficacy and impact of adverse reactions of this intervention compared to treatment as usual.
Type of trial:	A multi-site trial in patients who are taking or will be prescribed antipsychotic, antidepressant or antimanic drugs.
Trial design and methods:	This is a study investigating the use of pharmacogenetics tools in guiding the prescription of psychotropic drugs. This study will be conducted according to Good Clinical Practice and International Conference on Harmonisation guidelines. The study hypothesis to be tested is that personalised prescribing of psychotropic agents guided by genetic profiling results in higher efficacy and reduced side effects compared to treatment as usual. A total of 420 participants, plus 40 clinician-participants, will be recruited to the study.
Trial duration per participant:	Six months
Estimated total trial duration:	Six years from the recruitment of the first participant.
Planned trial sites:	Multi-site.
Total number of participants planned:	420 recruited to study, plus 40 clinician-participants. The 420 participants will be compared to a control group of patient data stored in the Clinical Record Interactive Search (CRIS) database.
Main inclusion/exclusion criteria:	Clinician participants must be consultant adult psychiatrists working within the NHS at an open site. Clinicians not willing to discuss the results of the genetic test with their patients will be excluded. Male and female patients ages 18 years or older with an ICD10 diagnosis of any "mental, behavioural or neurodevelopmental disorders" Codes F01 - F99 are included. Patients with current high risk of self-harm or harm to others, or those lacking the capacity to consent will be excluded from this study.
Statistical methodology and analysis:	Linear models, including CYP metabolic status, diagnosis, medication, baseline value of the outcome and experimental group as main effects, will be used to assess the groups against the primary and secondary outcome measures.

2 Background and Rationale

Schizophrenia and bipolar disorder constitute one of the leading causes of disability in young adults. Depression is a highly common condition affecting about one in four people. Antipsychotics and antidepressants are the most common treatment for these illnesses. Although they are effective for many patients they can also cause severe side effects. As a result of this patient adherence is low[1,2].

It is possible that genetic differences could explain why some patients respond to their treatment better than others and also why some patients experience severe side effects. By running this study, we seek to investigate if genetic profiling can help optimise the prescribing of psychotropic medications and lead to improved clinical outcomes and reduced side effects.

A large number of drugs have a marketing authorisation in the UK for the treatment of psychiatric disorders, but the evidence guiding choice for an individual patient is limited. In clinical practice, the selection of drug is effectively made by a trial and error approach. This can lead to several cycles of medications that fail until improvements are eventually reached, often several weeks or months later. In addition, standard doses are offered to all patients and doses are changed only in response to observed symptom changes and tolerability. Furthermore, many patients fail to show sufficient clinical improvement from psychotropic medication, and the side effect burden of these drugs is substantial. This contributes to the low levels of medication compliance seen in psychiatric conditions (e.g. depression, schizophrenia) and to the severe reduction in life expectancy among such patients[1–3].

Characterising the metabolic status of patients using genetic profiling could improve the prescribing of commonly used psychotropic medicines by helping clinicians to adjust the dose in an individualised, biologically-informed way[4,5]. Such pharmacogenetic interventions have been successful in oncology and haematology, and testing is already in use for the management of some drugs such as tamoxifen and warfarin. A handful of studies have undertaken clinical evaluations of CYP-testing in mental health patient populations. Some have shown clinical benefit for antidepressants, but other studies have not[6–11]. A Danish randomised controlled trial of CYP2D6/CYP2C19 testing in schizophrenia found it reduces treatment costs substantially[12]. Thus, for certain psychotropic medications, there is evidence of applications of pharmacogenetics data leading to significant cost savings[12], but there has been limited clinician uptake and there are no available data on efficacy or effectiveness[13,14].

This project aims to fill this gap by conducting a study to investigate the use of a pharmacogenetic testing to optimise the management of psychotropic drugs. We will examine feasibility and acceptability of pharmacogenetic interventions and gather data to test whether personalised prescribing of psychotropic agents results in higher efficacy and reduced side effects, compared to treatment as usual. Participants who consent to take part in the study will be offered genome-wide genotyping.

3 Objectives

Primary:

To investigate the use of pharmacogenetic tools in guiding the prescribing and dosing of psychotropic drugs. To prospectively investigate the efficacy and impact of adverse reactions of this intervention compared to treatment as usual.

Secondary:

- To set up a route for the introduction of pharmacogenetic testing for prescribing psychotropic drugs.
- To evaluate whether the pharmacogenetic-guided prescription of psychotropic drugs could be considered affordable in light of the clinical benefit it may provide.
- To evaluate clinician opinion on pharmacogenetic interventions in psychiatry.

4 Trial design

This is a study investigating the use of pharmacogenetic tools in guiding the prescription of psychotropic drugs. This study will be conducted according to Good Clinical Practice and International Conference on Harmonisation guidelines. The study hypothesis to be tested is that personalised prescribing of psychotropic agents guided by genetic profiling results in higher efficacy and reduced side effects compared to treatment as usual.

Up to 40 clinician-participants will be recruited, through whom 420 participants will be recruited to the study. All of these participants will provide a blood and/or saliva sample for pharmacogenetic analysis. Participants will be stratified according to diagnosis, baseline assessments and genetic metabolic status (see section 4.1). Participants will have the results of the pharmacogenetic test made available to their clinicians as soon as possible following sample collection. The clinician will then be able to review this and consider using the information to guide their treatment decisions. All clinicians will be asked to consent to discuss the results of their patients' genetic test with them and to explain how, if at all, they will use the results to guide treatment decisions. If a clinician does not consent to this, we will not recruit their patients to the study. All participants will be asked to consent to the storage of the pharmacogenetic data for future research.

If participants consent, the results of their genetic testing will be uploaded to their electronic health record to be used in fully anonymised research undertaken in the Clinical Record Interactive Search system. This will be optional and participants will still be able to join the study if they do not consent to this.

