GENE ASSOCIATION STUDIES OF CLOZAPINE RESPONSE: A DOPAMINE SYSTEM APPROACH

By

Eric Huang

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**Name:** Eric Huang

**Department:** Institute of Medical Science, University of Toronto

**ABSTRACT**

The antipsychotic clozapine (CLZ) is under-utilized due to its life-threatening side-effects despite superior efficacy for treatment-resistant schizophrenia (SCZ). Thus, predictors for response could help inform drug administration decisions. Given the genetic basis for response, we aimed to identify genetic predictors and build a preliminary genetic model for CLZ efficacy.

Since CLZ’s targets the DA system, we investigated a DA D2 receptor (DRD2) gene variant implicated in SCZ risk in a recent genome-wide association study. We observed that the A-allele was associated with better CLZ response. We also examined the association between catechol-O-methyltransferase gene (COMT) polymorphism Val158Met and antipsychotic response, observing that Met/Met was associated with greater improvement. Lastly, we developed a genetic model for response, consisting of four polymorphisms from the DRD2, serotonin-6 receptor (5-HT6), brain-derived neurotrophic factor (BDNF), and neurexin-1 genes (NRXN1) (Adjusted $R^2$=52%, sensitivity=70%, specificity=47%). While replication is required, our findings identify several potential genetic predictors of response.
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Contributions

The work presented in thesis is comprised of three original research studies. Chapter 3 was published in *Pharmacogenomics*. Chapter 4 was published in the *International Journal of Neuropsychopharmacology*. Chapter 5 is currently being prepared for submission to a peer-reviewed journal.

The following individuals contributed to the completion of this thesis:

**Dr. Arun Tiwari:** Provided critical expertise in support of the development of the genetic model in Chapter 5, as well as important feedback on the manuscripts for Chapters 3, 4, and 5.

**Dr. Clement Zai:** Contributed critically to the design of the meta-analysis and supervised the statistical analysis in Chapter 4, and provided important feedback on the manuscripts for Chapters 3 and 4.

**Ms. Amanda Lisoway:** Created the figures and provided important feedback on the manuscript in Chapter 4.

**Dr. Daniel Müller:** Collected the German Schizophrenia (GSZ) sample included in the meta-analysis in Chapter 4. Provided guidance on pharmacogenetics work in relation to both response and side-effects, as well as valuable feedback for the improvement of the manuscripts for Chapters 3 and 4.

**Dr. Gary Remington:** Provided valuable feedback for the improvement of the individual studies of the thesis. Also provided useful feedback on ways in which studies of clozapine response can be improved, methods for categorizing schizophrenia patients into different subtypes, and strategies for monitoring treatment adherence when designing studies.

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Dr. Jiang Li: Provided feedback on the manuscript in Chapter 3.

Dr. Jeffrey Lieberman: Collected a portion of the clozapine-treated sample used in Chapters 3-5. Led the collection of the CATIE sample used in Chapter 5, and provided the response data for this sample.

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<td>5-HT1A</td>
<td>5-Hydroxytryptamine (serotonin) Receptor 1A</td>
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<tr>
<td>5-HT2A</td>
<td>5-Hydroxytryptamine (serotonin) Receptor 2A</td>
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<td>5-Hydroxytryptamine (serotonin) Receptor 7</td>
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<td>5-HTT</td>
<td>5-Hydroxytryptamine (serotonin) Transporter</td>
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<td>5’-UTR</td>
<td>5’-Untranslated region</td>
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<td>ABCB1</td>
<td>ATP-binding Cassette Transporter 1</td>
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<td>ADHD</td>
<td>Attention Deficit Hyperactivity Disorder</td>
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<td>Adrenoceptor α-1A</td>
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<td>ADRA2A</td>
<td>Adrenoceptor α-2A</td>
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<tr>
<td>ARA</td>
<td>Affect, resistance, activation factors</td>
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<td>AUC</td>
<td>Area Under the Curve</td>
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<td>BD</td>
<td>Bipolar Disorder</td>
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<td>BDNF</td>
<td>Brain-Derived Neurotrophic Factor</td>
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<tr>
<td>BPRS</td>
<td>Brief Psychiatric Rating Scale</td>
</tr>
<tr>
<td>CADD</td>
<td>Combined Annotation Dependent Depletion</td>
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<tr>
<td>CATIE</td>
<td>Clinical Antipsychotic Trial of Intervention Effectiveness</td>
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<td>CHRM1</td>
<td>Muscarinic Receptor 1</td>
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<tr>
<td>CIA</td>
<td>Clozapine-Induced Agranulocytosis</td>
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<td>CLZ</td>
<td>Clozapine</td>
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<tr>
<td>CNV</td>
<td>Copy Number Variant</td>
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<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
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<td>CV</td>
<td>Cross-validation</td>
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<td>CYP</td>
<td>Cytochrome P450</td>
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<td>DA Transporter</td>
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<td>ddPCR</td>
<td>Digital-drop-PCR</td>
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<td>Disrupted In Schizophrenia 1</td>
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<td>DNA</td>
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<td>DRD1</td>
<td>Dopamine Receptor D1</td>
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<td>DSM-IV-TR</td>
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<td>Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition</td>
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<td>DTNB1</td>
<td>Dystrobrevin Binding Protein 1</td>
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<tr>
<td>DUP</td>
<td>Duration of Untreated Psychosis</td>
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<td>ECT</td>
<td>Electroconvulsive Therapy</td>
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<td>ENCODE</td>
<td>Encyclopedia of DNA Elements</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>EMSA</td>
<td>Electrophoretic Mobility Shift Assay</td>
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<tr>
<td>EPS</td>
<td>Extrapyramidal Symptoms</td>
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<tr>
<td>eQTL</td>
<td>Expression Quantitative Trait Loci</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
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<td>GABA</td>
<td>Gamma-aminobutyric Acid</td>
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<td>GDNF Family Receptor α-2</td>
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<td>GNB3</td>
<td>G-protein β-subunit 3</td>
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<td>GRID2</td>
<td>Glutamate Receptor, Ionotropic, Delta Subunit 2</td>
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<td>GRIN1</td>
<td>N-methyl-D-aspartate Ionotropic Receptor 1</td>
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<tr>
<td>GRIN2B</td>
<td>N-methyl-D-aspartate Ionotropic Receptor 2B</td>
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<tr>
<td>GTex</td>
<td>Genome Tissue Expression Project</td>
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<td>GWAS</td>
<td>Genome-Wide Association Study</td>
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<td>GWAVA</td>
<td>Genome Wide Annotation of Variants</td>
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<tr>
<td>HDAC2</td>
<td>Histone Deacetylase 2</td>
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<td>HRH1</td>
<td>Histamine Receptor 1</td>
</tr>
<tr>
<td>H2</td>
<td>Histamine Receptor 2</td>
</tr>
<tr>
<td>HWE</td>
<td>Hardy-Weinberg Equilibrium</td>
</tr>
<tr>
<td>ICD-10</td>
<td>International Statistical Classification of Diseases and Health-related Problems – 10th Revision</td>
</tr>
<tr>
<td>$K_{\text{off}}$</td>
<td>Dissociation Constant</td>
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<tr>
<td>LD</td>
<td>Linkage Disequilibrium</td>
</tr>
<tr>
<td>MAF</td>
<td>Minor Allele Frequency</td>
</tr>
<tr>
<td>MARTA</td>
<td>Multi-acting Receptor-targeted Antipsychotic</td>
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<tr>
<td>mCNV</td>
<td>Multi-allelic Copy Number Variant</td>
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<tr>
<td>MDD</td>
<td>Major Depressive Disorder</td>
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<tr>
<td>MDS</td>
<td>Multi-factor Dimensionality-scaling</td>
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<tr>
<td>Met</td>
<td>Methionine</td>
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<tr>
<td>mGlu2</td>
<td>Metabotropic Glutamate Receptor 2</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
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<tr>
<td>NDMC</td>
<td>N-desmethyl-clozapine</td>
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<td>NMDAR</td>
<td>N-methyl-D-aspartate receptor</td>
</tr>
<tr>
<td>NRXN1</td>
<td>Neurexin-1</td>
</tr>
<tr>
<td>OLZ</td>
<td>Olanzapine</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<tr>
<td>OXT</td>
<td>Oxytocin Prepropeptide</td>
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<tr>
<td>PCA</td>
<td>Principal Components Analysis</td>
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<tr>
<td>PCP</td>
<td>Phencyclidine</td>
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<td>PGC</td>
<td>Psychiatric Genomics Consortium</td>
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<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
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<tr>
<td>RFLP</td>
<td>Restriction Fragment Length Polymorphism</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operating Characteristics</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for DSM-IV</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>SCZ</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>SLC6A2</td>
<td>Norepinephrine Transporter</td>
</tr>
<tr>
<td>SMD</td>
<td>Standardized Mean Difference</td>
</tr>
<tr>
<td>SNP</td>
<td>Single-nucleotide Polymorphism</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>TRS</td>
<td>Treatment-resistant schizophrenia</td>
</tr>
<tr>
<td>TS</td>
<td>Tourette’s Syndrome</td>
</tr>
<tr>
<td>TS1</td>
<td>Toronto Sample 1</td>
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<tr>
<td>TS2</td>
<td>Toronto Sample 2</td>
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<td>Toronto Sample 5</td>
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<tr>
<td>Val</td>
<td>Valine</td>
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<tr>
<td>VNTR</td>
<td>Variable-number Tandem Repeat</td>
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<tr>
<td>WCST</td>
<td>Wisconsin Card Sorting Test</td>
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CHAPTER 1

INTRODUCTION

1.1 Schizophrenia (SCZ)

1.1.1 Definition

The term ‘schizophrenia’ was originally coined by Eugen Bleuler in 1911. The individual component phrases, ‘schiz’ and ‘frein’, mean ‘split’ and ‘mind’ respectively (Bleuler, 1911). The disorder was later referred to by Kraepelin as ‘dementia praecox’ and consisted of patients with catatonia, hebephrenia, and paranoid dementia with onset during adolescence or early adulthood (Kraepelin et al., 1919). Later, SCZ was re-defined based upon 11 symptoms that formed the basis of diagnosis for the disorder, including hallucinations and delusions (Schneider, 1959).

The current generally accepted definition of SCZ combines the work of Kraepelin, Bleuler, and Schneider. The disorder consists of three major categories of symptoms—positive, negative, and cognitive. Positive symptoms are defined as ones that healthy human beings do not usually experience but are present in SCZ patients. These include hallucinations where perception occurs without an external stimulus, and delusions, which are fixed false beliefs despite strong evidence to the contrary (Sadock, 2000). In contrast, negative symptoms are understood to be ones characterized by loss of common functions and behaviours that healthy individuals engage in. These include asociality, affective flattening, alogia, avolition, and anhedonia (Andreasen, 1982). Notably, the ‘deficit syndrome’ subtype of SCZ consists primarily of persistent forms of this category of symptom (Kirkpatrick et al., 2001). Lastly, cognitive symptoms are comprised of deficits in basic functions such as attention, working memory, verbal fluency, processing speed, executive function, and general IQ (Heinrichs and Buchanan, 1988; Rajji et al., 2009).
The diagnostic criteria for SCZ have evolved substantially since the term was first coined (Nasrallah and Smeltzer, 2002). The most recent change came with the publishing of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V). Whereas the earlier addition, DSM-IV-TR (DSM-IV-‘text revision’), outlined diagnostic subtypes of SCZ (e.g. paranoid, disorganized, catatonic), DSM-V eliminated these subtypes based on low diagnostic stability, reliability, and validity. In turn, DSM-V replaced these subtypes with a dimensional approach, where patients are evaluated based on severity in core symptoms (e.g. positive, negative). The special attribution given to Schneiderian auditory hallucinations and ‘bizarre delusions’ has also been taken out (Association).

1.1.2 Disease Progression

The progression of SCZ has recently been categorized into four phases: (1) premorbid, (2) prodromal, (3) psychotic or acute, and (4) stable (or ‘residual’) (Tandon et al., 2009). The ‘premorbid’ phase consists of cognitive, motor, and social deficits; individuals in this phase exhibit delayed motor development, language learning problems, poor academic achievement, and social and emotional detachment (Fish et al., 1992; Walker et al., 1994; Cornblatt et al., 1999; Cannon et al., 2002; Keshavan et al., 2005). The ‘prodromal’ phase is the period (on average: ~five years) prior to the onset of the first psychotic episode. Individuals in this phase usually present with relatively mild positive symptoms and functional decline (Klosterkötter et al., 2008). The ‘psychotic’ phase begins with the first psychotic episode and involves significant worsening of psychotic symptoms over a short period. This phase usually occurs between the ages of 15 and 45 (Gross, 1997). This is followed by the ‘stable’ phase, which is characterized by negative symptoms, social and cognitive deficits, and marked initial functional decline, but lessened positive symptom severity (Tandon et al., 2009).

1.1.3 Prevalence and Disease Burden

SCZ has a prevalence of ~1% of the population worldwide (Jablensky, 1995; Goeree et
The disorder possesses a substantial social, economic and personal burden (Knapp et al., 2004). The mortality gap between SCZ patients and healthy individuals is estimated to be between 15-25 years based on large population-cohort studies (Tiihonen et al., 2009) (Parks et al., 2006). SCZ patients have also been observed to have poorer outcomes compared to patients with other psychiatric disorders, including depression, mania, bipolar and other disorders (Winokur and Tsuang, 1975; Westermeyer et al., 1991). Poorer outcomes have been largely attributed to disorder’s negative and cognitive symptoms (Heinrichs and Buchanan, 1988; Milev et al., 2005; Rajji et al., 2009), such as amotivation and impaired social cognition (Medalia and Saperstein, 2011; Schmidt et al., 2011).

The illness has also been shown to bear a tremendous economic cost. In 2004, when accounting for days of productivity lost and healthcare costs, the total economic burden of SCZ in Canada was estimated to be ~$7 billion CDN (Goeree et al., 2005). The majority of these costs could be attributed to around ~30% of SCZ patients that are treatment-resistant (TRS) (Kennedy et al., 2014). SCZ also results in a significant burden for caregivers, since around a quarter of patients live with at least one relative following their diagnosis (Torrey et al. 2013).

1.1.4 Etiology

The specific cause of SCZ has yet to be identified. However, several main hypotheses have been proposed for its etiology; these focus on major neurotransmitter systems, including dopamine (DA), serotonin, and glutamate. Here, we will focus on the DA hypothesis, since it is the best supported by evidence from the literature.

The DA hypothesis was first described by Carlsson and Lindvist (1963). It postulates that SCZ symptoms result from a deficit in dopaminergic signaling in cortical regions and an excess of signaling in subcortical regions. The cortical deficit involves hypostimulation of D1 receptors, giving rise to negative and cognitive symptoms. On the other hand, the subcortical excess of DA activity results in hyperstimulation of D2 receptors, leading to
positive symptoms (Carlsson and Lindqvist, 1963; Seeman et al., 1976; Kebabian and Calne, 1979; Davis et al., 1991). The hypothesis is substantiated by findings that drugs that enhance DA signaling can induce positive symptoms similar to those experienced by SCZ patients. It is also supported by studies observing that all antipsychotics bind to D2 receptors and that their efficacy depends upon their affinity for the D2 receptor (Seeman et al., 1976). In addition, using positron emission tomography (PET) technology and radiolabelled D2-ligands, a minimum of 60-65% D2 binding was found to be required for antipsychotics’ therapeutic effect (Kapur and Mamo, 2003). This provided further evidence for the D2 receptor involvement in SCZ.

An updated version of the DA hypothesis of SCZ was recently proposed, consisting of four postulates: (1) there is a ‘final common pathway’ to psychosis, through which multiple ‘hits’ can contribute in an additive manner to DA system dysregulation; (2) DA dysregulation occurs predominantly at the presynaptic rather than at the post-synaptic dopaminergic level; (3) dysregulation of the DA system is not implicated in SCZ, but rather in psychosis; and (4) DA dysregulation influences the way in which stimuli are appraised through a process of ‘aberrant salience’ in which innocuous stimuli are perceived as salient in an unusual fashion (Howes and Kapur, 2009). However, further evidence is required to test the validity of this updated hypothesis.

1.1.5 Environmental Risk factors

While the causes of SCZ remain unknown, the disorder has been shown to have a number of environmental and genetic risk factors. Here, we provide an overview of the factors that have been consistently replicated.

A number of environmental risk factors have been consistently shown to be associated with SCZ. These include early-life biological risks such as male gender, birth during the winter months, advanced paternal age, pregnancy or birth complications, childhood-trauma, and prenatal stress, nutrition, and infection (Hare and Moran, 1981; Mednick et al., 1988; Torrey et al., 1988; O'callaghan et al., 1991; Hultman et al., 1997; Verdoux et
al., 1997b; Verdoux et al., 1997a; Mortensen et al., 1999; Brown et al., 2000; Malaspina et al., 2001; Brown and Susser, 2002; Cannon et al., 2002; Sipos et al., 2004; St Clair et al., 2005; Saha et al., 2006). Other risk factors consist of geographical factors, such as place of birth and upbringing, and migration. Specifically, growing up in urban regions and recent migration were associated with increased risk compared to rural areas (Lewis et al. 1992; Takei et al. 1992; Mortensen et al. 1999; Fearon et al. 2006; Bresnahan et al. 2007).

Later-life biological risk factors primarily center around the abuse of drugs such as amphetamines, methamphetamines, and cannabis (Andréasson et al., 1987; Chen et al., 2003). These drugs have been found to induce psychotic symptoms, which tend to be longer-lasting and more severe in individuals with a family history of SCZ. Interestingly, the effects of cannabis use on risk for psychosis were found to be modulated by the functional polymorphism, Val158Met, of the gene encoding catechol-O-methyltransferase (COMT) (Caspi et al., 2005). This enzyme plays a critical role in regulating DA levels in the PFC, and individuals with the minor allele (Met) have reduced enzymatic activity. Cannabis use does not increase risk of psychosis if the individual has the Met/Met genotype (Caspi et al., 2005), suggesting a strong gene-environment interaction.

1.1.6 Genetics

SCZ has also long been known to have a substantial genetic component. Studies of monozygotic twins revealed ~50% concordance for the illness, compared to ~17% in dizygotic twins (McGue and Gottesman, 1991; Cardno et al., 1999). Based on twin and family studies, the heritability of SCZ is estimated to be ~80% (Farmer et al., 1987; Sullivan et al., 2003), making genetic predisposition the greatest risk factor for SCZ. The methodology for twin and family studies has been reviewed extensively by Neale and Cardon (1992).
In turn, numerous linkage studies of SCZ have been conducted to identify specific regions of the genome implicated in risk for the disorder. These studies examine the segregation of alleles from individuals with SCZ to their progeny (Riley and McGuffin, 2000). While these studies have generally yielded mixed results, some replicated findings exist mapping SCZ risk to multiple different chromosomes. These have been reviewed in great detail elsewhere (Riley and McGuffin, 2000). Several replicated chromosomal regions include 6p24-22 and 1q21-22. These contain the genes encoding dystrobrevin binding protein 1 (DTNBP1) (involved in glutamatergic signaling) (Straub et al., 2002; Talbot et al., 2004) and disrupted in SCZ 1 (DISC1) (involved in cytoskeletal regulation) (St Clair et al., 1990) respectively.

Genetic association studies have also been performed to examine the involvement of specific genetic loci in SCZ at the population level. These studies have implicated a number of DA system genes in SCZ risk, including DA D2 (DRD2) and D3 (DRD3) receptors (Nanko et al., 1994; Mazia et al., 1995). Specifically, the DRD3 missense mutation, Ser311Cys, has been linked to SCZ in a number of meta-analyses (Glatt et al., 2003; Glatt and Jönsson, 2006). Evidence also exists implicating other DRD2 locus genetic variants including an insertion/deletion mutation -141C Ins/Del, Taq1A, and Taq1B in SCZ (Arinami et al., 1997; Breen et al., 1999; Dubertret et al., 2001; Dubertret et al., 2004). These findings suggest that the DA system is critical to SCZ’s etiology, providing support for the DA hypothesis described earlier.

Interestingly, the genes implicated in SCZ risk have also been linked to other neuropsychiatric disorders such as bipolar disorder (BD), major depressive disorder (MDD), attention deficit hyperactivity disorder (ADHD), and even Tourette’s syndrome (TS) (Diaz-Anzaldua et al., 2004; Laursen et al., 2007; Carroll and Owen, 2009). Important genes implicated are mostly from the dopaminergic, serotonergic, and glutamatergic systems.

The advent of genome-wide association studies (GWAS) provided the ability to efficiently examine large numbers of SNPs (on the order of millions) across the genome.
GWAS have provided further evidence supporting genetic overlap between SCZ and other psychiatric illnesses (Purcell et al., 2009; Huang et al., 2010). Notably, the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC) recently published the largest GWAS case-control study of SCZ (36,989 cases, 113,075 controls) (Ripke et al., 2014). The study identified 108 genetic loci implicated in SCZ susceptibility, encompassing 128 independent associations that met or exceeded the threshold for genome-wide significance ($p<5\times10^{-8}$). Importantly, the findings provided further support for the roles of the DA and glutamate systems in SCZ etiology, observing significant associations for previously implicated genes such as $DRD2$.

1.1.7 Treatments

Antipsychotic medications constitute the most effective and commonly used treatments for SCZ. The early 1950’s marked the discovery of the first antipsychotic, chlorpromazine, representing a major breakthrough in the treatment of a disorder for which no other therapies were effective (Miyamoto et al., 2012). This was followed by the discovery of clozapine (CLZ) in 1958 and multiple others since then.

Other than antipsychotic therapy, very few other treatments are currently employed. Of these treatments, perhaps the most common is electroconvulsive therapy (ECT). A randomized controlled trial (RCT) and recent meta-analysis revealed that ECT was an effective augmentative treatment for CLZ-resistant individuals (Petrides et al., 2015; Lally et al., 2016). Based on these findings, ECT is generally only used in cases in which all other antipsychotic therapy has failed. Thus, here we focus on antipsychotic therapies.

Efficacy

While antipsychotics have the highest efficacy for treating SCZ, response rates are $\sim$50-70% (Leucht et al., 2013b). Antipsychotics are known to be effective at treating positive symptoms, but less so for negative and cognitive symptoms (Leucht et al., 2009). A subset of $\sim$30% of patients are non-responsive to antipsychotic treatment and are
classified as ‘treatment-resistant’ (Meltzer, 1997). For these patients, the antipsychotic CLZ has been found to be the most effective (Kane et al., 1988a).

Typical vs. Atypical

Antipsychotics have traditionally been categorized into two main groups, typical (‘first-generation’) and atypical (‘second-generation’). While the dividing line remains ambiguous at times, the generally accepted difference between these two groups is their propensity to lead to extra-pyramidal symptoms (EPS) (Sharif et al., 2007). At therapeutic doses, typical antipsychotics possess higher risk of EPS compared to atypical ones (Leucht et al., 2009). Several different hypotheses have been proposed for this based on underlying differences in mechanisms between the two groups. Remington and Kapur (1999) observed that >80% antipsychotic occupancy of D2 receptors in striatal regions led to a substantial increase in EPS risk, while 65-70% occupancy was required for therapeutic effect. Based on these findings, Kapur and Seeman (2001) proposed a ‘fast-off’ D2 hypothesis for atypicality. They suggested that atypical antipsychotics have a higher D2 receptor dissociation constant (K_{off}) relative to typical ones, leading to lower occupancy levels and thus, decreased risk for EPS. In contrast, another theory attributed atypical antipsychotics’ lower EPS risk to a high ratio of serotonin 2A receptor (5-HT2A)-D2 antagonism (Janssen et al., 1988; Meltzer et al., 1989a). This hypothesis postulates that atypical antipsychotics’ stronger antagonism at 5-HT2A leads to increased DA signaling in the nigrostriatal pathway, lowering EPS risk.

However, exceptions to both theories remain. All antipsychotics have been shown to exert their therapeutic effects, at least in part, through binding to D2 receptors (Remington and Kapur, 1999). However, atypical antipsychotics such as CLZ and quetiapine are able to achieve therapeutic effects without meeting the 65-70% occupancy threshold described earlier (Farde and Nordström, 1992; Kapur et al., 2000). In addition, other atypical antipsychotics including risperidone, olanzapine (OLZ), ziprasidone, and sertindole have relatively lower D2 receptor K_{off} values, and thus can be seen as exceptions to the ‘fast-off D2’ hypothesis. The 5-HT2A-D2 antagonism ratio hypothesis
also has a number of exceptions. Notably, typical antipsychotics chlorpromazaine and loxapine have relatively high ratios of 5-HT2A-D2 antagonism despite having relatively high risk for EPS.

Overall, mechanisms of action vary widely particularly amongst atypical antipsychotics and the need for further subgrouping has been suggested (Figure 1.1) (Keltner and Johnson, 2002). For instance, drugs like aripiprazole act as partial D2 agonists rather than antagonists, dampening DA activity when it is high, and increasing activity when low (Kapur and Mamo, 2003). Others like amisulpride are combined D2/D3 receptor antagonists, binding preferentially to limbic system D2 receptors rather than those in the striatum (Table 1.1) (Scatton et al., 1997). Moreover, CLZ and OLZ are relatively unique in that they bind to a diverse range of receptor types, spanning the DA, serotonin, glutamate, and other systems (Table 1.2, 1.3, Figure 1.1) (Bymaster et al., 1996). The specific mechanism for CLZ is described in greater detail in the next section.

