POLYMORPHISMS IN THE DOPAMINE RECEPTOR 4 GENE: IS THERE AN ASSOCIATION WITH CLINICAL RESPONSE TO CLOZAPINE IN PATIENTS WITH TREATMENT-RESISTANT SCHIZOPHRENIA?

A DISSERTATION SUBMITTED TO THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY, CHENNAI, IN PARTIAL FULFILMENT OF THE REGULATIONS FOR THE AWARD OF M.D DEGREE IN BIOCHEMISTRY (BRANCH XIII) EXAMINATION TO BE HELD IN APRIL 2014

DEPARTMENT OF BIOCHEMISTRY
CHRISTIAN MEDICAL COLLEGE
VELLORE-632002
TAMIL NADU

CERTIFICATE

I approve and certify that the work presented in this dissertation entitled "**Polymorphisms in the dopamine receptor 4 gene: is there an association with clinical response to clozapine in patients with treatment-resistant schizophrenia?"** submitted by **Dr. R. Veera Manikandan** is a bonafide record of work done by him during the period of study under my supervision and guidance. This dissertation has not been submitted for any university previously in part or full for the award of any degree/diploma/or of any other similar title.

Prof. Molly Jacob M.D Ph.D.

Guide and Supervisor

Professor and Head

Department of Biochemistry

Christian Medical College

Vellore 632002

Tamil Nadu

Place: Vellore

Date:

DECLARATION

I certify that this work entitled "**Polymorphisms in the dopamine receptor 4 gene: is there an association with clinical response to clozapine in patients with treatment-resistant schizophrenia?"** was original research work carried out by me under the guidance of **Prof. Molly Jacob**, Professor and Head, Department of Biochemistry, Christian Medical College, Vellore. I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

Dr. R. Veera Manikandan

Department of Biochemistry

Christian Medical College

Vellore 632002

Tamil Nadu

Place: Vellore

Date:

ACKNOWLEDGMENTS

First and foremost, I would like to express my heartfelt gratitude to my mentor and guide, Prof. Molly Jacob, for giving me an opportunity to explore the world of genetics through pursuing a MD dissertation in pharmacogenetics. I am indebted to her for her constant guidance and encouragement, as well as her constructive criticisms, which have been invaluable to me. It has been an honor to pursue a post-graduate training under her supervision and I feel I am extremely fortunate in this regard. I could not wish for a better mentor and advisor. I admire her qualities of perfectionism, sincerity and dedication to her responsibilities.

I acknowledge my sincere gratitude to Prof. K.S. Jacob, Professor and Head of Psychiatry, my co-guide, for giving me an opportunity to work in collaboration with the Department of Psychiatry, CMC, Vellore and for his constant guidance and support.

I acknowledge my deepest appreciation for Dr. Anto P Rajkumar, who played a major role in my dissertation project. Without his guidance, training and support, this dissertation would not have been possible. I am grateful to him for his guidance in helping me understand statistical genetics and for inducing in me a passion for psychiatric genetics.

I am extremely grateful and indebted to my institution, Christian Medical College (CMC), Vellore that has sheltered me and helped me to grow as a professional. I acknowledge the institution for funding my dissertation project through its fluid research grants (Reference no. 7623).

I sincerely thank Prof. Anju Kuruvilla (Professor of Psychiatry), Prof. B. Poonkuzhali (Professor of Hematology) and Prof. Alok Srivastava (Professor of Hematology) for their guidance and support.

I would like to specially thank Ms. S. Lakshmi Kirupa, who has been very supportive and helpful during my laboratory work.

I would like to convey my appreciation to Ms. B. Chitra, who has been extremely helpful in collecting and maintaining socio-demographic data of patients for this project.

It is my duty to acknowledge my friend, Dr. Kaysina Vazhudhi, who has been a source of inspiration through his extraordinary computational talents. He has constantly encouraged me to learn R statistical software, expertise that proved extremely useful for my dissertation project.

I acknowledge my teachers Prof. Dhayakani Selvakumar, Prof. Premila Abraham, Prof. Minnie Faith and Dr. Joe Varghese for their support, guidance and encouragement and for their constant appreciation of my talents.

I am grateful to Dr. Abitha Sukumaran, my colleague, who taught me laboratory techniques and has been a source of moral support and an advisor throughout my post-graduate training.

I acknowledge Dr. Anand R, Dr. S.S. Prakash, Dr. Girish KS and Dr. Natraj B for their constant words of appreciation of my talents and for their moral support.

I thank Dr. Ramya R, Mr. Jithu James and Mr. Azharuddin Mohammed, who have been wonderful friends and for their moral support during times of ups and downs during my post-graduate training.

I acknowledge Mr. Arumugam SN, Dr. Kavita Rasalkar, Ms. Sree Rohini, Ms. Hemalatha R, Ms. Kasthuri N, Ms. Lekshmy R, Dr. Chinmai Jagadish, Dr. Gnana Prabha P, Dr. Jagadish R and Dr. T.S Arthi for their kind co-operation and support.

I sincerely thank staff in my department - Mr. Isaac Newton, Mr. Sridhar R, Mr. Lalu U and Mrs. Punitha Martin for their help and support.

Lastly, I would like to thank my parents and family for their blessings and sincere prayers.

TABLE OF CONTENTS

TABLES

FIGURES

1. ABSTRACT

Clozapine is the treatment of choice for treatment-resistant schizophrenia (TRS). Its use is, however, often associated with variable clinical outcomes. It acts as an antagonist of dopamine receptors, with a high affinity for dopamine receptor 4 (DRD4). Polymorphisms in the DRD4 gene have been suggested to contribute to variable drug responses seen. The aim of this study was to determine whether a 120 base-pair duplication polymorphism in the DRD4 gene affects response to clozapine. Patients diagnosed to have TRS, on stable doses of clozapine were the subjects of the study. Genomic DNA was isolated from peripheral venous blood from the patients, and genotyped for the polymorphism. Serum clozapine levels were also measured. Participants' socio-demographic and clinical profiles were recorded. Standard assessment schedules were used to assess premorbid adjustments, response to traumatic events, cognitive status and disability. Clozapine response was defined a priori; allelic and genotypic frequencies were determined and correlated with the clinical responses. No genotypic association was found between the polymorphism and serum clozapine levels or response to treatment with clozapine in patients with TRS. However, among the adverse effects, hyper-salivation is significantly associated with the polymorphism of interest (p=0.0009). Presence of the 120-bp duplication in DRD4 appears to confer risk for sialorrhea in response to clozapine therapy. These results suggest that routine screening for DRD4 120-bp repeat polymorphism before clozapine therapy may not be

useful as a predictor for clinical response to clozapine. It may, however, help to identify those at high risk for clozapine-induced hyper-salivation.

KEY WORDS:

Schizophrenia; clozapine; sialorrhea: adverse effects; DRD4; dopamine D4 receptor; polymorphism; pharmacogenetics; drug resistance; brief psychiatric rating scale; antipsychotic agents; tandem repeat sequences; genetic association studies;

2. REVIEW OF LITERATURE

Schizophrenia

INTRODUCTION

Schizophrenia is a chronic debilitating neuro-psychiatric disease characterized by abnormalities in cognition, affect and behavior. Lifetime prevalence of the disease is 1% (van Os and Kapur, 2009). Both males and females are equally affected. Females tend to present with symptoms later than males, with onset occurring in their late 20s or early 30s (Schultz et al., 2007). The disease is characterized by positive, negative and cognitive symptoms. Positive symptoms include delusions, hallucinations involving auditory, visual, olfactory, tactile and gustatory components, and disorganized speech and behavior (Tamminga and Holcomb, 2005). These positive symptoms are typically regarded as symptoms of psychosis. Negative symptoms include deficits of emotional responses, poverty of speech, blunted affect and emotion, lack of motivation and suicidal tendencies (Tamminga and Holcomb, 2005). Cognitive symptoms include disturbances in normal cognitive functions such as memory, attention, intelligence, executive function and motor skills (O'Carroll, 2000).

ETIOLOGY

The etiology of schizophrenia comprises genetic and environmental components. Though the genetic component play a major role, environmental factors also contribute to the risks by interacting with the genetic factors (Riley and Kendler, 2006). Multiple lines of evidence exist that demonstrate the key role of the genetic component in schizophrenia. Family and twin adoption studies have consistently showed that the risk of schizophrenia among the relatives of patients correlate with the degree of shared genes (Lewis and Lieberman, 2000). Risks ranged from ~2% in third degree relatives to ~17% in first-degree relatives. Among twins, the risk is estimated to be ~17% in dizygotic twins and ~50% in monozygotic twins (Lewis and Lieberman, 2000).

GENETICS OF SCHIZOPHRENIA

Genetic susceptibility for schizophrenia has been studied by three main approaches: linkages studies, genetic association studies and detection of chromosomal abnormalities (Owen et al., 2004). Linkage studies were done on family-based populations that included families containing two or more diseased individuals. The objective of linkage studies was to identify chromosomal regions that were co-transmitted in families, along with the disease-causing genes. Since it is possible to scan the whole genome with few hundred markers, such an approach has potential to map disease-causing genes precisely, even without prior knowledge of them (Owen et al., 2000). Nevertheless, successful mapping requires that the effect size of the disease-causing genes be very high, as in the case of

simple genetic disorders. Complex genetic diseases like schizophrenia, that have small to medium effect sizes, require large numbers of families to locate susceptibility genes with high statistical power; this is a major limitation for linkage studies (Owen et al., 2000). This has led to the increased frequency of genetic association studies that managed to deal with small effect sizes with acceptable statistical power (Owen et al., 2004). The genetic association studies hypothesizes that the disease-causing alleles occur more frequently in cases than in controls. Though such an approach appears promising, genetic association studies have their own limitations. While linkage studies required a few hundred evenly spaced genetic markers to scan the whole genome, genetic association studies required at least few million markers to scan the whole genome (Gabriel et al., 2002). Hence a prior selection of a specific set of candidate genes was done. Selection was done based on the assumption that the genes were either functional i.e., they encoded for protein(s) implicated in etiology of the disease, or positional i.e., they lay in the chromosomal region that proved to be a susceptibility locus, or both (Owen et al., 2004). The third approach to identify disease-causing genes is to locate chromosomal aberrations in diseased individuals. In doing so, chromosomal regions that carry genes that confer susceptibility can be identified. Aberrations include deletion, duplication and translocation (Owen et al., 2004). Such aberrations can contribute to pathogenesis by either disrupting a disease-related functional gene, forming a new functional gene or by fusing two unrelated genes.

Linkage studies:

Findings of genome-wide association studies have reported that multiple chromosomal regions are associated with schizophrenia. However, very few of these findings have been replicated. The loci identified so far by linkage studies were on chromosomes 2, 4, 5, 6, 7, 8, 9, 10, 13, 15, 18, 22, and X (Wong and Van Tol, 2003). A few postulated loci for which multiple lines of evidences exist are listed in Table 1.1.

Of these loci, the importance of 6p24-22, 1q21-22, 13q32-34, 8p21-22 and 6q21-25 are well supported by several studies (Blackwood et al., 2001; Blouin et al., 1998, 1998; Brzustowicz et al., 2000; Cao et al., 1997; Martinez et al., 1999; Straub et al., 1995).

Association studies of candidate genes:

Dysbindin (DTNB1)

The gene for dysbindin (*DTNB1*) is one that is most associated with schizophrenia, although the mechanism of association is unknown (Straub et al., 2002). It is located in the locus 6p24-21. Single nucleotide polymorphisms (SNPs) located in and around the *DTNB1* gene were analyzed for association with schizophrenia in an Irish population; it was found that most of the polymorphisms that were significantly associated with the disease were in the central 140 Kb region of the gene, highlighting the significant role of the dysbindin gene in schizophrenia. However, it is unclear whether this gene is itself

aberrant in schizophrenia or whether it is a marker of other aberrant genes in the vicinity (Straub et al., 2002).

Table 2.1 Chromosomal loci of significance in schizophrenia.

Chromosomal loci	References
6p24-22	Moises et al., 1995; Straub et al., 1995; Wildenauer et al., 1996
1q21-22	Bassett et al., 2002; Brzustowicz et al., 2000; Holliday et al., 2009; Hwu et al., 2003
13q32-34	Blouin et al., 1998; Brzustowicz et al., 1999; Levinson et al., 2000, p. 13
8p21-22	Blouin et al., 1998, 1998; Fallin et al., 2011; Tabarés-Seisdedos and Rubenstein, 2009
6q21-25	Cao et al., 1997; Levinson et al., 2000; Martinez et al., 1999
22q11-12	Gill et al., 1996; Liu, 2004; Mowry et al., 2004, p. 22; Takahashi et al., 2003a, 2003b
5q21-33	Gurling et al., 2001; Hovatta et al., 1998; Kendler et al., 2000; Levinson et al., 2000; Paunio et al., 2001
10p15-p11	Faraone et al., 1999; Kendler et al., 2000; Schwab et al., 2000, 1998; Straub et al., 1998
1q42	Bassett et al., 2002; Blackwood et al., 2001, p. 1; Hwu et al., 2003

Neuregulin (NRG1)

The gene for neuregulin (*Nrg1*) is located in the locus 8p22-21. Two haplotypes in the gene, spanning a region of 1 Mb, were found to be associated with schizophrenia in an Icelandic schizophrenia cohort. The findings were supported by reports from studies in

Scottish and Irish populations (Riley and Kendler, 2006). Neuregulin1 is a member of the epidermal growth factor (EGF) family of proteins. These proteins are known to play roles in the development of the central nervous system, particularly in the formation of

Table 2.2. Candidate genes of significance in schizophrenia.

Gene	Locus	Gene product	References
DTNB1	6p22	Dysbindin	Straub et al., 2002
NRG1	8p12-21	Neuregulin	Alaerts et al., 2009; Stefansson et al., 2002
COMT	22q11	Catechol-O-methyl transferase	Williams et al., 2007
PRODH	22q11	Proline dehydrogenase	Chakravarti, 2002
PPP3CC	8p21	Protein phosphatase 3 catalytic subunit	Berry et al., 2003
DISC1	1q42	Disrupted in schizophrenia 1	Brandon et al., 2009
G72	13q32-34	D-amino acid oxidase activator	Chumakov et al., 2002
RGS4	1q21-22	Regulator of G-protein signaling-4	Lipska et al., 2006
GRM3	7q21-22	Metabotropic glutamate receptor-3	Riley and Kendler, 2006
CHRNA7	15q13-14	α7 nicotinic receptor gene	Owen et al., 2004
AKT1	14q22-32	Serine threonine protein kinase	Berry et al., 2003

synapses and in expression and activation of neurotransmitter receptors (Stefansson et al., 2002).

Catechol-O-methyl transferase (COMT)

The gene for *COMT* is located at the locus 22q11. It is a functional candidate gene and is involved in methylation of catecholamines such as dopamine. A polymorphism characterized by the substitution of methionine for valine at codon 158 in the gene is well known for its functional effect on the *COMT* enzyme (Williams et al., 2007). The Val variant exhibits higher enzyme activity and thermo-stability. Several studies have reported associations between the Val158Met polymorphism and schizophrenia. Most showed a negative association. In addition, meta-analyses reports were inconclusive (Williams et al., 2007).

Proline dehydrogenase (PRODH2)

The gene for PRODH2 is located at locus 22q11. Deletion of the 22q11 region causes a congenital anomaly called velo-cardio-facial syndrome (VCFS) and is associated with schizophrenia (Harrison and Weinberger, 2005). The *PRODH2* gene is known to possess certain missense SNPs at a higher frequency among those with childhood schizophrenia. The gene was identified to have a duplicated pseudogene about 1.5 Mb downstream. The pseudogene contained missense SNPs similar to *PRODH2* and was found to be associated with schizophrenia. *Prodh2* gene knockout mice were found to have abnormal levels of

gamma amino butyric acid (GABA) and glutamate neurotransmitters in certain regions of brain and schizophrenia-like phenotype (Harrison and Weinberger, 2005).

Disrupted in schizophrenia (DISC1)

DISC1 is one of two genes identified at locus q42 in a linkage study involving a large Scottish family (Owen et al., 2004). The other gene, *Disc2*, is non-coding and shown to transcribe RNA that has a regulatory role. Numerous studies have explored the functional characteristics of *DISC1* since its identification (Brandon et al., 2009, p. 1; Ekelund et al., 2004, 2001; Hennah et al., 2003; Hodgkinson et al., 2004). It is found to be involved in several processes, such as neuronal development, differentiation and migration, cAMP signaling, cytoskeletal modulation and other signaling pathways (Brandon et al., 2009, p. 1, 2004; Camargo et al., 2007; Drerup et al., 2009; Duan et al., 2007). Studies that explored association of *DISC1* with schizophrenia produced inconsistent results (Brandon et al., 2009; Ekelund et al., 2004, 2001; Hennah et al., 2003; Hodgkinson et al., 2004). Hence, the role of *DISC1* in schizophrenia is not clear.