4.1 Assessment and Management of Risk

The table below summarise the risks and mitigations of all test above standard care that are being performed in a table:

Intervention	Potential Risk	Risk Management
Blood test	Bruising, Pain, Bleeding and Infection	Performed by trained phlebotomist. Follow trust standard operational procedures.
Clinical interview	The administration of the clinical questionnaires involves a semi structured interview taking place at two time points and takes approximately 90 minutes. These interviews have the potential to ask participants about topics which may be uncomfortable and in extreme cases may cause distress.	To minimise any potential distress, all clinical questionnaires will be administered by a trained clinical assessor trained in interviewing individuals with mental health difficulties. Participants will also be advised at the beginning of the each clinical assessment of their right to terminate the interview at any time without giving a reason and without jeopardising their clinical care.
Mental health questionnaire	The EQ-5D is a self-report questionnaire given to each participant and collected over two time periods (baseline, three months). This questionnaire is designed for self-completion by the patients and is cognitively undemanding and brief. However it does ask questions about current wellbeing which may cause discomfort to some participants.	To minimise any potential distress, the questionnaire will be fully explained by a trained researcher. Participants will also be advised at the beginning of their right to not complete the questionnaire at any time without giving a reason and without jeopardising their clinical care.
Clinical interviews and questionnaires	It is possible that a participant will disclose thoughts of harm to themselves or others in the course of these interventions.	All researchers will be trained to contact the CI and emergency services if a participant is thought to be at risk of harm to themselves or others. No at risk participant will be left alone.

4.2 Treatment group comparison

The 420 participants will be compared to an equivalent group within the CRIS database. This is a large anonymised collection of health records available within the UCL and KCL mental health associated trusts. Natural language processing has identified medication data for approximately 50,000 individuals, of whom at least 7,000 are taking an antipsychotic. Participants will be matched based on

age, sex and treatment. Genetic data are not available within CRIS yet, so it will not be possible to match according to metabolic phenotype. If genetic data become available prior to final analysis, participants will be matched according to this too.

4.3 Metabolic group comparison

Patients will be stratified according to their metabolic profile, which will be identified through their genetic test. Approximately half of the population is expected to be extensive (normal) metabolisers and these patients do not require any modification to their treatment in response to their genetic test result. Knowing that the metabolic status is normal may be reassuring and enhance medication adherence.

4.4 Clinician opinion

The clinician-participants will be asked to consent to answer a short questionnaire at the end of their involvement in the study. The questionnaire collects both quantitative and qualitative data on their opinion on the intervention and will be analysed using mixed-methods.

5 Selection of Participants

Up to 40 consultant adult psychiatrist from open sites will be approached to discuss the study. If they consent to take part, they will recommend potential participants from their case load. Clinician participants will seek verbal consent from their patients before sharing their contact details with the research team. Up to 420 participants will undergo screening assessments, including genotyping. Any patient that clinical staff believes to meet inclusion criteria will first be approached by a staff member to make a preliminary enquiry. If they are interested, the researchers will then fully explain the process of the study and gain informed consent. This will include an assessment of capacity where relevant. All participants will be paid for their time.

5.1 Inclusion criteria

1. Patients aged 18 years or older
2. Men or women
3. With an ICD10 diagnosis of any "mental, behavioural or neurodevelopmental disorders" Codes F01 - F99.

5.2 Exclusion criteria

1. Patients with current high risk of self-harm or harm to others.
2. Patients who lack capacity to consent to taking part in the research.

5.3 Recruitment

Potential participants will be identified at the patient's hospital by their clinical care team. They will make a preliminary enquiry and, if the patient is interested in learning more about the trial, will arrange a meeting with a researcher from the study. Patients will be provided with a patient information sheet at this meeting and will have ample time to consider their involvement in the trial. Patients will be encouraged to ask questions or raise any concerns with their clinician.

The clinical trial will be advertised to potential participants through a website, service user groups and posters in hospitals, outpatient clinics and GP surgeries.

Participant recruitment at a site will only commence when the trial has:

1. Been confirmed by the Sponsor (or it's delegated representative), and
2. Been issued an 'NHS permission letter'.

5.4 Informed consent

It is the responsibility of the Investigator, or a person delegated by the Investigator to obtain written informed consent from each participant prior to participation in the trial, following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the trial.

The person taking consent will be suitably qualified and experienced, and will have been delegated this duty by the CI/ PI on the Staff Signature and Delegation of Tasks.

"Adequate time" must be given for consideration by the participant before taking part. Consent will be sought at least 24 hours after being given the study documentation. It must be recorded in the medical notes when the participant information sheet (PIS) has been given to the participant.

The Investigator or designee will explain that participants are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason.

No trial procedures will be conducted prior to the participant giving consent by signing the Consent form. Consent will not denote enrolment into trial.

A copy of the signed Informed Consent form will be given to the participant. The original signed form will be retained in the trial file at site and a copy placed in the medical notes.

The PIS and consent form will be reviewed and updated if necessary throughout the trial (e.g. where new safety information becomes available) and participants will be re-consented as appropriate.

6 Interventions

6.1 Pharmacogenetic Intervention

List full details of all interventions under investigation. For mechanistic studies, this should include the name of the drug/supplement, pharmaceutical form/strength, the status of this (licensed/non-licensed), and where this will be sourced.

6.2 Storage and handling of drug at site (not applicable)

6.3 Accountability of drug (not applicable)

6.4 Concomitant medication (not applicable)

6.5 Dosages, modifications and method of administration

All participants will be treated within British National Formulary licensed limits (Joint Formulary Committee 2012). All participants will have their plasma levels of medication monitored. This is to confirm they are compliant and can be used to confirm appropriate dosage. Non-compliance and partial compliance will be accounted for in all statistical analyses.

Participants will be aware of what medication they have been prescribed and at what dose. The clinical teams will be directed not to discuss participant allocation or medication with the researcher carrying out follow-up assessments. All analyses will be described in a detailed statistical analysis plan.

7 Trial procedures

7.1 Pre-intervention assessments

Prior to registration, participants will be given study information and asked to sign an informed consent form. Patients will be assessed for capacity to consent at this time and their medical notes will be reviewed to confirm their diagnosis meets the criterion for inclusion.