Table 1.1 – DA Receptor Binding Affinities and Clinically Effectives Doses of Antipsychotic Medications

<table>
<thead>
<tr>
<th>Drug</th>
<th>clinically effective dose (mg)</th>
<th>$K_i$ Values (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$K_i$ short</td>
</tr>
<tr>
<td>Amisulpride</td>
<td>400–800</td>
<td>1.3</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>5–30</td>
<td>387</td>
</tr>
<tr>
<td>Benperidol</td>
<td>12–16</td>
<td>0.027</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>300–900</td>
<td>112</td>
</tr>
<tr>
<td>Chlorprothixene</td>
<td>50–400</td>
<td>3.3</td>
</tr>
<tr>
<td>Clozapine</td>
<td>300–900</td>
<td>189</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>2–15</td>
<td>24</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>2–15</td>
<td>83</td>
</tr>
<tr>
<td>Loxapine</td>
<td>25–100</td>
<td>54</td>
</tr>
<tr>
<td>Mesoridazine</td>
<td>100–400</td>
<td>4.3</td>
</tr>
<tr>
<td>Molindone</td>
<td>20–100</td>
<td>17.8</td>
</tr>
</tbody>
</table>
Adapted with permission from Richtand NM et al. (2007) Dopamine and serotonin receptor binding and antipsychotic efficacy. Neuropsychopharmacology 32:1715-1726. (Richtand et al., 2007)

Table 1.2 – Serotonin Receptor Binding Affinities of Antipsychotics

<table>
<thead>
<tr>
<th>Drug</th>
<th>5-HT&lt;sub&gt;1A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1B&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1D&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;1E&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2B&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2C&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;3&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;5A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;6&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;7&lt;/sub&gt;</th>
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</thead>
<tbody>
<tr>
<td>Aripiprazole</td>
<td>5.6</td>
<td>833</td>
<td>63</td>
<td>8000</td>
<td>17.5</td>
<td>0.36</td>
<td>22.4</td>
<td>628</td>
<td>1241</td>
<td>574</td>
<td>10</td>
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<tr>
<td>Chlorpromazine</td>
<td>3115</td>
<td>1489</td>
<td>452</td>
<td>344</td>
<td>3.32</td>
<td>15.55</td>
<td>977</td>
<td>118</td>
<td>12</td>
<td>21</td>
<td></td>
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<tr>
<td>Chlorprothixene</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>105</td>
<td>398</td>
<td>2132</td>
<td>966</td>
<td>130</td>
<td>9.15</td>
<td>7.38</td>
<td>14.9</td>
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<td>334</td>
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<td>21</td>
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<td>145</td>
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<tr>
<td>Haloperidol</td>
<td>1202</td>
<td>165</td>
<td>7606</td>
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<td>&gt;5000</td>
<td>118.6</td>
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<td>5580</td>
<td>&gt;10 000</td>
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<td>380</td>
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<td></td>
<td></td>
<td>4653</td>
<td></td>
<td>10 000</td>
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<tr>
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<td>2240</td>
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<td>3120</td>
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<td>364</td>
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<td>1356</td>
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<td>291</td>
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Adapted with permission from Richtand NM et al. (2007) Dopamine and serotonin receptor binding and antipsychotic efficacy. Neuropsychopharmacology 32:1715-1726. (Richtand et al., 2007)
<table>
<thead>
<tr>
<th>Antipsychotic</th>
<th>Classification</th>
<th>Criteria for atypical antipsychotics[^2]</th>
<th>D₂</th>
<th>D₂ dissociation (Kᵦ[^b])</th>
<th>5-HT₂ₐ</th>
<th>5-HT₂ₐ/5-HT₁ₐ</th>
<th>5-HT₂₀</th>
<th>α₂</th>
<th>α₁</th>
<th>H₁</th>
<th>M₃</th>
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<td>Amlodipine</td>
<td>D₂/D₃ antagonist</td>
<td>0EPS (7), 0PRL</td>
<td>1.3</td>
<td>0.02</td>
<td>2000</td>
<td>1588</td>
<td>&lt;10000</td>
<td>1600</td>
<td>7100</td>
<td>&lt;10000</td>
<td>&lt;10000</td>
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<tr>
<td>Aripiprazole</td>
<td>Partial dopamine receptor agonist[^c]</td>
<td>0EPS, 0PRL</td>
<td>2.3</td>
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<td>4160</td>
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<td>Chlorpromazine</td>
<td>Conventional</td>
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<td>0.012</td>
<td>12</td>
<td>1.8</td>
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</tr>
<tr>
<td>Iloperidone</td>
<td>SDA</td>
<td>0EPS</td>
<td>0.5</td>
<td>0.59</td>
<td>5.6</td>
<td>0.19</td>
<td>93</td>
<td>146</td>
<td>162</td>
<td>0.31</td>
<td>12.3</td>
</tr>
<tr>
<td>Loxapine</td>
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<td>NA</td>
<td>22</td>
<td>0.444</td>
<td>4</td>
<td>0.18</td>
<td>2900</td>
<td>17</td>
<td>2400</td>
<td>28</td>
<td>2.8</td>
</tr>
<tr>
<td>Molperone</td>
<td>Conventional[^d]</td>
<td>0EPS, 0PRL</td>
<td>143</td>
<td>2.27</td>
<td>102</td>
<td>0.71</td>
<td>2200</td>
<td>1342</td>
<td>150</td>
<td>180</td>
<td>580</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>MARTA</td>
<td>mEPS, mPRL</td>
<td>31</td>
<td>0.039</td>
<td>3.5</td>
<td>0.11</td>
<td>2720</td>
<td>14</td>
<td>314</td>
<td>109</td>
<td>0.65</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>MARTA</td>
<td>0EPS, 0PRL</td>
<td>700</td>
<td>3.013</td>
<td>96</td>
<td>0.13</td>
<td>320</td>
<td>1184</td>
<td>3630</td>
<td>22</td>
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</tr>
<tr>
<td>Remoxipride</td>
<td>D₂/D₃ antagonist</td>
<td>0EPS</td>
<td>51</td>
<td>1.23</td>
<td>&lt;10000</td>
<td>&lt;500</td>
<td>&lt;10000</td>
<td>5500</td>
<td>&lt;10000</td>
<td>&lt;10000</td>
<td>&lt;10000</td>
</tr>
<tr>
<td>Risperidone</td>
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<td>mEPS</td>
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<td>420</td>
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<td>151</td>
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<td>27</td>
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<tr>
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<td>SDA</td>
<td>0EPS, 0PRL</td>
<td>7</td>
<td>0.11</td>
<td>0.35</td>
<td>0.06</td>
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<td>640</td>
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<tr>
<td>Sulpiride</td>
<td>D₂/D₃ antagonist</td>
<td>0EPS, 0PRL</td>
<td>0.21-78</td>
<td>0.003</td>
<td>&lt;10000</td>
<td>&lt;500</td>
<td>&lt;10000</td>
<td>4300</td>
<td>&lt;10000</td>
<td>&lt;10000</td>
<td>&lt;10000</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>Conventional</td>
<td>NA</td>
<td>8.3</td>
<td>0.14</td>
<td>60</td>
<td>7.2</td>
<td>NA</td>
<td>46</td>
<td>453</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>SDA</td>
<td>0EPS</td>
<td>4.6</td>
<td>0.073</td>
<td>1.4</td>
<td>0.30</td>
<td>112</td>
<td>4.1</td>
<td>160</td>
<td>18</td>
<td>130</td>
</tr>
</tbody>
</table>

[^a]: Affinity constants (Kᵦ) for individual receptors involved in antipsychotic action are reviewed by Roth et al.[^2] and National Institute of Mental Health databases (http://pdp.cwru.edu/pdp.asp).

[^b]: Reflects the rate of unbinding from D₂ receptors.[^3]

[^c]: Also has 5-HT₂ₐ receptor antagonist properties.

[^d]: Molperone is classified as a conventional antipsychotic but its low affinity for D₂ receptors gives it a clinical profile similar to that of atypical agents.

0EPS = none or low induction of EPS; 0PRL = no prolactin elevation; EPS = extrapyramidal syndrome; MARTA = multi-acting receptor-targeted antipsychotics; mEPS = moderate induction of EPS; mPRL = moderate prolactin elevation; NA = not applicable; SDA = serotonin-dopamine antagonists.
Little evidence suggests that the two antipsychotic groups differ substantially in their clinical efficacy. A landmark meta-analysis observed that only four atypical antipsychotics (CLZ, OLZ, risperidone, amisulpride) had greater efficacy than typical antipsychotics for positive symptoms, and that the differences were generally small (Leucht et al., 2009). Nevertheless, CLZ has been shown to be the most effective for TRS (Kane et al., 1988a; Leucht et al., 2013b). The next chapter will delve further into CLZ’s unique efficacy, usage, and mechanism.

Figure 1.1 – Receptor affinity ratios for antipsychotic drugs

1.2 Clozapine (CLZ)

1.2.1 Efficacy

As described earlier, CLZ is an atypical antipsychotic that has been shown to have the highest efficacy for treating TRS, with 30-60% of these patients improving on this medication (Kane et al., 1988a; Meltzer et al., 1989b; Leucht et al., 2009). A meta-analysis recently observed that CLZ might also have the highest efficacy of all antipsychotics for the treatment of all types of SCZ (Leucht et al., 2013b). However, this finding could be due to the over-representation of TRS individuals in the RCTs included in the meta-analysis. The antipsychotic is effective at treating positive symptoms, but as with other atypical antipsychotics, its benefit for negative symptoms is not clear (Tandon et al., 1993; Lieberman et al., 1994). Studies have also yielded mixed results as to its cognitive effects, with results reporting beneficial, detrimental, and non-significant effects (Goldberg et al., 1993; Buchanan et al., 1994; Lindenmayer et al., 1998; Chakos et al., 2001).

Aside from treating the core symptoms of SCZ, CLZ has also been found to reduce levels of aggression and suicidality (Meltzer et al., 2003b; Spivak et al., 2003). It has also been observed to be effective for treating childhood-onset SCZ, which may be the result of substantial overlap between this population and TRS patients (Kasoff et al., 2016).

1.2.2 Side-effects

CLZ has an extremely low rate of EPS or tardive dyskinesia compared to older (‘typical’) antipsychotics. However, it possesses other more severe side-effects that have led to restrictions on its use. One of these side-effects, agranulocytosis, occurs in up to 1% of CLZ-treated patients and can be life-threatening. It is characterized by a significant decrease in absolute neutrophil count, resulting in immune system suppression and consequently higher risk of infections (Nielsen et al., 2009).
CLZ has also been observed to induce severe weight-gain (Henderson et al., 2000). This side-effect has been noted as a key reason for non-adherence to treatment (Lieberman et al., 2005a), with average weight gains as high as 13kg (Henderson et al., 2005). Severe weight gain leads to increased risk for diabetes and heart disease. Metformin, a drug for type 2 diabetes, and lifestyle interventions have been found to reduce the severity of this side-effect (Klein et al., 2006; Wu et al., 2008). Other serious side-effects include gastrointestinal hypomotility, ileus, seizures, myocarditis, and venous thromboembolism (Devinsky et al., 1991; Pacia and Devinsky, 1994; Erickson et al., 1995; Kilian et al., 1999; Hägg et al., 2001; Palmer et al., 2007; Rondla and Crane, 2007).

1.2.3 Regulations

The regulations governing CLZ use differ worldwide (Nielsen et al., 2016). Currently, in North America, CLZ is approved by the Food and Drug Administration (FDA) for treatment of TRS and for reducing suicide risk. Monitoring of neutrophil count is required weekly for the first six months of treatment, biweekly for the second six months, and once every four weeks after a year of treatment. CLZ treatment may be required to be either interrupted or discontinued depending on the severity of major side-effects, including agranulocytosis and myocarditis (Freudenreich et al., 2016).

1.2.4 Under-utilization

Given that TRS comprises ~30% of the SCZ population and that CLZ is the most effective treatment for TRS, CLZ prescription rates should in principle be ~30%. However, studies of prescription rates in multiple countries have found that the actual prescription rate is much lower than this (2-4% in the US, 10.2% in Denmark, and 4% in Malaysia) (Xiang et al., 2011; Nielsen et al., 2012). In Canada, CLZ recommendation rates appear to be on the rise, increasing 48% from 2005 to 2009 (Pringsheim et al.,
However, a recent study of hospitals in Quebec observed that CLZ was prescribed in only 6.7% of the 29,155 SCZ patients studied (Latimer et al., 2013).

CLZ use is estimated to cost ~$5500 per year, which is ~11 times more than most typical antipsychotics (Meltzer and Cola, 1994). Part of the reason for the higher cost is due to the need for blood monitoring (~$1000/year). However, recent studies have suggested that the subsequent reduction in hospital costs counterbalances the higher prices. Specifically, an RCT comparing CLZ’s cost-effectiveness to haloperidol found that the two drugs had similar overall costs, but that CLZ had greater efficacy and less side-effects. The trial also revealed that the CLZ use was highly cost-effective for patients with frequent hospital usage prior to the trial (Rosenheck et al., 1999). Together, these results suggest that CLZ is under-utilized.

1.2.5 Mechanism of action

Pharmacodynamics

The ‘pharmacodynamics’ of CLZ treatment refers to the drug’s biochemical and physiological effects on the body. As alluded to earlier, CLZ has been categorized as a multi-acting receptor-targeted antipsychotic (MARTA), because of its diverse receptor binding profile (Bymaster et al., 1996). CLZ binds with moderate to strong affinity to receptors from the dopaminergic, serotonergic, gamma-aminobutyric acid (GABA)-ergic, muscarinic, adrenergic, and histaminergic systems (Figure 1.1; Table 1.3). Notably, CLZ has relatively low D2 receptor affinity compared to other antipsychotics. The drug binds in a ‘fast-off’ manner to D2 receptors, which is hypothesized to explain its lower rates of EPS compared to higher-D2 affinity antipsychotics (Kapur et al., 2002). CLZ’s high ratio of 5-HT2A-D2 affinity may also contribute to its lower EPS risk and ‘atypical’ status (Figure 1.1) (Meltzer, 1989).

Of the DA receptors, CLZ has the highest affinity for the D4 receptor—previously hypothesized to underlie the drug’s relative efficacy for TRS (Van Tol et al., 1991). CLZ
also acts as a strong antagonist at the serotonin 2C (5-HT2C) receptor. Given that serotonin inhibits DA neuronal activity in the nucleus accumbens, this antagonistic effect has been hypothesized to increase DA levels in prefrontal regions, underlying CLZ’s purported amelioration of cognitive and negative symptoms (Kuroki et al., 1999; Di Matteo et al., 2002). Lastly, CLZ has been found to act as a partial serotonin 1A (5-HT1A) receptor agonist, which has been cited as a potential reason for its occasionally-observed beneficial effects for anxiety, depression, and negative symptoms (Coward, 1992; Newman-Tancredi et al., 1996).

**Pharmacokinetics**

The term ‘pharmacokinetics’ refers to the pathways through which the body metabolizes the drug. CLZ is primarily metabolized in the liver by cytochrome P450 isozyme 1A2 (CYP1A2), while other CYP450 isozymes 2D6 and 3A4 play secondary roles (Eiermann et al., 1997). The drug’s primary metabolite is N-desmethyl-CLZ (NDMC) (Linnet and Olesen, 1997).

**1.3 Pharmacogenetics of CLZ response**

CLZ pharmacogenetic studies investigate how genetic variation relates to variability in treatment outcome for patients taking the drug. The ultimate goal of these studies is to develop genetic tests to predict CLZ dosage, response, and risk for side-effects in individual patients. These tests would help reduce concerns surrounding the use of CLZ and could enable a larger proportion of patients to receive a more beneficial treatment. To this end, numerous pharmacogenetic studies have been conducted over the past two decades.

**1.3.1 Variability in drug response and social and economic consequences**
The discontinuation rate for first-line antipsychotics is estimated to be ~70% within one year of treatment (Kahn et al., 2008). Amongst patients that are resistant to treatment by first-line antipsychotics, between 30-60% respond to CLZ (Kane et al., 1988a; Meltzer et al., 1989b). A recent systematic review found that patients who were intolerant or non-responsive to treatment have ~20% lower quality of life compared to responders (Kennedy et al., 2014). With each failed trial of antipsychotic, these patients’ susceptibility to experiencing serious adverse side-effects also rises linearly, starting with ~4% for the first trial. Moreover, annual costs for patients that do not respond to treatment are estimated to be 3-11 times higher than those for responders, amounting to $34 billion in direct medical expenses in the US (Kennedy et al., 2014). The actual cost is likely much higher if indirect costs such as days of productivity lost are taken into account.

Thus, predictors for response to antipsychotic therapy could provide immense benefit to patients by (1) identifying medications with highest efficacy and lowest risk for side-effects, leading to optimal treatment outcomes and (2) screening out drugs to which the patients will not respond, thereby enabling them to forego failed trials, loss of valuable treatment time, unnecessary treatment expenses and adverse side-effects. In the case of CLZ, these predictors would be especially valuable for both physicians and patients weighing the costs and benefits of its use in light of its potentially life-threatening side effects (agranulocytosis, ileus etc.).

1.3.2 Predictors of response

A number of demographic variables have been consistently associated with response to antipsychotics. For instance, male gender and a family history of mental illness have been linked to poorer response (Robinson et al., 1999; Tabatabaei et al., 2008). Ethnicity has also more recently been linked to response as well as the drug dosage required for therapeutic effect (Emsley et al., 2002; Bigos et al., 2008). The latter is likely related to genetic differences in metabolic enzyme activity (Parkinson et al., 2004; McGraw and Waller, 2012).
Clinical variables have also been shown to predict treatment outcome. Longer duration of untreated psychosis (DUP) has been linked to poorer response (Perkins et al., 2004; Chiliza et al., 2012). Earlier age at onset, poorer premorbid level of functioning, and lower severity of symptomatology prior to treatment have also been correlated with decreased rates of response. There is also preliminary evidence implicating other variables such as illness characteristics (e.g. acute, chronic) and weight gained during treatment to response (Lieberman et al., 1993; Jalenques et al., 1996; Robinson et al., 1999; Hermes et al., 2011). These associations require further examination for confirmation.

Environmental factors such as substance abuse and diet have also been found to predict response (Green et al., 2004). In particular, an observational study noted that cannabis use amongst SCZ patients was associated with increased likelihood of hospital admission and having multiple trials of antipsychotic drugs (Patel et al., 2016). Smoking and use of multiple medications may also increase the risk of non-response. Smoking is known to induce CYP1A2 activity, potentially preventing a therapeutic dose from being reached. Conversely, taking additional medications that share the same metabolic pathway as the antipsychotic may lead to decreased rate of metabolism, resulting in more side-effects and reduced efficacy (Nakajima et al., 1999; Spina et al., 2003; Spina and De Leon, 2007).

Risk factors for TRS have also been examined in a recent Danish population-based cohort study (n=8,624) (Wimberley et al., 2016). The investigation confirmed prior findings that younger age and the use of multiple psychotropic medications were associated with TRS, while uncovering associations for other variables including primary education level, upbringing in less-urban areas, paranoid subtype and comorbid personality disorders. Overall, the study suggested that the predictive factors for TRS might differ from those for other subtypes of SCZ. This requires further examination in independent population-based samples.
A genetic component to antipsychotic response has also been observed. Prior studies have found concordance in response to CLZ and OLZ in monozygotic twins with SCZ, and to amisulpride in siblings with SCZ (Vojvoda et al., 1996; Mata et al., 2001; Hoyer et al., 2010). Given that non-genetic factors have been unsuccessful at predicting response with levels of clinical utility, a great deal of expectation and hope has been placed on genetics. This has led to the birth of the field of ‘pharmacogenetics’, which is examined in greater detail in the next section.

1.3.3 Prior studies

The majority of pharmacogenetic studies have focused on major neurotransmitter systems targeted by CLZ. These include the dopaminergic, serotonergic, and glutamatergic systems. Recent studies have also explored genes encoding enzymes involved in CLZ’s metabolism, as well as ones from other systems. Here, we review the best replicated as well as the most recent findings from each system.

Dopamine

CLZ exerts its therapeutic effects, in part, through antagonism at DA receptors. Thus, multiple studies have examined DA receptors (D1-D5) gene variants in relation to CLZ response, yielding mixed results. The DA D4 receptor (DRD4) gene has been frequently studied due to CLZ’s particularly strong affinity for this receptor. Studies have focused on a 48bp variable-number tandem repeat (VNTR) in the coding region of the gene. While significant associations between the 5-repeat and 7-repeat alleles have been observed with response in Caucasian and Han Chinese samples (Cohen et al., 1999; Zhao et al., 2005), multiple other studies did not detect these associations (Rao et al., 1994; Shaikh et al., 1995; Rietschel et al., 1996; Kohn et al., 1997; Hwang et al., 2012). Preliminary evidence also implicates other DRD4 polymorphisms across the gene including a 120bp insertion/deletion and a G-nucleotide repeat in response (Hwang et al., 2012), but replication in independent samples is required for confirmation.
The majority of studies of the D3 receptor have focused on rs6280 (Ser9Gly), a functional missense mutation in the gene’s first exon. This variant alters DA activity in D3-signaling pathways and is associated with differential binding affinity for D3 selective ligands (Lundstrom and Turpin, 1996). Two meta-analyses of these studies observed statistical trends for association with response, suggesting a small effect (Hwang et al., 2010; Gressier et al., 2015).

In addition, D1 (DRD1) and D2 receptor (DRD2) genetic variants have also been implicated in CLZ response, however with mixed results (Arranz et al., 1998b; Potkin et al., 2003; Reynolds et al., 2005; Hwang et al., 2007). An insertion/deletion in the promoter region was found to be associated with response (rs1799732) (-141C Ins/Del) (Malhotra et al., 1999), but a recent meta-analysis did not find an association (Gressier et al., 2015). Preliminary evidence has also implicated DRD2 variants Taq1A (rs1800497), Taq1B (rs1079597), and rs1125394 in response (Hwang et al., 2005a; Hwang et al., 2006). However, these findings require replication in independent samples. No significant associations have been reported for D5 (DRD5) receptor gene variation (Hwang et al., 2012).

The enzyme catechol-O-methyltransferase (COMT) regulates DA levels prefrontally, and to a lesser extent, subcortically. Variation in the COMT gene has thus been studied in relation to CLZ response. A functional polymorphism, Val158Met (rs4680), has been associated with improvement in negative and cognitive symptoms in two Caucasian samples (Woodward et al., 2007; Bosia et al., 2015). This finding is consistent with previous findings implicating Val158Met in response to other antipsychotics (Illi et al., 2003), but other studies were unable to replicate these results (Tybura et al., 2012). Given the limited number of studies of this gene in relation to CLZ response, findings needs to be confirmed in independent CLZ-treated samples.
**Serotonin**

CLZ’s antipsychotic effect is suggested to be due in part to its relatively high ratio of 5-HT2A-D2 receptor blockade as well as its affinity for 5-HT1A, 5-HT2C, serotonin receptor 6 (5-HT6), and serotonin receptor 7 (5-HT7) receptors (Meltzer, 1989). This has led many studies to examine serotonin receptor genes in relation to CLZ response (Malhotra et al., 1996; Arranz et al., 1998b; Masellis et al., 1998; Lee et al., 2012a; Rajkumar et al., 2012).

Two meta-analyses have found significant associations for serotonin receptor 2A (5-HT2A) gene variants rs6313 (T102C) and rs6314 (His452Tyr) (Gressier et al., 2015) (Table 1.4). rs6313 is a synonymous mutation, while rs6314 is a missense mutation, leading to histidine-to-tyrosine substitution. The Tyr form has been associated with altered 5-HT2A activity patterns (Ozaki et al., 1997) as well as lower rates of 5-HT2A dimerization with the D2 receptor (Łukasiewicz et al., 2011), potentially mediating differences in CLZ’s therapeutic effects.

Serotonin receptor 3A (5-HT3A) gene variation has also been studied in relation to CLZ response, given the antipsychotic’s moderate affinity for this receptor. Most studies have focused on a variant in the 5’-untranslated region (5’-UTR) of the gene, rs1062613 (C178T), which gives rise to a proline-to-serine substitution and may alter gene expression (Arranz et al., 2000; Gutiérrez et al., 2002; Souza et al., 2010c; Lee et al., 2012a; Rajkumar et al., 2012). A recent meta-analysis found that T-allele was associated with greater response (Gressier et al., 2015).

Other serotonin system genes have been implicated in response with mixed results, and require further examination in future studies. These include 5-HT1A, 5-HT2C, the serotonin transporter gene (5-HTT), and 5-HT6 (Table 1.5) (Yu et al., 1999; Arranz et al., 2000; Schumacher et al., 2000; Tsai et al., 2000; Kohlrausch et al., 2010; Lee et al., 2012a; Bosia et al., 2015).
Glutamate

Glutamatergic dysfunction has been suggested as one underlying mechanism for SCZ (Olney and Farber, 1995). This is primarily based upon the ability of N-methyl-D-aspartate receptor (NMDAR) antagonists, ketamine and phencyclidine (PCP), to induce SCZ-like positive and negative symptoms in patients (Krystal et al., 1994; Lahti et al., 1995). CLZ was observed to prevent effects of PCP and inhibit metabolic activation of ketamine in the brain, suggesting that it targets the glutamate system (Duncan et al., 2000). Thus, glutamate system gene variation has been examined in relation to CLZ response.