PATHOPHYSIOLOGY OF SCHIZOPHRENIA

Studies on the phenotype of schizophrenia have demonstrated that the symptoms experienced by patients are clustered into three distinct domains: positive symptoms, negative symptoms and cognitive symptoms (Liddle, 1987). These clusters of symptoms vary in their occurrence among patients. While some have predominantly positive symptoms, negative or cognitive symptoms may predominate in others. Whether these

clusters represent manifestations of distinct pathophysiology is uncertain (Tamminga and Holcomb, 2005). The course of illness in schizophrenia is life-long. The onset is insidious during the late teen or early adult years, with further course of disease being episodic, characterized by intervals of partial recovery from illness (Tamminga and Holcomb, 2005). Occasionally, a rapid onset and periods of remarkable recovery have also been seen. Overall, the course of illness remains unpredictable, reflecting the fact that pathophysiology and etiology are poorly understood.

Psychological assessment of brain activity in schizophrenia have proven informative, while the study of anatomical and biochemical differences between brains of those with schizophrenia and normal subjects have not (Tamminga and Holcomb, 2005). Several psychological and physiological parameters provided distinguishing features between the groups. Schizophrenic patients generally perform poorly in all neuropsychological tests. This may be attributed to poor motivation and distraction, commonly seen in such patients (Chapman and Chapman, 1973). In addition, subjects tend have cognitive deficits involving attention, working memory and verbal, visual and social learning (Gruzelier et al., 1988). These further explain the poor psychological performance. Though neuropsychological characteristics of patients with schizophrenia are abnormal in general, none of them served to localize the disease pathophysiology. Schizophrenic patients tend to have a global cortical dysfunction (Tamminga and Holcomb, 2005). It is shown that first-degree relatives of such patients show similar abnormalities in neuropsychological characteristics, even though they do not develop psychotic

symptoms. Magnetic resonance imaging studies have demonstrated an overall decrease in brain size with predominant cortical wasting and a relative ventricular enlargement in patients with schizophrenia. Positron emission tomography (PET) studies have demonstrated a reduced regional cerebral blood flow (rCBF) in schizophrenics, particularly in regions of the frontal cortex (Gur and Pearlson, 1993). Nevertheless, these findings may be a consequence of long-term anti-psychotic treatment rather than an effect of the disease (Tamminga and Holcomb, 2005). Post-mortem brain studies have shown abnormalities in terms of cell size and numbers, neuronal organizations, gross structure and neurochemistry profiles (Colter et al., 1987; Gao et al., 2000; Jeste and Lohr, 1989). However, consistent specific pathologic lesions, as seen in cases of Parkinson's and Alzheimer's disease, was not demonstrated so far (Tamminga and Holcomb, 2005).

The role of neurotransmitters and their receptors in the pathophysiology of schizophrenia was recognized when dopamine antagonists proved effective in treating psychotic symptoms of the disease (Kane and Correll, 2010). High levels of D2 receptors in specific regions of brain such as the putamen and caudate nucleus were demonstrated in earlier studies using ligand bindings techniques (Tamminga and Holcomb, 2005). However, later studies failed to replicate these findings (Farde et al., 1990; Martinot et al., 1991, 1990). Hietala et al suggested suggests that the finding of increased levels of D2 receptors might be confined to specific sub-groups of schizophrenics, such as those with a long-term illness (Hietala et al., 1994). In addition, the effect of antipsychotic treatment has been a confounder in all such studies. The significance of increased levels of D2

receptors in the brains of schizophrenics remains poorly understood (Tamminga and Holcomb, 2005).

The role of the serotonin neurotransmitter system in schizophrenia was considered when serotoninergic drugs such as LSD showed psychotomimetic actions in humans (Freedman, 1975). However, studies failed to demonstrate abnormalities in the serotonin system per se, both in terms of receptors as well as metabolites (Tamminga and Holcomb, 2005). Later evidence has shown that the serotonin system possibly relates to schizophrenia through their modulatory actions on the dopamine system (Marcus et al., 2000). Evidence also exists to support the role of the glutamatergic system in schizophrenia. Spinal fluid from schizophrenic subjects showed reduced levels of glutamate (Kim et al., 1980). In addition, anti-glutamatergic drugs, such as phencyclidine, induced schizophrenia-like symptoms (Domino and Luby, 2012). However, the roles of the glutamate and serotonin systems in schizophrenia are still poorly understood.

Treatment of schizophrenia

The era of pharmacological treatment for schizophrenia began more than half a century ago when chlorpromazine was first recognized to be effective in treating psychosis (Brunton et al., 2005). Thereafter a multitude of pharmacological agents evolved for the treatment of this condition. However, none proved curative of schizophrenia. All drugs so far for schizophrenia have been effective only in ameliorating the symptoms of the disease. This is mainly because the pathophysiology of schizophrenia is still unclear. Pharmacological treatment of schizophrenia consists mainly of two broad classes of drugs: first-generation anti-psychotics (FGA) and second-generation anti-psychotics (SGA).

First-generation anti-psychotics

First-generation anti-psychotics include drugs such as chlorpromazine, haloperidol, fluphenazine, flupentixol and clopentixol (Miyamoto et al., 2005). Chlorpromazine was the first of these. These drugs treat the positive symptoms of schizophrenia, with little or no effect on negative and cognitive symptoms (Miyamoto et al., 2005). They cause certain serious adverse-effects such as tardive dyskinesia, hyper-prolactinemia and extra-pyramidal symptoms (EPS). Extra-pyramidal symptoms include akinesia, akathisia, acute dyskinesias and dystonic reactions, parkinsonism and neuroleptic malignant syndrome (Miyamoto et al., 2012, 2005). These adverse effects result in poor compliance and consequently higher incidence of relapses. It was subsequently discovered that the anti-

psychotic efficacy of the first-generation anti-psychotics, as well their adverse-effects, were due to their antagonistic action against dopamine receptor type 2 (D2). D2 antagonism is thus considered a typical characteristic of first-generation anti-psychotics. These drugs are also called 'typical anti-psychotics' or 'classical anti-psychotics', with many studies providing evidence of their D2 antagonism (Ananth et al., 2001, 2001; Miyamoto et al., 2012, 2005). FGAs such as haloperidol bound to D2 with great affinity and dissociated slowly in in-vitro drug binding experiments (Kapur and Seeman, 2000). The therapeutic doses of these FGAs were correlated with their binding affinities for D2 receptors. The phenomenon was demonstrated in in-vivo experiments, employing positron emission tomography (PET) and single photon emission computed tomography (SPECT), where the dopamine receptor occupancy was shown to correlate well with the anti-psychotic response and incidence of adverse effects (Miyamoto et al., 2012; Remington and Kapur, 1999). These studies showed that 60-70% of D2 occupancy was associated with superior anti-psychotic effects and D2 occupancy greater than 80% was associated with higher incidence of extra-pyramidal symptoms. Thus, a therapeutic window of range 65-80% was suggested for optimal anti-psychotic dosing with minimal EPS (Kapur et al., 2000; Nyberg and Farde, 2000; Remington and Kapur, 1999). However, the reliability of this therapeutic window-based drug dosing is questionable, since patients receiving the same antipsychotic dose showed different degrees of occupancy of D2 receptors in brain imaging studies (Kapur et al., 2000).

SECOND-GENERATION ANTIPSYCHOTICS

Second generation antipsychotics include clozapine, olanzapine, risperidone, quetiapine, ziprasidone, sertindole and zotepine (Ananth et al., 2001). Introduction of clozapine was a major milestone in the history of pharmacotherapy of schizophrenia (Miyamoto et al., 2012). SGAs lacked the typical properties of FGAs and so were described as atypical antipsychotics. The advantages of SGAs over FGAs include greater efficacy in treating negative and cognitive symptoms, lower incidence of extra-pyramidal symptoms and tardive dyskinesia resulting in a better quality of life, and better drug compliance with fewer incidences of relapses (Miyamoto et al., 2005). However, these drugs have limitations as well. Although they cause less EPS, they produce adverse effects such as weight gain, hyperglycemia and dyslipidemia (Kang and Simpson, 2010; Miller, 2000; Safferman et al., 1991). The 'higher affinity of SGAs for 5-HT_{2A} receptors relative to D2 receptors has been postulated to explain their superior efficacy and lower propensity to cause EPS (Lieberman et al., 1998). However, monotherapy with $5HT_{2A}$ selective antagonists did not produce anti-psychotic effects (Lieberman et al., 1998). Therefore, the D2 receptor antagonism is believed to be essential for an antipsychotic effect, although the concept is still controversial (Lieberman et al., 1998). N-methyl D-aspartate (NMDA) receptors have also been thought to be involved in the pathophysiology of schizophrenia (Coyle, 1996; Coyle et al., 2012; Deutsch et al., 1989; Olney and Farber, 1995) Selective NMDA antagonists produced schizophrenia-like symptoms in humans and experimental animal models (Coyle, 1996; Krystal et al., 1994; Olney and Farber, 1995). Hence hypo

functioning of NMDA receptors was thought to be involved in pathophysiology of schizophrenia. Treatment with SGAs attenuated the effects of NMDA antagonists. Hence, correction of hypofunction of NMDA receptors is postulated to be one of the mechanisms by which SGAs exert their anti-psychotic action (Duncan et al., 1999). However, SGAs don't have any direct affinity for any of the glutamate receptors including NMDA receptors (Miyamoto et al., 2005). Thus exact role of glutamate system in schizophrenia is unclear. SGAs and FGAs interact with other neurotransmitter systems as well; these include muscarinic, histaminergic and adrenergic neurotransmitter systems (Raedler et al., 2007; Selvam et al., 2013; Smythies, 2002; Svensson, 2003). These interactions mainly result in adverse effects. However, recent studies claim that these interactions also contribute to the therapeutic efficacy of these drugs, although no clear evidence exists for this (Raedler et al., 2007; Smythies, 2002; Svensson, 2003).

Clozapine

Clozapine is a prototype atypical antipsychotic. It is a heterocyclic compound with the molecular formula $C_{18}H_{19}N_4Cl$ and a molecular weight of 326.8 (Fakra and Azorin, 2012). It was synthesized in 1958 by a Swiss pharmaceutical company and recognized as a potential drug to treat psychosis (Crilly, 2007). Clozapine was commercially marketed and used in clinical trials during the early 1970s. Later the drug was banned in 1975 when increasing deaths were reported due to agranulocytosis leading to infections among patients on clozapine medications (Crilly, 2007). Later, it was recognized that agranulocytosis is one of the adverse effects of clozapine. Clozapine has been found to be the most effective anti-psychotic drug of its class; its superior antipsychotic potential far exceeds disadvantage of its adverse effects. Clozapine was approved by the food and drug administration (FDA) in 1990, but it was restricted for use in treatment-resistant schizophrenia. Additionally, the FDA made it mandatory for patients who take clozapine to have regular blood cells count (Crilly, 2007; Fakra and Azorin, 2012).

MECHANISM OF ACTION

Clozapine acts on a wide range of receptors; these include those for dopamine (D1, D2, D3 and D4), serotonin ($5HT_{1A}$, $5HT_{1D}$, $5HT_{2A}$, $5HT_{2C}$, 5HT6 and 5HT7), catecholamine (adrenergic [α_1 and α_2] and muscarinic [M_1]) and histamine (H_1). It also possibly has effects on transporters of dopamine, serotonin and nor-adrenaline (Miyamoto et al., 2012, 2005). It has a much higher affinity (several-fold) for D4 and $5HT_{2A}$ receptors than

the D2 receptor. The lack of high affinity for D2 receptor in contrast to other typical antipsychotics is thought to explain the atypical profile of clozapine (Deutch, 1995; Seeman, 2004). The unique feature of clozapine that makes it stand out from the rest of the anti-psychotics is its ability to induce high Fos expression in the pre-frontal cortex selectively. Fos proteins are produced in neuronal cells as a result of induction of c-Fos gene. This is thought to modulate neurotransmitters in response to administration of anti-psychotic drugs (Ananth et al., 2001; Deutch, 1995). This unique property of clozapine is believed to make it highly efficacious in treating psychotic symptoms, particularly the negative and cognitive symptoms. In addition, its inability to induce Fos expression in other areas of brain, particularly in the striatum, explains the drug's lower propensity to cause extra-pyramidal symptoms (Deutch, 1995). The exact pathophysiology of this property of clozapine is unclear. None of the mechanisms underlying the action of other anti-psychotics that are common with those of clozapine is found to produce this effect. It is shown through PET imaging studies that for a antipsychotic effect to be observed at least 60% of D2 receptor occupancy is necessary. At therapeutic doses of clozapine, less than 60% of D2 receptors were blocked (Farde et al., 1992) Hence D2 receptor antagonism is minimally possible to explain clozapine's anti-psychotic actions. Clozapine has an antagonistic action against the $5HT_{2A}$ receptor. At therapeutic doses more than 80% of 5HT2A receptors were occupied by clozapine. Still is unclear whether 5HT2A antagonism can explain clozapine anti-psychotic efficacy. This is because of the observation that 5HT2A receptor specific antagonist failed to produce anti-psychotic effects (Lieberman et al., 1998). The effect of clozapine on adrenergic,

muscarinic and cholinergic receptors does not appear to be responsible for Fos induction in the prefrontal cortex (Deutch, 1995). Its higher affinity for D4 receptors, and consequently its higher D4:D2 affinity ratio, is postulated to explain drug's higher efficacy. However, selective D4 antagonists, namely L-745870, proved ineffective in producing anti-psychotic effects (Cao and Rodgers, 1997; Patel et al., 1997). Hence, the exact mechanism of clozapine's unique and potent antipsychotic effects is still unclear. It appears to be complex, with involvement of multiple neurotransmitter systems such as dopamine, serotonin, glutamate, muscarinic and cholinergic systems.

PHARMACOKINETICS

Clozapine is administered orally and is completely absorbed (Jann et al., 1993). The drug undergoes a variable first pass metabolism in the liver and hence oral bioavailability of the drug varies from 20% to 90%, with an average in the range of 50% to 60% (Jann et al., 1993). The drug is metabolized by the hepatic microsomal enzyme system involving the cytochrome P450 group of enzymes. The metabolites formed include N-desmethylclozapine and clozapine N-oxide. Peak levels are attained in 2 hours post drug intake. Steady state levels are attained after a week (Greenwood-Smith et al., 2003). Clozapine and its metabolites exist as both free and protein-bound forms in the circulation. The half-life of the drug varies between 3 and 33 hours, with an average around 12 hours (Greenwood-Smith et al., 2003). Of the total levels, the protein-bound fractions of clozapine, N-desmethyl clozapine and clozapine N-oxide were 95%, 90% and

75% respectively. The protein unbound free fractions undergo glomerular filtration as well as tubular secretion and get excreted in urine. (Jann et al., 1993).

DOSAGE

Clozapine therapy is initiated with small doses (12.5 – 25mg/day) given twice or thrice daily (Fakra and Azorin, 2012; Nielsen et al., 2011). The dosage is titrated gradually with stepwise increments each day, in order to avoid worrisome effects (orthostatic hypotension, sedation and sinus tachycardia) that might lead to early discontinuation (Falzer and Garman, 2012). Clozapine is licensed for a maximum dosage of 900mg/day, although most psychiatrists do not prefer to dose beyond 600mg/day due to high incidence of adverse effects (Falzer and Garman, 2012). Average doses for optimal effects are 400mg/day for men and 300mg/day for women. Tolerability and rate of discontinuation vary with the ages of the patients. It has been shown that the rate of discontinuation increases by 33% for every 10 years increase in a patient's age at the start of the treatment (Falzer and Garman, 2012). Hence, the elderly tend to have higher discontinuation rates and younger patients tolerate the drug better. Discontinuation of the drug happens commonly (40%) in the first year of the treatment (Nielsen et al., 2011).