7.2 Registration Procedures

Participant and clinician-participant registration will be undertaken centrally by the trial manager or delegate at UCL. All participants will be registered prior to providing a sample for genotyping.

Patients will be registered to the trial following participant consent and confirmation of eligibility (see section 8.1 for pre-treatment assessments). Patients will be allocated a unique trial number.

Participants will be stratified according to metabolic group (poor, intermediate, extensive and ultra-rapid) and duration of illness to eliminate these as sources of potential bias.

The prescribing clinicians and participants cannot be blind as the purpose of the trial is to guide prescription using the intervention (genetic test) to make an informed choice.

7.3 Intervention procedures

Baseline assessments will take place on the hospital wards.

At the baseline visit, all participants will provide a DNA sample for genotyping. DNA samples will be collected predominantly from blood samples, but saliva samples may also be collected. DNA will be extracted using standard commercial kits. A DNA aliquot from all participants will be frozen and stored for further study.

During these assessments, participants will be interviewed and asked to complete a set of validated questionnaires widely used in clinical trials in psychiatry. Participants will also complete quality of life questionnaires.

7.4 Subsequent assessments and procedures

Follow-up visits can take place either on the ward or in the community for participants being treated as outpatients. The final follow up assessment (6 months) will be undertaken based on clinical records and interview with the participant's primary nurse or care coordinator.

Schedule of trial assessments and procedures

Table 1: Required Investigations

Procedure	Baseline	3 months	6 months
Medical history	x		
Physical examination including height and weight	x	x	
Questionnaires (see sections 10.1 and 10.2)	x	x	
Blood sample collected for DNA analysis[1]	x		
Saliva sample collected for DNA analysis[1]	x		
Measurement of medication plasma levels		x	
Serum biochemistry	x	x	
Review of medical records by research team			x

[1] Both blood and saliva samples collected where possible.

7.5 Samples

7.5.1 Laboratory assessments

DNA samples will be collected from blood. For those participants who prefer it, DNA can be obtained from buccal swabs. DNA will be extracted using standard commercial kits. All study participants will donate a DNA sample to be used for genome-wide genotyping.

Both new and existing samples will be used in this study.

The following tests will be carried out at Local Laboratories:

LABORATORY TEST	PARAMETERS
HAEMATOLOGY	Full blood count Standard • Haemoglobin (Hb) • White Blood Count (WBC) • Platelet Count (Plt) • Red Cell Count (RBC) • Haematocrit (HCT) • Mean Cell Volume - Red cell (MCV) • Mean Cell Haemoglobin (MCH) Differential White Cell Count • Neutrophils • Lymphocytes • Monocytes • Eosinophils • Basophils
SERUM BIOCHEMISTRY	HbA1c, random glucose, fasting glucose. Liver function tests: Glutamate pyruvate transaminase (GPT / ALAT), glutamic-oxaloacetic transaminase (GOT / ASAT), gamma-glutamyl transferase (gamma-GT), alkaline phosphatase, total bilirubin. Kidney function: creatinine, GFR, total protein, albumin, Electrolytes: chloride, potassium, sodium Lipid profile: Total lipids, total cholesterol, HDL, LDL, triglycerides. Hormones: Prolactin, Thyroid function tests (TSH, T4).

Central laboratories:

Blood (and/or saliva) samples will be sent to a central laboratory for any storage and genotyping.

Central laboratory is the Molecular Psychiatry Laboratory

Dr Andrew McQuillin
Molecular Psychiatry Laboratory
Division of Psychiatry
University College London
Gower Street
London WC1E 6BT

7.5.2 Translational research samples

If participants consent, a DNA aliquot will be frozen and stored for use in future ethically approved research.

7.5.3 Sample storage and transfer

Blood and saliva samples will be collected from patients in accordance with the patient consent form and patient information sheet and shall include all tissue samples or other biological materials and any derivatives, portions, progeny or improvements as well as all patient information and documentation supplied in relation to them.

The blood and saliva samples will be appropriately stored at the UCL Molecular Psychiatry Laboratory. In addition DNA material may be sent to external collaborating laboratories for genotyping.

The laboratories will process, store and dispose of the blood and saliva samples, in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereto.

The extracted DNA or blood or saliva samples are not to be processed and/or transferred other than in accordance with the patients' consent. After ethics approval for the study has expired, the blood and saliva samples will be disposed of in accordance with the Human Tissue Act 2004 and any amendments thereto, or transferred to a licensed tissue bank.

7.6 Discontinuation/withdrawal of participants

In consenting to participate in the trial, participants are consenting to intervention, assessments, follow-up and data collection.

A participant may be withdrawn from trial whenever continued participation is no longer in the participant's best interests, but the reasons for doing so must be recorded. Reasons for discontinuing the trial may include:

- disease progression whilst on therapy
- loss of capacity during the trial
- intercurrent illness
- patients withdrawing consent
- persistent non-compliance to protocol requirements.

The decision to withdraw a participant from treatment will be recorded in the CRF and medical notes. If a participant explicitly states they do not wish to contribute further data to the trial their decision must be respected and recorded in the CRF and medical notes.

7.7 Definition of End of Trial

The expected duration of the study is 6 years from recruitment of the first participant.

The end of trial is defined as the point at which all subjects have had their records reviewed six months after their baseline visit.

8 Recording and reporting of adverse events

8.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or trial participant, which does not necessarily have a causal relationship with the intervention involved.
Serious Adverse Event (SAE).	Any adverse event that: • results in death, • is life-threatening*, • requires hospitalisation or prolongation of existing hospitalisation**, • results in persistent or significant disability or incapacity, or • consists of a congenital anomaly or birth defect.
* A life- threatening event, this refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. ** Hospitalisation is defined as an in-patient admission, regardless of length of stay. Hospitalisation for pre-existing conditions, including elective procedures do not constitute an SAE.	

8.2 Assessments of Adverse Events

Each adverse event will be assessed for severity and seriousness as described in sections 9.2.1 and 9.1 respectively.