The only significant associations observed have been for the gene encoding neurexin-1 (NRXN1), which is responsible for regulating NMDAR activity (Table 1.5). A putatively functional variant rs1045881 was associated with CLZ response in our own sample, while a trend was observed for rs12467557 (Souza et al., 2010b; Lett et al., 2011). No significant associations were reported for other glutamate system genes encoding NMDA ionotropic receptor 1 (GRIN1), 2A (GRIN2A), and 2B (GRIN2B) (Hong et al., 2001; Hwang et al., 2011; Taylor et al., 2016). Thus, current genetic findings do not provide much support for the role of the glutamate system in CLZ’s therapeutic effects. However, further study of glutamate system genes is required to confirm this.

Metabolic Pathways

CLZ pharmacokinetic studies have focused on a limited number of genes encoding the enzymes involved in the metabolism of this drug. Most studies have investigated the cytochrome P450 (CYP) liver enzymes—in particular, CYP1A2, the primary enzyme for metabolizing CLZ. Gene variants for CYP1A2 have previously been found to be associated with plasma levels of CLZ, suggesting these variants alter enzymatic activity (Eap et al., 2004; Jaquenoud-Sirot et al., 2008). The CYP1A2 *1F/*1F genotype (rs762551) has been observed to be associated with lower CLZ plasma levels as well as poorer treatment response in two studies (Eap et al., 2004; Balibey et al., 2011) (Table
1.4) In particular, Balibey et al. (2011) found that individuals homozygous for *1F were 2.4 times less likely to respond than patients with one or more wild type alleles (*I). This effect was greater for individuals that smoked; *1F homozygotes that smoked had a 15% lower response rate compared to those that did not. However, these findings were not replicated in two other studies (Lee et al., 2012b; Rajkumar et al., 2012). Thus, metabolic enzyme genes require further investigation to confirm associations with CLZ response.

*Other Systems*

Pharmacogenetic studies have also begun to examine genes from other systems. However, the majority of the associations observed have not been consistently replicated or have only been studied once. Genes with some evidence of implication in response include those encoding for: GDNF family receptor α-2 (GFRA-2), brain-derived neurotrophic factor (BDNF), G-protein β-subunit 3 (GNB3), oxytocin prepropeptide (OXT), norepinephrine transporter (SLC6A2), and ATP-binding cassette transporter 1 (ABCB1) (Table 1.5) (Hong et al., 2003; Müller et al., 2005; Kohlrausch et al., 2008; Souza et al., 2010a; Souza et al., 2010d; Lee et al., 2012a; Zai et al., 2012; Xu et al., 2015).

A variety of genes from other systems targeted by CLZ have also been investigated, but did not yield any significant findings. These include the genes encoding for muscarinic receptor 1 (CHRM1), adrenoreceptor 1A and 2A (ADRA1A, ADRA2A), and histamine receptor 1 (HRH1) (Bolonna et al., 2000; Tsai et al., 2001; Lee et al., 2012a).
Table 1.4 – Replicated associations between genetic polymorphisms and CLZ response

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Author</th>
<th>Ethnicity</th>
<th>Sample Size</th>
<th>p-value</th>
<th>Reported association</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT2A</td>
<td>rs6313</td>
<td>(Gressier et al., 2015)</td>
<td>Various (European/Asian/African-American)</td>
<td>868 (seven studies)</td>
<td>0.02</td>
<td>T-carriers experience greater improvement</td>
</tr>
<tr>
<td></td>
<td>(T102C)</td>
<td>(Arranz et al., 2000)</td>
<td>Caucasian</td>
<td>200</td>
<td>0.001</td>
<td>T with greater improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Arranz et al., 1995)</td>
<td>Caucasian</td>
<td>149</td>
<td>0.001</td>
<td>T with greater improvement</td>
</tr>
<tr>
<td></td>
<td>rs6314</td>
<td>(Gressier et al., 2015)</td>
<td>Various (European/Asian/African-American)</td>
<td>671 (five studies)</td>
<td>0.004</td>
<td>T with less improvement</td>
</tr>
<tr>
<td></td>
<td>(His452Tyr)</td>
<td>(Arranz et al., 2000)</td>
<td>Caucasian</td>
<td>200</td>
<td>0.01</td>
<td>TT with less improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Masellis et al., 1998)</td>
<td>African-American/European/Asian</td>
<td>185</td>
<td>0.01</td>
<td>T with less improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Arranz et al., 1998a)</td>
<td>Caucasian</td>
<td>160 (Sample 1)</td>
<td>0.03</td>
<td>TT with less improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>114 (Sample 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs6311</td>
<td>(Arranz et al., 1996)</td>
<td>Caucasian</td>
<td>153</td>
<td>0.008</td>
<td>TT with less improvement</td>
</tr>
<tr>
<td></td>
<td>(G-1438A)</td>
<td>(Arranz et al., 1998a)</td>
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<td>160 (Sample 1)</td>
<td>0.0006</td>
<td>GG with less improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(Arranz et al., 2000)</td>
<td>Caucasian</td>
<td>200</td>
<td>&lt;0.001</td>
<td>GG with less improvement</td>
</tr>
<tr>
<td>5-HT3A</td>
<td>rs1062613</td>
<td>(Gressier et al., 2015)</td>
<td>Various (European/Asian/African-American)</td>
<td>1026 (four studies)</td>
<td>0.03</td>
<td>C with less improvement</td>
</tr>
<tr>
<td></td>
<td>(T178C)</td>
<td>(Souza et al., Mostly Caucasian)</td>
<td>140</td>
<td>0.04</td>
<td>C with less improvement</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Polymorphism</td>
<td>Ethnicity</td>
<td>N</td>
<td>p-value</td>
<td>Improvement</td>
<td></td>
</tr>
<tr>
<td>----------</td>
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<td>-----</td>
<td>---------</td>
<td>-------------------------------</td>
<td></td>
</tr>
<tr>
<td>SLC6A4</td>
<td>HTTLPR</td>
<td>Indian</td>
<td>101</td>
<td>0.02</td>
<td>T with greater improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Rajkumar et al., 2012)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>200</td>
<td>0.04</td>
<td>Short allele with less</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Arranz et al., 2000)</td>
<td></td>
<td></td>
<td>improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brazilian</td>
<td>116</td>
<td>0.01</td>
<td>Short allele with less</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Kohlrusch et al., 2010)</td>
<td></td>
<td></td>
<td>improvement</td>
<td></td>
</tr>
<tr>
<td>DRD3</td>
<td>rs6280 (Ser9Gly)</td>
<td>Mostly Caucasian</td>
<td>133</td>
<td>0.04</td>
<td>TT with less improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Shaikh et al., 1996)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pakistani</td>
<td>32</td>
<td>0.033</td>
<td>C with greater improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Scharfetter et al., 1999)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Various (European/Asian/African-American)</td>
<td>852 (seven studies)</td>
<td>0.10</td>
<td>T with less improvement</td>
<td></td>
</tr>
<tr>
<td>DRD4</td>
<td>VNTR 48bp</td>
<td>Caucasian</td>
<td>32</td>
<td>0.002</td>
<td>7-repeat with greater</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(Cohen et al., 1999)</td>
<td></td>
<td></td>
<td>improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asian</td>
<td>81</td>
<td>0.05</td>
<td>5/5-repeat with less</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Zhao et al., 2005)</td>
<td></td>
<td></td>
<td>improvement</td>
<td></td>
</tr>
<tr>
<td>GNβ3</td>
<td>rs5443 (C825T)</td>
<td>Caucasian/African-American</td>
<td>89</td>
<td>0.03</td>
<td>CC with greater improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Müller et al., 2005)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brazilian</td>
<td>121</td>
<td>0.021</td>
<td>TT with less improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Kohlrusch et al., 2008)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CYP1A2</td>
<td>rs762551 (*1F)</td>
<td>Caucasian</td>
<td>33</td>
<td>0.01</td>
<td>*1F with less improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Eap et al., 2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs762551 (*1F)</td>
<td>Caucasian</td>
<td>100</td>
<td>0.02</td>
<td>*1F/*1F with less improvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Balibey et al., 2011)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *Denotes a meta-analysis, NS denotes non-significance. Only the positive findings for each polymorphism are shown above.
Table 1.5 – Positive findings for genetic polymorphisms and CLZ response awaiting replication

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Author</th>
<th>Ethnicity</th>
<th>Sample Size</th>
<th>p-value</th>
<th>Reported association</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT2C</td>
<td>rs6318 (Cys23Ser)</td>
<td>(Sodhi et al., 1995)</td>
<td>Caucasian</td>
<td>162</td>
<td>0.005</td>
<td>C with greater improvement</td>
</tr>
<tr>
<td>5-HT6</td>
<td>rs1805054 (T267C)</td>
<td>Yu et al. 1999 (Yu et al., 1999)</td>
<td>Asian</td>
<td>99</td>
<td>0.04</td>
<td>TT with greater improvement</td>
</tr>
<tr>
<td>DRD1</td>
<td>rs265976</td>
<td>(Hwang et al., 2007)</td>
<td>African-American</td>
<td>49</td>
<td>0.033</td>
<td>AA and CC associated with improved response compared to AC</td>
</tr>
<tr>
<td></td>
<td>rs4532 (A-48G)</td>
<td>(Potkin et al., 2003)</td>
<td>Caucasian</td>
<td>13</td>
<td>0.05</td>
<td>GG with greater improvement</td>
</tr>
<tr>
<td>DRD2</td>
<td>rs1799732 (-141C Ins/Del)</td>
<td>(Malhotra et al., 1999)</td>
<td>Caucasian/African-American</td>
<td>72</td>
<td>&lt;0.05</td>
<td>Del with less improvement</td>
</tr>
<tr>
<td></td>
<td>rs1800497 (Taq1A)/rs1079597 (Taq1B)/rs1125394</td>
<td>(Hwang et al., 2005b)</td>
<td>African-American</td>
<td>49</td>
<td>0.004</td>
<td>A/T/C haplotype with greater improvement</td>
</tr>
<tr>
<td>DRD4</td>
<td>120bp Ins/Del</td>
<td>(Hwang et al., 2012)</td>
<td>African-American</td>
<td>49</td>
<td>0.004</td>
<td>120bp Ins with greater improvement</td>
</tr>
<tr>
<td></td>
<td>Intron 1 G(n)</td>
<td>(Hwang et al., 2012)</td>
<td>African-American</td>
<td>49</td>
<td>0.014</td>
<td>142bp with greater improvement</td>
</tr>
<tr>
<td>SLC6A3</td>
<td>rs2652511/rs2975226/rs2963238</td>
<td>(Xu et al., 2010)</td>
<td>Asian</td>
<td>320</td>
<td>0.01</td>
<td>T/T/A with</td>
</tr>
<tr>
<td>Gene</td>
<td>SNP(s)</td>
<td>Reference</td>
<td>Sample</td>
<td>Participants</td>
<td>p-value</td>
<td>Comment</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>------------------------------------</td>
<td>---------------------------</td>
<td>--------------</td>
<td>---------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>COMT</td>
<td>rs4680 (Val158Met)</td>
<td>(Woodward et al., 2007)</td>
<td>Caucasian/African-American</td>
<td>84</td>
<td>&lt;0.01</td>
<td>Met-carriers with greater improvement</td>
</tr>
<tr>
<td>COMT/5-HT1A</td>
<td>rs4680 (Val158Met)/rs6295 (C-1019G)</td>
<td>(Bosia et al., 2015)</td>
<td>Caucasian</td>
<td>107</td>
<td>0.001</td>
<td>Val/Val, G/G with greater improvement in negative symptoms</td>
</tr>
<tr>
<td>NRXN1</td>
<td>rs1045881</td>
<td>(Lett et al., 2011)</td>
<td>Caucasian</td>
<td>169</td>
<td>0.03</td>
<td>CC with greater improvement in negative symptoms</td>
</tr>
<tr>
<td>OXT</td>
<td>rs2740204</td>
<td>(Souza et al., 2010a)</td>
<td>Caucasian/African-American</td>
<td>240</td>
<td>0.042</td>
<td>T allele with greater improvement</td>
</tr>
<tr>
<td>TNF-α</td>
<td>rs1800629 (G-308A)</td>
<td>(Zai et al., 2006)</td>
<td>Caucasian/African-American</td>
<td>190</td>
<td>0.015</td>
<td>A with greater improvement</td>
</tr>
<tr>
<td>DTNBP1</td>
<td>rs742105</td>
<td>(Zuo et al., 2009)</td>
<td>Caucasian/African-American</td>
<td>88</td>
<td>0.007</td>
<td>T allele with greater improvement</td>
</tr>
<tr>
<td>GFRA2</td>
<td>rs1128397/rs13250096/ rs4567028</td>
<td>(Souza et al., 2010d)</td>
<td>Mostly Caucasian</td>
<td>140</td>
<td>0.047</td>
<td>T/G/G with greater improvement</td>
</tr>
<tr>
<td>HRH2</td>
<td>rs2607474 (G-1018A)</td>
<td>(Mancama et al., 2010)</td>
<td>Caucasian/African-American</td>
<td>184</td>
<td>0.03</td>
<td>Not specified</td>
</tr>
<tr>
<td>Gene</td>
<td>SNP</td>
<td>Study</td>
<td>Population</td>
<td>N</td>
<td>p-value</td>
<td>Effect</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
<td>--------------------------------------------</td>
<td>------------</td>
<td>----</td>
<td>---------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>FKBP5</td>
<td>rs1360780</td>
<td>(Mitjans et al., 2015)</td>
<td>Caucasian</td>
<td>591</td>
<td>0.03</td>
<td>C with greater improvement</td>
</tr>
<tr>
<td>NTRK2</td>
<td>rs1778929</td>
<td>(Mitjans et al., 2015)</td>
<td>Caucasian</td>
<td>591</td>
<td>0.016</td>
<td>C with greater improvement</td>
</tr>
<tr>
<td></td>
<td>rs10465180</td>
<td>(Mitjans et al., 2015)</td>
<td>Caucasian</td>
<td>591</td>
<td>0.011</td>
<td>T with greater improvement</td>
</tr>
<tr>
<td>ABCB1</td>
<td>rs7787082</td>
<td>(Lee et al., 2012b)</td>
<td>Asian</td>
<td>96</td>
<td>0.0005</td>
<td>G with less improvement</td>
</tr>
<tr>
<td></td>
<td>rs10248420</td>
<td>(Lee et al., 2012b)</td>
<td>Asian</td>
<td>96</td>
<td>0.0013</td>
<td>A with less improvement</td>
</tr>
</tbody>
</table>

*Note: Each of the polymorphisms above have been observed to be associated with CLZ response in only one study.*
1.4 Aims and Hypotheses

Pharmacogenetic studies have identified a number of markers associated with CLZ response spanning the DA and serotonin systems, among others. However, the field has yet to yield robust, consistently replicated genetic predictors or a predictive test. Thus, the primary aim of this research is to develop a genetic model for CLZ response. A secondary aim was to re-examine DA system genetic variation in relation to response in light of the recent PGC GWAS revealing a genome-wide significant hit in the \textit{DRD2} locus for SCZ risk.

(1) The first study examines the PGC GWAS-identified \textit{DRD2} variant rs2514218 in relation to CLZ response. Given (a) that CLZ’s therapeutic mechanism is mediated in part through D2 receptor binding and (b) that genetic overlap has been observed between SCZ risk and antipsychotic response (Ikeda et al. 2015), we hypothesized that this variant would be implicated in response to this drug. To test this, we will genotype this marker in a sample of 208 SCZ patients who were treated with CLZ and evaluated prospectively over six months. This will be followed by association analysis to determine whether the SNP is implicated or not.

(2) The second study investigates the association between \textit{COMT} functional polymorphism, Val158Met, in relation to antipsychotic response. Numerous pharmacogenetic studies have examined this marker, yielding mixed results. Thus, the goal of this study is to perform a meta-analysis in order to clarify whether the marker is implicated in response. Given (a) the role of COMT in modulating DA levels in both the PFC and to a lesser extent in subcortical regions and (b) antipsychotics’ targeting of the DA system, we hypothesized that Val158Met would be associated with treatment outcome. The data for the meta-analysis will be obtained from extracting information from prior studies and contacting groups who have previously examined this association. We will also genotype and analyze the variant in our own CLZ-treated sample, and include this in the meta-analysis.
(3) The last study aims to achieve the primary goal of the thesis by building a preliminary genetic model for CLZ response. Given that a number of promising markers have been identified in relation to response, we hypothesized that the model would successfully predict response with high accuracy. To build this model, we will include all genetic variants from our group’s past pharmacogenetic studies that showed at least a statistical trend for association with response. This will be followed by cross-validation in order to limit over-fitting. Lastly, we will test this model in an independent sample of antipsychotic-treated patients to examine whether findings generalize to other antipsychotics.
2. Methodologies

2.1 Genotyping

Genotyping is the process of determining the genetic information possessed by an individual at a specific polymorphic site. This polymorphic site could be a SNP, a copy number variation (CNV), or a VNTR. A SNP is a substitution of one nucleotide for another, while CNVs and VNTRs are variations in the number of repeats of segments of the genome. Specifically, a CNV is a variation in the number of repeats in larger sections of the genome (up to millions of base-pairs), while a VNTR is usually a repeat in a short nucleotide sequence less than 10 nucleotides in length. This process involves first obtaining DNA from the individual, which is usually done by collecting blood, saliva, or cheek swabs. These samples are predominantly composed of epithelial cells and white blood cells. A number of procedures can then be used to extract the DNA. The one applied in this thesis is the high-salt method (Lahiri and Nurnberger, 1991).

The sequence containing the polymorphic region of interest must then be amplified so it can be detected during later steps. This is accomplished through polymerase chain reaction (PCR), which uses the enzyme DNA polymerase and short DNA fragments called primers that bind to regions flanking the polymorphism. The solution containing the DNA polymerase, primers, and DNA is heated to ~90 degrees Celsius, allowing the DNA to denature (strands separate), and then cooled to ~50 degrees Celsius, which allows the primers to anneal. The temperature is then increased to ~75 degrees Celsius to allow for the DNA polymerase to take effect and make new copies of the sequence containing the polymorphism. These steps are repeated multiple times to generate large amounts of DNA.

The genotyping approach used in this thesis is called the Taqman allele-specific assay. This method uses a modified form of PCR. In addition to the DNA polymerase and primers, two allele-specific probes (one for each allele) are added to the solution. Each probe has a fluorescing and anti-fluorescing molecule attached. The anti-fluorescing
effect only occurs if the anti-fluorescing molecule is within close proximity of the fluorescing one. The probes bind to the specific alleles, with one or both binding depending on whether the individual is homozygous or heterozygous. Next, since the DNA polymerase cleaves double-stranded DNA, it pushes off the probe bound to the allele, causing the fluorescing molecule to be released. The resultant fluorescent signal is then measured. Different genotypes give rise to different amounts of fluorescence, which are used to identify the genotype of the individual (Holland et al., 1991).

2.2 Meta-analysis

Meta-analysis is a powerful statistical method that combines results from multiple studies in order to answer a specific question with increased power. It can be effective for clarifying whether an effect exists when mixed results have been observed by various studies (Rothman et al., 2008). The method can also help examine whether findings extrapolate to a wider population, given that it incorporates data from a number of potentially different samples.

Since the statistical procedures behind meta-analysis are complex and beyond the scope of this thesis, this section will provide only brief overview of the steps involved. More about meta-analysis can be found elsewhere (Rothman et al., 2008).

After designing a research question of interest, the first step of the meta-analysis involves identifying an effect size measure that is helpful in answering the research question. For instance, in pharmacogenetics, the question is often whether a specific variant is associated with drug response. Thus, the effect size measure of interest is usually either the difference in symptom improvement between genotype groups or the odds ratio (OR) for response/non-response depending on genotype.

The next step is to decide on specific inclusion and exclusion criteria for studies. In pharmacogenetic meta-analyses, common inclusion criteria often include: specific diagnoses for patients, treatment duration, and availability of the genetic data of interest.
Based on the criteria, a systematic search of the literature is performed and studies obtained.

Prior to analysis, the specific model to be applied must be decided. When studying the effect of one independent variable on one dependent variable, either fixed-effects or random-effects models are employed. A fixed-effects model assumes that the studies included were performed under similar conditions with similar subjects. A random-effects model, on the other hand, does not have this assumption, assuming instead that the effects of each study are randomly distributed. The process of deciding which model to apply is described in greater detail elsewhere (Egger et al., 1997).

During analysis, a pooled effect size is computed. The effect size measure is most often the OR for binary dependent variables, and the standardized mean difference (SMD) for continuous ones. The Mantel-Haenszel method is generally considered the most robust for computing pooled effect sizes. The results are then illustrated in ‘forest plots’, which plot the effect size of each study along with a pooled effect size (see Figure 4.3). The line intersecting the x-axis represents the line of ‘no effect’.

Next, heterogeneity across studies is assessed. This is most often done using the Cochran’s Q test. The test yields a Cochran’s Q statistic, reflecting the average difference between individual study effects and the pooled effect. The test also yields an $I^2$, defined as the percentage variation across studies attributed to heterogeneity. This is followed by using either an Egger’s test or Harbord test along with the funnel plot method to evaluate potential publication bias. These methods are described in greater detail elsewhere (Egger et al., 1997; Harbord et al., 2006). Lastly, sensitivity analysis is conducted whereby studies are removed from analysis in order to assess their contribution to the pooled effect.

2.3 Polygenic risk scores
Polygenic risk score analysis is a powerful method for examining the combined genetic effect of numerous markers. The method first involves determining the risk allele for a given polymorphism with two alleles through association analysis. Each individual is then given a score of 0, 1, or 2 corresponding to the number of risk alleles they have at that polymorphism (Plomin and Deary, 2015). This is done for each marker tested. An individual’s score at each SNP is added together to yield a total genetic ‘risk score’. Individuals with the highest score should, in principle, be most likely to have the trait of interest (e.g. be a responder rather than a non-responder, or have a SCZ diagnosis). Risk scores can either be weighted or unweighted. Weighted scores assume that each SNP has a different contribution to the phenotype of interest. They are calculated by multiplying the risk allele count (0, 1, 2) by the effect size for each SNP. Unweighted scores, on the other hand, assume that all SNPs have the same contribution to a specific phenotype. This method is discussed in greater detail elsewhere (Dudbridge, 2013).

2.4 Cross validation (CV)

CV is a commonly-used method for evaluating model performance. It involves dividing the dataset into training and test subsets, then fitting the model to the training set and testing its accuracy in the test set. The three common types of CV include the holdout method, K-fold CV, and leave-one-out CV (LOOCV). The difference between the three methods lies in the number of subsets into which the data is divided. The holdout method divides the dataset into two subsets, K-fold CV into K subsets, and LOOCV into N subsets, where N is the number of data points in the dataset (Schneider, 1997).

Here, we will focus on K-fold CV, as this is the only CV method used in this thesis. After dividing the dataset into K subsets (or ‘folds’), a randomly selected subset is treated as the test set, while the remaining subsets are together used as the training set. Dividing the subset into between 10-20 subsets has been found to yield the best performance for smaller sample sizes (Kohavi, 1995). The model is then evaluated for its performance in the test set based on accuracy, sensitivity, and specificity measures. These steps are repeated a specified number of times (usually 100), and the averages of the
aforementioned three measures obtained. The advantage to this approach is that each data point will be in the test set exactly once, so it is less important how the data is divided (Schneider, 1997). More information on CV methods can be found elsewhere (Schneider, 1997).
CHAPTER 3

Preliminary Evidence for Association of Genome-Wide Significant \textit{DRD2} Schizophrenia Risk Variant with Clozapine Response

Eric Huang$^{1,2}$, Malgorzata Maciukiewicz$^2$, Clement C. Zai$^2$, Arun K. Tiwari$^2$, Jiang Li$^5$, Steven G. Potkin$^3$, Jeffrey A. Lieberman$^4$, Herbert Y. Meltzer$^5$, Daniel J. Müller$^{2,6}$, James L. Kennedy$^{2,6}$

$^1$King’s College Circle, Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, ON M5S 1A8, Canada; $^2$250 College St, Pharmacogenetic Research Clinic, Centre for Addiction and Mental Health, Toronto, ON M5T 1R8, Canada; $^3$5251 California Ave, Department of Psychiatry and Human Behavior, University of California, Irvine, Irvine, CA 92617, USA; $^4$1051 Riverside Dr, Department of Psychiatry, Columbia University Medical Center, New York, NY 10032, USA; $^5$303 E Chicago Ave, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA; $^6$250 College St, Department of Psychiatry, University of Toronto, Toronto, ON M5T 1R8, Canada

**Corresponding Author:**
James L. Kennedy, MD, FRCP, FRSC
Director, Neuroscience Research
Head, Neurogenetics Section
Centre for Addiction and Mental Health (CAMH);
Professor and Co-Director Div. of Brain and Therapeutics
Department of Psychiatry
University of Toronto
jim.kennedy@camh.ca
www.camh.ca

Adapted from Huang et al. 2016, *Pharmacogenomics*. 
3.1 Abstract

**Aim:** The recent PGC GWAS study identified a SNP, rs2514218, located 47kb upstream of the DRD2 gene to be associated with risk for SCZ ($p=2.75\times10^{-11}$). Since all antipsychotics bind to D2 receptors, we examined rs2514218 in relation to response to antipsychotic treatment. **Patients & Methods:** We investigated the SNP in relation to treatment response in a prospective study consisting of 208 patients (151 Caucasians, 42 African-Americans, 15 others) treated with CLZ for six months. **Results:** rs2514218 was associated with total score change in the Brief Psychiatric Rating Scale (BPRS) under an additive model ($p_{corr}=0.033$). **Conclusion:** Our finding provides evidence for the association between rs2514218 and antipsychotic response, but further replication is required before firm conclusions can be drawn.