THERAPEUTIC DRUG MONITORING

Therapeutic drug monitoring in clozapine therapy is not well established and is controversial (Greenwood-Smith et al., 2003). Although literature supports the use of serum clozapine levels in guiding dosage and evaluating response, drug doses are most

often adjusted on the basis of clinical improvement in psychotic symptoms rather than serum clozapine levels (Falzer and Garman, 2012; Nielsen et al., 2011). Clozapine levels can be measured either in plasma or serum, although serum levels are more frequently measured. Serum levels of clozapine tend to be higher than plasma levels by approximately 10% (Greenwood-Smith et al., 2003). Specific standard methods are employed for each and the reference values differ among laboratories (Greenwood-Smith et al., 2003; Raaska and Neuvonen, 1998). Levels are usually expressed as nanograms per milliliter (ng/ml) (Nielsen et al., 2011). Levels are characterized by extensive inter-individual variations. No clear thresholds have been established for characterizing patients' clinical response to clozapine therapy. However, most studies suggest an average threshold range of 350–420 ng/ml for a positive clinical response to clozapine. The lower threshold suggested for a clinical response was 250 ng/ml. However, a higher threshold limit has not been suggested so far (Nielsen et al., 2011). The relationship between the administered dose and the attained serum clozapine levels is not well characterized and tends to be complex due to extensive inter individual variation (Greenwood-Smith et al., 2003).

ADVERSE EFFECTS

Although clozapine causes less extra-pyramidal symptoms and minimal changes on serum prolactin levels, it is characterized by its own list of adverse effects that restricts its wide usage (Iqbal et al., 2003). Life-threatening adverse effects of clozapine include agranulocytosis, seizures, myocarditis, diabetic ketoacidosis and

cardiovascular/respiratory arrest (Safferman et al., 1991). Other common side effects are sedation, hyper-salivation, tachycardia, dizziness, constipation, nausea, hypotension, dry mouth, sweating, urinary problems, tremors, visual disturbances, fever, hypertension and weight gain (Safferman et al., 1991). The incidences of various adverse effects of clozapine are given in Table 1.3

Agranulocytosis

Agranulocytosis is the most serious life-threatening adverse effect induced by clozapine administration. This is defined as a granulocyte count of less than 500 cells/mm^3. Its incidence is shown to be 0.8% in the first year of treatment and 0.91% at $1^1/_2$ years (Alvir et al., 1993). Most patients who develop neutropenia do so in the first three months of treatment. The incidence tends to increase with age (Alvir et al., 1993). Female subjects showed a higher risk than males, with female to male incidence ratio of 1: 0.56 (Alvir et al., 1993). The risk of agranulocytosis has made leukocyte monitoring mandatory for patients on clozapine therapy. The mechanism underlying development of agranulocytosis is poorly understood. One postulated mechanism is that clozapine undergoes bio-activation in neutrophils to become a toxic intermediate that causes neutrophil cell death (Pereira and Dean, 2006). In 1990, Lieberman et al reported higher frequency of HLAB38 among Jewish patients with clozapine-induced agranulocytosis (Lieberman et al., 1990). Since then, multiple reports have emerged on associations between clozapine-induced agranulocytosis and genetic polymorphisms and haplotypes such as those in genes coding for human leukocyte antigen (HLA), cytochrome P450 2D6

(CYP2D6), myeloperoxidase (MPO), NADPH oxidase genes, dihydronicotinamide and riboside quinone oxidoreductase 2 gene (NQO2); however the findings have not been consistent (Amar et al., 1998; Claas et al., 1992; Dettling et al., 2000; Mosyagin et al., 2004; Valevski et al., 1998; Yunis et al., 1995).

Sedation

Sedation is the most common adverse effect of clozapine therapy, with an incidence of 39% (Safferman et al., 1991). It occurs early during treatment and patients frequently develop tolerance over period of time. The sedation is caused by the anti-histaminic and anti-adrenergic properties clozapine (Safferman et al., 1991). Administration of methylphenidate has proved to be helpful in ameliorating clozapine-induced sedation (Miller, 1996). However, patients tend to develop tolerance with methylphenidate. In addition, worsening of movement disorders have been reported in patients treated with methylphenidate for clozapine-induced sedation (Miller, 1996).

Hyper-salivation

Hyper-salivation or sialorrhea is the second most common adverse effect of clozapine therapy, with an incidence of 31% (Safferman et al., 1991). Hyper-salivation affects patients' quality of life (Davydov and Botts, 2000). Patient with sialorrhea experience sensations of choking at night and incidents of aspiration pneumonia as a consequence of aspiration of salivary secretions have been reported (Abdelmawla and Ahmed, 2009). Therefore, untreated sialorrhea may be life threatening. The pathophysiology of clozapine

Table 2.3 Incidence of adverse effects of clozapine therapy (Safferman et al., 1991)

Adverse effect	Incidence
Sedation	39%
Salivation	31%
Tachycardia	25%
Dizziness	19%
Constipation	14%
Nausea/vomiting	11%
Hypotension	9%
Sweating	6%
Dry mouth	6%
Urinary problems	6%
Tremor	6%
Visual disturbance	5%
Fever	5%
Hypertension	4%

Adverse effect	Incidence
Weight gain	4%
Seizures	3%
Akathisia	3%
Rigidity	3%
Agranulocytosis	1%

induced sialorrhea is poorly understood. Several mechanisms have been postulated. Clozapine, with anti-cholinergic properties, lead one to expect dryness of the mouth rather than hyper-salivation (Davydov and Botts, 2000). Indeed, dryness of the mouth occurs among patients on clozapine therapy, but the incidence is small (6%) in comparison to that of sialorrhea (31%) (Safferman et al., 1991). Later it was reported that clozapine has strong agonistic activity on M4 muscarinic receptor that might account for hyper salivation (Zorn et al., 1994). However, contribution of M4 receptors towards clozapine induced salivary secretion is not clear. It has been postulated that the anti-adrenergic action of clozapine against alpha-2 receptors in salivary glands may be one of the mechanisms of sialorrhea (Davydov and Botts, 2000). In support of this, clonidine, which is a selective alpha-2 agonist proved effective in treating clozapine-induced sialorrhea (Grabowski, 1992). However, alpha-2 selective antagonists, such as minaserin, failed to produce sialorrhea (Corrigan et al., 1995). Hence, it is unclear whether the beneficial effects of clonidine over sialorrhea are mediated through alpha-2 receptors. In 1996, Ben-Aryeh et al reported that the rate of salivary secretion per se was not affected in patients with clozapine-induced sialorrhea (Ben-Aryeh et al., 1996). Later it was postulated that sialorrhea is a consequence of defect in the swallowing reflex in patients on clozapine (Davydov and Botts, 2000). In support of this, reports of esophageal hypo-motility among patients with sialorrhea, as evidenced by barium swallow studies, have emerged (Boyce and Bakheet, 2005; Maddalena et al., 2004; McCarthy and Terkelsen, 1994; Pearlman, 1994). To summarize, the exact mechanism behind clozapine-induced

sialorrhea is poorly understood. Possible explanations include antagonism against alpha-2 receptors, agonism of M4 receptors and dysfunctional swallowing reflex.

Seizures and other central nervous system (CNS) effects

Clozapine produces dose-related changes in the electro-encephalogram of patients (Koukkou et al., 1979; Schmauss et al., 1989; Small et al., 1987). Approximately 1-2% of those who take 300mg of clozapine per day develop seizures (Safferman et al., 1991). The incidence increases with dose: 3-4% for 300-600 mg/day, 5% for 600-900 mg/day (Safferman et al., 1991). Patients with a history of a seizure disorder are at very high risk of developing this adverse effect; extra caution needs to be taken in these cases (Haller and Binder, 1990; Juul Povlsen et al., 1985). Other adverse effects in the CNS that have been observed include dizziness, confusion and syncope. The dizziness and syncope are the consequences of orthostatic hypotension caused by the drug (Safferman et al., 1991). In general, patients tend to develop tolerance in due course (Safferman et al., 1991). Sometimes patients develop states of confusion and delirium (Grohmann et al., 1989). This is believed to be due to central anti-cholinergic effects of the drug (Safferman et al., 1991).

Dopamine receptor D4

STRUCTURE

The dopamine receptor D4 (*DRD4*) is a subtype of dopamine receptor and grouped under D2-like receptors. It was identified in 1991 (Van Tol et al., 1991). It is coded by the gene *DRD4* that is located on chromosome 11 at locus 11p15.5 (Oak et al., 2000). The *DRD4* gene is 3.399 kilo-base pairs in length and consists of four exons. The genomic organization of the *DRD4* gene is highly conserved in humans and mice. The D4 receptor protein contains 467 amino acids and has a structure similar to any other G-protein coupled receptor (Oak et al., 2000). The structure consists of seven transmembrane domains that are hydrophobic in nature, an extracellular amino terminus and an intracellular carboxyl terminus (Kobilka, 2007). The receptor has structural homology in the trans- membrane domains with other G-protein coupled receptors, which is considered as the molecular signature of G-protein coupled receptors. The highest homology is found with D2 subtype and alpha-2 adrenoceptor family (Oak et al., 2000). The diversity occurs mainly in the extra- and intracellular terminals of the receptor, with the greatest diversity seen in the amino terminus. The conformational organization of the membrane-spanning regions of D4 receptors is highly similar to that of the membrane spanning alpha helices of G-protein coupled receptor rhodopsin (Baldwin et al., 1997). The receptor has consensus amino acid sequences in the extracellular amino acid tail that are potential sites for post-translation modifications such as N-linked glycosylation and

phosphorylation (Van Tol et al., 1991). The receptor has intracellular sequences that are involved in G-protein activation. The sequence Asp-Arg-Phe present in the second intra cellular loop is the key determinant in receptor activation (Oak et al., 2000).

EXPRESSION PROFILE

Tissue distribution of D4 receptors in the brain and other regions of the body were explored using multiple approaches such as northern blotting and RT-PCR that measure the mRNA transcripts, immuno-histochemistry and western blot that measure protein levels, and also using various ligand binding techniques (Oak et al., 2000). However, each of the above-mentioned approaches had both merits as well as demerits. For example, ligand-binding techniques were done using subtraction approaches with multiple ligands of varied affinities (Oak et al., 2000). This is due to lack of specific ligands for D4 receptors, as they are structural homologues of other D2-like receptors (D2 and D4). Consequently, studies produced conflicting results that were poorly replicated. Recently, transgenic animal models that express green fluorescent protein (GFP) under transcriptional control of the *DRD4* gene were generated to study the expression of *DRD4* (Noaín et al., 2006). However, the information available regarding the expression profile from the above-mentioned approaches was mainly from the animal models that cannot be extrapolated to humans. Data in humans were limited to studies done on post-mortem brain tissue (Helmeste et al., 1996; Mulcrone and Kerwin, 1996; Roberts et al., 1996; Seeman et al., 1995). The available information about *DRD4* expression in various tissues is briefly summarized below.

The most important site in the brain that is concerned with the action of anti-psychotics is the striatum, where the dopamine receptors subtype-2 (D2) are highly expressed (Seeman, 2004). Earlier studies that used subtraction ligand binding and RT-PCR approaches claimed presence of D4 receptors in the striatum, but at lower levels than D2 receptors (Oak et al., 2000; Van Tol et al., 1991). However, recent transgenic animal models showed absence of *DRD4*-expressing neurons in the striatum (Noaín et al., 2006). This supports the finding that *DRD4* antagonists, such as olanzapine and clozapine that has minimal D2 occupancy at therapeutic concentrations, are devoid of extra-pyramidal side effects, one of the debilitating consequences of D2 receptors blockade in the striatum (Seeman, 2004). Highest expression of *DRD4* is seen in the retina and pineal gland (Jackson et al., 2011; Kim et al., 2010; Matsumoto et al., 1995). They are also highly expressed in the prefrontal cortex and thought to be involved in complex cognitive behaviors (Noain et al., 2006). Defects in dopamine transmission in the prefrontal cortex cause cognitive behavioral disorders, such as attention deficit hyperactivity disorder (ADHD) (Depue et al., 2010; Lempp et al., 2013; Vasic et al., 2012). Certain *DRD4* genetic variations are implicated in such complex behavioral disorders. In addition, modulation of D4 receptors in the prefrontal cortex is considered to explain clozapine's superior efficacy over first generation anti-psychotics in treating cognitive symptoms of schizophrenia (Youngren et al., 1999). Apart from the brain region, D4 receptors are also detected in peripheral sites such as lymphocytes, cardiac atrium, cortical and medullary collecting ducts of kidneys (Oak et al., 2000).

GENETIC POLYMORPHISMS IN *DRD4*

One of the well-known facts about D4 receptors is that the *DRD4* gene that codes for the receptor is highly polymorphic (Oak et al., 2000). It is the most variable gene ever known in the human genome (Hattori et al., 2009). Numerous polymorphisms exist in the coding and upstream region of *DRD4* gene (Table 1.4).

The 48 base pairs tandem repeat

Of the polymorphisms reported in *DRD4*, the 48 base pairs variable number tandem repeats (VNTR), located in the third exon that codes for the third cytoplasmic loop of the receptor protein, have been extensively studied in the field of psychiatric genetics (Hattori et al., 2009). These base pairs code for 16 amino acids; the length of the cytoplasmic loop varies according to the number of repeats. The number of repeats observed in humans so far ranges from two to eleven; this varies the length of the cytoplasmic loop from 32 to 176 amino acids respectively (Naka et al., 2011). The most commonly observed variants are the 4R and 7R alleles, the global mean frequencies of which are 64.3% and 20.6% respectively (Chang et al., 1996). The next commonly observed variant is the 2R allele, the global mean frequency of which is 8.7%, though the frequency is considerably higher (18.1%) among South and East Asians (Chang et al., 1996). This polymorphism has been studied for genetic association with attention deficit hyper-activity disorder (ADHD) (Bellgrove et al., 2005, p. 48; Eisenberg et al., 2000; Kirley et al., 2004, p. 4; Li et al., 2013).

Table 2.4 Polymorphisms in the *DRD4* gene

Polymorphism	Location	Major allele	Minor allele	References
-1106T>C	Promoter	T	C	Nakajima et al., 2007
-906T>C	Promoter	T	C	Nakajima et al., 2007; Oades et al., 2008; Pal et al., 2009; Zhang et al., 2012; Zheng et al., 2012
-809G>A	Promoter	G	A	Nakajima et al., 2007
-616G>C	Promoter	G	C	Nakajima et al., 2007; Nemoda et al., 2010; Simpson et al., 2010
-521T/C	Promoter	T	C	Bhowmik et al., 2011; Das et al., 2011; Li et al., 2013; Nakajima et al., 2007; Nemoda et al., 2010; Okuyama et al., 1999a; Tsutsumi et al., 2009
-376C>T	Promoter	C	T	Li et al., 2013; Nakajima et al., 2007
-291C>T	Promoter	C	T	Nakajima et al., 2007
-768G>A	Promoter	G	A	Mitsuyasu et al., 2001, 1999
48-bp VNTR	Exon-III	4R, 7R	others	Bhaduri et al., 2007; Eisenberg et al., 2000; Hattori et al., 2009
120-bp VNTR	Promoter	240-bp	120-bp	Kereszturi et al., 2007, p. 5; Paredes et al., 2013; Seaman et al., 1999

The most accepted and replicated finding is the high prevalence of 7R allele among ADHD probands, although conflicting studies do exist (Bellgrove et al., 2005; Eisenberg et al., 2000, 2000). Recently studies have explored single nucleotide polymorphisms (SNP)

within the tandem repeat regions and have demonstrated a higher frequency of variations within the 7R alleles of ADHD probands (Tovo-Rodrigues et al., 2012). Among the variations observed within the 7R alleles, synonymous substitutions tended to be higher than non-synonymous substitutions among the ADHD probands (Tovo-Rodrigues et al., 2012). Ding et al reported that the 7R variant of this VNTR polymorphism arose as a mutational event of recent origin that was positively selected to occur in high frequencies among all populations of the world (Ding et al., 2002). However, the postulated positive selection hypothesis by Ding et al was challenged by a recent report where the authors have compared the heterozygosity of single nucleotide polymorphisms located within 25-kilo base pairs on either side of the *DRD4* gene on chromosome 11 (Naka et al., 2011). The results showed that the heterozygosity of this VNTR polymorphism is not significantly different from that of other SNPs around 25 kbp distance suggesting the possibility for recent positive selection to be very minimal (Naka et al., 2011).