8.2.1 Severity

Category	Definition
Mild	The adverse event does not interfere with the participant's daily routine, and does not require further intervention; it causes slight discomfort

Moderate	The adverse event interferes with some aspects of the participant's routine, or requires further intervention, but is not damaging to health; it causes moderate discomfort
Severe	The adverse event results in alteration, discomfort or disability which is clearly damaging to health

8.2.2 Causality (not applicable)

8.2.3 Expectedness (not applicable)

8.3 Recording adverse events

All adverse events will be recorded in the medical records in the first instance. Medical notes will be reviewed at 3 months and 6 months after the first participant visit.

8.4 Procedures for recording and reporting Serious Adverse Events

All serious adverse events will be recorded in the medical records and the CRF, and the sponsor's AE log. The AE log of SAEs will be reported to the sponsor at least once per year.

All SAEs (except those specified in section 9.5 as not requiring reporting to the Sponsor) must be recorded on a serious adverse event (SAE) form. The CI/PI or designated individual will complete the sponsor's SAE form and the form will be preferably emailed to the Sponsor within 5 working days of becoming aware of the event. The Chief or Principal Investigator will respond to any SAE queries raised by the sponsor as soon as possible.

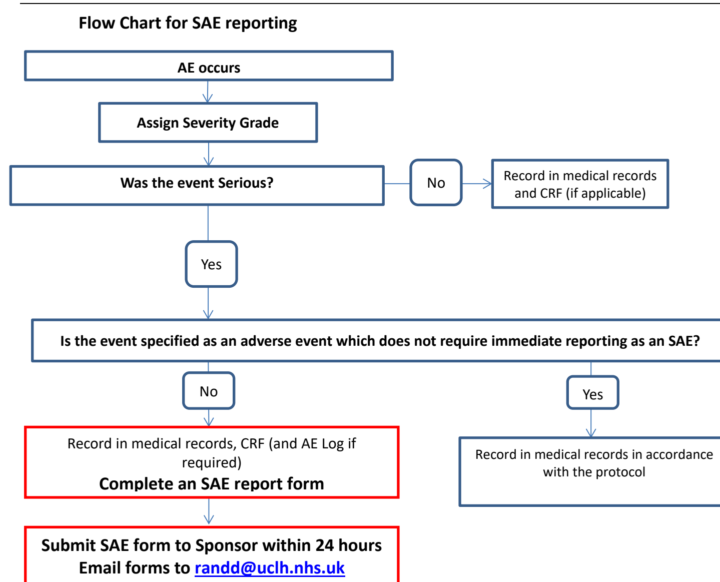
Completed SAE forms must be sent within 5 working days of becoming aware of the event to the Sponsor
Email forms to randd@uclh.nhs.uk

SAEs will be reported to the sponsor until the end of the trial.

Participants must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline values, or until the event has stabilised. Follow-up should continue after completion of protocol treatment and/or trial follow-up if necessary.

Follow-up SAE forms (clearly marked as follow-up) should be completed and emailed to the JRO as further information becomes available.

SUSAR reporting is not applicable for this trial.


PGx in Mental Health, Protocol version 1.2, 27th September 2019
18/0557
Flow Chart for SAE reporting
AE occurs
Assign Severity Grade
Was the event Serious?
No
Record in medical records and CRF (if applicable)
Yes
Is the event specified as an adverse event which does not require immediate reporting as an SAE?
No
Yes
Record in medical records, CRF (and AE Log if required)
Complete an SAE report form
Record in medical records in accordance with the protocol
Submit SAE form to Sponsor within 24 hours
Email forms to randd@uclh.nhs.uk
UCL interventional protocol template version 1 (30/11/2015)
Page 21

8.5 Serious Adverse Events that do not require reporting (not applicable)

8.6 Unblinding (not applicable)

8.7 Reporting Urgent Safety Measures

If any urgent safety measures are taken the CI shall immediately and in any event no later than three days from the date the measures are taken, give written notice to the relevant REC and Sponsor of the measures taken and the circumstances giving rise to those measures.

8.8 Reporting at-risk participants

Some of the questionnaires used in this study could lead to the identification of participants at risk of self-harm, or harm to others. If a researcher observes a participant displaying concerning behaviour, or responding to structured interview questions in a manner suggesting they may be a risk to themselves or others, they will report it to the CI immediately. They will also be able to contact the local crisis care team (see contact details below). The researcher will remain with the participant until a clinician or emergency service staff arrive to ensure the safety of the participant.

The participant's clinician will be informed of any concerning observations within three working days.

8.9 Notification of reportable protocol violations

A reportable protocol violation is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the participants of the trial; or

(b) the scientific value of the trial.

The sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase.

8.10 Reporting incidents involving a medical device(s) (not applicable)

8.11 Trust Incidents and Near Misses

Incidents and near misses must be reported to the Trust through DATIX as soon as the individual becomes aware of them. An incident or near miss is any unintended or unexpected event that could have or did lead to harm, loss or damage that contains one or more of the following components:

It is an accident or other incident which results in injury or ill health.
It is contrary to specified or expected standard of patient care or service.

It places patients, staff members, visitors, contractors or members of the public at unnecessary risk.
It puts the Trust in an adverse position with potential loss of reputation.
It puts Trust property or assets in an adverse position or at risk.

9 Data management

9.1 Confidentiality

All data will be handled in accordance with the General Data Protection Regulation.

The Case Report Forms (CRFs) will not bear the participant's name or other personal identifiable data. The participant's initials, date of birth and trial identification number will be used for identification.

Samples sent for genotyping will include patient identifiers. However, these samples will be transferred in accordance with approved NHS processes and researchers not directly involved in the patient care will not have access to patient identifiable information.

This will be clearly explained to the patient in the Patient information sheet. Patient consent for this will be sought.

9.2 Data collection tools and source document identification

Data will be collected from sites through review of the patient medical notes, the administration of the LUNSERS (Liverpool University Side Effect Rating Scale) and UKU Side Effect Rating Scale and any SAE forms.

It is the responsibility of the investigator to ensure the accuracy of all data entered in the CRFs. The delegation log will identify all those personnel with responsibilities for data collection and handling, including those who have access to the trial database.