**Keywords:** Dopamine D2 receptor, antipsychotic, genetics, pharmacogenetics, schizophrenia, clinical response, clozapine
3.2 Introduction

Recently, the Schizophrenia Working Group of the PGC conducted the largest GWAS on SCZ risk. Of the 128 independent associations identified, the study discovered one SNP, rs2514218, located 47kb upstream the DRD2 gene (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Specifically, they found the T allele was associated with decreased SCZ risk (OR=0.927, p=2.75e-11). This finding was especially interesting, as previous studies have strongly implicated the D2 receptor in SCZ etiology (Seeman et al., 1976; Seeman, 2010). Moreover, D2 receptor blockade is an important component of the mechanism of action of all first generation antipsychotics (Carlsson, 1978). Second-generation antipsychotics like CLZ also likely exert part of their therapeutic effect through D2 receptor binding (Kapur and Mamo, 2003), while exhibiting a strong affinity for serotonin, muscarinic, adrenergic and histaminergic receptors (Meltzer et al., 2003a). Additionally, in silico evidence from GTEx Portal suggests a cis-eQTL effect for rs2514218 on D2 expression in the basal ganglia (Consortium, 2013). The variant has also been associated with impaired striatal functioning in the unaffected siblings of SCZ patients (Vink et al., 2015), providing further support for its functional role.

Based on strong evidence for D2 involvement in antipsychotic drug mechanisms, numerous studies have investigated whether associations between polymorphisms known to modify D2 receptor density and/or function can explain inter-individual variability in antipsychotic treatment response. In particular, four polymorphisms have been observed to modify DRD2 expression or D2 density. The -141C Ins/Del polymorphism (rs1799732) has been reported to alter D2 receptor expression in vitro (Arinami et al., 1997) and to be associated with increased striatal D2 density in healthy subjects (Jönsson et al., 1999). Another variant Taq1A (rs1800497) has also been observed to influence DRD2 expression, with the A1 allele associated with reduced expression in vivo (Ritchie and Noble, 2003) and in vivo (Pohjalainen et al., 1998). In addition, the T allele of variant C957T (rs6277) and B1 allele of variant Taq1B (rs1079598) have been reported to decrease striatal D2 density in healthy subjects (Jönsson et al., 1999; Ritchie and Noble, 2003; Hirvonen et al., 2004).
A meta-analysis of this polymorphism revealed the Del allele of -141C Ins/Del to be associated with poorer treatment response (Zhang et al., 2010b). The other three variants have also been implicated in the prediction of antipsychotic response, albeit with mixed results (Suzuki et al., 2000; Hwang et al., 2005b; Hwang et al., 2006; Kwon et al., 2008; Shen et al., 2009; Lee et al., 2012b).

However, the above studies interrogated only a limited portion of the variation in \textit{DRD2}; other \textit{DRD2} variants affecting gene expression or receptor function may still explain inter-individual variability in antipsychotic treatment response. Moreover, a very recent study by Ikeda \textit{et al.} (2015) revealed a trend for significant enrichment of SCZ risk variants among SNPs associated with risperidone treatment response, suggesting possible overlap between the genes implicated in SCZ susceptibility and antipsychotic response (Ikeda \textit{et al.}, 2015). Thus, we hypothesize that the genome-wide significant SCZ-risk SNP, rs2514218, may be associated with response to antipsychotic treatment. Our study examined the association between this SNP and CLZ response in a treatment refractory/intolerant schizophrenia population. We also conducted an exploratory investigation of the interaction between this SNP and the four putatively functional \textit{DRD2} polymorphisms described above (rs6277, rs1800497, rs1079597, and rs1799732).

### 3.3 Methods

#### Subjects

Our sample consisted of 208 patients obtained from three research clinics: Case Western Reserve University in Cleveland, Ohio (HYM, N=86); Hillside Hospital in Glen Oaks, New York (JAL, N=90); and University of California at Irvine (SGP, N=32) \textit{(Table 3.1)}. Our Caucasian subsample included 151 patients. Inclusion criteria included DSM-III-R or DSM-IV (Association, 1994) diagnoses of SCZ. Almost all subjects met criteria for treatment refractoriness (non-response to at least two antipsychotics) or intolerance to traditional antipsychotic therapy as defined by Kane \textit{et al.} (1988) (Kane \textit{et al.}, 1988a). After a two to four week washout period, patients were treated with CLZ and evaluated
prospectively for six months, during which blood levels of CLZ were monitored, reducing the probability of treatment non-adherence. Treatment response was evaluated using the 18-item BPRS scale (Overall and Gorham, 1962) at the beginning and end of treatment. Differences in response rates between clinical sites were not observed ($p=0.10$). Therefore, data from the three clinical sites were pooled and analyzed together. The Caucasian subsample ($N=151$) was analyzed separately from the African-American subsample, as rs2514218 genotype distributions were significantly different between the two ($p=0.031$). The Caucasian sample had 80% power to detect an odds ratio of 1.65 at a responder frequency of 47.7% and minor allele frequency of 0.358 (unmatched case-control design; $\alpha=0.05$; Quanto v1.2.4) (Gauderman, 2002). Responders were defined as those experiencing a reduction >20% in overall BPRS score or a BPRS score <35 after six months of treatment, based on criteria proposed by Kane et al. (1988) (Kane et al., 1988a). Of the 151 Caucasian patients, we had BPRS total and subscale score data for a subset (BPRS total: $N=86$, positive symptom subscale: $N=81$, negative symptom subscale: $N=83$) (*Table 3.2*). Our continuous response subset of the Caucasian sample had a power of 80% to detect an effect that explains 8.7% of the variance in total score change (Gauderman, 2002). Our sample is described in greater detail elsewhere (Hwang et al., 2005b).

*Table 3.1 – Demographics of Caucasian subsample ($N=151$), separated by site of collection or ethnicity.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Responder/Non-responder</th>
<th>Caucasian/African-American/Other</th>
<th>Males/Females</th>
<th>Mean Age ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meltzer ($N=86$)</td>
<td>37/49</td>
<td>63/23/0</td>
<td>62/24</td>
<td>32.4 ± 8.4</td>
</tr>
<tr>
<td>Lieberman ($N=90$)</td>
<td>55/35</td>
<td>65/14/11</td>
<td>66/24</td>
<td>34.9 ± 8.6</td>
</tr>
<tr>
<td>Potkin ($N=32$)</td>
<td>12/20</td>
<td>23/5/4</td>
<td>28/4</td>
<td>36.0 ± 6.7</td>
</tr>
<tr>
<td>Caucasian ($N=151$)</td>
<td>72/79</td>
<td></td>
<td>113/38</td>
<td>34.0 ± 7.9</td>
</tr>
</tbody>
</table>

*Table 3.2 – Demographics of subset of Caucasian subsample with continuous response data.*

<table>
<thead>
<tr>
<th>Group</th>
<th>$\Delta$BPRS ± SD</th>
<th>$\Delta$BPOS ± SD</th>
<th>$\Delta$BNEG ± SD</th>
<th>Males/Females</th>
<th>Mean age ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meltzer ($N=63$)</td>
<td>-9.3 ± 11.1</td>
<td>-3.2 ± 4.9</td>
<td>-1.1 ± 3.3</td>
<td>48/16</td>
<td>32.0 ± 8.0</td>
</tr>
</tbody>
</table>
### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Potkin (N=23)</th>
<th>Total (N=86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPRS</td>
<td>-5.2 ± 11.8</td>
<td>-8.2 ± 11.4</td>
</tr>
<tr>
<td>BPOS</td>
<td>-1.7 ± 6.3</td>
<td>-2.8 ± 5.4</td>
</tr>
<tr>
<td>BNEG</td>
<td>-1.0 ± 4.5</td>
<td>-1.0 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>20/3</td>
<td>68/19</td>
</tr>
<tr>
<td></td>
<td>35.9 ± 6.6</td>
<td>33.1 ± 7.8</td>
</tr>
</tbody>
</table>

Note: BPRS = Brief psychiatric rating scale; BPOS = positive symptom subscale; BNEG = negative symptom subscale.

### Genetics

Genomic data were extracted using the high-salt method from venous blood (Lahiri and Numbringer, 1991). The DRD2 rs2514218 variant was genotyped using Taqman 5’ nuclease assay (Applied Biosystems; Foster City, CA, USA). Samples were subjected to 50 cycles consisting of heating to 95°C for 10 minutes, 92°C for 15 seconds, and then 60°C for 60 seconds in a 2720 thermal cycler (Applied Biosystems). Allelic discrimination was conducted using the Applied Biosystems Viia7 real-time PCR system. All ambiguous genotypes were retyped. Genotypes that remained ambiguous were excluded from further analyses. Genotyping accuracy was assessed by repeating genotyping for 10% of the sample, and results showed 100% concordance. The accuracy calls for genotyping were all above 97%. DRD2 gene variants rs6277, rs1800497, rs1079597, and rs1799732 had already been genotyped for a previous study using a 5’-exonuclease fluorescence assay (Shi et al., 1999; Hwang et al., 2005b). SNP rs1079598, which is in complete linkage disequilibrium (LD) with rs1079597 (D’=1), was genotyped as a proxy for this SNP.

### Statistical analyses

In our primary analysis, we examined dichotomous and continuous response data in the Caucasian subsample separate from the rest of the sample to avoid confounding effects due to population stratification. For the dichotomous response data, logistic regression was conducted to examine the association between genotype and numbers of responders/non-responders under an additive model. We also analyzed response as a continuous variable, looking at six-month change in total BPRS score under an additive model using linear regression. Baseline score was found to be the sole covariate associated with score change (p<0.05), and was thus included as a covariate in all
analyses. Other potential confounding variables such as sex were not associated with response (Table 3.6). We did not have complete data for age at onset, and therefore, could not control for this variable. For all significant associations, secondary analyses were carried out to determine whether the association was specific to improvement on a particular subscale (positive or negative) and whether the association existed in the total sample. We did not conduct analysis on the African-American subsample separately due to sample size limitations (n=42).

Table 3.6 – Potential Confounding Factors and Response

<table>
<thead>
<tr>
<th>Factor</th>
<th>Strength of Association (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.201</td>
</tr>
<tr>
<td>Illness duration</td>
<td>0.597</td>
</tr>
<tr>
<td>Baseline score</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

For the DRD2 SNP interaction analysis, logistic regression and ANCOVA were used to analyze dichotomous response and quantitative score change, respectively, with baseline score as a covariate. We did not apply a multifactor dimensionality-scaling (MDS) model, as we examined only four pre-defined pairs of SNPs with goal of specifically investigating the influence of rs1800497, rs6277, rs1079597, and rs1125394 on rs2514218’s association with response. The Statistical Package for the Social Sciences (SPSS) 22.0 was used to conduct these computations (Chicago, IL, USA). LD analysis was performed using Gabriel’s method (Gabriel et al., 2002) in the program Haploview 4.2 (Barrett et al., 2005). To correct for multiple testing, we used Conneely and Boehnke’s (2007) correction method, which takes into account correlation between phenotypes and genotypes (Conneely and Boehnke, 2007). This method was selected to achieve the most appropriate level of correction, given the correlated phenotypes we examined (ie responder/non-responder and BPRS score change).

3.4 Results

No variants deviated from HWE (p>0.05) in the Caucasian subsample or total sample. No significant difference in gender, age at onset, or illness duration was observed across genotypes for rs2514218 in Caucasians.
In the Caucasian subsample, no significant association between responder/non-responder status and rs2514218 genotype was observed (Table 3.3). For the continuous association analysis, two subjects in the Caucasian group were identified to be significant outliers (>3 standard deviations away from predicted value) and were thus excluded. In the Caucasian group, our continuous response analysis revealed a significant association between genotype and total score change ($p=0.020$, $\beta=-3.28$, Adjusted $R^2=0.34$) (Table 3.3). Specifically, risk allele A was associated with greater reduction in BPRS scores (Fig. 3.1). This association remained significant after correction for multiple testing ($p_{corr}=0.033$). Additionally, this association with BPRS score change became more significant when examined in the total sample ($p=0.013$, $\beta=-3.07$, Adjusted $R^2=0.37$) (Table 3.4).

Table 3.3 – rs2514218 Primary Association Analysis Results (Caucasian Subsample)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>R/NR</th>
<th>$p_{corr}$</th>
<th>$\Delta$BPRS ± SD</th>
<th>$p_{corr}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>27/36</td>
<td>0.12</td>
<td>-6.26 ± 10.3</td>
<td>0.033</td>
</tr>
<tr>
<td>A/G</td>
<td>32/36</td>
<td></td>
<td>-7.15 ± 12.2</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>13/7</td>
<td></td>
<td>-15.13 ± 9.8</td>
<td></td>
</tr>
</tbody>
</table>

Note: R/NR = number of responders/non-responders.
Figure 3.1 – BPRS score change and rs2514218 genotype in Caucasian subsample. BPRS six-month score change (six-month – baseline score) was plotted after adjustment for baseline score. rs2514218 was examined under an additive model using linear regression.

Table 3.4 – rs2514218 Association Analysis Results (Total Sample)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>R/NR</th>
<th>p-value</th>
<th>ΔBPRS ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>49/53</td>
<td>0.056</td>
<td>-7.61 ± 12.8</td>
<td>0.013</td>
</tr>
<tr>
<td>A/G</td>
<td>43/42</td>
<td></td>
<td>-6.64 ± 12.0</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>13/8</td>
<td></td>
<td>-14.75 ± 9.6</td>
<td></td>
</tr>
</tbody>
</table>

Note: R/NR = number of responders/non-responders. ΔBPRS = change in BPRS score (six-month – baseline score).

Given the association with BPRS total score change, we conducted secondary analyses to examine whether this association was driven by an association with score change in either the positive or negative symptom subscale. No significant association with either subscale was observed (pos: p=0.16; neg: p=0.36), suggesting that one or more of the remaining ten items of the BPRS scale was responsible for the association with total
score change (Table 3.5). These items resemble the ‘general psychopathology’ subscale of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). A meta-analysis of BPRS factor analyses classified these items under three subcategories – affect, resistance, and activation (ARA) (Shafer, 2005). Hence, in our secondary analysis, we examined the association between the change in the sum of these items’ scores and genotype (individual item scores unavailable), observing a significant association under the additive model ($p=0.038$, $\beta=-1.69$, $R^2=0.110$) (Table 3.5). Specifically, the minor allele A was associated with greater improvement in score (Fig. 3.2).

Figure 3.2 – Change in sum score of affect, resistance, and activation (ARA) factors of the BPRS scale and rs2514218 genotype in Caucasian subsample. ARA constitutes the remaining items of the BPRS scale not included in either positive or negative symptom subscales. ARA score change was plotted after adjustment for baseline score. rs2514218 was examined under an additive model using linear regression.

LD analyses using Haploview 4.2 revealed that rs2514218 was not in LD ($D’<0.9$) with any of the other four $DRD2$ SNPs examined in the interaction analysis. Thus, we decided
not to conduct haplotype analysis. The interaction analysis in the Caucasian subsample yielded no significant findings.

Table 3.5 – rs2514218 BPRS Subscale Association Analysis Results (Caucasian Subsample)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ΔBPOS ± SD</th>
<th>p-value</th>
<th>ΔBNEG ± SD</th>
<th>p-value</th>
<th>ΔARA ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>-2.16 ± 4.4</td>
<td>0.16</td>
<td>-0.51 ± 3.4</td>
<td>0.36</td>
<td>-3.41 ± 7.3</td>
<td>0.038</td>
</tr>
<tr>
<td>A/G</td>
<td>-2.30 ± 6.1</td>
<td></td>
<td>-1.56 ± 3.9</td>
<td></td>
<td>-3.34 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>-5.13 ± 5.2</td>
<td></td>
<td>-1.20 ± 3.8</td>
<td></td>
<td>-8.80 ± 6.4</td>
<td></td>
</tr>
</tbody>
</table>

Note: BPOS = positive symptom subscale; BNEG = negative symptom subscale; ARA = affect, resistance, and activation factors of the BPRS scale. ARA constitutes the ten items of the BPRS scale not belonging to either BPOS or BNEG.

*Figure 3.3 – BPRS score change by rs2514218 genotype in total sample. BPRS six-month score change was plotted after adjustment for baseline score. rs2514218 was examined under an additive model using linear regression.*
3.5 Discussion

To our knowledge, this is the first study to investigate PGC-GWAS-identified DRD2 SNP rs2514218 in relation to antipsychotic response. Our finding that rs2514218 is significantly associated with CLZ response is consistent with antipsychotics’ antagonism at D2 receptors (Kapur and Mamo, 2003). Our finding is also concordant with previous studies implicating DRD2 locus genetic variants such as Taq1A and -141C Ins/Del in antipsychotic response in Caucasians (Hwang et al., 2005b; Hwang et al., 2006; Zhang et al., 2010b). Nevertheless, it should be noted that studies of DRD2 association with antipsychotic response have yielded mixed results (Arranz et al., 1998b; Xing et al., 2007). Possible explanations of these mixed results may be due to factors including study heterogeneity in sample size, ethnicity, and antipsychotic prescribed.

Post hoc analyses revealed that rs2514218 was nominally associated with change in the sum of the items not included in the positive or negative subscales. A meta-analysis of BPRS factor structure by Shafer (2005) revealed that these items formed three clusters, named the affect, resistance, and activation factors (Shafer, 2005). This finding suggests that one or more of these items is driving the association with total score change. In particular, it is worth noting that the affect factor consists of four measures of depressed state: anxiety, guilt, somatic concern, and depressive mood. CLZ has previously been observed to lead to improvements in these symptoms (Lindenmayer et al., 2004), with potential benefits for patients’ quality of life and wellbeing. Moreover, previous imaging studies have shown that improvement in these symptoms correlates with D2 receptor occupancy in individuals being treated with atypical antipsychotics (de Haan et al., 2000; Mizrahi et al., 2007). Thus, it is possible that improvement in these four items may be driving the observed association with BPRS total score change. Additionally, CLZ has been observed to reduce levels of hostility, a symptom encompassed by the ‘resistance’ factor (Citrome et al., 2001). Thus, it is possible that rs2514218 is associated with improvement in these symptoms. Additionally, the strength of the association increased
when examined in the total sample (includes Caucasians, African-Americans, others) \((p=0.013)\), suggesting that the association may not be limited to individuals of Caucasian ancestry.

*In silico* evidence from the non-coding genome annotation tool Haploreg suggests rs2514218 is an enhancer for the *DRD2* gene, as it was predicted to alter four known regulatory motifs near the gene (Ward and Kellis, 2012). These findings are consistent with the expression data from the GTexPortal expression quantitative trait loci (eQTL) gene expression data bank, which revealed a significant association between this SNP and D2 receptor mRNA levels in the basal ganglia \((p=0.030)\) (Consortium, 2013), the region with the highest concentration of D2 receptors (Hall et al., 1994; Xiberas et al., 2001). Together, these findings suggest that the SNP may play a role in altering D2 expression, contributing to differential CLZ response.

It should be noted that the six-month length and prospective design of the study may enable it to assess response to CLZ in a more accurate manner than shorter studies, since it has previously been suggested that full response to CLZ may require up to six months or more to take effect (Rosenheck et al., 1999). Moreover, blood CLZ levels were monitored throughout the six months, reducing probability of treatment non-adherence.

Our exploratory interaction analysis did not identify any significant associations, suggesting little cumulative contribution of the studied *DRD2* variants. However, limited sample size may be the most important factor influencing these results.

Our finding that the A-allele was associated with greater improvement in symptoms was not in agreement with another study of the same variant, which observed an association for the opposite allele with antipsychotic response (Zhang et al. 2015). Several possible reasons may explain these mixed results. First, the other study’s sample was treated with aripiprazole or risperidone, both of which possess different receptor binding profiles compared CLZ. Notably, aripiprazole and risperidone show a substantially stronger affinity for the D2 receptor compared to CLZ (Richtand et al. 2007). In principle, this
difference may lead to different effects of D2 SNPs on the antipsychotics’ therapeutic mechanisms. Secondly, Zhang et al.’s sample consisted of first-episode patients, while ours included patients that had received prior treatment with typical antipsychotics. This may be important given that antipsychotic treatment has been observed to affect brain function and structure (Keshavan et al., 1994; Lui et al., 2010; Ho et al., 2011), and receptor expression (Wilmot and Szczepanik, 1989). Thirdly, the two studies’ samples differed in terms of ethnicity: Zhang et al.’s sample consisted of both Caucasians and African-American patients, while ours included only Caucasian individuals. Given the confounding effects of population stratification and differing LD structures across ancestries (Cardon, 2003), this difference may have contributed to different effects observed. Lastly, it is important to note that the two studies observed associations with different sets of symptoms—Zhang et al with improvement in positive symptoms and our study with improvement in general psychopathological symptoms. Thus, the findings should not be seen as being contradictory to one another; rather, the difference in symptoms supports the potential variation of the role of the DRD2 genetic variant in response to different antipsychotics and in non-overlapping clinical populations. Alternatively, the differing allele effects of our two studies may hint that rs2514218 is not the true causal variant, but is rather in incomplete LD with the functional SNP.

There are several limitations to our study. First, our sample size was fairly small, with the Caucasian subsample consisting of 151 patients and total sample 208 patients. Nevertheless, our analysis of continuous response had a power of 80% to detect an effect that explained 8.7% of the variance in BPRS score change. This was sufficient to allow us to detect the association observed. Secondly, our study did not have access to individual item scores of the BPRS scale, preventing us from examining each item individually to identify the one(s) driving the association with total score change. Ideally, future studies would have access to this data and conduct further analyses to better characterize the rs2514218’s possible association with response.
3.6 Conclusion

Overall, our results provide preliminary evidence for an association between DRD2 rs2514218 and response to CLZ. However, these results require replication in independent samples before firm conclusions can be drawn. Our finding also suggests it may be worthwhile (1) to closely re-examine DRD2 genetic variation in relation to antipsychotic response and (2) to investigate whether specifically rs2514218 is associated with response to antipsychotics other than CLZ. Moreover, as this is one of the first studies to identify a significant association between a PGC SCZ genome-wide significant SNP and antipsychotic response, further investigation of PGC genome-wide significant SCZ variants in relation to antipsychotic treatment outcome are warranted. If replicated, this variant may be considered for inclusion as one variable in genetic predictive tests for CLZ response.

Financial and Competing Interests Disclosure

JL Kennedy has acted as a consultant or received honoraria from GlaxoSmith-Kline, Sanofi-Aventis, Dainippon-Sumitomo, Novartis, Shire, Eli Lilly, and Roche; he is on a Scientific Advisory Board member (unpaid) of AssureRx Health Inc. JA Lieberman has received research funding from and/or is a member of the advisory board of Allon, Alkermes Bioline, GlaxoSmith-Kline Intracellular Therapies, Lilly, Merck, Novartis, Pfizer, Pierre Fabre, Psychogenics, F Hoffman-La Roche Ltd, Sunovion, and Targacept. HY Meltzer has received grants from or acted as consultant to Abbott Labs, ACADIA, Alkemdes, Bristol-Myers Squibb, Dai-Nippon Sumitomo, Eli Lilly, EnVivo, Janssen, Otsuka, Pfizer, Roche, Sunovion and BiolineRx; he is a shareholder of ACADIA and GlaxoSmithKline. SG Potkin has served as a consultant or advisory board member, or received research support from Otsuka, Roche/Genentech, Novartis, Sunovion, Lundbeck and Alkermes. CC Zai is supported by the Brian and Behavior Research Foundation, American Foundation for Suicide Prevention, and Eli Lilly. E Huang, J Li, M Maciukiewicz, and AK Tiwari have no financial disclosures. All authors have no other relevant financial involvement or competing interests in conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.
### Executive Summary

#### Background
- Schizophrenia Working Group PGC conducted the largest genome-wide association study on schizophrenia risk; one of the genome-wide significant SNPs, rs2514218, was located 47kb upstream of the DRD2 gene (T allele, OR=0.927, \( p=2.75\times10^{-11} \)).
- All antipsychotic drugs exert at least part of their effect through D2 receptor binding.
- This novel SNP has not previously been investigated in relation to response to CLZ.

#### Results
- DRD2 genome-wide significant rs2514218 was associated with antipsychotic response in a prospectively studied Caucasian sample treated for six months with CLZ (\( p_{\text{corr}}=0.033 \)) (minor allele A associated with improved response under an additive model) and in an overall sample of mixed-ethnicity (\( p=0.013 \)).
- rs2514218 was not associated with either positive or negative symptom subscale score change, but rather improvement in the remaining BPRS items, which are comprised of mostly general psychopathological symptoms (\( p=0.038 \)).
- No significant interaction effects were observed between rs2514218 and previously studied DRD2 gene variants -141CIns/Del (rs1799732), C957T (rs6277), Taq1A (rs1800497), and Taq1B (rs1079597).