Studies have also explored possible associations between the *DRD4* 48-bp VNTR and schizophrenia, although the results were inconclusive (Barr et al., 1993; Macciardi et al., 1994; Serretti et al., 1999; Shaikh et al., 1995, p. 4). The fact that *DRD4* receptors have a high affinity for clozapine has prompted researchers to explore the association between the 48-bp VNTR and clinical response to clozapine. Initial reports published in the early 1990s claimed that there was no significant association between the 48-bp VNTR variants and response to clozapine in patients with schizophrenia and schizoaffective disorders (Cohen et al., 1999; Hwu et al., 1998; Rao et al., 1994; Serretti et al., 1999; Shaikh et al.,

1995, 1993). However, it is to be noted that the sample sizes were inadequate. Later Kaiser et al, in 2000, replicated the findings of previous reports that the VNTR polymorphism is not associated with clozapine response with an adequate sample size (Kaiser et al., 2000). Zhao at al (2005) showed that 5R variants were significantly less frequent among responders, raising the possibility of an association of 5R variants with a poor response to clozapine. In fact, these findings were supported by the reports of Hwang et al (2012) in African-American and Caucasian populations, where they have shown that 4R variants are associated with a better response to clozapine. Considering all the studies together, it seems that though reports supporting a negative association predominate, the possibility of an association of the 48-bp VNTR with clozapine response cannot be ignored since all the reports were inadequately powered. This warrants further replicative studies with larger sample sizes.

-521C/T polymorphism

Another variant that has been studied is one that is located 521 base pairs upstream of the initiation codon (Okuyama et al., 2000). This polymorphism is characterized by replacement of single nucleotide thymine by cytosine (C/T) and is shown to affect the promoter activity. In vitro transfection experiments have demonstrated that T and C alleles are associated with low and high transcriptional activities respectively (Okuyama et al., 2000). The polymorphism is highly studied in relation to a personality trait called 'novelty seeking' in humans (Jönsson et al., 1997; Lusher et al., 2001; Mitsuyasu et al., 2001, p. 4; Okuyama et al., 2000; Ronai et al., 2001; Schinka et al., 2002). Meta-analysis

reports showed that the SNP is associated with increased novelty-seeking behavior in humans (Schinka et al., 2002). Being a functional polymorphism, it has been studied for association with schizophrenia (Lai et al., 2010; Nakajima et al., 2007; Okuyama et al., 1999b). Meta- analysis showed a positive association between the polymorphism and schizophrenia (Jönsson et al., 2003).

120 base pair duplication

This polymorphism is located 1.24 kilo base pairs upstream (5') of the initiation codon of the *DRD4* gene and is characterized by a tandem repeat of 120 base pairs (Paredes et al., 2013). One copy (120 bp) and two copy alleles (240 bp), also described as short and long alleles, are the ones that occur most commonly in humans. The polymorphism is thus usually described as 'tandem duplication'. The term 'tandem repeat' was very rarely used to describe this polymorphism (Paredes et al., 2013). Since the polymorphism was located in the promoter region of the gene, the duplication was expected to influence the promoter activity of the gene. In 2004, D'souza et al showed that in K-N-MC, SH-SY5Y, HEK293 and Hela cell lines, the long allele exhibited a lower transcriptional activity than the short allele, when transiently expressed using a luciferase reporter gene construct. However, later in 2013, the same authors have reported that the effect was not replicated in primary neuronal cell cultures from neonatal rats (Paredes et al., 2013). In addition, it was demonstrated that the two alleles might differ in their affinities towards transcriptional factors that bind to this region and that the variants might have a cell-specific or developmental stage-specific effect on the transcription of the *DRD4* gene

(Paredes et al., 2013). Despite the lack of solid evidence for a functional consequence of this polymorphism, various studies have been done to look for associations with multiple human behavioral and neurological disorders. Among those attention deficit hyperactivity disorder (ADHD) and schizophrenia have been more frequently studied. The studies in ADHD were mostly family based studies, where the transmission disequilibrium test (TDT) was employed to look for any preferences that might exist in the transmission of the *DRD4* alleles (long and short) from parent to the offspring. Most demonstrated a preferential transmission of the long allele of the *DRD4* 120-bp polymorphism from parent to offspring, either alone or as a haplotype with a 4R variant of the 48-bp VNTR polymorphism (Kereszturi et al., 2007; Kirley et al., 2004; Kustanovich et al., 2004, 2004; Sánchez-Mora et al., 2011).

Association studies have reported a higher frequency of the long allele (240-bp) among schizophrenics than the general population (Lai et al., 2010; Xing et al., 2003). In addition, a homozygous genotype for the short allele (120-bp/120-bp) has been shown to increase the risk for tardive dyskinesia (an adverse effect induced by anti-psychotics in north Indian subjects (Srivastava et al., 2006). With regard to anti-psychotic drug responses, very few reports have been published on the *DRD4* 120-bp duplication polymorphism. A study from north India reported that the long allele (240-bp) is associated with a better response to olanzapine, an atypical antipsychotic used in the treatment of TRS (Thomas et al., 2008). Another study that was done in two different populations (Caucasians and African-Americans) showed mixed results of association

with clozapine response (Hwang et al., 2012). Supporting the findings of Thomas et al. (2008) on olanzapine response, the authors demonstrated a similar trend in African-Americans; the homozygous genotype for the short allele (120/120) was found to be associated with a poor response to clozapine, which was defined based on the improvement in the brief psychiatric rating scale (BPRS) scores, over 6 months of treatment. However, the same study did not find any significant association between the 120-bp duplication and clozapine responses in a Caucasian population (Hwang et al., 2012). These mixed results raised questions about the role of ethnicity in modulating interactions of genetic factors with phenotypes such as clozapine response.

3. AIMS AND OBJECTIVES

Hypothesis

The hypothesis of this study was that a 120-bp duplication variant in the dopamine receptor-4 (*DRD4)* gene might influence clinical response to and adverse effects of clozapine therapy in patients with treatment-resistant schizophrenia.

Objectives and aim of the study

PRIMARY OBJECTIVES

To investigate the association between a 120 base pair duplication polymorphism of the *DRD4* gene and clinical response to clozapine in patients with treatment- resistant schizophrenia

To investigate the association between the 120 base pair duplication polymorphism of the *DRD4* gene and clozapine-induced adverse effects in patients with treatment- resistant schizophrenia

SECONDARY OBJECTIVES

1. To investigate association of the 120 base pair duplication polymorphism of the *DRD4* gene and serum level of clozapine in patients with treatment- resistant schizophrenia

2. To investigate association of the 120 base pair duplication polymorphism of the *DRD4* gene and oral dose of clozapine in patients with treatment- resistant schizophrenia

3. To investigate the association of 120 base pair duplication polymorphism of the *DRD4* gene and cognitive function, disability and psychopathology of patients with treatment-resistant schizophrenia.

4. To investigate the association between clozapine-induced adverse effects and serum levels of clozapine in patients with treatment-resistant schizophrenia

5. To investigate the association between clozapine-induced adverse effects and oral doses of clozapine in patients with treatment-resistant schizophrenia

4. METHODOLOGY

RECRUITMENT OF PATIENTS

The subjects of this study were part of a previous larger pharmacogenetic study done in the Department of Psychiatry, Christian Medical College, Vellore. DNA from blood samples from this study that had been stored were used for this study. Information on their recruitment and clinical assessment is given below.

Patients were recruited from among the outpatients of the Department of Psychiatry, Unit 1, at Christian Medical College, Vellore. Inclusion and exclusion criteria used were as follows.

Inclusion criteria

1. Patients with a diagnosis of schizophrenia as per the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV TR) (Quinn, 1999)
2. Patients with a diagnosis of resistance to treatment of schizophrenia, which was defined as failure to respond to at least two adequate anti-psychotic trials. An anti-psychotic trial was defined as treatment with 600mg of chlorpromazine or its equivalents for 6 weeks, with good drug compliance.
3. Patients of South Indian ethnicity

Exclusion criteria

1. Patients with severe neurological deficits
2. Patients with intellectual disability and sensory impairment
3. Patients who did not give informed consent

All subjects were recruited after getting informed consent from them in the presence of at least one of their relatives and a social worker.

CLINICAL ASSESSMENT

All recruited subjects were assessed for the following clinical variables, using standard instruments, as detailed below.

1. Brief Psychiatric Rating Scale (BPRS) for assessment of pyschopathology (Overall and Gorham, 1962)
2. Abnormal Involuntary Movements Scale (AIMS) for assessment of drug-induced dyskinesia (GUY and EDCEU, 1976)
3. Addenbrooke's Cognitive Examination Scale (ACE-R) for assessment of cognitive status (Mioshi et al., 2006)
4. World Health Organization Disability Assessment Scale-II (WHODAS-II) for assessment of disability (WHO, n.d.)
5. Childhood Traumatic Events Scale for assessment of traumatic experiences prior to the age of seventeen (CTES) (Goldberg et al., 1999)

6. Recent Traumatic Events Scale (RTES) for assessment of recent traumatic experiences in the past 3 years (Pennebaker and Susman, 1988)
7. Premorbid Assessment Scale (PAS) for assessment of premorbid functioning (Rabinowitz et al., 2007)

Therapeutic response to clozapine was determined using BPRS (Overall and Gorham, 1962). Those with a score equal to or less than 35 on this scale were considered responders. Those with scores above 35 were considered non-responders.

COLLECTION OF SOCIO-DEMOGRAPHIC DATA

Information on various socio-demographic and clinical variables was obtained by taking detailed histories from patients and their relatives. The proforma used for this is included in the Appendix.

MEASUREMENT OF SERUM CLOZAPINE LEVELS

Blood samples were collected from the patients 12 hours after their last clozapine dose. Serum was separated immediately. The clozapine dosages received by the study participants was recorded. The serum samples were stored at -20°C. Estimation of clozapine levels was done together in a single batch in order to avoid inter-assay variability. They were measured using high pressure liquid chromatography (HPLC), with ultra-violet detection (Chung et al., 1993). Clozapine levels were expressed as ng/ml. The ratio of serum clozapine levels to the dose of clozapine was also calculated.

GENOTYPING

The following steps were involved in genotyping.

1. Isolation of DNA from blood samples
2. Amplification of DNA sequence of interest by polymerase chain reaction (PCR)
3. Separation of PCR products by agarose gel electrophoresis

Isolation of DNA

Peripheral venous blood samples were used as the source of DNA, which was isolated using a commercially available kit (Qiagen DNA Mini Kit, Qiagen GmBH, Hilden, Germany). The steps carried out as per the manufacturer's protocol were as follows.

1. Three ml of blood sample and 9 ml of RBC lysis solution were added to a 15 ml tube.
2. The contents were incubated at -20°C for 10 minutes. This causes the RBCs to lyse and releases haemoglobin.
3. After incubation, the tubes were centrifuged at 4000 rpm at 4°C for 10 minutes; the supernatant obtained was discarded.
4. The residual pellet obtained at the end of the above step often still contained residual haemoglobin. RBC lysis solution was added as described above and the

step repeated twice more. At the end of these steps, the pellet that remained was that of WBC.

5. One ml of cell lysis solution was then added to each tube; the contents were mixed to disrupt WBC pellet at the bottom of the tubes.
6. After the pellet was completely re-suspended, each tube was incubated at 37°C overnight. This step resulted in lysis of the WBC.
7. The contents were gently mixed and 1 ml of protein precipitating solution was added and mixed again.
8. The tubes were centrifuged at 4000 rpm for 5 minutes.
9. The supernatant obtained in each tube was transferred to a 15 ml tube. Four millilitres of 99% isopropranolol was added to this; the contents were mixed gently by inverting the tube. DNA was seen to precipitate during this step.
10. The DNA threads obtained were transferred to a 2 ml microtube using a micropipette tip. Absolute alcohol (750 µl) and water (250 µl) were added to this.
11. After washing the DNA by gentle repeated inversions, the DNA thread was transferred to a dry 2 ml micro tube, using a micropipette tip. It left to dry at room temperature for 15 minutes.
12. DNA hydration solution (50-200 µl) was added to the DNA pellet (depending on its size). The contents of the tube were mixed by tapping; it was kept in a 55°C water bath for 1 hour.
13. The tubes containing the DNA samples were removed from the bath and were stored at 4°C till further use.

The purity of the DNA isolated was assessed. For this, the optical density (OD) of the DNA solution was measured spectrophotometrically at 260nm and 280nm. The ratio of the OD at these 2 wavelengths was calculated. A value between 1.8 and 2 obtained from the ratio of the OD at 260 nm to the OD at 280 nm was considered to be an indication of purity. The concentration of DNA in each sample was estimated. For this, the OD of each sample was measured at 260 nm. The concentration of DNA was calculated using the following formula.

DNA concentration (μg/ml) = OD at 260 nm x dilution factor x 50

(an OD of 1 at 260nm indicates a concentration of 50 μg/ml)

Amplification of DNA sequence of interest by polymerase chain reaction (PCR)

The forward and reverse primer sequences used for the amplification of the region spanning the *DRD4* 120-bp duplication polymorphism were taken from published literature (McCracken et al., 2000a) and were as follows.

- Forward primer: 5'-GTT GTC TGT CTT TTC TCA TTG TTT CCA TTG -3'
- Reverse primer: 5'-GAA GGA GCA GGC ACC GTG AGC -3'

The conditions used for the PCR assays were as follows (McCracken et al., 2000).

A 20 μl reaction, containing 10 μl of master mix, 1 μl of forward and reverse primers, 1 μl of DNA and 8 μl of deionized water, was used for the PCR.

Steps:

1. Initial denaturation at 95°C for 15 minutes
2. Denaturation at 95°C for 10 seconds
3. Annealing at 66°C for 30 seconds
4. Extension at 72°C for 90 seconds
5. Steps 2 to 4 were repeated for 10 cycles
6. Denaturation at 95°C for 10 seconds
7. Annealing at 55°C for 30 seconds
8. Extension at 72°C for 90 seconds
9. Steps 6 to 8 were repeated for 25 cycles
10. Final extension at 72°C for 10 minutes

Agarose gel electrophoresis

The amplified products obtained at the end of the PCR were subjected to agarose gel electrophoresis, using 1.5% agarose gels containing ethidium bromide. Each PCR product (10 µl) was loaded in to the wells in the gel. The samples were electrophoresed at 100 volts for 60 minutes. After the electrophoresis, the DNA bands were visualized under ultra-violet light, using a gel documentation system (Alpha Innotech, Fluor Chem SP). The genotype of each sample was deduced from the pattern of bands observed.

STATISTICAL ANALYSIS

Types of variables

The genotypes studied (namely wild type – 120/120, heterozygous – 120/240 and homozygous – 240/240), socio-demographic variables and clozapine side effects were coded as categorical variables. Serum clozapine levels and all the clinical variables that were assessed and scored, using scales, were coded as continuous variables.

Statistical tests

Data on continuous variables were checked for normality of distribution by Kolmogorov-Smirnov test. All these data were found to have a skewed distribution. Hence, these data were analyzed by Kruskal Wallis test or Wilcoxon-Mann-Whitney test. Categorical variables were analyzed by chi square test. Spearman's correlation coefficients were used to determine correlations among the variables studied. A p value < 0.05 was considered to indicate statistical significance in all cases.

Allele frequencies in the patients were checked to determine if they were in keeping with Hardy Weinberg equilibrium (HWE). Pearson's Chi-squared Test was used to compare allele frequencies that were observed and those that were expected. The allele frequencies were considered to follow Hardy Weinberg equilibrium when a p value of more than 0.05 was obtained.

Allele frequencies and clozapine responses were analyzed using Pearson's Chi-Squared test. They were also studied under five genetic models of inheritance: co-dominant, dominant, recessive, over-dominant and log-additive. The three genotypes (120/120, 120/240, 240/240) were given a specific score under each model. A Chi-squared trend test with genotype scores was done under each model to compute the significance. A p value < 0.05 was considered to be statistically significant. Logistic regression analysis was also done under each model. Odds ratios and 95% confidence intervals were calculated.

Associations between clozapine side effects and genotypes were analyzed using Pearson's Chi-squared test. A p value < 0.05 was considered statistically significant. The side effects that proved significant were again analyzed using Chi-squared trend test and logistic regression analysis with appropriate genotype scores under aforementioned genetic models of inheritance. All statistical analyses were performed using R statistical software (R Development Core Team, 2013).

5. RESULTS

CLINICAL AND SOCIO-DEMOGRAPHIC PROFILE OF THE PATIENTS

The socio-demographic and clinical profiles of the participants in the study were as follows.