9.3 Completing Trial Documentation

All trial documents must be completed and signed by staff that are listed on the site staff delegation log and authorised by the CI/ PI to perform this duty. The CI/PI is responsible for the accuracy of all data reported in the trial documents. Documents should be sent via email to the trial office:

- E.bramon@ucl.ac.uk
- Isabelle.zimmerman.11@ucl.ac.uk

Once completed the original documents must be sent to the Division of Psychiatry and a copy kept at site. The documents must be returned within 6 weeks of the participant visit.

9.4 Data handling

In the study, data will be collected from participants in accordance with the patient consent form, patient information sheet and this protocol.

The study is compliant with the requirements of General Data Protection Regulation (2016/679) and the Data Protection Act (2018). All investigators and study site staff will comply with the requirements of the General Data Protection Regulation (2016/679) with regards to the collection, storage, processing and disclosure of personal information, and will uphold the Act's core principles. UCL is the data controller; the UCL Data Protection Officer is Lee Shailer (data-protection@ucl.ac.uk). The study will be collecting the following personal data: Initials, date of birth, sex.

We will make following security arrangements for storage of personal data during the study:

- Hard copies will be locked filing cabinets in locked rooms with only authorised researchers holding keys. These files will be stored in building with security and access by swipe card.
- Electronic data with be pseudo-anonymised.
- Patient data will within the UCL Data Safe Haven. The Data Safe Haven has been certified to the ISO27001 information security standard and conforms to NHS Digital's Information Governance Toolkit. No data will be stored in any local drive of any computer (desktop or laptop). No data to be stored on laptops. All data and databases will be held in one dedicated centralised drive accessible only to researchers working in the project. Such drives are held in central servers at UCL and are managed by the IT services team. The data are backed up daily in two remote sites making it very safe for long term storage. Access is limited and highly secure. Only approved researchers have access to the shared drive and their use can be monitored. When a researcher leaves the team access is removed by the IT team.

10 Statistical Considerations

10.1 Primary Outcome

Number of days under acute service care.

10.2 Secondary outcomes

- Recruitment rate
- Further measures of efficacy and side effects at 3 months including:
  - Body mass index (BMI)
  - Clinical global impression score
  - Global assessments of function
  - Movement disorder scale (AIMS)
  - Glasgow antipsychotic side effect scale,
  - Clinical assessment of treatment score (CAT),
  - Plasma levels of medication

- Positive and negative symptom scale (PANSS)
- Liverpool University Neuroleptic Side Effect Rating Scale (LUNSERS)
- Quality of life with EQ-5D
- Service usage and costs with the client services receipt inventory (CSRI)
- HbA1c, cholesterol and prolactin levels

10.3 Sample size calculation

A total of up to 420 participants will be recruited to the study. They will be compared to a group of 6,000 participants in the CRIS database, with the groups being matched on age, sex and treatment distribution.

Recently published data from the Clinical Record Interactive Search (CRIS) system in a sample of 17,666 patients treated by the crisis resolution teams, shows that the average duration of crisis team care was 20.2 days per patient with a standard deviation of 20.8 days[15]. An intervention achieving a 3.5-day average change in crisis team care gives an effect size of 0.17 (Cohen's D)[16]. This is a small yet clinically meaningful change in the duration of crisis team care. Therefore, the sample we propose to collect (420 intervention and 6,000 treatment as usual) gives 92% power to detect an effect size as small as 0.17 (two-sided test, 5% significance).

10.4 Planned recruitment rate

We estimate we can recruit three to six patients per week (thus 150 to 300 patients in a year). Thus recruiting a total of 420 patients, can be achieved in two to three years.

10.5 Statistical analysis

10.5.1 Treatment group comparisons

Linear models, including CYP metabolic status, diagnosis, medication, baseline value of the outcome and experimental group as main effects, will be used to compare the two treatment groups (trial group and CRIS sample) on the outcome measures.

The principal aim of the study is to undertake a combined analysis including all participants. In addition, we will undertake supporting analyses to examine the effect of further covariates that are known to influence medication response including duration of illness, concurrent medication, smoking status and illegal substance use. We will explore the effects of experimental group within registration strata, including exploratory tests for interaction between stratum and experimental group.

10.5.2 Metabolic group comparison

We will conduct linear models, controlling for age and sex as main effect, to compare the four metabolic groups on the outcome measures.

10.5.3 Clinician questionnaire

The questionnaires returned by the clinician-participants will be analysed to assess the opinion of the recruited clinicians on the value of pharmacogenetic interventions for their patients.

10.5.4 Sensitivity and other planned analyses

We will undertake sensitivity analyses to missing data. We will be aware of compliance and non-compliance with medication as we will monitor blood levels of medication during the trial.

Once we have collected our first 50 participants we will write a more detailed statistical analysis plan. In this document, a more technical and detailed elaboration of the principal features stated in the protocol will be included. This plan will cover the primary and secondary analyses and will be updated as a result of the blind review of the initial data and will be finalised before breaking the blind. We will keep records of analyses conducted and note when blind is broken.

11 Record keeping and archiving

At the end of the trial, all essential documentation will be archived securely by the CI for a minimum of 25 years from the declaration of end of trial.

Essential documents are those which enable both the conduct of the trial and the quality of the data produced to be evaluated and show whether the site complied with all applicable regulatory requirements.

The sponsor will notify sites when trial documentation can be archived. All archived documents must continue to be available for inspection by appropriate authorities upon request.

12 Oversight Committees

12.1 Trial Management Group (TMG)

The TMG will include the Chief Investigator and trial staff. The TMG will be responsible for overseeing the trial. The group will meet regularly and will send updates to clinical members involved in the trial.

The TMG will review recruitment figures, SAEs and substantial amendments to the protocol prior to submission to the REC. All PIs will be kept informed of substantial amendments through their nominated responsible individuals.