#### Conclusion
- Although replication in independent samples is required, our findings provide preliminary evidence for DRD2 gene variant rs2514218 being associated with antipsychotic response.
- Our findings provide reason for re-visiting the DRD2 genetic variation in pharmacogenetic studies of antipsychotic response.
CHAPTER 4

Catechol-O-methyltransferase Val158Met polymorphism and clinical response to antipsychotic treatment in schizophrenia and schizoaffective disorder patients: a meta-analysis.

Eric Huang, BSc\textsuperscript{1,14}, Clement C. Zai, PhD\textsuperscript{1}, Amanda Lisoway, BSc\textsuperscript{1,14}, Malgorzata Maciukiewicz, PhD\textsuperscript{1}, Daniel Felsky, BSc\textsuperscript{1,14}, Arun K. Tiwari, PhD\textsuperscript{1}, Jeffrey R. Bishop, PharmD MSc\textsuperscript{2}, Masashi Ikeda, MD PhD\textsuperscript{3}, Patricio Molero, MD PhD\textsuperscript{4}, Felipe Ortuno, MD PhD\textsuperscript{4}, Stefano Porcelli, MD\textsuperscript{5}, Jerzy Samochowiec, MD PhD\textsuperscript{6}, Pawel Mierzejewski, MD PhD\textsuperscript{7}, Shugui Gao\textsuperscript{8}, Benedicto Crespo-Facorro, MD PhD\textsuperscript{9}, José M Pelayo-Terán, MD PhD\textsuperscript{9}, Harpreet Kaur, PhD\textsuperscript{10}, Ritushree Kukreti, PhD\textsuperscript{10}, Herbert Y. Meltzer, MD\textsuperscript{11}, Jeffrey A. Lieberman, MD\textsuperscript{12}, Steven G. Potkin, MD\textsuperscript{13}, Daniel J. Müller, MD PhD\textsuperscript{1}, James L. Kennedy, MD\textsuperscript{1}

\textsuperscript{1}Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada; \textsuperscript{2}Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, MN; \textsuperscript{3}Department of Psychiatry, Fujita Health University, Toyoake, Aichi, Japan; \textsuperscript{4}Departamento de Psiquiatría, Clínica Universidad de Navarra, Pamplona, Spain; \textsuperscript{5}Department of Biomedical and NeuroMotor Sciences, University of Bologna, Bologna, Italy; \textsuperscript{6}Department of Psychiatry, Pomeranian Medical University, Szczecin, Poland; \textsuperscript{7}Department of Pharmacology, Institute of Psychiatry and Neurology, Warsaw, Poland; \textsuperscript{8}Department of Psychiatry, Ningbo Kangning Hospital, Ningbo, China; \textsuperscript{9}Department of Psychiatry, CIBERSAM, University Hospital Marqués de Valdecilla-IDIVAL, School of Medicine, University of Cantabria, Santander, Spain. \textsuperscript{10}Institute of Genomics and Integrative Biology, Delhi, India. \textsuperscript{11}Feinberg School of Medicine, Northwestern University, Chicago, IL; \textsuperscript{12}Department of Psychiatry, Columbia University Medical Center, New York, NY; \textsuperscript{13}Department of Psychiatry and Human Behavior, University of California, Irvine, Irvine, CA; \textsuperscript{14}Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, ON; \textsuperscript{15}Department of Psychiatry, Faculty of Medicine, University of Toronto, Toronto, ON.

Corresponding Author:
James L. Kennedy, MD, FRCPC, FRSC
Director, Neuroscience Research
Head, Neurogenetics Section
Centre for Addiction and Mental Health (CAMH);
Professor and Co-Director Div. of Brain and Therapeutics
Department of Psychiatry
University of Toronto
jim.kennedy@camh.ca
www.camh.ca

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4.1 Abstract

**Background** – The COMT enzyme plays a crucial role in DA degradation. A functional polymorphism, Val158Met (rs4680), in the coding region of the COMT gene is associated with significant differences in enzymatic activity and consequently DA concentrations in the PFC. Multiple studies have analyzed the COMT Val158Met variant in relation to antipsychotic response. Here, we conducted a meta-analysis examining the relationship between COMT Val158Met and antipsychotic response.

**Methods** – Searches using the PubMed, Web of Science, and PsycInfo databases (03/01/2015) yielded 23 studies investigating COMT Val158Met variation and antipsychotic response in SCZ and schizo-affective disorder. Responders/non-responders were defined using each study’s original criteria. If no binary response definition was used, authors were asked to define response according to at least 30% PANSS score reduction (or equivalent in other scales), as this was the most common definition used in the studies collected. Analysis was conducted under a fixed-effects model.

**Results** – Ten studies met inclusion criteria for the meta-analysis. Five additional antipsychotic-treated samples were analyzed for Val158Met and response, and included in the meta-analysis (n<sub>total</sub>=1416). Met/Met individuals were significantly more likely to respond than Val-carriers \( p=0.039, \text{OR}_{\text{Met/Met}}=1.37, 95\% \text{ CI: 1.02-1.85} \). Met/Met patients also experienced significantly greater improvement in positive symptoms relative to Val-carriers \( p=0.030, \text{SMD}=0.24, 95\% \text{ CI: 0.024-0.46} \). Post hoc analyses on patients treated with atypical antipsychotics (n=1207) showed that Met/Met patients were significantly more likely to respond relative to Val-carriers \( p=0.0098, \text{OR}_{\text{Met/Met}}=1.54, 95\% \text{ CI: 1.11-2.14} \), while no difference was observed for typical-antipsychotic-treated patients (n=155) \( p=0.65 \).

**Conclusions** – Our findings suggest that the COMT Val158Met polymorphism is associated with response to antipsychotics in schizophrenia and schizo-affective disorder patients. This effect may be more pronounced for atypical antipsychotics.
4.2 Introduction

SCZ is a debilitating neurodevelopmental disorder with high heritability (~0.8) (McGuffin et al., 1995). While antipsychotic drugs are currently the most effective treatment, clinical response varies significantly between patients, with an overall response rate of only ~50% (Lieberman et al., 2005b). The field of pharmacogenetics aims to optimize treatment outcome at the individual level by leveraging our knowledge of how genetic variants influence clinical response. Since all antipsychotics target the DA system to various extents by binding to D2 receptors, pharmacogenetic studies have largely focused on DA system-related genes, including DRD2 and COMT. Previous studies have identified associations for genes such as DRD2 and DRD3 (D3 receptor) in relation to response to antipsychotics (Hwang et al., 2010; Zhang et al., 2010b). Other genes implicated in response include 5-HT2A and 5-HT2C (Pouget and Müller, 2014). This is reviewed in greater detail in the thesis’ Introduction section.

The COMT gene is located within the 22q11 chromosomal locus, a region highly implicated in SCZ risk (Badner and Gershon, 2002; Lewis et al., 2003). The COMT enzyme plays a significant role in cortical DA degradation (Lachman et al., 1996). The majority of pharmacogenetic studies examining COMT in relation to antipsychotic response have focused on the functional variant Val158Met (rs4680). Other putatively functional variants have also been examined, though relatively infrequently (Chen et al., 2004; Molero et al., 2007). The Val158Met variant is located within exon 4 of the COMT gene and involves a G-to-A nucleotide switch, leading to a valine (Val) to methionine (Met) substitution at codon 158. The Met-allele form of the enzyme has lower thermostability and has been observed to exhibit reduced enzymatic activity, contributing to increased levels of cortical DA (Chen et al., 2004); the Met/Met homozygote has 3-4 fold lower enzymatic activity than the Val/Val homozygote, while the heterozygote has intermediate activity (Chen et al., 2004).

Studies of COMT Val158Met and antipsychotic response in SCZ and schizoaffective disorder have yielded mixed results. A number of studies have observed that the Met-allele is associated with better overall clinical response as well as greater improvement in negative symptoms and cognitive abilities such as working memory.
(Bertolino et al., 2004; Bertolino et al., 2007; Molero et al., 2007; Woodward et al., 2007; Pelayo-Terán et al., 2011). However, other studies have either been unable to replicate these findings (Yamanouchi et al., 2003; Gao et al., 2012; Tybura et al., 2012; Bishop et al., 2015) or observed the opposite effect, with the Met-allele predicting non-response to antipsychotic therapy (Illi et al., 2003; Porcelli et al., 2009). In an effort to clarify whether COMT Val158Met is associated with antipsychotic response, we conducted a meta-analysis of the existing literature and analyzed the variant in our own samples. In our primary analysis, we examined the association between this variant and both binary and continuous measures of response. The meta-analysis focused solely on clinical response to antipsychotics, since the overwhelming majority of Val158Met pharmacogenetic studies examined this phenotype.

4.3 Methods

**Literature Search**

A literature search was conducted for articles written in English published up to March 1, 2015, examining the COMT Val158Met variant and antipsychotic response in SCZ and schizoaffective disorder patients. We performed searches on PubMed, PsychInfo, and Web of Science using all combinations of the key terms ‘COMT’, ‘Catechol O-methyltransferase’, and ‘Val158Met’, with the phrases ‘antipsychotic response’ and ‘SCZ treatment response’. Criteria for inclusion in the current study consisted of: 1) the association between the COMT Val158Met polymorphism and antipsychotic response was assessed; 2) the patients were diagnosed with SCZ or schizoaffective disorder based on DSM-III, DSM-IV, DSM-V, or the International Statistical Classification of Diseases and Health-related Problems-10th Revision (ICD-10) criteria and their diagnoses were verified through a standardized structured clinical interview; 3) antipsychotic response was evaluated according to a standardized clinical rating scale, such as the PANSS or BPRS; 4) drug response (symptom severity) was evaluated at a minimum of two time points, one of which was directly prior to starting antipsychotic therapy and 5) patients were evaluated over a minimum of two weeks for response to treatment. Studies investigating non-clinical aspects of response were
excluded. We also searched through the reference sections of articles meeting these criteria in order to find other articles fit for inclusion. Authors were contacted for data when the data necessary for analysis was not reported in articles that met inclusion criteria.

In total, the literature search yielded 23 peer-reviewed articles published before March 1, 2015. Two of these articles were identified through searching the reference sections of the other 21 articles (Porcelli et al., 2009; Kang et al., 2010). The literature search process is outlined in Fig. 4.1. In total, 15 articles met inclusion criteria for the current study. However, 11 of the 15 studies did not have group-level data (i.e., number of responders/non-responders) available online (Molero et al., 2007; Ikeda et al., 2008; Fijal et al., 2009; Need et al., 2009a; Porcelli et al., 2009; Kang et al., 2010; Gao et al., 2012; Tybura et al., 2012; Zhao et al., 2012; Bishop et al., 2015; Bosia et al., 2015). Another two studies focused on first episode psychosis, and did not provide the response/non-response data for its subset of SCZ or schizo-affective disorder patients (Pelayo-Terán et al., 2011; Prata et al., 2012). We attempted to contact each of these groups and obtained data from seven of these studies (Molero et al., 2007; Ikeda et al., 2008; Porcelli et al., 2009; Pelayo-Terán et al., 2011; Gao et al., 2012; Tybura et al., 2012; Bishop et al., 2015). The studies for which we were unable to retrieve the required data were excluded (Fijal et al., 2009; Need et al., 2009a; Kang et al., 2010; Prata et al., 2012; Zhao et al., 2012; Bosia et al., 2015). We added data from five additional samples that our group has published on previously (Hwang et al., 2012; Zai et al., 2012; Tiwari et al., 2013; Gonçalves et al., 2014) to these 10 studies’ samples to give a total of 15 samples ($n_{\text{total}}=1416$). Two of these samples had previously been analyzed for Val158Met and antipsychotic response (Nolan et al., 2006), while the other three had not been. Fig. 4.1 illustrates the literature search process. The demographic and clinical characteristics of the 15 samples are described in Table 4.1. All 15 samples included data for the number of responders and non-responders. Ten of these samples also had data for change in total score (%). Of these 10 samples, six further included positive and negative symptom subscale score change data. We later contacted the authors for studies with a mix of atypical and typical-antipsychotic-treated patients to obtain response data for each antipsychotic type separately.
Figure 4.1 Literature Search Process Results

Subjects from Toronto Samples

The five samples from Toronto in this analysis included Toronto samples 1-5. Toronto Sample 1 (TS1) (Lieberman, N=58) was collected at four psychiatric state hospitals in New York and North Carolina. These patients met DSM-IV criteria for chronic SCZ and or schizo-affective disorder. They exhibited sub-optimal response to prior treatment, defined primarily by persistent positive symptoms and poor functioning.
over the past two years. The patients were recruited as part of a 14-week randomized double-blinded study and response was evaluated using the PANSS scale. This sample is also described in greater detail elsewhere (Volavka et al., 2004). Toronto Sample 2 (TS2) (Müller, N=89) was collected at Charité University Medicine, Berlin, Germany. Patients were diagnosed with SCZ or schizo-affective disorder according to DSM-IV or ICD-10 criteria and treated with several different antipsychotics. This sample is described in greater detail elsewhere (Müller et al., 2012). Toronto Samples 3 (TS3) (Meltzer, N=90), 4 (Lieberman, N=90), and 5 (TS5) (Potkin, N=32) were collected from Case Western Reserve University in Cleveland, Ohio, Zucker Hillside Hospital in Glen Oaks, New York, and University of California at Irvine, respectively. Subjects from TS3, TS4, and TS5 had DSM-III-R or DSM-IV diagnoses of SCZ and met criteria for treatment refractoriness or intolerance to traditional antipsychotic therapy. After a two to four week washout period, patients were treated with CLZ and evaluated prospectively for six months, during which blood levels of CLZ were monitored. Treatment response was evaluated using the 18-item BPRS (Overall and Gorham, 1962) at the beginning and end of treatment. These samples are described in greater detail elsewhere (Hwang et al., 2005b). Analysis of response in each of these samples is discussed in the section below, ‘Definition of Response’. The ethnicities of patients from all five samples are described in Table 4.1.

Ethical Considerations

The scientific work described in this article is in compliance with the ethical standards stated in the 1964 Declaration of Helsinki. Informed consent was obtained prior to patient participation, and this work was approved by the Ethics Committee of the Centre for Addiction and Mental Health.

Definition of Clinical Response

Clinical responders and non-responders were defined based on the criteria applied within each original study separately. If a study did not report the antipsychotic response data in binary responder/non-responder form, study investigators were contacted and
requested to apply a response threshold of 30% change in PANSS score or the equivalent in the clinical rating scale used in their study. The 30% cutoff was adopted for the current study, as it was the most commonly applied criteria in the studies included in the meta-analysis. We used Leucht et al.’s (2013) conversion scale to calculate corresponding response thresholds for other rating scales (Leucht et al., 2013a). For instance, according to this scale, the equivalent score change in the BPRS scale is 35%. For Toronto Sample 4 (TS4), only responder/non-responder data was available, for which response was defined as >20% reduction in overall BPRS score after 6 months of treatment based on criteria proposed by Kane et al. (1988). Thus, we had no option but to utilize this response definition for this sample. Overall, 14 out of the 15 samples used the 30% PANSS score change (or equivalent) response criteria. Non-clinical dimensions of response, such as improvement in cognition, were not analyzed.

Genotyping

For the Toronto samples, genomic data were extracted using the high-salt method from venous blood (Lahiri and Nurnberger, 1991). The COMT Val158Met (rs4680) variant was genotyped using Taqman 5’ nuclease assay (Applied Biosystems; Foster City, CA, USA). All ambiguous genotypes were retyped. Genotypes that remained ambiguous were excluded from further analyses. Genotyping accuracy was assessed by repeating genotyping for 10% of the sample, and results showed 100% concordance.

Statistical Analysis

All data were analyzed using R statistical software (v3.1.1). Meta-analysis was performed using the ‘meta’ package, version 4.1-0 (Guido Schwarzer, University of Freiburg). Fixed effects models were used for each analysis, consistent with approaches commonly used in pharmacogenetic meta-analyses (Serretti et al., 2007; Zhang et al., 2010b). In our primary analysis, separate meta-analyses were conducted assuming three genetic models: recessive (Met/Met vs. Val-allele carriers), dominant (Val/Val vs. Met-allele carriers), and allelic (Val vs. Met allele). All three models were used to test the association of COMT Val158Met with numbers of treatment responders vs. non-responders. Odds ratios and 95% confidence intervals were computed and pooled using
the Mantel-Haenszel method to determine overall effect sizes. We also examined each genetic model in relation to total symptom score change (%), positive subscale score change (%), and negative subscale score change (%) in a subset of study samples for which data were available (total: studies=10, n=1085; subscales: studies=6, n=616). Effect sizes were analyzed by computing standardized mean differences (SMD).

Heterogeneity was assessed using Cochran’s Q test. Meta-regression was used to check for confounding effects due to the following variables: self-reported ethnicity, SCZ type (first-episode vs. non-first episode), antipsychotic type (atypical-only vs. mixed; specific drugs), age of onset, sex, study design (e.g. prospective, naturalistic), duration of study, drug naïve vs. prior exposure, and criteria for response. Publication bias was evaluated using the Harbord test (Harbord et al., 2006) and the funnel plot method (“metabias” and “funnel” commands, respectively). Sensitivity analyses were conducted on any significant findings (p<0.05) by removing the studies with the largest and smallest effect sizes, as in other antipsychotic pharmacogenetic meta-analyses (Zhang et al., 2010b).

In order to dissect the factors (e.g. ancestry, antipsychotic type) contributing to the observed genetic effect, we examined the association between genotype and number of responders/non-responders in samples of European ancestry (studies=10, n_total=929). We focused on this ancestry group, as it constituted the majority of the samples. Ancestry may be important due to significant differences in Val158Met allele frequency between ethnicities. Moreover, since antipsychotic type showed a trend-level association with response, we examined the association between Val158Met and number of responders vs. non-responders in atypical and typical antipsychotic-treated patients separately.

With regards to correction for multiple testing, our approach is in line with that of prior pharmacogenetic meta-analyses in that we did not correct for the multiple genetic models tested when analyzing single genetic variants due to high correlations between the models (Bakker et al., 2006; Bakker et al., 2008; Zhang et al., 2010b).

Table 4.1 – Characteristics of studies investigating the association between COMT polymorphism Val158Met and antipsychotic drug response in schizophrenic patients

<table>
<thead>
<tr>
<th>Study/Sample</th>
<th>N</th>
<th>Ethnicity</th>
<th>MAF</th>
<th>Design</th>
<th>Medication</th>
<th>Response Criteria</th>
<th>Responders/Non-Responders</th>
</tr>
</thead>
</table>


### 4.4 Results

*Primary Analyses*

We observed a significant association with number of responders/non-responders under the recessive model (Met/Met vs. Val-allele carriers) \((p=0.039, \text{OR}_{\text{Met/Met}}=1.37, 95\% \text{ CI}: 1.02-1.85)\) (Fig. 4.2; Table 4.2). We also observed a trend-level association for total score change \((p=0.087, \text{SMD}=0.14, 95\% \text{ CI}: -0.020-0.30)\) under a recessive model.
Upon examination of the subscales, we observed a significant effect of Val158Met on positive symptom response also under a recessive model ($p=0.030$, SMD=0.24, 95% CI: 0.024-0.46). No significant associations of Val158Met genotype were observed for any other response measures, under any genetic model (all $p>0.05$). The Cochran chi-squared test did not reveal any significant heterogeneity for the recessive model in relation to number of responders/non-responders ($p=0.37$, $Q=15.07$, df=14, $I^2=7.1\%$), or for the other genetic models and response measures (all Cochran test $p>0.05$). Funnel plot analysis and the Harbord test revealed no evidence of publication bias for the recessive model in relation to number of responders/non-responders ($p=0.35$) (Fig. 4.3), or for the other genetic models.

Figure 4.2 All studies - Meta-analysis of the association between the Val158Met polymorphism (Met/Met versus Val carrier) and antipsychotic drug response: Forest plot

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Antipsychotic</th>
<th>Odds Ratio</th>
<th>OR</th>
<th>95%-CI</th>
<th>W(fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bertolino et al., 2004</td>
<td>30</td>
<td>Olanzapine</td>
<td>6.00</td>
<td>6.00</td>
<td>[0.58; 61.84]</td>
<td>0.9%</td>
</tr>
<tr>
<td>Bertolino et al., 2007</td>
<td>29</td>
<td>Olanzapine</td>
<td>4.00</td>
<td>4.00</td>
<td>[0.77; 20.67]</td>
<td>2.0%</td>
</tr>
<tr>
<td>Bishop et al., 2014</td>
<td>61</td>
<td>Various</td>
<td>1.17</td>
<td>1.17</td>
<td>[0.21; 6.44]</td>
<td>3.2%</td>
</tr>
<tr>
<td>Gao et al., 2012</td>
<td>83</td>
<td>Risperidone</td>
<td>0.77</td>
<td>0.77</td>
<td>[0.19; 3.19]</td>
<td>5.6%</td>
</tr>
<tr>
<td>Gupta et al., 2009</td>
<td>117</td>
<td>Risperidone</td>
<td>4.53</td>
<td>4.53</td>
<td>[1.24; 16.54]</td>
<td>3.7%</td>
</tr>
<tr>
<td>Ikeda et al., 2008</td>
<td>107</td>
<td>Risperidone</td>
<td>0.68</td>
<td>0.68</td>
<td>[0.19; 2.37]</td>
<td>8.1%</td>
</tr>
<tr>
<td>Molero et al., 2007</td>
<td>205</td>
<td>Various</td>
<td>0.68</td>
<td>0.68</td>
<td>[0.33; 1.40]</td>
<td>23.8%</td>
</tr>
<tr>
<td>Pelayo-Teran et al., 2011</td>
<td>99</td>
<td>Various</td>
<td>1.33</td>
<td>1.33</td>
<td>[0.15; 11.59]</td>
<td>2.1%</td>
</tr>
<tr>
<td>Porcelli et al., 2009</td>
<td>144</td>
<td>Various</td>
<td>1.07</td>
<td>1.07</td>
<td>[0.43; 2.65]</td>
<td>12.1%</td>
</tr>
<tr>
<td>Toronto Sample 1</td>
<td>58</td>
<td>Various</td>
<td>0.83</td>
<td>0.83</td>
<td>[0.04; 17.23]</td>
<td>1.3%</td>
</tr>
<tr>
<td>Toronto Sample 2</td>
<td>89</td>
<td>Various</td>
<td>1.28</td>
<td>1.28</td>
<td>[0.46; 3.55]</td>
<td>8.8%</td>
</tr>
<tr>
<td>Toronto Sample 3</td>
<td>90</td>
<td>Clozapine</td>
<td>3.04</td>
<td>3.04</td>
<td>[1.01; 9.19]</td>
<td>4.7%</td>
</tr>
<tr>
<td>Toronto Sample 4</td>
<td>90</td>
<td>Clozapine</td>
<td>1.35</td>
<td>1.35</td>
<td>[0.45; 4.00]</td>
<td>7.8%</td>
</tr>
<tr>
<td>Toronto Sample 5</td>
<td>35</td>
<td>Clozapine</td>
<td>5.40</td>
<td>5.40</td>
<td>[0.20; 142.71]</td>
<td>0.5%</td>
</tr>
<tr>
<td>Tybura et al., 2012</td>
<td>179</td>
<td>Various</td>
<td>1.43</td>
<td>1.43</td>
<td>[0.67; 3.07]</td>
<td>15.2%</td>
</tr>
</tbody>
</table>

1.37 [1.02; 1.85] 100%

Table 4.2 – Primary Analysis Results – All studies (n=15; total sample size=1416)

<table>
<thead>
<tr>
<th>Genetic Model</th>
<th>Association Effect Size (Odds Ratio, 95% CI)</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recessive</td>
<td>1.37 (1.02-1.85)</td>
<td>0.039*</td>
</tr>
</tbody>
</table>
(M/M vs. V/M and V/V) |  |  
---|---|---
Dominant (M/M and V/M vs. V/V) | 1.07 (0.83-1.37) | 0.58 |
Allelic (M vs. V) | 1.13 (0.96-1.34) | 0.14 |

CI = Confidence interval; M/M = Met/Met genotype, V/M = Val/Met genotype, V/V = Val/Val genotype; *denotes significance (p<0.05).

Figure 4.3 All studies - Meta-analysis of the association between the Val158Met polymorphism (Met/Met versus Val carrier) and antipsychotic drug response: Funnel plot and regression plot

To screen for potentially undetected heterogeneity, we employed the sensitivity analysis conducted by Zhang et al. (2010) in their pharmacogenetic meta-analysis (Zhang et al., 2010b), excluding the two studies with the largest (Bertolino et al., 2004) and smallest effect sizes (Ikeda et al., 2008). Sensitivity analysis was conducted for the
association between the Val158Met variant and number of responders/non-responders under the recessive model. This revealed no undetected heterogeneity and no significant influence on overall effect. After exclusion of the two studies, the significance of the association increased slightly ($p=0.012$), while the effect size remained the same (OR=1.54, 95% CI: 1.10-2.15) and the heterogeneity decreased ($I^2=0\%$, $p=0.65$).