Socio-demographic profile

Ninety-five patients were recruited for the study. Of these, 69 were males and 26 were females (Table 5.1). The subjects' ages were in the range of 20 – 60 years, with a mean age of 35.2 years (±9.4) (Table 5.1). A majority of the subjects (66.31%) were single or separated from the spouse and were unemployed (57.9%). The study participants comprised of people from both urban (60%) and rural (40%) areas. Twenty one percent of subjects were found to drink at least 3 cups of coffee or tea per day and 16.8% were active smokers.

Table 5.1. Socio-demographic profile of study participants.

Variables	Mean (SD)	Median	Range
Age (years)	35.2 (9.4)	34	20 – 60
No. of years of education	12 (3.8)	12	0 – 19
Monthly family income (Rs.)	4916 (6206)	3000	300-50000

Table 5.2 Socio-demographic profile of study participants (cont'd)

Characteristic	Categories	Number of patients (%)
Sex	Male	69 (72.63%)
	Female	26 (27.36%)
Marital status	Single/separated	63 (66.31%)
	Married	32 (33.68%)
Current employment status	Employed	40 (42.1%)
	Unemployed	55 (57.9%)
Residence	Urban	57 (60%)
	Rural	38 (40%)
Caffeine intake	≥ 3 cups/day	20 (21.1%)
	< 3 cups/day	75 (78.9)
Current smokers	Smokers	16 (16.8%)
	Non-smokers	79 (83.2%)

Clinical profile

The majority of the subjects (83.2%) had non-paranoid type of schizophrenia (Table 5.3). Among them, 17.9% had a positive family history of schizophrenia and 5.3% had a past history of catatonia (Table 5.3).

Table 5.3 Clinical profile of the study subjects

Characteristic	Categories	Number of patients (%)
Family history of schizophrenia	Present	17 (17.9%)
	Absent	78 (82.1%)
Type of schizophrenia	Paranoid subtype	79 (83.2%)
	Non-paranoid subtype	16 (16.8%)
Past history of catatonia	Present	5 (5.3%)
	Absent	90 (94.7%)

The mean age of onset of disease was 22.94 (± 9) years, with 17 (17.9 %) of the subjects having had their first psychotic episode before 18 years of age (early onset schizophrenia) (Table 5.4). The duration of illness among the subjects varied widely from a minimum of 1 year to a maximum of 33 years (Table 5.4). The average duration of illness was 12.31 (± 6.8) years. The average duration of treatment with clozapine among the subjects was 41.1 (± 38.7) months, with a range of 4 months to 174 months (Table 5.4). The majority of them (87.4%) had had more than 36 weeks of clozapine treatment. The maximum dose of clozapine received by the study participants ranged from 100 mg/day to 650 mg/day, with an average of 346.1 (± 117.9) mg/day. The serum clozapine levels of the study participants ranged widely (from 104 ng/ml to 2547 ng/ml), with an average of 562.6 ng/ml (± 385.75). The mean body mass index of the study participants was 34.48 (±11) that ranged from 24 to 69.

Table 5.4 Clinical profile of subjects (cont'd)

Variables	Mean (SD)	Median	Range
Age of onset of disease (years)	22.94 (6.98)	21	10 - 46
Duration of illness (years)	12.31 (6.8)	11	1 – 33
Duration of treatment (months)	111.2 (78.4)	96	4 – 360
Duration of clozapine treatment (months)	41.1 (38.7)	28	4 – 174
Maximum oral dose of clozapine (mg/day)	346.1 (117.9)	350	100 – 650
Serum clozapine level (ng/ml)	562.6 (385.75)	385.8	104 – 2547
Serum clozapine level/dose ratio	1.7 (1.2)	1.5	0.3 – 8.3
Body mass index (BMI) (Kg/m^2)	24.4 (4.6)	23.9	16.7 – 38
BPRS total score	34.48 (11)	31	24 – 69
ACE-R total score	63.56 (20.1)	68	7 – 96
WHODAS-II total score	17.43 (12.9)	15	0 – 46
CTES total score	8.15 (10.1)	4	0 – 42
RTES total score	6.08 (8.9)	2	0 – 44
PAS total score	55 (21.8)	54	8 – 116

BPRS - Brief Psychiatry Rating Scale; ACE-R - Addenbrooke's Cognitive Examination Scale; WHODAS-II - World Health Organization – Disability Assessment Schedule-II; CTES- Childhood Traumatic Events Scale; RTES-Recent Traumatic Events Scale; PAS-Premorbid adjustment Scale.

CORRELATIONS AMONG CLINICAL SCORES OF PATIENTS

The correlations between the various clinical scores (BPRS, ACE-R, RTES, CTES, WHODAS, PAS) of the subjects were found to be as follows

Correlation of BPRS with other scales used

Brief Psychiatric Rating Scale (BPRS) scores of the subjects were checked for correlation with other clinical scales in the subjects (Figures 5.1). The BPRS scores, which is a measure of pyschopathology in the subjects, showed a significant negative correlation with scores obtained from Addenbrooke's Cognitive Examination – Revised (ACE-R) ($r = -0.37$, $p = 0.0003$) (Figure 5.1C). This shows that with increase in psychopathology (indicated by increases in BPRS scores), cognitive functions decline (indicated by decreases in ACE-R scores). BPRS scores did not correlate with the other scales (RTES, CTES, WHODAS and PAS) used.

Correlation of ACE-R with other scales used

ACE-R scores (a measure of the cognitive function) of the patients were significantly correlated with WHODAS, PAS, CTES and BPRS scores (Figures 5.1) ACE-R scores correlated negatively ($r = -0.46$; $p < 0.0001$) with the WHODAS scores, suggesting that as cognitive function decreased (as indicated by low ACE-R scores), the level of disability in the subjects worsened (as indicated by high WHODAS scores) (Figure 5.1F). ACE-R and CTES scores showed positive correlations with one another ($r = 0.27$; $p = 0.007$) (Figure

5.1M). This shows that people who experienced more childhood trauma (as indicated by high CTES scores) had better cognition (as indicated by high ACE-R scores). However it is unclear if this is just an incidental finding or a real correlation. ACE-R and PAS scores were negatively correlated ($r = -0.31$; $p = 0.002$), suggesting that people with poor premorbid adjustment (as indicated by high PAS scores) had poor cognition (as indicated by low ACE-R scores) (Figure 5.1L). ACE-R scores were not significantly correlated with RTES scores (Figure 5.1K).

Correlation of WHODAS with other scales used

WHODAS scores were significantly negatively correlated with ACE-R ($r = -0.46$; $p < 0.0001$) and RTES scores ($r = -0.22$; $p = 0.03$). WHODAS scores were not significantly correlated with other clinical scores obtained (Figure 5.1F&G)

Figure 5.1 Correlations among clinical scores of the patients

CLOZAPINE-INDUCED ADVERSE EFFECTS AMONG THE STUDY PARTICIPANTS

Adverse effects observed in the subjects

All the study subjects were evaluated for the presence of adverse effects of clozapine. These included sedation, salivation, seizures, sexual dysfunction (erectile dysfunction), gastro-intestinal dysfunction (nausea, vomiting and abdominal pain), constipation, enuresis, metabolic side effects (dyslipidemia) and agranulocytosis (Table 5.5). Sedation was the most commonly reported adverse effect among the subjects (77%). The second most commonly reported adverse effect was salivation, which was present in 47% of subjects. Seizures, sexual dysfunction, gastro-intestinal dysfunction, constipation, enuresis and metabolic adverse effects were reported in 9.5%, 13.7%, 21%, 20%, 5.2% and 10.5% of patients, respectively. Agranulocytosis was not found in any of the study participants.

Age and sex distribution of subjects with and without adverse effects

Age and sex distribution of the study participants were analyzed to look for statistical differences between subjects with and without adverse effects (Table-5.5). The results showed that enuresis and seizures occurred more frequently in older subjects. Subjects with seizures and enuresis were found to be significantly older than those without these adverse effects. Sexual dysfunction was found to occur only in male subjects.

Table 5.5 Age and sex distribution of study subjects with respect to adverse effects

Adverse effects			Age (years)		Sex		
		Number of subjects	Mean (SD)	P value	Male	Female	P value
Hyper-salivation	Present	45	34.73 (9.32)	0.59	34	11	0.71
	Absent	50	35.62 (9.57)		35	15	
Sedation	Present	73	34.8 (9.41)	0.44	51	22	0.4
	Absent	22	36.4 (9.54)		18	4	
Seizures	Present	9	41.55 (7.94)	0.02*	6	3	0.9
	Absent	86	34.53 (9.35)		63	23	
Sexual dysfunction	Present	13	36.61 (5.53)	0.26	13	0	0.01*
	Absent	82	34.98 (9.9)		56	26	
Gastro-intestinal effects	Present	20	36.65 (9.75)	0.43	15	5	1
	Absent	75	34.81 (9.35)		54	21	
Constipation	Present	19	34.31 (8.90)	0.8	16	3	0.32
	Absent	76	35.42 (9.58)		53	23	

Table 5.5 (cont'd)

Adverse effects			Age (years)		Sex		
		Number of subjects	Mean (SD)	P value	Male	Female	P value
Enuresis	Present	5	44.8 (7.63)	0.02*	3	2	0.89
	Absent	90	34.67 (9.2)		66	24	
Dyslipidemia	Present	10	34.1 (9.3)	0.69	6	4	0.56
	Absent	85	35.33 (9.5)		63	22	

* indicates a p value < 0.05; Age distribution between groups was analyzed by Wilcoxon-Mann-Whitney test; Sex distribution between groups was analyzed by Chi-squared test or Fisher's exact test.

Association of adverse effects with dose and serum levels of clozapine.

The maximum doses of clozapine used per day and the serum levels of the drug were analyzed to determine associations of these with the occurrence of adverse effects. It was found that the adverse effects observed were not dependent on dose or on the serum levels attained (Table 5.6).

Table 5.6. Comparisons of serum clozapine levels and oral dose of clozapine between subjects with and without adverse effects

Adverse effects		Mean Serum clozapine levels in mg/day (SD)	P value	Mean Oral dose of clozapine in ng/ml (SD)	P value
Hyper-salivation	Present	605.23 (437.4)	0.41	358.9 (110.3)	0.25
	Absent	524.2 (332.4)		334.5 (124.3)	
Sedation	Present	535.33 (311.43)	0.72	339.4 (120.2)	0.28
	Absent	653.1 (567.8)		368.2 (109.7)	
Seizures	Present	846.6 (686.1)	0.07	361.1 (106.1)	0.73
	Absent	532.9 (332.9)		344.5 (119.5)	
Sexual dysfunction	Present	568.0 (375.1)	0.96	382.7 (141.9)	0.27
	Absent	561.7 (389.7)		340.2 (113.6)	
Gastro-intestinal effects	Present	698.3 (593.4)	0.49	368.8 (115.8)	0.24
	Absent	526.4 (303.8)		340.0 (118.5)	
Constipation	Present	568.1 (395.6)	0.67	332.9 (93.6)	0.69
	Absent	540.6 (352.8)		349.4 (123.6)	

Table 5.6 Cont'd

Adverse effects		Mean Serum clozapine levels (SD)	P value	Mean Oral dose of clozapine (SD)	P value
Enuresis	Present	597.1 (235.9)	0.42	450.0 (117.3)	0.05
	Absent	560.7 (393.2)		340.28 (115.9)	
Dyslipidemia	Present	518.4 (157.8)	0.54	375.0 (150.0)	0.69
	Absent	567.8 (404.5)		342.7 (114.2)	

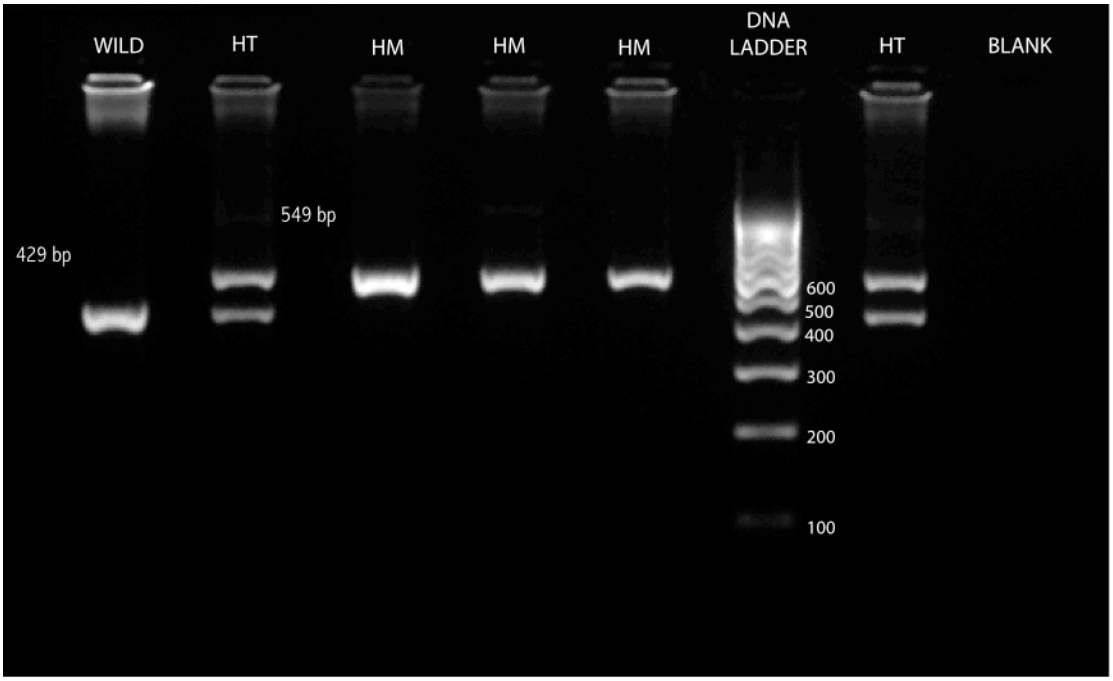
Data were analyzed using Wilcoxon-Mann-Whitney test.

GENOTYPES AND HARDY-WEINBERG EQUILIBRIUM

Interpretation of genotypes

The PCR reaction yielded an amplicon of size 429 base pairs in case of a wild type allele and 549 base pairs in case of a duplicated allele. Accordingly, in agarose gel electrophoresis a wild type sample yielded a single band of size 429 base pairs, a heterozygous sample yielded two bands, one of 429 base pairs and the other of 549 base pairs and a homozygous sample yielded single band of 549 base pairs (Figure 5.2). The genotype status of the subjects was determined based on this.

Figure 5.2 Agarose gel electrophoresis.

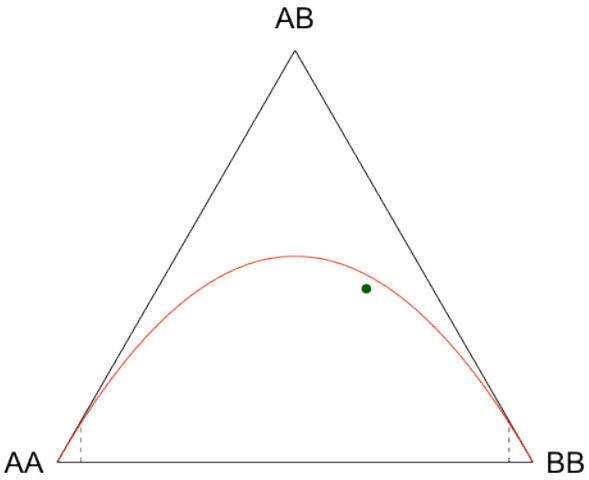


Lane 1 shows the wild type sample with a single band of 429 bp, lane 2 shows a heterozygous sample with one band of 429 bp and another of 549 bp, and lane 3 shows a homozygous sample with a single band of size 549 bp. Lane with the label "DNA LADDER" shows a DNA ladder for purposes of comparison.

Hardy-Weinberg equilibrium

The genotype and allele frequencies seen are shown in Table 5.7. The allele frequencies were found to lie within Hardy Weinberg equilibrium. The Hardy Weinberg equilibrium was also determined by using a ternary plot (Figure 5.3), which is also called the *de-finneti* diagram.

Figure 5.3 Ternary plot (de finetti diagram).



The green dot indicates the *DRD4* 120-bp duplication polymorphism. When the dot falls below the red line of the parabola, the gene is said to lie within Hardy Weinberg equilibrium. AA – wild type (120/120); AB – heterozygous (120/240); BB – homozygous (240/240)

Table 5.7 Genotypes, allelic frequencies and value of Hardy Weinberg equilibrium

Genotypes			Alleles		Hardy Weinberg equilibrium		
120/120	120/240	240/240	120-bp	240-bp	X^2	df	p value
13	40	42	66	124	0.48	1	0.51

The p and X^2 values were computed by Chi-squared test between observed and expected genotype frequencies.