12.2 Other committees (not applicable)

13 Ethical requirements and patient and public involvement

Ethics

The sponsor will ensure that the trial protocol, participant information sheet, consent form, GP letter and submitted supporting documents have been approved by the appropriate research ethics committee, prior to any participant recruitment. The protocol, all other supporting documents including and agreed amendments, will be documented and submitted for ethical and regulatory approval as required. Amendments will not be implemented prior to receipt of the required approval(s).

Before any NHS site may be opened to recruit participants, the Chief Investigator/Principal Investigator or designee must receive NHS permission in writing from the Trust Research & Development (R&D). It is the responsibility of the CI/ PI or designee at each site to ensure that all subsequent amendments gain the necessary approvals, including NHS Permission (where required) at the site. This does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual participants (see section 9.6 for reporting urgent safety measures).

An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. The chief investigator will prepare the APR.

Within 90 days after the end of the trial, the CI/Sponsor will ensure that the main REC is notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial.

The CI will supply the Sponsor with a summary report of the trial, which will then be submitted to the REC within one year after the end of the trial.

Patient and public involvement (PPI)

We have presented this research project to the Service User Research Forum (SURF). This advisory board have advised us on recruitment strategies and study design. The SURF at UCL have also agreed to be involved in the management of the study as it progresses.

14 Monitoring

The sponsor will determine the appropriate level and nature of monitoring required for the trial. Risk will be assessed on an ongoing basis and adjustments made accordingly.

The degree of monitoring will be proportionate to the risks associated with the trial.

A trial specific oversight and monitoring plan will be established for studies. The trial will be monitored in accordance with the agreed plan.

15 Finance

Funding for this study has been provided by the organisations listed below. Additional funding may be applied for from non-commercial groups over the course of the study.

1 Medical Research Council

2 Biomedical Research Centre
3 British Medical Association

16 Insurance

University College London holds insurance against claims from participants for injury caused by their participation in the trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this trial without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this trial shall provide negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

17 Publication policy

The results of this study will be published in a peer reviewed scientific journal, presented at relevant conferences and shared on the UCL Division of Psychiatry website. Results will be shared as soon as possible after the completion of trial and statistical analysis, and no later than within 6 months of trial completion.

18 Intellectual property

All background intellectual property rights (including licences) and know-how used in connection with the study shall remain the property of the party introducing the same and the exercise of such rights for purposes of the study shall not infringe any third party's rights.

All intellectual property rights and know-how in the protocol and in the results arising directly from the study, but excluding all improvements thereto or clinical procedures developed or used by each participating site, shall belong to UCL. Each participating site agrees that by giving approval to conduct the study at its respective site, it is also agreeing to effectively assign all such intellectual property rights ("IPR") to UCL and to disclose all such know-how to UCL.

Each participating site agrees to, at the request and expense of UCL execute all such documents and do all acts necessary to fully vest the IPR in UCL.

Nothing in this section shall be construed so as to prevent or hinder the participating site from using know-how gained during the performance of the study in the furtherance of its normal activities of providing or commissioning clinical services, teaching and research to the extent that such use does not result in the disclosure or misuse of confidential information or the infringement of an intellectual property right of UCL. This does not permit the disclosure of any of the results of the study, all of which remain confidential.

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E.2 Participant information documents

The patient information documents are some of the most important pieces of trial documentation, as they are the main source of information for prospective participants. It is a legal requirement that any potential participant be fully informed of all that is expected of them before they consent to take part in the trial. Therefore it is necessary to explain the background, objectives and trial design in detail, but in language that is understandable to a lay audience. For this trial we will recruit two groups of participants; clinicians and patients. I have drafted separate documents for each of these groups. For the clinician group, the information document and informed consent form were combined into one document.

LONDON'S GLOBAL UNIVERSITY

Pharmacogenetics in Mental Health

Chief investigator: Prof Elvira Bramon | tel. 020 3549 5873

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

Why have I been invited to take part?

We are inviting people over the age of 18 who have a clinical diagnosis of a mental illness (such as depression, schizophrenia or bipolar disorder) to take part in this study to help us to understand more about the treatment of these illnesses. We plan to recruit approximately 420 participants in total.

Why are we doing this study?

The purpose of this study is to see if genetic testing could be useful for patients who are going to be given antidepressant, antipsychotic or mood stabilising medicine as treatment for their mental illness.

Our genes are made up of DNA. DNA is a complex chemical that carries genetic information. It determines how proteins and enzymes are made in the body and is the material passed on from parents to children that results in inherited traits such as eye colour and hair colour. DNA is made of a long chain of chemicals which can change in sequence. These changes can either be inherited or they can happen spontaneously. Changes in DNA sequence can alter the way the body makes proteins and enzymes, including the enzymes that break down the medicines we take. Some people's enzymes work faster than usual, whereas other people's may work slower than usual. If this is the case, some medicines might not work as well or it could mean you experience more side effects.

Since your genes can change how your body responds to medicines, it is possible that treatment could be more effective, and result in fewer side effects, if your genetics are taken into consideration. In this study, we will do genetic testing on patients before they are prescribed treatment to help understand if it could be beneficial. At the moment there is not enough evidence to know if this is the case. This study therefore aims to add to what we know in this field and hopefully improve the treatment of patients with mental illness in the future.

What does participating in this study involve?

The first step in taking part in this study will be to read this information sheet, think it over and, if you want to take part, sign an informed consent form. After that, there are two visits required of you, detailed below. Wherever possible, we will schedule these visits alongside your usual medical appointments, or come to your home. We would like to inform your GP if you decide to take part in this study. If you agree we will send them a letter, but you do not have to agree to this in order to take part in the study.

If your clinical team has concerns that you are no longer able to understand the research and why you are taking part you will be withdrawn from the study. This will always be discussed with you.

<u>Baseline visit</u>

After you have agreed to take part in this study you will be asked to attend a baseline visit with the study team. We will assign you a unique trial number which will be used, along with your initials and sometimes your date of birth, to identify any samples or notes about you. Your full name will not be recorded for this study.