Meta-regressions including potential confounding variables revealed a trend for antipsychotic type (atypical-only vs. mixed) as a predictor of response rate across studies ($p=0.055$). We could not include the variable of atypical vs. typical antipsychotic-treated samples, as there were no studies with patients exclusively treated with typical antipsychotics. None of the remaining potential confounders tested were associated with response rate: gender, ethnicity, first-episode vs. non-first episode, treatment duration, study design, age at onset, drug-naïve vs. prior exposure, and criteria for response.

**Post Hoc Analyses**

Since the meta-regression revealed antipsychotic type (atypical vs. mixed) to be a potential predictor of response ($p=0.055$), analyses were conducted separately on patients treated with atypical antipsychotics (studies=15; $n_{\text{total}}=1207$) and typical antipsychotics (studies=7; $n_{\text{total}}=155$). The seven studies with typical antipsychotic-treated patients also included patients treated with atypical antipsychotics, but these were excluded for this analysis. In the subsample of patients treated with atypical antipsychotics, significant differences in the number of responders/non-responders were observed under a recessive genetic model (Met/Met vs. Val-allele carriers), whereby patients with Met/Met genotype experienced improved clinical response relative to Val carriers ($p=0.0098$, OR=1.54, 95% CI: 1.11-2.14) (Fig. 4.4; Table 4.2). Additionally, allelic analyses revealed that the Met-allele was associated with greater likelihood of response compared to the Val-allele ($p=0.043$, OR=1.20, 95% CI: 1.01-1.44) (Fig. 4.6; Table 4.2). No association was observed between Val158Met and response/non-response in the subsample of patients treated with typical antipsychotics (Table 4.3).
Table 4.3 – Secondary Analysis Results – Atypical and Typical-Antipsychotic Patient Subsamples

<table>
<thead>
<tr>
<th>Genetic Model</th>
<th>Atypical Association Effect Size (Odds Ratio, 95% CI) (n=1207)</th>
<th>Significance (p-value)</th>
<th>Typical Association Effect Size (Odds Ratio, 95% CI) (n=155)</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recessive (M/M vs. V/M and V/V)</td>
<td>1.54 (1.11-2.14)</td>
<td>0.0098*</td>
<td>0.78 (0.36-1.70)</td>
<td>0.54</td>
</tr>
<tr>
<td>Allelic (M vs. V)</td>
<td>1.20 (1.01-1.44)</td>
<td>0.043*</td>
<td>0.78 (0.49-1.22)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

CI = Confidence interval; M/M = Met/Met genotype, V/M = Val/Met genotype, V/V = Val/Val genotype; *denotes significance (p<0.05).

Figure 4.4 – Atypical antipsychotic-treated samples - Meta-analysis of the association between the Val158Met polymorphism (Met/Met versus Val carrier) and antipsychotic drug response: Forest plot
In the subsample of atypical antipsychotic-treated patients, Cochran’s chi-square test revealed that heterogeneity was not significant under the recessive ($I^2=0\%, p=0.52$) or allelic models ($I^2=0\%, p=0.50$). After excluding the two studies with the largest (Bertolino et al., 2004) and smallest effect sizes (Molero et al., 2007), sensitivity analyses revealed no undetected heterogeneity and no significant influence on overall effect under the recessive or allelic models. Further analyses revealed no significant publication bias (Fig. 4.5). The effect remained significant when analyzing only samples for which response was defined as 30% PANSS score reduction (studies=14, n=1317) ($I^2=13.8\%, p=0.046$).

Due to significant differences in Val158Met allele frequency between ethnicities (ASN: 0.29, EUR: 0.52, AFR: 0.31), we conducted an analysis on studies with samples of European-ancestry (studies=10, n$_{total}=929$). No significant effects were observed for binary response/non-response under the recessive model ($p=0.29$, OR=1.21, 95% CI=0.85-1.72); however, consistent with whole group analyses, a trend was observed for atypical-antipsychotic-treated patients of European ancestry (studies=10, n$_{total}=745$) ($p=0.061$, OR=1.45, 95% CI=0.98-2.15), with Met/Met patients experiencing better response than Val-allele carriers. The Cochran test showed no significant heterogeneity
under any of the genetic models (all tests \( p > 0.05 \)). Furthermore, funnel plot analysis and the Harbord test did not reveal any significant publication bias (Fig. 4.7).

**Figure 4.6** – Atypical antipsychotic-treated samples – Meta-analysis of the association between the Val158Met polymorphism (Met vs. Val Allele) and antipsychotic drug response: Forest Plot

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Odds Ratio</th>
<th>OR</th>
<th>95%-CI</th>
<th>W(fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bertolino et al., 2007</td>
<td>29</td>
<td></td>
<td>2.86</td>
<td>[0.96; 8.49]</td>
<td>1.8%</td>
</tr>
<tr>
<td>Bertolino et al., 2004</td>
<td>30</td>
<td></td>
<td>3.40</td>
<td>[1.17; 9.86]</td>
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</tr>
<tr>
<td>Bishop et al., 2014</td>
<td>53</td>
<td></td>
<td>0.90</td>
<td>[0.30; 2.69]</td>
<td>3.1%</td>
</tr>
<tr>
<td>Gao et al., 2012</td>
<td>83</td>
<td></td>
<td>0.70</td>
<td>[0.31; 1.58]</td>
<td>6.1%</td>
</tr>
<tr>
<td>Gupta et al., 2009</td>
<td>117</td>
<td></td>
<td>1.76</td>
<td>[1.01; 3.07]</td>
<td>8.7%</td>
</tr>
<tr>
<td>Ikeda et al., 2008</td>
<td>107</td>
<td></td>
<td>0.98</td>
<td>[0.55; 1.76]</td>
<td>10.3%</td>
</tr>
<tr>
<td>Moiero et al., 2007</td>
<td>137</td>
<td></td>
<td>0.88</td>
<td>[0.52; 1.48]</td>
<td>13.6%</td>
</tr>
<tr>
<td>Pelayo-Teran et al., 2011</td>
<td>67</td>
<td></td>
<td>0.92</td>
<td>[0.41; 2.09]</td>
<td>5.4%</td>
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<tr>
<td>Porcelli et al., 2009</td>
<td>117</td>
<td></td>
<td>1.05</td>
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<td>8.9%</td>
</tr>
<tr>
<td>Toronto Sample 1</td>
<td>46</td>
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<td>[0.20; 3.94]</td>
<td>1.7%</td>
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<tr>
<td>Toronto Sample 2</td>
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<td></td>
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<tr>
<td>Toronto Sample 3</td>
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</tr>
<tr>
<td>Toronto Sample 4</td>
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<td></td>
<td>1.09</td>
<td>[0.60; 2.01]</td>
<td>9.1%</td>
</tr>
<tr>
<td>Toronto Sample 5</td>
<td>35</td>
<td></td>
<td>1.25</td>
<td>[0.41; 3.83]</td>
<td>2.5%</td>
</tr>
<tr>
<td>Tybura et al., 2012</td>
<td>124</td>
<td></td>
<td>1.29</td>
<td>[0.78; 2.14]</td>
<td>12.1%</td>
</tr>
</tbody>
</table>

\( 1.20 \ [1.01; 1.44] \) 100%

**Figure 4.7** – Atypical antipsychotic-treated samples – Meta-analysis of the association between the Val158Met polymorphism (Met vs. Val Allele) and antipsychotic drug response: Funnel plot and regression line
4.5 Discussion

To our knowledge, this study is the most comprehensive meta-analysis to investigate the relationship between COMT Val158Met and antipsychotic clinical response. Our meta-analysis includes data from eight studies not reported in the original manuscripts and kindly provided to us by the authors (Molero et al., 2007; Ikeda et al., 2008; Gupta et al., 2009; Porcelli et al., 2009; Pelayo-Terán et al., 2011; Gao et al., 2012; Tybura et al., 2012; Bishop et al., 2015), as well as three samples previously not investigated for Val158Met and antipsychotic response (TS2, TS3, TS5). Thus, our study provides a much-expanded sample compared to a previous meta-analysis (Chen et al., 2015). More importantly, all samples included in our meta-analysis were analyzed for response/non-response according to uniform criteria (PANSS 30% reduction or equivalent), with the exception of one (TS4), minimizing inter-study heterogeneity.

Overall, significant effects of Val158Met on response vs. non-response, as well as positive symptom improvement, were observed. The association was stronger when restricting the analysis to studies with atypical antipsychotic-treated samples (e.g., CLZ, olanzapine, risperidone). Overall, Met-allele homozygotes experienced improved response compared to Val-allele carriers.

These results are consistent with previous studies finding that the Met-allele is associated with improved cognitive response to atypical antipsychotics (Bertolino et al., 2004; Weickert et al., 2004; Woodward et al., 2007). These studies evaluated cognitive response using tests such as the Wisconsin Card Sorting Test (WCST), which evaluate cognitive abilities including working memory, executive function, and attention (Weickert et al., 2004). Conceivably, improvements in clinical symptoms, particularly positive symptoms, may contribute to cognitive improvement. Alternatively, improvements in clinical symptoms may be mediated by improvement in cognitive function (Censits et al., 1997; Velligan et al., 1997; Gold et al., 1999; Hoff et al., 1999); in particular, improved working memory and executive function could lead to improved treatment compliance and adherence to psychiatrist recommendations, resulting in improved clinical outcome. Cognitive response data was available in three of the studies included in the meta-analysis. Consistent with previous findings, Bertolino et al. (2004)
observed that the Met-allele predicted better working memory response to olanzapine as measured by the N-back task (Bertolino et al., 2004). The two other studies did not observe an association between Val158Met and improvement in memory function or cognitive flexibility (Gao et al., 2012), and spatial working memory (Bishop et al., 2015) in response to antipsychotic treatment, respectively. The reasons for this are not clear, but may relate to antipsychotic drug-type (both these samples were treated either largely or entirely with risperidone) and cognitive assessment methods (i.e., Bishop et al. assessed spatial working memory). However, it should be noted that Gao et al. (2012) observed the same direction effect as previous studies, with the Met-allele appearing to experience greater improvement in orientation, comprehension, and experiential and number memory (Gao et al., 2012).

The findings are also consistent with the tonic-phasic DA hypothesis as applied to antipsychotic response. According to this hypothesis, DA regulation in limbic striatal regions occurs through (1) transient, high-amplitude, phasic DA release and (2) persistent low-level tonic DA release modulated by corticostratial glutamatergic afferents. In these regions, tonic DA release also decreases phasic release through the stimulation of D2 auto-receptors at the synapse (Bilder et al., 2004). Importantly, COMT is involved in modulating the tonic, extracellular pool of DA subcortically, as it breaks down DA that is not re-absorbed by DA transporters. Thus, the Met-allele leads to lower COMT activity, higher tonic DA, and lower phasic DA release (more negative symptoms), while the Val-allele gives rise to lower tonic and higher phasic DA release (more positive symptoms) (Bilder et al., 2004). Bilder et al. (2004) have also suggested that D1 receptors are more important for modulating tonic release, while D2 receptors are more critical for regulating phasic DA release (Bilder et al., 2004). Therefore, the COMT Val158Met variant may have different effects on response to different classes of antipsychotics. Specifically, they suggest that the Met-allele may predict better response to agents enhancing D1-transmission (5-HT2A-antagonists and/or 5-HT1A-agonists or partial agonists), since they are thought to increase tonic DA transmission (Bilder et al., 2004). These agents include the majority of atypical antipsychotics. The Val-allele, in contrast, is hypothesized to predict better response to conventional D2-blocking agents (i.e., typical antipsychotics), as they decrease phasic DA transmission.
Our findings for positive symptoms may also be consistent with the tonic-phasic DA hypothesis. Based on Bilder et al.’s predictions, the Met-allele may also predict improved response in positive symptoms due to lower phasic DA transmission subcortically, which could potentiate the effects of antipsychotic-induced D2 receptor blockade. This prediction is supported by previous findings that the Met-allele is associated with greater DA synthesis in the midbrain, and thus, lower striatal dopaminergic stimulation (Meyer-Lindenberg et al., 2005). Moreover, consistent with this theory, reduced COMT activity has been observed to potentiate CLZ-induced DA release in the PFC (Tunbridge et al., 2004). By the tonic-phasic DA hypothesis, increased DA release in the PFC leads to greater D1 receptor activation, stimulating greater glutamate release from pyramidal neurons onto the nucleus accumbens. This, in turn, increases tonic DA release, subsequently decreasing phasic DA release, thus reducing positive symptom severity (Bilder et al., 2004). Specific atypical antipsychotics such as risperidone and olanzapine have also been observed to up-regulate COMT mRNA expression in the rat PFC (Chen and Chen, 2007), suggesting a direct effect of these drugs on COMT activity.

The observed null genetic effect in typical-antipsychotic-treated patients may be a product of lack of statistical power, as the majority of patients were treated with atypical antipsychotics (typical: n=155, atypical: n=1207). Ultimately, it is possible that analyses conducted in larger samples could reveal associations of Val158Met with response in subjects treated with typical antipsychotics. Further, we acknowledge that differences among specific drugs within each class of antipsychotics, rather than broader class differences between atypical and typical antipsychotics, may be driving the results. However, due to limited data, this could not be tested.

The direction of genetic effect was consistent across 11 of the 15 samples in both the analysis of total sample as well as of atypical-antipsychotic-treated patients alone. The four samples observing a genetic effect in the opposite direction included TS1, Molero et al. (2007), Gao et al. (2007), and Ikeda et al. (2008). Notably, these four samples’ effects were all non-significant (95% CI of OR encompasses 1), leaving open the possibility that the opposite directions of effect may be due to random chance. It is possible that ancestry-related differences in the frequencies of COMT genetic variants
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other than Val158Met may explain these results. For instance, the functional variant Ala72Ser (rs6267), which is not in LD with Val158Met, also alters COMT activity and exists only in East-Asian populations (MAF: 0.07-0.11) (Gupta et al., 2011). Functional investigations suggest that this variant may exert its effect additively together with Val158Met, whereby presence of both low-activity alleles results in lower COMT activity than possession of either allele alone (Lee et al., 2005). Thus, while ancestry-related differences in MAF and COMT genetic variation may provide insight into the opposite direction of effect observed, we did not have sufficient samples of Asian ancestry to test for ancestry-specific associations. Another sample showing an opposite direction of effect, TS1, was highly treatment-resistant and included only four responders compared to 54 non-responders; this lack of statistical power likely contributed to the null genetic effect observed in this sample. Finally, none of the four samples deviated from Hardy-Weinberg equilibrium (HWE) ($p<0.05$), ruling this out as a possible explanation.

In addition, Molero et al. (2007) may have observed a non-significant effect for Val158Met in their sample due to several reasons. First, their sample differed from the majority of other samples in that the study specifically included patients who had recently experienced an acute worsening of psychotic symptoms, leading to uniquely high initial scores (avg PANSS score: ~90). Secondly, the Val-allele carriers had significantly higher PANSS scores compared to Met-allele homozygotes at the study’s beginning. Since baseline score has been observed to predict overall response, this score differential may have contributed to greater score change for Val-allele carriers, and thus a higher number of responders. Importantly, Met-allele homozygotes had significantly lower PANSS scores than Val-allele carriers at the end of the six months of treatment, which is consistent with findings from other studies in the meta-analysis.”

The present meta-analysis had several limitations. First, while we attempted to test and control for possible confounding factors, several factors could not be accounted for, due to lack of data availability. These include pharmacological aspects of treatment response, such as drug dose or concentrations, as well as environmental factors, such as significant stress or lifetime exposure to psychosis-inducing drugs. Nevertheless, it should be noted that no confounding effects were observed for the variables tested,
except for antipsychotic type (trend observed). Moreover, heterogeneity across studies was not significant for any of the genetic models or response measures evaluated, suggesting minimal confounding by unidentified factors. Second, although we did not observe a significant influence of ethnicity in our samples, it should be noted non-European ethnicities were underrepresented. This imbalance may be important, since, as mentioned previously, Val158Met allele frequencies differ considerably among ethnic groups. Nevertheless, the fact that the sample consisted of mostly patients of European ancestry helps reduce heterogeneity in the sample. Third, it should also be noted that the genotypes in the sample from Pelayo-Teran et al. (2011) were not in HWE \((p<0.05)\). However, the skewing effect due to non-HWE should be minimal, as removing this sample did not impact the overall genetic effect observed or the level of heterogeneity. Lastly, if we were to apply a Bonferroni correction threshold, the association observed between Val158Met and number of responders/non-responders in the total sample would be non-significant. However, given the high correlation between the genetic models applied and phenotype measures of response, this method for correction would be overly conservative.

Overall, our meta-analysis provides evidence that \(COMT\) Val158Met is associated with response to antipsychotics, with a stronger effect observed for atypical antipsychotics. Prospective studies with complete categorical and continuous response measure data that differentiate between atypical and typical antipsychotics would help in strengthening the evidence of this functional variant’s relationship with antipsychotic response. Future studies of \(COMT\) should also include other putatively functional variants such as rs6267, rs737865, and rs4818 (SZGene Data Base, Nature Genetics, 2008) (Bray et al., 2003; Chen et al., 2004) in order to obtain a more comprehensive understanding of how genetically-predicted COMT function may influence antipsychotic response. Moreover, given the complexity of the antipsychotic response phenotype, it is likely that other genetic, environmental, and potentially epigenetic factors are involved, which were beyond the scope of this study. Notably, several findings provide evidence for multiple gene-gene interaction and gene-environment interactions involving \(COMT\) (Nicodemus et al., 2007). Moving forward, investigations of epistatic and gene-environment interactions involving \(COMT\) should further improve our understanding of this gene’s effect on
antipsychotic response. Nevertheless, our findings suggest that \textit{COMT} Val158Met may warrant closer consideration in randomized clinical trials. Since this variant appears to associate with response, balancing each arm of a RCT so that the same percentage of Met/Met are represented in each arm may prevent COMT genetic imbalance from confounding the efficacy results.

\textbf{Funding}

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\textbf{Statement of Interest}

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Forest, Merck, Novartis, Otsuka, and Sunovion. The remaining authors have no potential conflicts of interests to disclose.
CHAPTER 5

Preliminary genetic model for clozapine response in schizophrenia

Eric Huang¹,², Clement C. Zai¹,³, Vanessa Gonçalves¹,³, Marcos Sanches³, Arun K. Tiwari¹,³, James L. Kennedy¹,³

¹Neurogenetics Section, Campbell Family Research Institute, Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada; ²Institute of Medical Science, University of Toronto, Toronto, ON, Canada; ³Department of Psychiatry, University of Toronto, Toronto, ON, Canada

Corresponding Author:
James L. Kennedy, MD, FRCPC, FRSC
Director, Neuroscience Research
Head, Neurogenetics Section
Centre for Addiction and Mental Health (CAMH);
Professor and Co-Director Div. of Brain and Therapeutics
Department of Psychiatry
University of Toronto
Tel: (416) 979-4987
Fax: (416) 979-4666
jim.kennedy@camh.ca
www.camh.ca

Currently in preparation for submission to peer-reviewed journals.
5.1 Abstract

**Background** – Despite its clinical utility, a pharmacogenetic test for response to the antipsychotic CLZ has yet to be developed. Arranz et al (2000) proposed the first model of this kind, but it was not replicated in independent samples (Schumacher et al., 2000). Since then, to our knowledge, no other studies have sought to create a genetic panel predicting treatment response to this antipsychotic. Thus, we sought to create a preliminary genetic model for CLZ response. In the past 20 years, multiple genetic variants (e.g. DA, serotonin) have been linked to CLZ response. We developed a model incorporating the most promising findings from our group’s repository of CLZ response studies.

**Methods** – Our sample consisted of 151 Caucasian subjects with SCZ (DSM-III) treated with CLZ for six months. Response was assessed using the BPRS, and evaluated using (1) absolute score change and (2) binary response (Kane et al. 1988 criteria), with baseline score as a covariate. Variants showing at least a nominal statistical trend ($p<0.1$) were included in the model. An unweighted risk score was calculated for each SNP, and the total risk score assessed for association with response. Ten-fold CV was performed to limit model overfitting. The model was then tested in an independent sample of antipsychotic-treated SCZ patients of European ancestry (N=390) to examine generalizability of findings to other antipsychotics.

**Results** – Four markers were included in the model: **DRD2** rs2514218, **5-HT6** rs1805054, **BDNF** rs6265 (Val66Met), **NRXN1** rs1045881. We observed a statistically significant association between genetic risk score with (1) BPRS score change ($p = 0.000039$, Adjusted $R^2 = 0.565$) and (2) binary response ($p = 0.004$, Nagelkerke $R^2 = 0.097$) assuming a linear increase in response for each additional risk allele. The model had an accuracy of 62%, a sensitivity of 70%, and a specificity of 47%. Individuals with the lowest genetic risk score experienced the smallest decrease in BPRS score and were least likely to respond to CLZ. The model was not significantly associated with response in the independent sample treated with other antipsychotics ($p = 0.10$).
Discussion – We have developed a preliminary genetic model for CLZ response. However, replication in independent studies of CLZ response in SCZ is required to confirm the validity of these findings.

5.2 Introduction

Amongst antipsychotic medications, CLZ has long been identified to be the most efficacious for TRS (Kane et al., 1988b; Chakos et al., 2001). Currently, CLZ is approved for treatment for TRS and recurrent suicidal behavior in SCZ and schizo-affective disorder patients, having been shown to be effective at reducing suicidal thoughts (Spivak et al. 2013). Interestingly, a recent meta-analysis on antipsychotic efficacy and adverse side-effects observed that CLZ results in the greatest symptom reduction for SCZ patients (both refractory and non-refractory) and the lowest rate of EPS (Leucht et al., 2013b).

Despite these benefits, CLZ prescription rates remain very low in North America (~ 4.4% in the USA) (Meltzer, 2012). A large antipsychotic trial observed that CLZ was only prescribed in 14% of eligible patients (Stroup et al., 2009). This is primarily due to the drug’s potentially life-threatening side-effect of agranulocytosis, a sharp decrease in the body’s neutrophil count (Nielsen et al., 2009). Other important side-effects include myocarditis, seizures, ileus and severe weight gain (Iqbal et al., 2003). Thus, predictive tests for response and side-effects to CLZ would be especially beneficial for patients and valuable for physicians weighing the costs and benefits of prescribing the antipsychotic. Specifically, these tests should reduce the chance of patients experiencing CLZ’s adverse side-effects and non-response.

sample of 200 SCZ patients, the model successfully predicted response with an accuracy of 76.9%, a sensitivity of 95%, and a specificity of 38%. However, this model was not replicated in another sample of 163 European patients treated with CLZ (Schumacher et al. 2000). Since then, pharmacogenetic studies have implicated numerous genetic variants across multiple systems (e.g. DA, serotonin) in CLZ response. Recent meta-analyses revealed significant associations for functional 5-HT2A genetic variants rs6313 (T102C) and 6314 (His452Tyr), and 5-HT3A variant rs1062613 (C178T) (Gressier et al., 2015). Meta-analyses also found statistical trends for functional DRD3 variant rs6280 (Ser9Gly) (Gressier et al., 2015). In addition, DRD2 genetic variants such as an insertion/deletion in the promoter region (-141C Ins/Del) have also been implicated in CLZ response, albeit with mixed results (Arranz et al., 1998b; Hwang et al., 2005b; Hwang et al., 2006; Huang et al., 2016). Despite these findings, a genetic predictive test has yet to be developed.

Our study sought to build such a test based upon the most promising findings from our group’s repository of CLZ response studies. We performed a risk score analysis of all genetic variants showing at least a statistical trend for association with response, followed by cross-validation. The model was then tested in an independent sample of antipsychotic-treated SCZ patients.

5.3 Methods

Subjects
Sample 1 – CLZ-treated SCZ patients

This sample is described in greater detail in the Methods in Chapter 3 (Table 3.1). The study examined the 151 subjects of Caucasian ethnicity in order to avoid confounding effects due to population stratification (Cardon and Palmer, 2003). All subjects met criteria for treatment refractoriness or intolerance to traditional antipsychotic therapy as defined by Kane et al. (1988) (Kane et al., 1988a). Subjects were treated with CLZ for six months, during which time they were evaluated prospectively. Response was assessed
using the 18-item BPRS. Responders were defined using the same criteria employed by Kane et al. (1988).

Sample 2 – Clinical Antipsychotic Trials for Intervention Efficacy (CATIE) Sample

We also tested the model in relation to antipsychotic response in a sample from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study (Lieberman et al., 2005a). The goal of this was to evaluate whether the model could predict response to antipsychotics other than CLZ. The total sample consisted of 741 chronic SCZ patients ranging between 18 and 65 years of age. Patients were diagnosed with SCZ according to the Structured Clinical Interview for DSM-IV (SCID) and assessed for symptom severity using the PANSS. We limited our analysis to patients of European ancestry, in order to reduce heterogeneity in ethnicity and to maintain consistent ethnic distribution with the CLZ sample analysis. Principal components analysis (PCA) was used to identify patients of European ancestry (N=390). In addition, we focused our analysis on Phase I data (N=309), where patients are treated with olanzapine, risperidone, perphenazine, quetiapine, or ziprasidone for up to 18 months under double-blinded conditions. Patients were evaluated for response every visit for up to 18 months until discontinuation of drug treatment (average treatment duration = 435.7 +/- 125 days).

**Genetics**

SNPs from genes across multiple neurotransmitter systems were genotyped as part of previous studies. Genomic DNA was extracted using the high-salt method from venous blood (Lahiri and Nurnberger, 1991). Individual SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis or Taqman 5’ nuclease assays (Applied Biosystems; Foster City, CA, USA). The genes from which SNPs were genotyped are listed in Table 5.1.