CLINICAL CHARACTERISTICS IN THE DIFFERENT GENOTYPES

Table 5.8 shows the clinical variables across the three genotypes. There was a significant difference among the maximum doses received by subjects in the three different

genotypes (p-0.004). In case of serum clozapine level-dose ratio, the values tended to be different among the three genotypes, with a p value of 0.06, which is close to statistical significance

Table 5.8 Clinical characteristics of patients with the various genotypes

Clinical Variables		Genotypes			Kruskal Wallis test		
		120/120	120/240	240/240	X^2	df	p
Age at onset (years)	Mean (SD)	22.4 (12)	23 (9.2)	23 (8.6)	0.5	2	0.8
	Median	19	21.5	21.5			
Duration of illness (years)	Mean (SD)	14 (10.2)	10.6 (6.2)	13.3 (5.9)	5.3	2	0.07
	Median	10	9	12			
Duration of clozapine (months)	Mean (SD)	46.3 (43)	32.5 (30.5)	48 (43.3)	1.89	2	0.25
	Median	30	22.5	35			
Body mass index (kg/m^2)	Mean (SD)	25.1 (3.9)	24.12 (5.6)	24.3 (3.7)	1.89	2	0.25
	Median	24.8	22.5	24.2			

Table 5.8 (cont'd)

Clinical Variables		Genotypes			Kruskal Wallis test		
		120/120	120/240	240/240	X^2	df	p
Maximum dose of clozapine used per day (mg/day)	Mean (SD)	253.85 (144.2)	378.75 (112.6)	343.45 (99.85)	10.9	2	**0.004***
	Median	250	375	350			
Serum clozapine levels (ng/ml)	Mean (SD)	565.3 (481.1)	531.8 (297.1)	591.1 (433.2)	0.44	2	0.801
	Median	413	438	444			
Serum clozapine level/dose ratio	Mean (SD)	2.3 (1.25)	1.45 (0.71)	1.8 (1.38)	5.6	2	0.06
	Median	1.5	1.45	2.3			
BPRS total score	Mean (SD)	32.08 (7.93)	35.73 (12.62)	34.05 (10.27)	0.21	2	0.9
	Median	31	31.5	31			
WHODAS-II total score	Mean (SD)	15.4 (10)	18.2 (12.6)	7.2 (13.9)	0.45	2	0.79
	Median	17	16	13			
ACE-R total score	Mean (SD)	65.2 (20.7)	62 (20.4)	64.6 (19.9)	0.98	2	0.611
	Median	72	66.5	69			

* Indicates a p value < 0.05; the distributions of the clinical variables among the three genotypes were analyzed using Kruskal-Wallis test.

ASSOCIATION BETWEEN THE VARIOUS GENOTYPES (*DRD4* 120-BP REPEAT) AND CLOZAPINE RESPONSE

Genotypic association

Table 5.9 Associations of *DRD4* 120-bp repeat with clozapine response

Model	Genotype	Genotype score	Responders	Non-responders	Chi-squared test with trend scores		
			N	N	X^2	df	p value
Co - dominant	120/120	0	10	3	1.26	2	0.59
	120/240	0	24	16			
	240/240	0	26	16			
Dominant	120/120	0	10	3	0.64	1	0.36
	120/240	1	24	16			
	240/240	1	26	16			
Recessive	120/120	0	10	3	<0.01	1	0.9
	120/240	0	24	16			
	240/240	1	26	16			

Table 5.9 (cont'd)

Model	Genotype	Genotype score	Responders	Non-responders	Chi-squared test with trend scores		
			N	N	X^2	df	p value
Over-dominant	120/120	0	10	3	0.1	1	0.74
	120/240	1	24	16			
	240/240	0	26	16			
Log-additive	120/120	0	10	3	0.5	1	0.48
	120/240	1	24	16			
	240/240	2	26	16			

The p values were computed using Chi-squared test, with a specific score for each group as mentioned in the table.

Genotypic association of *DRD4* 120-bp repeat polymorphism with clozapine response was studied under 5 genetic models of inheritance: co-dominant, dominant, recessive, over-dominant and log-additive (Tables 5.9 and 5.10). Analysis showed that there was no significant association between the genotypes and response to clozapine under any of the genetic models of inheritance. Logistic regression analysis was also done under each model to compute the odds ratio and 95% confidence interval (Table 5.10). The odds ratios obtained were not statistically significant.

Table 5.10 Associations of *DRD4* 120-bp repeat with clozapine response

Model of inheritance	Genotype score	Logistic regression analysis			
		Odds ratio	**95% CI**	**p value**	**AIC**
Co-dominant	-	1.019[f] 0.860[†]	0.82-1.25 0.63-1.16	0.86 0.33	137.82
Dominant	0,1,1	1.17	0.88 – 1.55	0.273	135.85
Recessive	0,0,1	1.022	0.83-1.24	0.824	137.03
Over-dominant	0,1,0	1.056	0.86-1.28	0.591	136.79
Log-additive	0,1,2	1.051	0.91 - 1.21	0.485	136.58

AIC – Akaike Information Criterion (AIC value gives the measurement of information loss from a statistical model. The one with less AIC score tends to have less information loss); CI – confidence interval; [f]odds ratio of heterozygous (120/240) genotype in comparison with wild type (120/120); [†]odds ratio of homozygous genotype (240/240) in comparison with wild type (120/120)

ASSOCIATIONS OF THE VARIOUS GENOTYPES (*DRD4* 120-BP REPEAT) AND ALLELES WITH CLOZAPINE-INDUCED ADVERSE EFFECTS

Crude analysis

All the recorded adverse effects were analyzed to see if any of them was significantly associated with the *DRD4* 120-bp repeat polymorphism (Tables 5.11 and 5.12). Results showed that clozapine-induced hyper-salivation was significantly associated with the *DRD4* 120-bp repeat ($p = 0.004$). None of the other adverse effects were significantly associated with the polymorphism. Allelic association analysis showed that the long allele (240-bp) is significantly associated with increased risk of hyper-salivation (Table 5.12). The 240-bp allele was found to increase the risk of hyper-salivation by 3.6 times, in comparison with the 120-bp allele (odds ratio of 3.6 as seen in Table 5.12).

Association of clozapine-induced hyper-salivation with *DRD4* 120-bp repeat

In order to further explore the association between hyper-salivation and *DRD4* 120-bp repeat, the genotypic association between the 2 was studied under five genetic models of inheritance - co-dominant, dominant, recessive, over-dominant and log-additive models. Significant associations were found between the 2 under all the models except the over-dominant one (Table-5.13). Logistic regression analysis, using generalized linear model under each mode of inheritance with appropriate genotype scores, also showed significant odds ratios for all the models except the over-dominant one. The results, thus, show that possessing a single copy of 240-bp allele (heterozygotes) increased the risk for

hyper-salivation by 1.279 times; this risk doubled to 2.56 when both the alleles were 240-bp (240/240). The log-additive model had the lowest AIC (135.06) and was the most informative (relative likelihood of other models in comparison to log-additive was less than 0.5) of all the models in explaining the association of clozapine-induced sialorrhea with *DRD4* 120-bp duplication polymorphism. Thus, the *DRD4* 120-bp duplication polymorphism appears to follow an additive mode of inheritance for the phenotype of clozapine-induced hyper-salivation, with the homozygotes at double the risk of heterozygotes for developing clozapine-induced sialorrhea.

Table 5.11 Genotypic association between clozapine-induced adverse effects and the *DRD4* 120-bp repeat

Adverse effects of clozapine		**Genotype**			**Chi-squared test**		
		120/120	120/240	240/240	X^2	df	p
Hyper-salivation	Present	2	16	27	11.02	2	0.004*
	Absent	11	24	15			
Sedation	Present	9	31	33	0.503	2	0.77
	Absent	4	9	9			

Table 5.11 (cont'd)

Adverse effects of clozapine		Genotype			Chi-squared test		
		120/120	120/240	240/240	X^2	df	p
Seizures	Present	2	3	4	0.71	2	0.71
	Absent	11	37	38			
Sexual dysfunction	Present	1	6	6	0.89	2	0.63
	Absent	12	34	36			
Gastro-intestinal effects	Present	3	10	7	0.89	2	0.69
	Absent	10	30	35			
Constipation	Present	2	7	10	0.71	2	0.77
	Absent	11	33	32			
Enuresis	Present	0	2	3	1.02	2	1
	Absent	13	38	39			
Dyslipidemia	Present	0	5	5	1.7	2	0.47
	Absent	13	35	37			

*Indicates a p value < 0.05

Table 5.12 Allelic associations between clozapine-induced adverse effects and *DRD4* 120-bp repeat

Adverse effects of clozapine		Alleles		Chi-squared test		Logistic regression	
		120-bp	240-bp	X^2	p value	OR	95% CI
Salivation	Present	120	70	30.11	<0.0004*	3.6	2.3 – 5.7
	Absent	46	97				
Sedation	Present	49	97	0.192	0.6	0.80	0.4 – 1.63
	Absent	17	27				
Seizures	Present	7	11	0.016	0.9	1.22	0.43 – 3.32
	Absent	59	113				
Sexual dysfunction	Present	8	18	0.05	0.813	0.82	0.32 – 1.96
	Absent	58	106				
Gastro-intestinal effects	Present	16	24	0.35	0.55	1.33	0.64 - 2.73
	Absent	50	100				

Table 5.12 (cont'd)

Adverse effects of clozapine		Alleles		Chi-squared test		Logistic regression	
		120-bp	240-bp	X^2	p value	OR	95% CI
Constipation	Present	11	27	0.42	0.52	0.72	0.32 – 1.55
	Absent	55	97				
Enuresis	Present	2	8	0.44	0.51	0.48	0.06 – 2.03
	Absent	64	116				
Dyslipidemia	Present	5	15	0.52	0.47	0.60	0.19 – 1.67
	Absent	61	109				

*Indicates a p value < 0.05

Table 5.13 Association analysis of clozapine induced hyper-salivation and *DRD4* 120-bp repeat

Models of inheritance	Chi-squared test with trends			Logistic regression analysis			
	Genotype Scores (AA, AB, BB)	df	p value	Odds ratio	95% Confidence intervals	p value	AIC
Co-dominant	-	2	0.004*$^{\Delta}$	1.627	1.21 - 2.19	0.002*	137.05
				1.261	0.93 - 1.71	0.14	
Dominant	0, 1, 1	1	0.028*	1.441	1.08 - 1.92	0.01*	140.84
Recessive	0, 0, 1	1	0.006*	1.368	1.12 - 1.66	0.002*	137.41
Over-dominant	0, 1, 0	1	0.308	0.869	0.71 - 1.06	0.18	145.25
Log-additive	0, 1, 2	1	0.0009*^{f}	1.279	1.11 - 1.47	0.0007*	135.06

df – degrees of freedom**;** AIC – Akaike Information Criterion; ^{f}Cochran Armitage Trend Test (CATT); Genotypes: AA – 120/120; AB – 120/240; BB – 240/240; * p = < 0.05; $^{\Delta}$Chi-squared test with no trend scores

6. DISCUSSION

The hypothesis of the present study was that the 120-bp repeat polymorphism, located upstream of the *DRD4* gene, might influence clinical response to clozapine therapy in patients with refractory schizophrenia. The basis of this hypothesis was as follows. Clozapine has a very high affinity for the D4 receptor, with a dissociation constant (K_i) that is 15.3 times less than that for the D2 receptor (D2 receptor is the common target of most antipsychotics) (Van Tol et al., 1991). The *DRD4* gene codes for the D4 receptor; numerous polymorphisms have been reported in this gene, with the variations reported to account for certain phenotypic variations observed in human populations, such as novelty-seeking traits, drug addiction and alcohol dependence (McCracken et al., 2000b; Oak et al., 2000c; van den Wildenberg et al., 2007). Hence, it was hypothesized that variations in *DRD4* may account for variable clinical responses to clozapine. The 120-bp repeat variant has been less studied than the others, with regard to response to clozapine. In-vitro and post-mortem human brain studies have shown that duplication of the 120 base pairs resulted in decreases in mRNA levels for *DRD4* receptors, reflecting a decrease in the promoter activity of the gene (D'Souza et al., 2004; Simpson et al., 2010). This would be expected to result in decreased levels of the protein, D4 receptor, in the brain; however, such a decrease has not been documented. It was, nevertheless, postulated that presence of the 120-bp repeat might be associated with fewer D4 receptors. For a given dose of clozapine, this would be expected to produce a greater extent of receptor blockade, in comparison with those in whom there were no repeats. It would, thus, be

expected that the presence of the 120-bp duplication would produce a better response to clozapine. On the other hand, Hwang et al (2012) have shown that in a Caucasian population, the 120-bp short allele is associated with a better clozapine response (Hwang et al., 2012); however, such an association was not seen in an African-American population (Hwang et al., 2012). Thomas et al (2008) have shown, in a North-Indian population, that the long allele was associated with a non-response to olanzapine. These contradictory observations show that there is no clear consensus on this question. There is also no data of this nature in a South Indian population. This was a starting point for the present study. In this study, it has been found that there is no significant association between *DRD4* 120-bp duplication and response to clozapine. Neither the genotypes nor the alleles were significantly associated with clinical response to clozapine.

Maximum doses of clozapine received by the patients differed significantly among the genotypes (p=0.004). Those with heterozygous (120/240) and homozygous (240/240) genotypes had received significantly higher doses of clozapine than those with the wild genotype. However, the doses of the drug used were similar in both the responders and non-responders. Association analyses were carried out between genotypes and response to clozapine, adjusting for the confounding effects of clozapine doses. No association was found between these 2 variables.

The results of the present study differ from those reported earlier. The differences in the definition of clozapine response used might partially account for the observed differences in results of these studies. In the present study, clozapine response was defined by a BPRS score less than or equal to 35 at the time point when the patient was recruited. Hwang et al (2012) measured the percentage of reduction in the BPRS scale; they defined a good response as a reduction in BPRS score of more than 20 percent over baseline. Heterogeneity in clinical definitions used are likely to be a major contributory factor that may account for variable findings in pharmacogenetic studies done to assess response to clozapine. In addition, schizophrenia is a multifactorial disease, with both environmental and genetic etiological components. The effect of a single gene would be difficult to elucidate, due to multiple genetic and environmental factors involved in pathogenesis of the disease (van Os and Kapur, 2009). This fact, along with sample size constraints, may account for the conflicting reports seen in pharmacogenetic studies of single genes with regard to clinical response to clozapine. Ethnicity also contributes to the phenotypic variations in schizophrenia (Opolka et al., 2003). Hence, pharmacogenetic findings in one population may not be same for another population.

In the second part of the study, the associations of clozapine-induced adverse effects with *DRD4* 120-bp repeat polymorphism was analyzed. It was seen that there was a significant association between clozapine-induced sialorrhea and the long allele (240-bp) of the *DRD4* gene. Participants with the long allele developed sialorrhea 2.95 times more frequently than those with the short allele (OR=2.95; CI=1.58-5.67; p=0.001). Genotypic

associations were found to be statistically significant and found to fit a log-additive mode of inheritance. Clozapine- induced sialorrhea has been shown to be a consequence of dysfunction of deglutition, rather than dysfunction of salivary glands, where it has been demonstrated that patients with or without sialorrhea did not differ in their salivary flow rates (Ben-Aryeh et al., 1996).. The possible link between *DRD4* and sialorrhea may be in the regions of brain that control pharyngeal and esophageal phases of swallowing. They include the human motor cortex, pre-frontal cortex and nuclei of the brain stem, namely solitarius and ambiguus. Strong evidence exists for the role of these regions in the regulation of deglutition (Bieger and Neuhuber, 2006; Hamdy, 2006; Hamdy et al., 1999; Kern et al., 2001). D4 receptors are highly expressed in the above-mentioned regions of the brain and have been thought to be likely to contribute to regulation of deglutition, though no experimental evidences exist so far to support this postulate (Hyde et al., 1996; Noaín et al., 2006). Blockade of D4 receptors, by clozapine, in these regulatory regions might contribute to the dysfunction in deglutition. This is a possible explanation behind the association between the 120-bp duplication (that reduces levels of D4 receptor) and the occurrence of sialorrhea in patients on clozapine.