During the baseline visit we will ask about your medical history, conduct a physical examination, ask you to complete some questionnaires about your health and well-being. We will also do a blood test to check the

following measures: glucose (blood sugar), cholesterol and other lipid (fat) levels, prolactin (a hormone), liver and kidney function and the amount of prescribed medication found in your blood. We are looking at these substances because some medications can change the amount of them in your blood, and it is possible that your genetics can affect the extent to which this happens.

You are asked to provide a blood or saliva or a mouth swab sample for genetic analysis. A single blood sample of up to 30 millilitres (ml) (3 tubes) or a saliva sample of approximately 4 ml (1 teaspoon) is taken.

Information on your family, medical and mental health history will be obtained from your hospital case notes or GP records and from a short interview, if needed. With your permission, we will contact a member of your clinical team and tell them in confidence that you have agreed to participate.

Some patients will be asked to have the results of the genetic test shared with their clinician, who may use this information to guide how they treat you. This will always be discussed with you in detail.

3 month follow-up visit

You will be seen again by the study team three months after your recruitment. Here we will repeat some, but not all, of the test you had at the baseline visit. The tests we will do at this visit are: a physical examination, questionnaires on your health and well-being, a blood test to measure the amount of medication in your blood and to measure glucose, cholesterol and prolactin levels.

6 month review of your medical notes

Once it has been 6 months since you were enrolled to the study, we will check on your well-being by reviewing your hospital case notes and GP record. You will not have to come in for another visit.

After you have finished taking part in the study, your treatment will continue as usual.

Taking part in this study will not stop you from participating in other research projects, providing the other research projects will not require you to change your medication. If you have been involved in any research in the past you will be eligible to take part.

What will happen to the samples I give?

The blood or saliva sample provided will be pseudo-anonymised and labelled with your trial number and date of birth. It will be sent to the laboratory by secure courier or delivered in person by a member of the study team. In the event that your blood or saliva sample is taken by someone outside of the study team, for example by your GP, it will be pseudo-anonymised and sent to the laboratory for genetic analysis.

We would like to request to keep your blood or saliva or mouth swab sample and genetic test results for future research, and store them for a maximum of 30 years. You do not have to consent to this in order to be eligible for this study and you are free to change your mind at any time without giving a reason. Samples kept for future research will always be anonymised and any future research conducted using the anonymised sample will require ethical approval.

If you consent to the use of your sample in future research, the blood or saliva sample, or cells taken from your blood sample, will be tested in the laboratory at University College London and may be made available world-wide to other scientific collaborators, charities or commercial companies for further ethically approved medical research. White blood cells from your blood sample may be made into a living growth of white cells that can be cultured and multiplied. This sample will be used indefinitely or until you make a request for it to be withdrawn (which you can do at any point in time). You should be aware that in the unlikely event that your DNA was to become of commercial value in the future, you would not be able to claim financial reimbursement. If your sample or your data are sent outside of the United Kingdom it will always be anonymised.

Other possible future research:

We would also like to ask your permission to be contacted in the future. Again, this is optional and agreeing to be contacted does not mean you have to take part in future research if you don't want to.

If you consent, we may enter the results of your genetic test to your medical record, as it may be useful for your future treatment and also for additional fully-anonymised research. This is optional and if you do not wish to have the genetic results added to your medical records you can still take part in this study.

When you agree to take part in a research study, the fully-anonymised information about your health and care may be provided to researchers running other research studies in this organisation and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research.

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of health and care research, and cannot be used to contact you or to affect your care. It will not be used to make decisions about future services available to you, such as insurance.

How your information is stored?

All the information related to you and to your blood or saliva sample will be confidential – your sample will be labelled with your unique trial number, initials and date of birth only. All information relating to you will be stored in a secure and safe place and only people directly involved in the research will have access to it. Your name will not be used in any way when the results of this research are made known or identified in relation to any DNA samples.

UCL is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. UCL will keep identifiable information about you for a maximum of 1 year after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information by contacting Dr Elvira Bramon.

Camden and Islington NHS Foundation Trust will use your name, date of birth, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Members of the study team from University College London and regulatory organisations may look at your medical and research records to check the accuracy of the research study. Camden and Islington NHS Foundation Trust will pass these details to University College London along with the information collected from you and your medical records. The only people in University College London who will have access to information that identifies you will be people who need to contact you as part of the study or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.

Camden and Islington NHS Foundation Trust will keep identifiable information about you from this study for 1 year after the study has finished.

What happens if you no longer want to be involved?

You do not have to take part in this study if you do not want to. If you decide to take part you may withdraw by speaking to your local researcher or contacting the Chief Investigator of the study without having to give a reason. Your decision whether to take part or not will not affect your future care and management in any way. You can withdraw your blood sample and DNA and ask us to destroy your DNA and any personal information about you that we hold at any time. Your blood sample and DNA will not be used at any time for any other purpose than for research which aims to improve our understanding of mental illness and its treatment.

Risks and Benefits

This trial is minimally invasive and the risk associated with blood samples is extremely low. All blood samples will be taken by a trained phlebotomist. There is a risk you may experience slight bruising.

If you are selected to have your genetic results given to your clinician it is possible that your treatment might be changed and result in a reduction in side effects, but this cannot be guaranteed. Any changes made to your treatment will be decided upon by your clinician and discussed with you.

We will cover all travel expenses related to your participation in this study. We can also offer you £5 as reimbursement for your time.

Who is organising and funding this study?

This research study is being organised and lead by Professor Elvira Bramon at University College London. Funding has been provided by the following organisations:

- Medical Research Council, the non-departmental government organisation responsible for co-coordinating and funding medical research in the United Kingdom
- NIHR Biomedical Research Centre at UCL and UCLH, a body dedicated to the support of experimental medical research at UCL and UCLH.
- British Medical Association, the professional association for doctors in the UK

It is possible that other similar organisations to those listed above may provide additional funding over the course of the study.

Our research approval

All proposals for research using human subjects are reviewed by a research ethics committee before they can proceed. This proposal was reviewed by the London Camden and Kings Cross Research Ethics Committee and has been given its approval.

We have presented this research project to the Service User Research Forum (SURF) at UCL. SURF at UCL have also agreed to be involved in the management of the study as it progresses.