Table 5.1 – Genes analyzed previously for association with CLZ response
<table>
<thead>
<tr>
<th>System</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td><em>DRD1, DRD2, DRD3, DRD4, DRD5, COMT, DBH</em></td>
</tr>
<tr>
<td>Serotonin</td>
<td><em>5HT2A, 5HT2C, 5HT3A, 5HT3B, 5HT4, 5HT5, 5HT6, 5HTT</em></td>
</tr>
<tr>
<td>Glutamate</td>
<td><em>GRIN1, GRIN2A, GRIN2B, SLC1A1</em></td>
</tr>
<tr>
<td>Metabolic Enzyme</td>
<td><em>CYP1A2, CYP2D6, CYP3A4, CYP3A43, ABCB1</em></td>
</tr>
<tr>
<td>Other</td>
<td><em>OXT, OXTR, NRXN1, GSK3, BDNF, GFRA1, GFRA2, GFRA3, GNB3</em></td>
</tr>
</tbody>
</table>

The proteins encoded by each of these genes are delineated in the List of Abbreviations.

In the CATIE sample, *DRD2* rs2514218 and *BDNF* rs6265 have been previously genotyped. The two other markers, *5-HT6* rs1805054 and *NRXN1* rs1045881, were not available, so we attempted to impute the genotypes using IMPUTE v2.2 (Howie et al., 2012). Pre-phasing was conducted using SHAPEIT2 (Delaneau et al., 2013). Imputation was performed in segments 5Mb in length using 1000 Genomes Project Phase 3 as the reference panel (Sudmant et al., 2015). The output was converted to PLINK format using the program GTOOL (Genetics Software Suite © 2007, Oxford University).

**Statistical Methods**

Individual SNP Analysis

All markers were analyzed assuming an additive model. A linear regression model was applied to test for the association between each SNP and BPRS score change, while a logistic regression model was used to examine association with response/non-response. Baseline score was included as a covariate, as it was significantly associated with response (p<0.05). As discussed in Chapter 3, gender and age at onset were not associated with treatment response, and were thus not included as covariates.
Risk Score Analysis

Variants showing at least a statistical trend ($p>0.1$) for association with either measure of response were included in the predictive model. Each SNP included was coded as a ‘2’ for having both risk alleles, ‘1’ for one risk allele, and ‘0’ for no risk alleles, as done in other polygenic risk score analyses (Plomin and Deary, 2015). A subject’s genetic risk score was calculated by summing the number of risk alleles at each of the four SNPs. Genetic risk scores were calculated in an unweighted fashion (not accounting for the effect sizes of each genetic variant). This approach has been observed to be more useful in order to avoid overfitting particularly in case of error in estimating true effect sizes (Dudbridge, 2013). A linear regression model was used to test for an association between risk score and BPRS score change. A logistic regression model was used to examine association with response/non-response. In all analyses, baseline score was included as a covariate. If a significant association with BPRS score change was observed, secondary analyses were performed to determine whether the association was specific to score change on particular subscale (positive or negative). Risk score analyses were conducted using SPSS 22.0 (Chicago, IL, USA).

Sensitivity Analysis

In the final model, sensitivity analysis was performed in order to evaluate the contribution of each genetic variant to the model’s performance. This was done by conducting the statistical analyses mentioned above while leaving one genetic variant out at a time.

Cross Validation

After identifying markers that showed at least a statistical trend, ten-fold CV was performed to limit potential overfitting of the model to the sample data, and obtain a
more accurate estimate of the model’s prediction performance. This method has been suggested to provide the optimal balance between variance explained and bias (Kohavi, 1995). This method is described in greater detail in Chapter 1 (Methodologies). Ten-fold CV was repeated 100 times with random independent dataset partitions to reduce the variation across testing datasets. Repetition of CV has been suggested to optimize the stability of results (Kim, 2009). For BPRS score change, the model’s performance was measured using $R^2$, as used in other studies (Iniesta et al., 2016). For response/non-response, the model’s performance was evaluated by computing the area-under the curve (AUC), averaged from the 100 repeats from the cross-validation. Cross-validation was performed using R statistical software (v3.1.1) package ‘cvTools’, version 0.3.2 (Alfons, 2012). Model performance was evaluated using package ‘pROC’, version 1.8 (Robin et al., 2011).

Testing Model in Independent Sample

In order to maintain consistency in the scales used to evaluate response, we converted the CATIE sample’s PANSS scores into BPRS scores using a standard conversion table (Leucht et al., 2013a). Response was defined using the same criteria as the CLZ-treated sample—based on criteria proposed by Kane et al. (1988). A linear regression model was then applied to analyze the association between BPRS score change and risk score. A logistic regression model was used to examine the association between response/non-response and risk-score. Baseline BPRS score, treatment duration (Phase I), and medication type were included as covariates in both models.

5.4 Results

CLZ Response

All variants were in HWE ($p>0.05$) in the sample. The variants that showed at least a trend ($p<0.1$) for association with response were $DRD2$ SNP rs2514218, 5-HT6
rs1805054, *NRXN1* rs1045881 and *BDNF* rs6265 (Table 5.2). The alleles conferring improved response (‘risk alleles’) can be found in Table 5.2.

**Table 5.2 – Top SNP Association Analysis Results**

<table>
<thead>
<tr>
<th>Gene &amp; SNP</th>
<th>Risk Allele</th>
<th>Non-risk Allele</th>
<th>Minor Allele Frequency (MAF)</th>
<th>ΔBPRS p-value</th>
<th>ΔBPRS Variance Explained (Adjusted $R^2$)</th>
<th>R/NR p-value</th>
<th>R/NR Variance Explained (Nagelkerke $R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRD2 rs2514218</td>
<td>A</td>
<td>C</td>
<td>0.25</td>
<td>0.033*</td>
<td>0.43</td>
<td>0.12</td>
<td>0.021</td>
</tr>
<tr>
<td>5HT6 rs1805054</td>
<td>T</td>
<td>C</td>
<td>0.16</td>
<td>0.002*</td>
<td>0.44</td>
<td>0.073</td>
<td>0.032</td>
</tr>
<tr>
<td>BDNF rs6265</td>
<td>A</td>
<td>G</td>
<td>0.21</td>
<td>0.13</td>
<td>0.39</td>
<td>0.095</td>
<td>0.028</td>
</tr>
<tr>
<td>NRXN1 rs1045881</td>
<td>A</td>
<td>G</td>
<td>0.14</td>
<td>0.16</td>
<td>0.41</td>
<td>0.082</td>
<td>0.028</td>
</tr>
</tbody>
</table>

*Denotes significance (p<0.05). The p-values provided are not corrected for multiple tests. The adjusted variance explained for ΔBPRS is adjusted for baseline score. The variance explained for R/NR is not adjusted for covariates, since this data was not available for all subjects.*

In our primary analysis, we observed a statistically significant association between total risk score with (a) ΔBPRS ($p = 3.9 \times 10^{-5}$, $\beta = 0.36$, SE = 0.02, $R^2 = 0.655$) (Fig. 5.1, 5.2) and (b) numbers of responders/non-responders ($p = 0.004$, Nagelkerke $R^2 = 0.097$) (Fig. 5.2). Ten-fold CV revealed that the risk score model was able to explain 52% of the variance in ΔBPRS, and predict response with an accuracy (AUC) of 62%, a sensitivity of 70%, and a specificity of 47% (Fig. 5.4). Individuals with the lowest risk score (score = 3) experienced the smallest decrease in BPRS (Avg. ΔBPRS = -0.40 points, 95% CI: -10.16-9.37) and were least likely to respond to CLZ (Response Rate = 25%) (Fig. 5.1, 5.3). Conversely, subjects with the highest risk score (score = 8) improved the most (Avg. ΔBPRS = 17.52 points, 95% CI: 7.70-27.34).
The sensitivity analysis revealed that all four variants contributed to the model’s performance in relation to response. 5-HT6 rs1805054 provided the greatest contribution to the model (when removed, $R^2$ decreased from 52% to 44%, AUC decreased from 0.62 to 0.57). The sensitivity analysis results are provided in Table 5.3.

Table 5.3 – Model Sensitivity Analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No 5-HT6 rs1805054</td>
<td>0.44</td>
<td>0.57</td>
</tr>
<tr>
<td>No NRXN1 rs1045881</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>No BDNF rs6265</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>No DRD2 rs2514218</td>
<td>0.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Each of the variants was excluded one by one. AUC denotes the area-under-the-curve, which represents the accuracy of the model.

Given the association with BPRS score change, we performed secondary analyses examining whether genetic risk score was associated with any of the symptom subscales. A significant association was observed between risk score and improvement in the positive symptom subscale ($p=0.001, \beta = 0.36, R^2= 0.34$) but not for negative symptoms ($p = 0.74$). In addition, risk score was significantly associated with improvement in the general psychopathological symptoms not belonging to either the positive or negative subscales ($p = 0.003, \beta = 0.26, R^2= 0.58$). No multiple testing correction was applied as only one model was tested ($\Delta$BPRS phenotype).
Figure 5.1 – ΔBPRS vs. Genetic Risk Score

Note: Error bars represent standard error. Numbers of patients with each risk score: 3 – N=3; 4 – N=5; 5 – N=18; 6 – N=28; 7 – N=11; 8 – N=3.

Figure 5.2 – ΔBPRS vs. Genetic Risk Score

Numbers of patients with each risk score: 3 – N=3; 4 – N=5; 5 – N=18; 6 – N=28; 7 – N=11; 8 – N=3.
Figure 5.3 – % Responder/Non-responder vs. Genetic Risk Score

Numbers of patients with each risk score: 3 – N=4; 4 – N=11; 5 – N=32; 6 – N=52; 7 – N=18; 8 – N=6.

Figure 5.4 – Receiver Operating Characteristics (ROC) Curve – Predicting Response/Non-response using Genetic Risk Score

AUC = 0.62
Sensitivity = 70%
Specificity = 47%
Note: Green line denotes an AUC of 0.50, or random chance. Blue line denotes the performance of the genetic model.

**CATIE Sample Results**

In the CATIE sample, 5-HT6 rs1805054 had good imputation accuracy (INFO score > 0.8). However, NRXN1 rs1045881 had low imputation accuracy and was thus excluded from analysis.

The resultant genetic risk score consisted of the individual risk scores from rs1805054, rs2514218, and rs6265. All three SNPs were in HWE ($p>0.05$) in the CATIE sample. A potential trend toward association was observed between risk score and antipsychotic response ($p = 0.11, \beta = -0.083, \text{SE} = 0.58, R^2=0.17$) (Fig. 5.5). However, the trend disappeared when stratifying for medication type (Fig. 5.6-5.10). No significant association was observed between risk score and number of responders/non-responders ($p = 0.38$).

All statistical assumptions were met for the linear and logistic regression models. Under the linear model, three outliers were found to be greater than three standard deviations away from the regression line. After careful examination of these data points, they were kept in the analysis. Removing the three outliers did not have a significant effect on the results ($p = 0.10, \beta = -0.085, \text{SE} = 0.88, R^2 = 0.19$).
Figure 5.5 – ΔBPRS Score vs. Genetic Risk Score – CATIE

Note: Error bars represent standard error. Numbers of patients with each risk score: 0-1 – N=23; 2 – N=99; 3 – N=119; 4 – N=53; 5 – N=15

Figure 5.6 – ΔBPRS Score vs. Genetic Risk Score for Olanzapine-treated Patients

Note: Error bars represent standard error. Numbers of patients with each risk score: 1 – N=9; 2 – N=24; 3 – N=32; 4 – N=10; 5 – N=1
Figure 5.7 – ΔPANSS Score vs. Genetic Risk Score for Risperidone-treated Patients

Note: Error bars represent standard error. Numbers of patients with each risk score: 0-1 – N=6; 2 – N=22; 3 – N=23; 4 – N=13; 5 – N=3

Figure 5.8 – ΔPANSS Score vs. Genetic Risk Score for Quetiapine-treated Patients

Note: Error bars represent standard error. Numbers of patients with each risk score: 1 – N=4; 2 – N=17; 3 – N=26; 4 – N=12; 5 – N=4
Figure 5.9 – ΔPANSS Score vs. Genetic Risk Score for Perphenazine-treated Patients

Note: Error bars represent standard error. Numbers of patients with each risk score: 2 – N=19; 3 – N=27; 4 – N=13; 5 – N=5

Figure 5.10 – ΔPANSS Score vs. Genetic Risk Score for Ziprasidone-treated Patients

Note: Error bars represent standard error. Numbers of patients with each risk score: 1 – N=4; 2 – N=17; 3 – N=11; 4 – N=5; 5 – N=2
5.5 Discussion

The current study presents the first genetic model for CLZ response since Arranz et al. (2000) published their six-marker model. Built upon four markers, the model significantly predicted improvement in symptoms for CLZ-treated SCZ patients, explaining 56.5% variance in response. In our secondary analysis, the model was significantly associated with improvement in positive and general psychopathological symptoms, but not negative symptoms. However, the model (excluding NRXN1 rs1045881) did not significantly predict response in a sample of SCZ patients treated with quetiapine, olanzapine, risperidone, perphenazine, or ziprasidone.

Of the markers included in Arranz et al.’s (2000) CLZ response model, our study did not observe significant associations for 5-HT2A rs6313 and 5-HT2C Cys23Ser (rs6318). Genetic data were not available for 5-HT2C 330-GT/244-CT repeat, 5-HT2A His452Tyr (rs6314), 5-HTTLPR, and H2 1018G/A (rs2067474). None of the markers included in our model were investigated by Arranz et al (2000).

Nevertheless, all four SNPs included in the model for CLZ response have previously been associated with response to either CLZ or other antipsychotics. 5-HT6 rs1805054 was observed to be associated with response to CLZ and risperidone, respectively, in two samples of Asian ancestry (Yu et al., 1999; Lane et al., 2004). The only study to report a negative result for this SNP and antipsychotic response was published by our group, but that study (Masellis et al., 2001) only had access to a portion of the sample used in this current study. NRXN1 rs1045881 has previously been found to be associated with response to CLZ by our group (Lett et al., 2012). BDNF rs6265 Val-allele has also been associated with improved response to antipsychotics in SCZ patients in a number of studies (Hong et al., 2003; Zai et al., 2012; Perkovic et al., 2014), but was not replicated in others (Anttila et al., 2005; Xu et al., 2010; Pae et al., 2012; Xu et al., 2015). Lastly, DRD2 rs2514218 has previously been found to associate with response to CLZ in our sample (Huang et al., 2016) and with response to risperidone and aripiprazole in a smaller sample of first-episode psychosis patients, albeit with opposite directions of effect (Zhang
et al., 2015). The reasons for this opposite direction of effect have previously been discussed in the Discussion in Chapter 3.

The four genes have also previously been implicated in SCZ pathology as well as antipsychotic mechanisms. NRXN1 is a synaptic membrane cell-adhesion protein that regulates synaptic transmission and is implicated in neuronal differentiation, maturation, and plasticity (Varoqueaux et al., 2006; Zhang et al., 2010a). The protein has been suggested to modulate NMDA receptor activity by binding to another protein (LRRTM2) that influences differentiation of glutamatergic synapses (De Wit et al., 2009). Given CLZ’s effects on preventing PCP-induced elevation in NMDA activity (Ninan et al., 2003) (see Introduction), NRXN1 may foreseeably be involved in CLZ’s mechanism. Genetic deletions in NRXN1 have also previously been commonly implicated in SCZ (Kirov et al., 2009b; Kirov et al., 2009a; Rujescu et al., 2009). Moreover, the NRXN1 marker investigated, rs1045881, is located in a potential micro-RNA (miRNA) binding site and is correlated with frontal lobe white matter volume as well as sensorimotor function (Voineskos et al., 2011). Together, these prior findings provide strong support for the association of rs1045881 with CLZ response.

The 5-HT6 receptor has also been implicated in SCZ and antipsychotic mechanisms. The receptor has been found to be involved in neurite growth (Duhr et al., 2014), and has lower expression in the hippocampus of SCZ patients compared to healthy controls (East et al., 2002). 5-HT6 receptors were also observed to mediate cognitive function and anxiety response in animal models of SCZ (Otano et al., 1999; Branchek and Blackburn, 2000). Notably, 5-HT6 receptor antagonists effectively reduced positive symptoms but not negative symptoms in animal models of SCZ (Pouzet et al., 2002). This is consistent with our findings that 5-HT6 rs1805054 was associated with positive symptom improvement in our CLZ-treated sample.

Lastly, the association between brain-derived neurotrophic factor (BDNF) gene variant rs6295 (Val66Met) and CLZ response is reasonable, given prior evidence implicating BDNF in SCZ and CLZ’s mechanism. BDNF is known to play an important role in
dopaminergic and serotonergic transmission in the brain. Specifically, it has been observed to modulate dopaminergic neuron differentiation (Spencer et al., 1995), as well as neuronal maturation and plasticity in the adult brain (Notaras et al., 2015). The protein has also been found to have lower expression in the PFC and hippocampus of SCZ patients compared to healthy controls (Zhang et al., 2012). In addition, antipsychotics including CLZ have been observed to lead to reduced serum BDNF levels (Grillo et al., 2007). Thus, since the valine-to-methionine substitution (rs6295) is associated with deficient BDNF activity-dependent secretion, it is plausible that this variant would be implicated in CLZ response.

Our genetic model consisting of 5-HT6 rs1805054, DRD2 rs2514218, and BDNF rs6265 did not replicate in the CATIE sample. This may have been due to several key differences in sample characteristics. First, the CATIE sample was treated with a number of antipsychotics that possessed notably different receptor binding profiles from CLZ. Risperidone, ziprasidone, and perphenazine show much stronger affinity for D2 receptors than CLZ (Horacek et al., 2006). Quetiapine also has a much weaker affinity for the 5-HT6 receptor relative to CLZ (Richtand et al., 2007). OLZ was the only drug to resemble CLZ in binding profile to DRD2 and 5-HT6. However, notable differences in these two drugs’ affinity for 5-HT1A and adrenergic receptors may have contributed to differences in findings (Horacek et al., 2006).

Second, the two samples differed in treatment history. The CLZ sample was naïve to atypical antipsychotic therapy, whereas CATIE consisted of chronic patients that had received prior treatment in some cases with atypical antipsychotics. This may be important given that prior antipsychotic drug exposure may alter receptor expression (Wilmot and Szczepanik, 1989) as well as brain function and structure (Keshavan et al., 1994; Lui et al., 2010; Ho et al., 2011).

There were several limitations to our study. First, as discussed previously, CATIE was not a true replication sample for our specific genetic model, due to non-overlap in medication and differences in sample characteristics. Thus, the lack of replication sample
means that findings must be taken as preliminary until further validation. Secondly, the number of genetic variants examined in our study was relatively limited, particularly compared to genome-wide association studies. Given the complexity of the CLZ response phenotype, there are likely multiple small genetic effects not detected. This may explain the relatively low specificity (47%) of our model, which limits clinical usefulness. Lastly, the size of our CLZ sample was relatively small. Nevertheless, the sample had sufficient power to detect the observed effect of the model.

Overall, our study presents a preliminary genetic model of CLZ response. Moving forward, future studies of larger and well-characterized CLZ response samples are required to validate these findings.
CHAPTER 6

DISCUSSION

Since studies on twins and families with SCZ treated with antipsychotics revealed a likely genetic component to antipsychotic response (Sautter et al., 1993; Vojvoda et al., 1996; Mata et al., 2001), numerous studies have examined genetic variation in relation to treatment outcome. These studies aimed to find robust predictors for response, which would yield immense clinical benefits for patients while reducing treatment costs. However, despite several suggestive findings, studies have yet to identify clinically useful genetic predictors for response. Thus, we sought to uncover genetic variation associated with CLZ efficacy. To do so, we used (1) a hypothesis-driven targeted gene approach and (2) meta-analysis of published studies. We then developed a genetic model to predict CLZ response by combining the most promising genetic effects.

6.1 Summary of Findings and Implications

6.1.1 Chapter 3 – Genome-wide significant DRD2 SCZ risk marker and CLZ response

Chapter 3 of the thesis investigated the association between a SNP (rs2514218) located 47kb upstream of the DRD2 gene in relation to CLZ response. This genetic variant was found to be associated with SCZ risk at a genome-wide significant level in the PGC’s most recent GWAS of 36,989 cases and 113,075 controls. We hypothesized that this variant would be implicated in CLZ response since the drug’s antipsychotic effects are partly mediated by its binding to the D2 receptor. Previously, several DRD2 gene variants have also shown significant associations with response to CLZ in a smaller sample of African-American ancestry (Hwang et al., 2005b; Hwang et al., 2006). Additionally, a prior study had observed significant genetic overlap between SCZ risk and response to the antipsychotic risperidone (Ikeda et al., 2015). We observed a significant association
between *DRD2* rs2514218 and CLZ response. The association appeared to be specific to improvement in general psychopathological symptoms (e.g. anxiety, depressive mood), rather than positive or negative ones.

This study is the first to implicate a genome-wide significant SCZ-risk variant in response to CLZ. The finding is consistent with those of a recent study observing enrichment of SCZ risk markers in antipsychotic target genes (Ruderfer et al., 2016). To do so, Ruderfer et al created 167 sets of genes based on the targets of pharmacologically-similar medications. They then examined the frequency of genome-wide significant SCZ risk loci identified by the PGC GWAS and loss-of-function variants detected through the Swedish SCZ exome study. Our results also provide further support for the involvement of *DRD2* genetic variation in CLZ’s therapeutic effects. Moreover, our finding that rs2514218 is associated with improvement in general psychopathological symptoms is plausible given that D2 receptor occupancy has been observed to correlate with anxiety and depressive mood symptom severity in patients treated with atypical antipsychotics (de Haan et al., 2000; Mizrahi et al., 2007). CLZ treatment has also been shown to lead to improvement in these symptoms (Lindenmayer et al., 2004).

The finding also highlights the importance of re-examining previously studied antipsychotic target genes in the DA, serotonin, and glutamate systems. Prior studies of these systems focused on genetic variants within or nearby the genes’ coding regions. However, the variant investigated in this study, rs2514218, is located 47kb upstream of *DRD2* and still appears to alter expression while being implicated in both SCZ risk and CLZ response. Thus, our study’s findings suggest that the window of genetic variants surrounding important genes such as *DRD2* that ought to be examined should be expanded. Moreover, the creation of functional annotation and gene expression databases such as ENCODE and GTex Portal now provide immense information for studying functional variants (Consortium, 2012; Consortium, 2013). These tools should be used in selecting genetic variants when re-examining the genes of important antipsychotic targets.
Our finding that the A-allele was associated with greater improvement in symptoms was not in agreement with another study of the same variant, which observed an association for the opposite allele with antipsychotic response (Zhang et al., 2015). This may be attributed to several factors including differences in the antipsychotics studied (aripiprazole and risperidone vs. CLZ), patient diagnoses (first-episode psychosis vs. chronic schizophrenia), and sample ethnicity (mixed sample vs. Caucasians alone). Moreover, it is important to note that the two studies observed associations with different sets of symptoms—Zhang et al with improvement in positive symptoms and our study with improvement in general psychopathological symptoms. Thus, the findings should not be seen as being contradictory to one another; rather, the difference in symptoms supports the potential variation of the role of the DRD2 genetic variant in response to different antipsychotics and in non-overlapping clinical populations. Alternatively, the differing allele effects of our two studies may hint that rs2514218 is not the true causal variant, but is rather in incomplete LD with the functional SNP.

Overall, the observed association between DRD2 rs2514218 and CLZ response provides support that despite CLZ’s diverse receptor binding profile, its mechanism involves D2 receptor binding. The finding also provides a possible genetic variant to be included in a predictive test for CLZ response. However, replication of this finding in independent CLZ-treated samples of SCZ patients is required for validation.

6.1.2 – Chapter 4 – COMT Val158Met genetic variant and antipsychotic response

Chapter 4 examined whether COMT functional genetic variant Val158Met was associated with antipsychotic response. COMT plays a critical role in modulating DA levels in the PFC, and to a lesser extent in subcortical regions. Since antipsychotics all target the DA system, multiple studies have investigated COMT genetic variants in relation to antipsychotic response, yielding mixed results. Thus, we performed a meta-analysis to clarify whether there was an association. We observed that Val158Met was associated with response to antipsychotics. In particular, Met-allele homozygotes were more likely
to respond to treatment and experienced greater improvement in positive symptoms compared to Val-allele carriers. After stratifying for typical and atypical antipsychotics, we observed that the association was only significant for the atypical group. However, this may be due to the limited sample size of typical antipsychotic-treated patients.

Our findings were consistent with the tonic-phasic DA hypothesis of antipsychotic response, proposed by Bilder et al. (2004). Based on COMT’s differing roles in cortical and subcortical regions, the hypothesis predicted that the Met-allele would be associated with greater response to drugs enhancing D1 transmission (5-HT2A antagonists, 5-HT1A agonists) (including the many atypical antipsychotics), and the Val-allele with better response to conventional D2-blocking drugs (Bilder et al., 2004). We observed that Met-allele homozygotes responded better to atypical antipsychotics compared to Val-allele carriers. In turn, we observed the opposite direction of effect for typical antipsychotics, although this was not significant.