7. CONCLUSION

The present study looked for an association between a 120-bp repeat in the *DRD4* gene and response to and adverse effects of clozapine in a group of treatment-resistant schizophrenia in a South Indian population. No significant association was found between *DRD4* 120-bp repeat and clinical response to clozapine. Patients with the 120-bp repeat had significantly higher incidence of sialorrhea in response to clozapine therapy. These results suggest that routine screening for *DRD4* 120-bp repeat polymorphism before clozapine therapy may not be useful as a predictor for clinical response to clozapine. It may, however, help to identify those at high risk for clozapine-induced hyper-salivation.

8. BIBLIOGRAPHY

Abdelmawla, N., Ahmed, M.I., 2009. Clozapine and risk of pneumonia. Br. J. Psychiatry 194, 468–469.

Alaerts, M., Ceulemans, S., Forero, D., Moens, L.N., De Zutter, S., Heyrman, L., Lenaerts, A.-S., Norrback, K.-F., De Rijk, P., Nilsson, L.-G., Goossens, D., Adolfsson, R., Del-Favero, J., 2009. Support for NRG1 as a susceptibility factor for schizophrenia in a northern Swedish isolated population. Arch. Gen. Psychiatry 66, 828–837.

Alvir, J.M.J., Lieberman, J.A., Safferman, A.Z., Schwimmer, J.L., Schaaf, J.A., 1993. Clozapine-Induced Agranulocytosis – Incidence and Risk Factors in the United States. N. Engl. J. Med. 329, 162–167.

Amar, A., Segman, R.H., Shtrussberg, S., Sherman, L., Safirman, C., Lerer, B., Brautbar, C., 1998. An association between clozapine-induced agranulocytosis in schizophrenics and HLA-DQB1*0201. Int. J. Neuropsychopharmacol. Off. Sci. J. Coll. Int. Neuropsychopharmacol. CINP 1, 41–44.

Ananth, J., Burgoyne, K.S., Gadasalli, R., Aquino, S., 2001. How do the atypical antipsychotics work? J. Psychiatry Neurosci. 26, 385–394.

Baldwin, J.M., Schertler, G.F., Unger, V.M., 1997. An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. J. Mol. Biol. 272, 144–164.

Barr, C.L., Kennedy, J.L., Lichter, J.B., Van Tol, H.H., Wetterberg, L., Livak, K.J., Kidd, K.K., 1993. Alleles at the dopamine D4 receptor locus do not contribute to the genetic susceptibility to schizophrenia in a large Swedish kindred. Am. J. Med. Genet. 48, 218–222.

Bassett, A.S., Chow, E.W.C., Vieland, V.J., Brzustowicz, L., 2002. Is schizophrenia linked to chromosome 1q? Science 298, 2277; author reply 2277.

Bellgrove, M.A., Hawi, Z., Lowe, N., Kirley, A., Robertson, I.H., Gill, M., 2005. *DRD4* gene variants and sustained attention in attention deficit hyperactivity disorder (ADHD): effects of associated alleles at the VNTR and -521 SNP. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 136B, 81–86.

Ben-Aryeh, H., Jungerman, T., Szargel, R., Klein, E., Laufer, D., 1996. Salivary flow-rate and composition in schizophrenic patients on clozapine: subjective reports and laboratory data. Biol. Psychiatry 39, 946–949.

Berry, N., Jobanputra, V., Pal, H., 2003. Molecular genetics of schizophrenia: a critical review. J. Psychiatry Neurosci. JPN 28, 415–429.

Bhaduri, N., Das, M., Das, A.B., Mukhopadhyay, K., 2007. Dopamine receptor D4 exon 3 variable number of tandem repeat polymorphism: Distribution in eastern Indian population. Indian J. Hum. Genet. 13, 54–58.

Bhowmik, A.D., Chaudhury, S., Dutta, S., Shaw, J., Chatterjee, A., Choudhury, A., Saha, A., Sadhukhan, D., Kar, T., Sinha, S., Mukhopadhyay, K., 2011. Role of functional dopaminergic gene polymorphisms in the etiology of idiopathic intellectual disability. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1714–1722.

Bieger, D., Neuhuber, W., 2006. Neural circuits and mediators regulating swallowing in the brainstem. GI Motil. Online.

Blackwood, D.H., Fordyce, A., Walker, M.T., St Clair, D.M., Porteous, D.J., Muir, W.J., 2001. Schizophrenia and affective disorders--cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. Am. J. Hum. Genet. 69, 428–433.

Blouin, J.L., Dombroski, B.A., Nath, S.K., Lasseter, V.K., Wolyniec, P.S., Nestadt, G., Thornquist, M., Ullrich, G., McGrath, J., Kasch, L., Lamacz, M., Thomas, M.G., Gehrig, C., Radhakrishna, U., Snyder, S.E., Balk, K.G., Neufeld, K., Swartz, K.L., DeMarchi, N., Papadimitriou, G.N., Dikeos, D.G., Stefanis, C.N., Chakravarti, A., Childs, B., Housman, D.E., Kazazian, H.H., Antonarakis, S., Pulver, A.E., 1998. Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. Nat. Genet. 20, 70–73.

Boyce, H.W., Bakheet, M.R., 2005. Sialorrhea: a review of a vexing, often unrecognized sign of oropharyngeal and esophageal disease. J. Clin. Gastroenterol. 39, 89–97.

Brandon, N.J., Handford, E.J., Schurov, I., Rain, J.-C., Pelling, M., Duran-Jimeniz, B., Camargo, L.M., Oliver, K.R., Beher, D., Shearman, M.S., Whiting, P.J., 2004. Disrupted in Schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: implications for schizophrenia and other major neurological disorders. Mol. Cell. Neurosci. 25, 42–55.

Brandon, N.J., Millar, J.K., Korth, C., Sive, H., Singh, K.K., Sawa, A., 2009. Understanding the role of DISC1 in psychiatric disease and during normal development. J. Neurosci. Off. J. Soc. Neurosci. 29, 12768–12775.

Brunton, L., Lazo, J., Parker, K., 2005. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed. McGraw-Hill, New York.

Brzustowicz, L.M., Hodgkinson, K.A., Chow, E.W.C., Honer, W.G., Bassett, A.S., 2000. Location of a Major Susceptibility Locus for Familial Schizophrenia on Chromosome 1q21-q22. Science 288, 678–682.

Brzustowicz, L.M., Honer, W.G., Chow, E.W., Little, D., Hogan, J., Hodgkinson, K., Bassett, A.S., 1999. Linkage of familial schizophrenia to chromosome 13q32. Am. J. Hum. Genet. 65, 1096–1103.

Camargo, L.M., Collura, V., Rain, J.-C., Mizuguchi, K., Hermjakob, H., Kerrien, S., Bonnert, T.P., Whiting, P.J., Brandon, N.J., 2007. Disrupted in Schizophrenia 1 Interactome: evidence for the close connectivity of risk genes and a potential synaptic basis for schizophrenia. Mol. Psychiatry 12, 74–86.

Cao, B.J., Rodgers, R.J., 1997. Dopamine D4 receptor and anxiety: behavioural profiles of clozapine, L-745,870 and L-741,742 in the mouse plus-maze. Eur. J. Pharmacol. 335, 117–125.

Cao, Q., Martinez, M., Zhang, J., Sanders, A.R., Badner, J.A., Cravchik, A., Markey, C.J., Beshah, E., Guroff, J.J., Maxwell, M.E., Kazuba, D.M., Whiten, R., Goldin, L.R., Gershon, E.S., Gejman, P.V., 1997. Suggestive evidence for a schizophrenia susceptibility locus on chromosome 6q and a confirmation in an independent series of pedigrees. Genomics 43, 1–8.

Chakravarti, A., 2002. A compelling genetic hypothesis for a complex disease: PRODH2/DGCR6 variation leads to schizophrenia susceptibility. Proc. Natl. Acad. Sci. U. S. A. 99, 4755–4756.

Chang, F.M., Kidd, J.R., Livak, K.J., Pakstis, A.J., Kidd, K.K., 1996. The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus. Hum. Genet. 98, 91–101.

Chapman, L.J., Chapman, J.P., 1973. Problems in the measurement of cognitive deficit. Psychol. Bull. 79, 380–385.

Chumakov, I., Blumenfeld, M., Guerassimenko, O., Cavarec, L., Palicio, M., Abderrahim, H., Bougueleret, L., Barry, C., Tanaka, H., La Rosa, P., Puech, A., Tahri, N., Cohen-Akenine, A., Delabrosse, S., Lissarrague, S., Picard, F.-P., Maurice, K., Essioux, L., Millasseau, P., Grel, P., Debailleul, V., Simon, A.-M., Caterina, D., Dufaure, I., Malekzadeh, K., Belova, M., Luan, J.-J., Bouillot, M., Sambucy, J.-L., Primas, G., Saumier, M., Boubkiri, N., Martin-Saumier, S., Nasroune, M., Peixoto, H., Delaye, A., Pinchot, V., Bastucci, M., Guillou, S., Chevillon, M., Sainz-Fuertes, R., Meguenni, S., Aurich-Costa, J., Cherif, D., Gimalac, A., Van Duijn, C., Gauvreau, D., Ouellette, G., Fortier, I., Raelson, J., Sherbatich, T., Riazanskaia, N., Rogaev, E., Raeymaekers, P., Aerssens, J., Konings, F., Luyten, W., Macciardi, F., Sham, P.C., Straub, R.E., Weinberger, D.R., Cohen, N., Cohen, D., Ouelette, G., Realson, J., 2002. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 99, 13675–13680.

Chung, M.C., Lin, S.K., Chang, W.H., Jann, M.W., 1993. Determination of clozapine and desmethylclozapine in human plasma by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. 613, 168–173.

Claas, F.H., Abbott, P.A., Witvliet, M.D., D'Amaro, J., Barnes, P.M., Krupp, P., 1992. No direct clinical relevance of the human leucocyte antigen (HLA) system in

clozapine-induced agranulocytosis. Drug Saf. Int. J. Med. Toxicol. Drug Exp. 7 Suppl 1, 3–6.

Cohen, B.M., Ennulat, D.J., Centorrino, F., Matthysse, S., Konieczna, H., Chu, H.M., Cherkerzian, S., 1999. Polymorphisms of the dopamine D4 receptor and response to antipsychotic drugs. Psychopharmacology (Berl.) 141, 6–10.

Colter, N., Battal, S., Crow, T.J., Johnstone, E.C., Brown, R., Bruton, C., 1987. White matter reduction in the parahippocampal gyrus of patients with schizophrenia. Arch. Gen. Psychiatry 44, 1023.

Corrigan, F.M., MacDonald, S., Reynolds, G.P., 1995. Clozapine-induced hypersalivation and the alpha 2 adrenoceptor. Br. J. Psychiatry J. Ment. Sci. 167, 412.

Coyle, J.T., 1996. The glutamatergic dysfunction hypothesis for schizophrenia. Harv. Rev. Psychiatry 3, 241–253.

Coyle, J.T., Basu, A., Benneyworth, M., Balu, D., Konopaske, G., 2012. Glutamatergic synaptic dysregulation in schizophrenia: therapeutic implications. Handb. Exp. Pharmacol. 267–295.

Crilly, J., 2007. The history of clozapine and its emergence in the US market: a review and analysis. Hist. Psychiatry 18, 39–60.

D'Souza, U.M., Russ, C., Tahir, E., Mill, J., McGuffin, P., Asherson, P.J., Craig, I.W., 2004. Functional effects of a tandem duplication polymorphism in the 5'flanking region of the *DRD4* gene. Biol. Psychiatry 56, 691–697.

Das, M., Das Bhowmik, A., Bhaduri, N., Sarkar, K., Ghosh, P., Sinha, S., Ray, A., Chatterjee, A., Mukhopadhyay, K., 2011. Role of gene-gene/gene-environment interaction in the etiology of eastern Indian ADHD probands. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 577–587.

Davydov, L., Botts, S.R., 2000. Clozapine-induced hypersalivation. Ann. Pharmacother. 34, 662–665.

Depue, B.E., Burgess, G.C., Willcutt, E.G., Ruzic, L., Banich, M.T., 2010. Inhibitory control of memory retrieval and motor processing associated with the right lateral prefrontal cortex: evidence from deficits in individuals with ADHD. Neuropsychologia 48, 3909–3917.

Dettling, M., Sachse, C., Müller-Oerlinghausen, B., Roots, I., Brockmöller, J., Rolfs, A., Cascorbi, I., 2000. Clozapine-induced agranulocytosis and hereditary polymorphisms of clozapine metabolizing enzymes: no association with myeloperoxidase and cytochrome P4502D6. Pharmacopsychiatry 33, 218–220.

Deutch, A., 1995. Mechanisms of action of clozapine in the treatment of neuroleptic-resistant and neuroleptic-intolerant schizophrenia. Eur. Psychiatry J. Assoc. Eur. Psychiatr. 10 Suppl 1, 39s–46s.

Deutsch, S.I., Mastropaolo, J., Schwartz, B.L., Rosse, R.B., Morihisa, J.M., 1989. A "glutamatergic hypothesis" of schizophrenia. Rationale for pharmacotherapy with glycine. Clin. Neuropharmacol. 12, 1–13.

Ding, Y.-C., Chi, H.-C., Grady, D.L., Morishima, A., Kidd, J.R., Kidd, K.K., Flodman, P., Spence, M.A., Schuck, S., Swanson, J.M., Zhang, Y.-P., Moyzis, R.K., 2002. Evidence of positive selection acting at the human dopamine receptor D4 gene locus. Proc. Natl. Acad. Sci. 99, 309–314.

Domino, E.F., Luby, E.D., 2012. Phencyclidine/Schizophrenia: One View Toward the Past, The Other to the Future. Schizophr. Bull. 38, 914–919.

Drerup, C.M., Wiora, H.M., Topczewski, J., Morris, J.A., 2009. Disc1 regulates foxd3 and sox10 expression, affecting neural crest migration and differentiation. Dev. Camb. Engl. 136, 2623–2632.

Duan, X., Chang, J.H., Ge, S., Faulkner, R.L., Kim, J.Y., Kitabatake, Y., Liu, X., Yang, C.-H., Jordan, J.D., Ma, D.K., Liu, C.Y., Ganesan, S., Cheng, H.-J., Ming, G., Lu, B., Song, H., 2007. Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. Cell 130, 1146–1158.

Duncan, G.E., Sheitman, B.B., Lieberman, J.A., 1999. An integrated view of pathophysiological models of schizophrenia. Brain Res. Brain Res. Rev. 29, 250–264.

Eisenberg, J., Zohar, A., Mei-Tal, G., Steinberg, A., Tartakovsky, E., Gritsenko, I., Nemanov, L., Ebstein, R.P., 2000. A haplotype relative risk study of the dopamine D4 receptor (*DRD4*) exon III repeat polymorphism and attention deficit hyperactivity disorder (ADHD). Am. J. Med. Genet. 96, 258–261.

Ekelund, J., Hennah, W., Hiekkalinna, T., Parker, A., Meyer, J., Lönnqvist, J., Peltonen, L., 2004. Replication of 1q42 linkage in Finnish schizophrenia pedigrees. Mol. Psychiatry 9, 1037–1041.

Ekelund, J., Hovatta, I., Parker, A., Paunio, T., Varilo, T., Martin, R., Suhonen, J., Ellonen, P., Chan, G., Sinsheimer, J.S., Sobel, E., Juvonen, H., Arajärvi, R., Partonen, T., Suvisaari, J., Lönnqvist, J., Meyer, J., Peltonen, L., 2001. Chromosome 1 loci in Finnish schizophrenia families. Hum. Mol. Genet. 10, 1611–1617.

Fakra, E., Azorin, J.-M., 2012. Clozapine for the treatment of schizophrenia. Expert Opin. Pharmacother. 13, 1923–1935.

Fallin, M.D., Lasseter, V.K., Liu, Y., Avramopoulos, D., McGrath, J., Wolyniec, P.S., Nestadt, G., Liang, K.-Y., Chen, P.-L., Valle, D., Pulver, A.E., 2011. Linkage and association on 8p21.2-p21.1 in schizophrenia. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 156, 188–197.

Falzer, P.R., Garman, D.M., 2012. Optimizing clozapine through clinical decision making. Acta Psychiatr. Scand. 126, 47–58.

Faraone, S.V., Meyer, J., Matise, T., Svrakic, D., Pepple, J., Malaspina, D., Suarez, B., Hampe, C., Chan, G., Aelony, A., Friedman, J.H., Kaufmann, C., Cloninger, C.R., Tsuang, M.T., 1999. Suggestive linkage of chromosome 10p to schizophrenia is not due to transmission ratio distortion. Am. J. Med. Genet. 88, 607–608.