What if there is a problem?

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff you may have experienced due to your participation in the research, National Health Service or UCL complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this.

In the unlikely event that you are harmed by taking part in this study, compensation may be available.

If you suspect that the harm is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation. After discussing with your doctor, please make the claim in writing to Dr Elvira Bramon who is the Chief Investigator for the study and is based at Camden and Islington NHS Foundation Trust. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office.

You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Insurance matters

Your participation in this research should not have any effect on life insurance or private medical insurance. However, if you have any doubts please check with your insurer first.

Questions

If you have any questions about the research project, please contact Prof Elvira Bramon, who will be responsible for security and access to your data. Further information about this trial can be found on www.clinicaltrials.gov. No personal information about you or any other participants will ever be added to this database.

Contacts

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Trial manager:
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LONDON'S GLOBAL UNIVERSITY

Pharmacogenetics in Mental Health

Chief investigator: Prof Elvira Bramon | tel. 020 3549 5873

Please confirm that you have read the following statements by initialling the boxes on the right hand side of the page.

1. I confirm that I have read and understood the information sheet dated 27th September 2019 (Version 1.2) for the above study and have had the opportunity to ask questions. ☐
2. I also confirm that I have had sufficient time to consider whether or not to be included in the study. ☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
4. I consent to being contact by phone, email and/or letter during the study, with information relating to my involvement in the study. ☐
5. I consent to provide a blood or saliva sample for genetic testing. ☐
6. I understand that my sample will be linked-anonymised, which means that it will be anonymous to the research team but not to Prof Bramon. ☐
7. I understand that researchers for this study may contact my GP, other healthcare providers or emergency services if they feel there is a risk to my safety. ☐
8. I confirm that I am willing to have the results of my genetic test added to my hospital notes and GP records. I understand that this is optional and if I do not wish to have my genetic test results added to my medical record I can still take part in this study.

Yes	
No	

9. I understand that my hospital notes and GP records may be viewed to obtain information relevant to the research. I give permission for copies of my notes to be released to Prof Bramon and her research team by secure 'safe haven' fax or by electronic upload of encrypted data to a secure server at UCL. ☐

10. I confirm that I am happy for a letter to be sent to my GP informing them that I am taking part in this study, and for this letter to be kept in my medical records. I understand that this is optional and if I do not wish my GP to be informed of my involvement I can still take part in this study.

Yes	
No	

11. I understand that, in the event that my blood sample is not taken by the research team, my sample may be forwarded to the study team, via post, with only my trial number on it.

12. I agree to be contacted by the study team to be invited to participate in further research. I understand that I can chose not to take part in this additional research without giving a reason. My medical care or legal rights will not be affected. I understand that this is optional and if I do not wish to be contacted in the future I can still take part in this study.

Yes	
No	

13. I consent to my sample being donated as a gift and may be retained for up to 30 years, or until I ask for it to be destroyed. It may be kept for future research, subject to review of NHS or UCL/UCLH Research Ethics Committees. I understand that this is optional and if I do not wish my sample to be stored I can still take part in this study.

Yes	
No	

14. I agree to take part in this study.

....	....
Signature of Volunteer	Signature of Researcher/Clinical Studies Officer
....	....
Print Name of Volunteer	Print Name of Researcher/ Clinical Studies Officer
Date	Date ....

A copy of this completed and signed consent form will be stored in the research file and in your medical records. You will also be given a copy to keep.

LONDON'S GLOBAL UNIVERSITY

Pharmacogenetics in Mental Health

Chief investigator: Professor Elvira Bramon

We would like to inform you about a research study and invite you and your patients to participate. Please read this study summary as well as the attached patient information sheet and study protocol. If you have any questions, please contact us using the details overleaf.

Study summary

The purpose of this study is to investigate the use of pharmacogenetic testing to inform the prescribing of psychotropic medications. We aim to examine the benefit of genetic testing on adverse effects and treatment cost. Guidelines from the Clinical Pharmacogenetics Implementation Consortium, as well as some medication labels published by the Food and Drug Administration or the European Medicines Agency, recommend dose adjustments based on CYP2D6/CYP2C19 genotype for many psychotropic drugs. However, pharmacogenetic testing is not used routinely in clinical psychiatric practice.

We will recruit up to 420 adult patients taking antipsychotics or antidepressants. All patients will be offered a genetic test, the results of which will be sent to you for your information. The treatment decisions always remain at your discretion –taking part in the study does not require you to change treatment based on any genetic results.

What does participating in this study involve?

We will ask you to sign this consent form to allow us to contact any of your patients with whom you have discussed the study and who have given verbal consent to be contacted by us. You will be required to discuss the genetic test results with your patients. However, is not mandatory that you change your patient's treatment in light of certain results. The chief investigator will be available to provide guidance on the interpretation of the results if desired.

At the end of the study, we will ask you to complete a short questionnaire. The CI and research team will also be happy to discuss any feedback or concerns you may wish to raise throughout the study.

Our research approval

This proposal was approved by the London Kings Cross and Camden Research Ethics Committee (19/LO/1403).

If you would like to take part, please initial and sign below, or confirm consent via email:

1. I authorise Prof Bramon and her team to access my case load and confirm I will only provide contact details of patients who have given their verbal consent to be contacted for this study. ☐
2. I understand that any changes to my patients' treatment based on their genetic test results are entirely at my discretion. ☐
3. I confirm that I am willing to discuss the results of my patient's genetic test result with them if required, and to explain any changes to their treatment I make based on the genetic results. ☐
4. I agree to take part in this study. ☐

Contacts

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E.3 NHS and Genomics England Pharmacogenetics Working Group: Reports

As described in chapter 5, I was invited to join the NHS-Genomics England Pharmacogenetics working group. Along with colleagues in this working group, I drafted the first evidence based guidelines for the implementation of pharmacogenetic testing in the NHS. I was tasked with drafting guidelines for four antidepressants: citalopram, escitalopram, nortriptyline and paroxetine. The final versions of these documents are provided below.