Overall, our findings suggest a possible candidate for inclusion in predictive tests for atypical antipsychotics. Given the limited number of subjects treated with typical antipsychotics, future studies of this variant in response to those drugs is required to assess whether the observed effect is specific to atypical antipsychotics. Moreover, additional studies in SCZ patients of other ancestries not represented in the meta-analysis are warranted to examine whether the effect generalizes to all ancestry groups.

6.1.3 – Chapter 5 – Genetic model for CLZ response

Chapter 5 aimed to develop a preliminary genetic model for predicting CLZ response based on the multitude of genetic response studies conducted by our group over the past twenty years (Masellis et al., 1998; Hwang et al., 2005a; Hwang et al., 2006; Hwang et al., 2007; Souza et al., 2010b; Hwang et al., 2011; Hwang et al., 2012; Souza et al., 2012; Zai et al., 2012; Huang et al., 2016). Despite evidence implicating multiple genetic markers in CLZ response, a replicated genetic model has yet to be assembled. Arranz et
al’s (2000) six-marker model remains the only published attempt to fill this gap, but it was not replicated in an independent sample (Schumacher et al. 2000). In our study, we developed a four-marker model explaining 56.5% of variance in symptom improvement in our CLZ-treated sample. The model consists of 5-HT6 rs1805054 (267T/C), DRD2 rs2514218 (investigated in Chapter 3), BDNF rs6265 (Val66Met), and NRXN1 rs1045881. The model did not significantly predict response in an independent sample (CATIE) treated with five different antipsychotics (CLZ not included), suggesting that our model may not generalize to antipsychotics other than CLZ. This is a possibility given these drugs’ differences in receptor binding profiles and mechanisms of action (Horacek et al., 2006; Richtand et al., 2007). Other reasons for non-replication have been discussed in Chapter 5. Alternatively, it is possible that our model is a spurious one.

We were unable to directly test Arranz et al’s model, as we did not have access to genetic data for all six markers. We were able to examine the association for the two markers for which we had data, 5-HT2A rs6313 and 5-HT2C Cys23Ser (rs6318), and did not observe significant associations with response.

Our findings provide additional support for the implication of each of the four markers’ in response to antipsychotics. With the exception of NRXN1 rs1045881, each marker in our model has previously been associated with antipsychotic response in independent samples (Yu et al., 1999; Hong et al., 2003; Lane et al., 2004; Zai et al., 2012; Perkovic et al., 2014; Zhang et al., 2015). For BDNF rs6265, the association analysis results have been mixed, with a number of studies unable to replicate the association (Anttila et al., 2005; Xu et al., 2010; Pae et al., 2012; Xu et al., 2015). Moreover, as discussed earlier, an opposite allele effect was observed for DRD2 rs2514218 by another study (Zhang et al., 2015).

Overall, our study presents a genetic model of CLZ response. Further examination of our model in independent CLZ-treated samples is required for validation.
6.2 Limitations and Considerations

There are several key limitations to the three studies included in this thesis. The limitations fall into four broader categories: (1) sample characteristics, (2) limitations of the candidate gene approach, (3) limited access to response phenotype data and (4) lack of true replication samples.

Sample Characteristics

Several limitations relating to the samples studied in this thesis need to be taken into account. First, the analyses in Chapters 2 and 4 focused purely on patients of European ethnicity in order to avoid confounding effects due to population stratification (Cardon and Palmer, 2003). Thus, the genetic effects observed for \textit{DRD2} rs2514218 and the four-marker model may not generalize to other ethnicities. This is possible given that LD structures as well as MAF are known to differ across ethnicities (Petryshen et al., 2010).

Secondly, since drug response is a relatively complex phenotype, the genetic effects are smaller in size and involve numerous polymorphisms. Thus, the small sample size of our CLZ-treated sample studied may have prevented the detection of small genetic effects when examining numerous genetic variants for association in Chapter 5. Similarly, insufficient sample size prevented us from conducting drug-specific analyses in the meta-analysis in Chapter 4 and may have affected our ability to detect an effect for Val158Met in typical-antipsychotic treated patients.

Thirdly, our CLZ-treated sample included patients prescribed CLZ due to either severe side-effects from previous treatment (‘treatment-intolerant’) or treatment-resistance as defined by Kane et al. (1988). It is possible that these two groups of patients differ in terms of their underlying illness pathology, leading to differences in both response to CLZ and genetic associations with response. Unfortunately, we were unable to assess the effect of this variable on response or control for it during analyses due to lack of data each patient’s treatment-intolerant or treatment-resistant status.
Lastly, differences in study design amongst the fifteen samples included in the meta-analysis in Chapter 4 may have confounded the results. The studies consisted of both naturalistic and RCTs as well as both prospective and retrospective study designs. We attempted to assess the effect of differences in study design using meta-regression and did not observe a significant association with treatment response. However, it is still possible that there were confounding effects that were not taken into account.

**Limitations to the candidate gene approach**

The candidate gene approach was used in all the chapters of this thesis and has been largely employed in pharmacogenetic studies of antipsychotic response. While it possesses advantages such as reduced multiple testing burden, strong focused hypotheses, the ability to conduct in-depth analyses, and less technically-challenging statistical methods, it also has a number of important limitations. First, the candidate gene studies are hypothesis-driven and utilize available biological information to identify possible genetic associations. This precludes the identification of novel genetic variations in genes that have less biological plausibility based on current evidence. Thus, the use of this approach alone makes it difficult to develop robust predictive models, which can be utilized universally. Such studies often also do not examine the epistatic interactions across genes, which have been suggested to be important for complex phenotypes (Moore, 2003).

**Limited access to phenotype and genotype data**

In our three studies, useful phenotype and genotype data were not available, preventing us from conducting more specific analyses. For instance, our CLZ sample did not have access to the scores for the individual items of the BPRS scale, preventing us from mapping observed genetic effects with CLZ response to improvement on individual symptoms (e.g. hallucinations). This would have been especially useful in parsing out which of the general psychopathological symptoms was responsible for the overall effect.
observed in Chapter 3. Moreover, access to individual items scores would have also been helpful for understanding the specific symptoms for which our genetic model predicts improvement in Chapter 5.

Like the majority of antipsychotic pharmacogenetic studies, our study also did not have access to data for important non-genetic factors implicated in drug response. These include clinical variables such as DUP and premorbid level of functioning and environmental ones such as substance abuse and degree of social support (Green et al., 2004; Perkins et al., 2004; Chiliza et al., 2012). Thus, we could not account for their potential confounding effects in our analyses. However, it should also be noted that some of these variables, particularly DUP and premorbid level of functioning, have not been consistently observed to predict treatment response (Robinson et al., 1999).

The development of the genetic model for CLZ response in Chapter 5 was also limited by lack of access to additional genetic data. As previously mentioned, we were unable to test Arranz et al’s six-marker model due to lack of data for four of the markers. Moreover, given the complexity of the drug response phenotype, access to genome-wide data would have been useful for identifying the multitude of smaller genetic and haplotypic associations with response. Nevertheless, our sample size may be underpowered to detect significant effects using the GWAS approach, given the increased multiple testing burden.

*Lack of true replication samples*

An important limitation to our three studies was the lack of an independent sample for replication of findings. Lack of replication leaves open the possibility that our findings for rs2514218 and the four-marker genetic model were spurious. In Chapter 5, we attempted to address this issue by examining the model’s association with response in the CATIE sample. However, CATIE was not a true replication sample, since the patient treatment history and the antipsychotic medications differed from our CLZ sample. We also applied ten-fold CV in analyzing the genetic model’s performance in order to obtain
a better estimate of its accuracy, but this approach is still limited by the small size of our sample and cannot replace independent replication (Rao et al., 2008).

Difficulty finding true replication samples is an important issue facing the field of pharmacogenetics. It stems from the extensive heterogeneity in both treatments and patient characteristics across studies. Studies often differ in terms of antipsychotic prescribed, treatment duration, monitoring for treatment adherence, polypharmacy, patient treatment history, comorbidities, social support, and multiple other variables, all of which may influence response (Lieberman et al., 1993; Robinson et al., 1999; Spina et al., 2003; Perkins et al., 2004; Spina and De Leon, 2007; Alvarez-Jimenez et al., 2011). Studies may also use varying cutoff thresholds to define response, which has been observed to influence whether a significant association is observed (Leucht et al., 2007; Rajkumar et al., 2012).

6.3 Future Directions

The results presented in this thesis identify several promising genetic variants that may predict response to CLZ for SCZ patients. However, the findings leave several important questions unanswered, paving the way for future studies of CLZ response. Here, we review these questions, providing possible avenues for addressing them.

*Would our findings replicate in independent samples of CLZ-treated SCZ patients?*

First, as highlighted earlier, we did not have access to a replication sample to validate our findings for *DRD2* rs2514218 and our four-marker model for CLZ response. Thus, an important next step would be to examine these markers in independent CLZ-treated samples. To qualify as true replications, independent samples should be comprised of treatment-refractory/intolerant SCZ patients of European ancestry evaluated prospectively for CLZ response over six months. Ideally, response would be evaluated according to the same scale and analyzed using the same definitions (i.e. Kane et al. 1988
criteria), as differing definitions have previously been shown to affect the results of pharmacogenetic studies (Leucht et al., 2007; Rajkumar et al., 2012). Stringent monitoring of treatment adherence would also be important to avoid confounding effects due to non-compliance (Subotnik et al., 2014). Unfortunately, CLZ-treated samples are exceedingly rare, given the low prescription rates of this antipsychotic (~4.4% in the US) (Meltzer, 2012). However, were the findings to replicate, the next step would be to examine whether the genetic effects are robust across all ancestry groups. This step is important given that differences in LD structure across ancestries can give rise to effects that are present in one group, but not another (Petryshen et al., 2010). Investigating whether effects generalize to all ancestries is also vital for assessing the clinical application of the finding.

*What underlying biological mechanisms link these variants to CLZ response?*

If our findings are replicated, a critical next step would be to understand the underlying biological mechanisms through which these genetic variants are implicated in response. As a first step, it would be important to determine whether the four SNPs in the model are the actual causal variants or rather are in LD with the true causal variants—defined as the variant that gives rise to phenotypic differences influencing treatment outcomes. One of the genetic variants in our model, *BDNF* rs6265, results in a valine-to-methionine substitution at position 66 of the protein. This variation in amino acid sequence has been previously shown to result in deficient BDNF translocation and secretion (Egan et al., 2003), which has been implicated in risk for a number of different neuropsychiatric phenotypes. Thus, the potential for this variant to be causal is high. However, the remaining three variants require further investigation to determine their functional roles. Online gene expression databases such as GTex Portal provide preliminary evidence for effects of rs2514218 on D2 receptor expression in the basal ganglia (Consortium, 2013). The ENCODE database suggests that it alters four regulatory motifs. However, the role of these motifs remains unknown. ENCODE also indicates that *NRXN1* rs1045881 alters four known regulatory motifs, but these motifs have yet to be explored (Consortium, 2012). The last of the four markers, *5-HT6* rs1805054, gives rise to a synonymous
mutation in the gene’s coding region. While synonymous substitutions do not alter the amino-acid sequence, they have been observed to have physiological consequences through their effects on mRNA splicing and stability, and protein conformation (Nackley et al., 2006; Kimchi-Sarfaty et al., 2007). Moreover, ENCODE suggests that this variant influences binding of a transcription factor.

Thus, each of these markers warrants further investigation through *in vitro* and *in vivo* studies. A well-established approach could involve using site-directed mutagenesis to construct plasmids that possess these specific variants, followed by transfection into neuroblastoma cell lines. Subsequent effects on gene expression could be observed using luciferase reporter gene assays. Since ENCODE predicted that rs1805054 was located within a transcription factor binding site, an electrophoretic mobility shift assay (EMSA) could be used to analyze binding activity, as done in earlier studies (Maeda et al., 2006).

In the event that the variants are not causal, high throughput sequencing could be used to obtain genetic data for all SNPs in the same LD block. Examining each SNP in relation to CLZ response should provide further insight into determining the causal variant, since the causal one in principle should show the strongest association. In turn, this could be further investigated through functional studies.

*Could the genetic associations observed be specific to one or more distinct subtypes of SCZ patients?*

A recent study indicated that SCZ might actually be comprised of eight genetically distinct subtypes or ‘schizophrenias’ (Arnedo et al., 2015). Another recent study used an array of biomarker data ranging from neuropsychological tests to auditory-stimulation-evoked brain responses to uncover three biologically distinct phenotypes (‘biotypes’) for psychosis (Clementz et al., 2015). This study found that these biotypes did not overlap with DSM-diagnoses of BP, schizoaffective disorder, and SCZ; in fact, there were similar distributions of each of these diagnoses within each biotype. The biotypes also differed significantly in brain structure and social functioning. These two studies reflect a shift
away from diagnosing psychiatric patients based on clinical phenomenology towards
doing so based on neurobiological differences. While these studies’ findings await
replication, an important implication is that genetic associations with response may differ
across subtypes or biotypes. This may explain the difficulty replicating genetic
associations with response to antipsychotics. In addition, our sample included patients
that were either refractory or intolerant to first-generation antipsychotics, which may also
confound genetic studies of response.

Future studies may thus benefit from assessing genetic associations with CLZ response in
different subtypes or biotypes of SCZ separately. While this approach may lead to greater
difficulties in recruiting large samples of patients, the subsequent reduction in sample
heterogeneity could increase statistical power.

Moreover, it may be worthwhile for future pharmacogenetics studies to consider recent
proposals of classifying SCZ patients into three categories: SCZ-antipsychotic-responsive
(70-80% of patients), SCZ-CLZ-responsive (15-20%), and SCZ-CLZ-resistant (5-10%)
(Farooq et al., 2013). Clinical studies of antipsychotic treatment have suggested that these
classes possess strong face validity (Agid et al., 2011). A recent large Danish population-
based cohort study’s findings supported this classification, observing that the clinical risk
factors for SCZ and TRS differed substantially such that established risk factors for SCZ
did not predict TRS (Wimberley et al., 2016).

*How else can the genome help us predict CLZ response?*

While targeted genetics studies like ours have identified multiple genetic variants
implicated in CLZ response, the question remains as to what other genomic elements can
serve as biomarkers for treatment outcome.

Recent genome-wide association analyses have uncovered significant genetic overlap
between SCZ risk and antipsychotic response (Ikeda et al., 2015; Ruderfer et al., 2016).
This overlap is supported by our finding that a genome-wide significant SCZ risk marker
is implicated in response to CLZ. Thus, future pharmacogenetic studies should further examine SCZ risk variants in relation to CLZ response. Targeted studies like these have the advantage of reduced multiple testing burden and smaller required sample sizes, making them more feasible.

Moreover, CLZ response genetic associations have yet to be explored using a genome-wide approach. To date, GWAS studies have been performed in relation to response to risperidone (Stevenson et al., 2016), iloperidone (Lavedan et al., 2009), and five different antipsychotics (CATIE) (McClay et al., 2011). From these studies, one genome-wide significant association was identified in the glutamate-system gene, GRID2, in relation to risperidone response (Stevenson et al., 2016), and multiple suggestive findings for other antipsychotics. Taken together, these results support the use of this approach in studying response to CLZ. GWAS provides the advantage of efficiently examining millions of variants across the genome. A genome-wide approach is important, given the finding from the ENCODE project that biological functions are influenced by 80% of the non-coding genome rather than merely the 1% coding DNA (Consortium, 2012). Large consortia examining CLZ treatment outcome such as CRESTAR and CLOZUK are currently in the process of conducting GWAS. Their findings should provide further insights into the genetic effects for treatment response.

After obtaining genome-wide association results, future studies would benefit from examining top findings in both gene expression databases such as GTex Portal (Consortium, 2013) and Braineac (Ramasamy et al., 2014) and gene annotation databases such as ENCODE to identify variants with strong evidence for functional roles. For the latter, novel effective gene annotation tools such as GWAVA (Genome Wide Annotation of Variants) and CADD (Combined Annotation Dependent Depletion) can be used to prioritize functional variants (Kircher et al., 2014; Ritchie et al., 2014).

However, given the complexity of the drug response phenotype, studying single gene effects even at a genome-wide level may have limits to its predictive ability. These limitations were highlighted by the fact that despite a large sample size, single-SNP
effects from the PGC GWAS could only explain 25% of the variance in SCZ risk (Ripke et al., 2014). The remaining unexplained variance, named ‘missing heritability’, has since been attributed to genetic phenomena such as epistasis—long known to be important in complex phenotypes (Moore, 2003). For instance, Arnedo et al. (2015) observed that analyzing interactive gene sets allowed them to explain greater amount of variance in SCZ risk than single variant analyses (Arnedo et al., 2015). Thus, future studies of CLZ response should also investigate epistatic interactions both within (haplotypes) and across genes (gene-gene interactions). In particular, these studies should target the genes of proteins that are known to interact biologically. For instance, a recent in vitro study revealed that multiple antipsychotics enhanced formation of heterodimers between serotonin 1A (5-HT1A) and D2 receptors in human HEK 293 cells (Łukasiewicz et al., 2016). The study observed that of the antipsychotics tested, CLZ had the strongest enhancing effect. The study also observed that this D2-5HT1A complex possessed distinct functional, biochemical, and pharmacological properties that differ from each receptor alone. Since CLZ’s therapeutic effects have previously been attributed in part to its binding to these receptors, studying the interaction between variants in these genes would be a reasonable next step. Moreover, previous studies of this kind have already revealed significant gene-gene interactions in relation to CLZ response (D1-D3 and D1-GRIN2A) (Hwang et al., 2012), suggesting this approach holds promise.

Other types of genetic variation also warrant investigation in relation to drug response. With the development of whole-genome sequencing, it has become possible to obtain genetic data for rare variants, which have been observed to have larger effect sizes and higher penetrance across the genome (Need and Goldstein, 2010). The effects of these variants have already been observed in several studies in relation to other neuropsychiatric phenotypes (Need et al., 2009b; Rujescu et al., 2009). Moreover, advances in sequencing and bioinformatics tools have paved the way for analysis of other forms of genetic variation such as copy number variants (CNVs). A subset of the CNV mutations are multi-allelic (mCNV) and were recently found to constitute greater than 85% of the variation in human gene dosage due to CNV’s. These elements were observed to be highly prevalent throughout the human genome, and to possess higher mutation
rates compared to SNPs due to evolution through non-allelic homologous recombination (Gu et al., 2008; Handsaker et al., 2015). A recent groundbreaking finding implicated one such mCNV in the C4 complement gene in SCZ risk (Sekar et al., 2016). The study sequenced the mCNV using recently-developed digital-drop-PCR (ddPCR) and analyzed the data using an imputation-based approach called Genome STRiP (Genome Structure in Populations). Given the significant genetic overlap between SCZ and antipsychotic response, mCNV’s like this one should be investigated using the same tools in relation to CLZ response. Moreover, this technology paves the way for the analysis of other known hyper-variable mCNV’s across the genome, such as the \((G)^n\) repeat polymorphism in the coding region of the gene encoding DRD4. This polymorphism is an especially promising candidate, given that CLZ binds to DRD4 with relatively strong affinity (ten times stronger than DRD2) (Van Tol et al., 1991).

*How can genomic data be integrated with other modalities to develop biomarkers for CLZ response?*

Recent advances in our understanding of endophenotypes such as protein levels and brain structure have begun to provide further avenues of developing predictors for drug response. The integration of this information with genomic data holds immense potential for not only discovering novel genetic predictors, but also understanding the mechanisms through which DNA sequence variation influences treatment outcome.

A recent study of antidepressant response serves as a model for this type of integration (Gupta et al., 2016). The study first conducted an analysis of the metabolome (the levels of metabolites present in the human body), observing that plasma serotonin concentration was associated with treatment outcome. A GWAS was then performed to identify genetic variants associated with plasma serotonin levels. Two genome-wide significant SNPs were discovered, and their effects on serotonin-pathway gene expression validated \textit{in vitro} through knockdown and over-expression experiments in neuroblastoma cell lines. These markers also appear to be associated with treatment outcome. The approach used
in this study presents a powerful addition to methods studying the genome alone for detecting markers associated with response and uncovering their mechanisms.

Additionally, a functional study recently found that CLZ altered the expression of 122 proteins in human oligodendrocytes (Cassoli et al., 2016). While these findings require replication, the genes of these proteins could serve as targets to study in relation to CLZ response. In addition, conducting a GWAS in relation to protein expression across different cell types in CLZ-treated patients may reveal further insights into the mechanism of CLZ’s effects at the molecular level, while identifying potential predictors of response.

Neuroimaging studies have also provided promising endophenotypes to study in conjunction with the genome. The strongest findings have been obtained through functional imaging studies using functional magnetic resonance imaging (fMRI) and PET. For instance, CLZ efficacy in SCZ patients has been linked with lower metabolism in the basal ganglia and thalamus through the use of $^{18}$F fluoro-deoxy-glucose-PET (Rodriguez et al., 1996; Rodríguez et al., 1997; Molina et al., 2005). Improved response to antipsychotics has also been observed to be associated with reduction in grey matter and perfusion of frontotemporal regions, and increases in white matter and perfusion of the basal ganglia (Anderson et al. 2015). These endophenotypes provide promising targets for further investigation through GWAS studies. As noted earlier, a GWAS approach would identify important SNPs underlying these differences and predicting drug response. And while sample size may be a limitation given the cost of PET scans, this obstacle could be overcome by either (1) studying multiple smaller samples followed by meta-analysis or (2) pooling resources and establishing a consortium.

Epigenetic modifications also present a promising avenue for studying CLZ response. Epigenetics has increasingly been observed to play important roles in SCZ etiology (Mill et al., 2008; Dempster et al., 2011). The ‘epigenetic code’ consists primarily of two types of modifications – histone modifications and DNA methylation. Together, these changes can alter the structure of chromatin, leading to increased or decreased gene expression.
The epigenome has also been shown to change throughout life and to be influenced by environmental factors such as significant stressors and drug exposure (Murgatroyd et al., 2010; Horvath, 2013). Antipsychotics including CLZ have been found to alter DNA methylation in mice (Dong et al., 2008; Melas et al., 2012). In particular, CLZ was observed to increase H3K4 trimethylation of an important GABA-synthesis gene GAD67 (Pinal and Tobin, 1997; Dong et al., 2008). CLZ was also shown to increase histone acetylation and reduce hypermethylation at the promoter for RELN, the gene encoding reelin, a matrix protein that regulates neuronal migration (Dong et al., 2008). Lastly, response to atypical antipsychotics was found to be regulated by histone deacetylase 2’s (HDAC2) modulation of metabotropic glutamate receptor 2 (mGlu2) activity in the mouse and human PFC (Kurita et al., 2012). Overall, these findings highlight the importance of studying DNA acetylation and methylation in conjunction with genetic effects in relation to drug response.

Recently, a pioneering ‘pharmaco-epigenetic’ study observed that the amount of methylation in the 5-HT1A promoter region was associated with antipsychotic response in first-episode SCZ (Tang et al., 2014). Importantly, a SNP in this region, -1019C/G, has previously been observed to be associated with negative symptom response in other antipsychotic-treated samples (Reynolds et al., 2006; Wang et al., 2008; Mössner et al., 2009) and to influence the binding of several transcription factors that influences 5-HT1A expression (Lemonde et al., 2003; Jacobsen et al., 2008). Thus, Tang et al’s (2014) findings suggest that methylation studies can complement and provide additional insight into previous pharmacogenetic study results. However, it should be noted that current human epigenetic studies involving brain-related phenotypes such as drug-response are limited by the difficulty of obtaining brain samples. Because of this, current pharmaco-epigenetic studies have focused on peripheral cells (blood, saliva), whose methylation patterns may differ substantially from those of the brain (Meissner et al., 2008; Ziller et al., 2013). Nonetheless, a number of studies have suggested relatively higher degrees of concordance in DNA methylation between peripheral and brain tissue for psychiatric disorders (Masliah et al., 2013; Smith et al., 2015). Thus, integration of epigenetic and
genetic variation is a promising direction for future research on biomarkers of CLZ response.

6.4 Concluding Remarks

Overall, the three studies in this thesis have yielded several promising candidate genes as well as a preliminary genetic model for CLZ response. All findings require further investigation in larger, prospectively-studied samples. Currently, the field of pharmacogenetics has yet to yield consistently replicated markers for antipsychotic response. The strongest genetic findings have instead been identified in relation to major side-effects such as agranulocytosis and weight gain, which may soon be translated into genetic tests in the clinic. Nevertheless, pharmacogenetics continues to hold strong potential for identifying biomarkers for treatment response and improving understanding of the mechanisms of antipsychotic drugs. Future studies integrating genomic analysis with epidemiological factors, proteomics, neuroimaging, and epigenetics hold immense promise for identifying robust predictors for CLZ response.
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<th>COMT Val158Met Genotype</th>
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<td>Met (low COMT activity)</td>
<td>↑ tonic DA release, ↓ phasic DA release</td>
<td>Excessive stability of neuronal activation states</td>
<td>↑ negative symptoms</td>
<td>Better response to D1-transmission enhancers (5-HT2A antagonists, 5-HT1A agonists)</td>
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<tr>
<td>Val (high COMT activity)</td>
<td>↓ tonic DA release, ↑ phasic DA release</td>
<td>Excessive instability of neuronal activation states</td>
<td>↑ positive symptoms</td>
<td>Better response to conventional D2-blocking drugs</td>
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