Farde, L., Nordström, A.L., Wiesel, F.A., Pauli, S., Halldin, C., Sedvall, G., 1992. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch. Gen. Psychiatry 49, 538–544.

Farde, L., Wiesel, F.A., Stone-Elander, S., Halldin, C., Nordström, A.L., Hall, H., Sedvall, G., 1990. D2 dopamine receptors in neuroleptic-naive schizophrenic patients. A positron emission tomography study with [11C]raclopride. Arch. Gen. Psychiatry 47, 213–219.

Freedman, D.X., 1975. LSD, psychotogenic procedures, and brain neurohumors. Psychopharmacol. Bull. 11, 42–43.

Gabriel, S.B., Schaffner, S.F., Nguyen, H., Moore, J.M., Roy, J., Blumenstiel, B., Higgins, J., DeFelice, M., Lochner, A., Faggart, M., Liu-Cordero, S.N., Rotimi, C., Adeyemo, A., Cooper, R., Ward, R., Lander, E.S., Daly, M.J., Altshuler, D., 2002. The structure of haplotype blocks in the human genome. Science 296, 2225–2229.

Gao, X.M., Sakai, K., Roberts, R.C., Conley, R.R., Dean, B., Tamminga, C.A., 2000. Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. Am. J. Psychiatry 157, 1141–1149.

Gill, M., Vallada, H., Collier, D., Sham, P., Holmans, P., Murray, R., McGuffin, P., Nanko, S., Owen, M., Antonarakis, S., Housman, D., Kazazian, H., Nestadt, G., Pulver, A.E., Straub, R.E., MacLean, C.J., Walsh, D., Kendler, K.S., DeLisi, L., Polymeropoulos, M., Coon, H., Byerley, W., Lofthouse, R., Gershon, E., Read, C.M., 1996. A combined analysis of D22S278 marker alleles in affected sib-pairs: support for a susceptibility locus for schizophrenia at chromosome 22q12. Schizophrenia Collaborative Linkage Group (Chromosome 22). Am. J. Med. Genet. 67, 40–45.

Goldberg, R.T., Pachas, W.N., Keith, D., 1999. Relationship between traumatic events in childhood and chronic pain. Disabil. Rehabil. 21, 23–30.

Grabowski, J., 1992. Clonidine treatment of clozapine-induced hypersalivation. J. Clin. Psychopharmacol. 12, 69–70.

Greenwood-Smith, C., Lubman, D.I., Castle, D.J., 2003. Serum clozapine levels: a review of their clinical utility. J. Psychopharmacol. Oxf. Engl. 17, 234–238.

Grohmann, R., Rüther, E., Sassim, N., Schmidt, L.G., 1989. Adverse effects of clozapine. Psychopharmacology (Berl.) 99 Suppl, S101–104.

Gruzelier, J., Seymour, K., Wilson, L., Jolley, A., Hirsch, S., 1988. Impairments on neuropsychologic tests of temporohippocampal and frontohippocampal functions and word fluency in remitting schizophrenia and affective disorders. Arch. Gen. Psychiatry 45, 623–629.

Gur, R.E., Pearlson, G.D., 1993. Neuroimaging in schizophrenia research. Schizophr. Bull. 19, 337–353.

Gurling, H.M., Kalsi, G., Brynjolfson, J., Sigmundsson, T., Sherrington, R., Mankoo, B.S., Read, T., Murphy, P., Blaveri, E., McQuillin, A., Petursson, H., Curtis, D., 2001. Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. Am. J. Hum. Genet. 68, 661–673.

GUY, EDCEU, 1976. assessment manual for psychopharmacology. Wash. DC US Dep. Health Educ. Welf.

Haller, E., Binder, R.L., 1990. Clozapine and seizures. Am. J. Psychiatry 147, 1069–1071.

Hamdy, S., 2006. Role of cerebral cortex in the control of swallowing. GI Motil. Online.

Hamdy, S., Mikulis, D.J., Crawley, A., Xue, S., Lau, H., Henry, S., Diamant, N.E., 1999. Cortical activation during human volitional swallowing: an event-related fMRI study. Am. J. Physiol. - Gastrointest. Liver Physiol. 277, G219–G225.

Harrison, P.J., Weinberger, D.R., 2005. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol. Psychiatry 10, 40–68; image 5.

Hattori, E., Nakajima, M., Yamada, K., Iwayama, Y., Toyota, T., Saitou, N., Yoshikawa, T., 2009. Variable number of tandem repeat polymorphisms of *DRD4*: re-evaluation of selection hypothesis and analysis of association with schizophrenia. Eur. J. Hum. Genet. EJHG 17, 793–801.

Helmeste, D.M., Tang, S.W., Bunney, W.E., Jr, Potkin, S.G., Jones, E.G., 1996. Decrease in sigma but no increase in striatal dopamine D4 sites in schizophrenic brains. Eur. J. Pharmacol. 314, R3–5.

Hennah, W., Varilo, T., Kestilä, M., Paunio, T., Arajärvi, R., Haukka, J., Parker, A., Martin, R., Levitzky, S., Partonen, T., Meyer, J., Lönnqvist, J., Peltonen, L., Ekelund, J., 2003. Haplotype transmission analysis provides evidence of association for DISC1 to schizophrenia and suggests sex-dependent effects. Hum. Mol. Genet. 12, 3151–3159.

Hietala, J., Syvälahti, E., Vuorio, K., Någren, K., Lehikoinen, P., Ruotsalainen, U., Räkköläinen, V., Lehtinen, V., Wegelius, U., 1994. Striatal D2 dopamine receptor characteristics in neuroleptic-naive schizophrenic patients studied with positron emission tomography. Arch. Gen. Psychiatry 51, 116–123.

Hodgkinson, C.A., Goldman, D., Jaeger, J., Persaud, S., Kane, J.M., Lipsky, R.H., Malhotra, A.K., 2004. Disrupted in schizophrenia 1 (DISC1): association with schizophrenia, schizoaffective disorder, and bipolar disorder. Am. J. Hum. Genet. 75, 862–872.

Holliday, E.G., McLean, D.E., Nyholt, D.R., Mowry, B.J., 2009. Susceptibility locus on chromosome 1q23-25 for a schizophrenia subtype resembling deficit schizophrenia identified by latent class analysis. Arch. Gen. Psychiatry 66, 1058–1067.

Hovatta, I., Lichtermann, D., Juvonen, H., Suvisaari, J., Terwilliger, J.D., Arajärvi, R., Kokko-Sahin, M.L., Ekelund, J., Lönnqvist, J., Peltonen, L., 1998. Linkage analysis of putative schizophrenia gene candidate regions on chromosomes 3p, 5q, 6p, 8p, 20p and 22q in a population-based sampled Finnish family set. Mol. Psychiatry 3, 452–457.

Hwang, R., Tiwari, A.K., Zai, C.C., Felsky, D., Remington, E., Wallace, T., Tong, R.P., Souza, R.P., Oh, G., Potkin, S.G., Lieberman, J.A., Meltzer, H.Y., Kennedy, J.L., 2012. Dopamine D4 and D5 receptor gene variant effects on clozapine response in schizophrenia: replication and exploration. Prog. Neuropsychopharmacol. Biol. Psychiatry 37, 62–75.

Hwu, H.-G., Liu, C.-M., Fann, C.S.-J., Ou-Yang, W.-C., Lee, S.F.-C., 2003. Linkage of schizophrenia with chromosome 1q loci in Taiwanese families. Mol. Psychiatry 8, 445–452.

Hwu, H.G., Hong, C.J., Lee, Y.L., Lee, P.C., Lee, S.F., 1998. Dopamine D4 receptor gene polymorphisms and neuroleptic response in schizophrenia. Biol. Psychiatry 44, 483–487.

Hyde, T.M., Knable, M.B., Murray, A.M., 1996. Distribution of dopamine D1-D4 receptor subtypes in human dorsal vagal complex. Synap. N. Y. N 24, 224–232.

Iqbal, M.M., Rahman, A., Husain, Z., Mahmud, S.Z., Ryan, W.G., Feldman, J.M., 2003. Clozapine: a clinical review of adverse effects and management. Ann. Clin. Psychiatry Off. J. Am. Acad. Clin. Psychiatr. 15, 33–48.

Jackson, C.R., Chaurasia, S.S., Hwang, C.K., Iuvone, P.M., 2011. Dopamine D_4 receptor activation controls circadian timing of the adenylyl cyclase 1/cyclic AMP signaling system in mouse retina. Eur. J. Neurosci. 34, 57–64.

Jann, D.M.W., Grimsley, S.R., Gray, E.C., Chang, W.-H., 1993. Pharmacokinetics and Pharmacodynamics of Clozapine. Clin. Pharmacokinet. 24, 161–176.

Jeste, D.V., Lohr, J.B., 1989. Hippocampal pathologic findings in schizophrenia. A morphometric study. Arch. Gen. Psychiatry 46, 1019–1024.

Jönsson, E.G., Nöthen, M.M., Gustavsson, J.P., Neidt, H., Brené, S., Tylec, A., Propping, P., Sedvall, G.C., 1997. Lack of evidence for allelic association between personality

traits and the dopamine D4 receptor gene polymorphisms. Am. J. Psychiatry 154, 697–699.

Jönsson, E.G., Sedvall, G.C., Nöthen, M.M., Cichon, S., 2003. Dopamine D4 receptor gene (*DRD4*) variants and schizophrenia: meta-analyses. Schizophr. Res. 61, 111–119.

Juul Povlsen, U., Noring, U., Fog, R., Gerlach, J., 1985. Tolerability and therapeutic effect of clozapine. A retrospective investigation of 216 patients treated with clozapine for up to 12 years. Acta Psychiatr. Scand. 71, 176–185.

Kaiser, R., Könneker, M., Henneken, M., Dettling, M., Müller-Oerlinghausen, B., Roots, I., Brockmöller, J., 2000. Dopamine D4 receptor 48-bp repeat polymorphism: no association with response to antipsychotic treatment, but association with catatonic schizophrenia. Mol. Psychiatry 5, 418–424.

Kane, J.M., Correll, C.U., 2010. Pharmacologic Treatment of Schizophrenia. Dialogues Clin Neurosci 12, 345–357.

Kang, X., Simpson, G.M., 2010. Clozapine: more side effects but still the best antipsychotic. J. Clin. Psychiatry 71, 982–983.

Kapur, S., Seeman, P., 2000. Antipsychotic agents differ in how fast they come off the dopamine D2 receptors. Implications for atypical antipsychotic action. J. Psychiatry Neurosci. JPN 25, 161–166.

Kapur, S., Zipursky, R., Jones, C., Remington, G., Houle, S., 2000. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am. J. Psychiatry 157, 514–520.

Kendler, K.S., Myers, J.M., O'Neill, F.A., Martin, R., Murphy, B., MacLean, C.J., Walsh, D., Straub, R.E., 2000. Clinical features of schizophrenia and linkage to chromosomes 5q, 6p, 8p, and 10p in the Irish Study of High-Density Schizophrenia Families. Am. J. Psychiatry 157, 402–408.

Kereszturi, E., Kiraly, O., Csapo, Z., Tarnok, Z., Gadoros, J., Sasvari-Szekely, M., Nemoda, Z., 2007. Association between the 120-bp duplication of the dopamine D4 receptor gene and attention deficit hyperactivity disorder: genetic and molecular analyses. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 144B, 231–236.

Kern, M.K., Jaradeh, S., Arndorfer, R.C., Shaker, R., 2001. Cerebral cortical representation of reflexive and volitional swallowing in humans. Am. J. Physiol. Gastrointest. Liver Physiol. 280, G354–360.

Kim, J.-S., Bailey, M.J., Weller, J.L., Sugden, D., Rath, M.F., Møller, M., Klein, D.C., 2010. Thyroid hormone and adrenergic signaling interact to control pineal expression of the dopamine receptor D4 gene (*DRD4*). Mol. Cell. Endocrinol. 314, 128–135.

Kim, J.S., Kornhuber, H.H., Schmid-Burgk, W., Holzmüller, B., 1980. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. Neurosci. Lett. 20, 379–382.

Kirley, A., Lowe, N., Mullins, C., McCarron, M., Daly, G., Waldman, I., Fitzgerald, M., Gill, M., Hawi, Z., 2004. Phenotype studies of the *DRD4* gene polymorphisms in ADHD: association with oppositional defiant disorder and positive family history. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 131B, 38–42.

Kobilka, B.K., 2007. G protein coupled receptor structure and activation. Biochim. Biophys. Acta 1768, 794–807.

Koukkou, M., Angst, J., Zimmer, D., 1979. Paroxysmal EEG activity and psychopathology during the treatment with clozapine. Pharmakopsychiatr. Neuropsychopharmakol. 12, 173–183.

Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers, M.B., Jr, Charney, D.S., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51, 199–214.

Kustanovich, V., Ishii, J., Crawford, L., Yang, M., McGough, J.J., McCracken, J.T., Smalley, S.L., Nelson, S.F., 2004. Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with *DRD4* and DRD5. Mol. Psychiatry 9, 711–717.

Lai, J.H., Zhu, Y.S., Huo, Z.H., Sun, R.F., Yu, B., Wang, Y.P., Chai, Z.Q., Li, S.B., 2010. Association study of polymorphisms in the promoter region of *DRD4* with schizophrenia, depression, and heroin addiction. Brain Res. 1359, 227–232.

Lempp, T., Toennes, S.W., Wunder, C., Russe, O.Q., Möser, C.V., Kynast, K.L., Freitag, C.M., Niederberger, E., 2013. Altered gene expression in the prefrontal cortex of young rats induced by the ADHD drug atomoxetine. Prog. Neuropsychopharmacol. Biol. Psychiatry 40, 221–228.

Levinson, D.F., Holmans, P., Straub, R.E., Owen, M.J., Wildenauer, D.B., Gejman, P.V., Pulver, A.E., Laurent, C., Kendler, K.S., Walsh, D., Norton, N., Williams, N.M., Schwab, S.G., Lerer, B., Mowry, B.J., Sanders, A.R., Antonarakis, S.E., Blouin, J.L., DeLeuze, J.F., Mallet, J., 2000. Multicenter linkage study of schizophrenia candidate regions on chromosomes 5q, 6q, 10p, and 13q: schizophrenia linkage collaborative group III. Am. J. Hum. Genet. 67, 652–663.

Lewis, D.A., Lieberman, J.A., 2000. Catching up on schizophrenia: natural history and neurobiology. Neuron 28, 325–334.

Li, Y., Baker-Ericzen, M., Ji, N., Chang, W., Guan, L., Qian, Q., Zhang, Y., Faraone, S.V., Wang, Y., 2013. Do SNPs of *DRD4* gene predict adult persistence of ADHD in a Chinese sample? Psychiatry Res. 205, 143–150.

Liddle, P.F., 1987. The symptoms of chronic schizophrenia. A re-examination of the positive-negative dichotomy. Br. J. Psychiatry J. Ment. Sci. 151, 145–151.

Lieberman, J.A., Mailman, R.B., Duncan, G., Sikich, L., Chakos, M., Nichols, D.E., Kraus, J.E., 1998. Serotonergic basis of antipsychotic drug effects in schizophrenia. Biol. Psychiatry 44, 1099–1117.

Lieberman, J.A., Yunis, J., Egea, E., Canoso, R.T., Kane, J.M., Yunis, E.J., 1990. HLA-B38, DR4, DQw3 and clozapine-induced agranulocytosis in Jewish patients with schizophrenia. Arch. Gen. Psychiatry 47, 945–948.

Lipska, B.K., Mitkus, S., Caruso, M., Hyde, T.M., Chen, J., Vakkalanka, R., Straub, R.E., Weinberger, D.R., Kleinman, J.E., 2006. RGS4 mRNA expression in postmortem human cortex is associated with *COMT* Val158Met genotype and *COMT* enzyme activity. Hum. Mol. Genet. 15, 2804–2812.

Liu, A., 2004. [Advances in the study of relation between mutation of chromosome 22q and schizophrenia]. Zhonghua Liu Xing Bing Xue Za Zhi Zhonghua Liuxingbingxue Zazhi 25, 808–810.

Lusher, J.M., Chandler, C., Ball, D., 2001. Dopamine D4 receptor gene (*DRD4*) is associated with Novelty Seeking (NS) and substance abuse: the saga continues.. Mol. Psychiatry 6, 497–499.

Macciardi, F., Petronis, A., Van Tol, H.H., Marino, C., Cavallini, M.C., Smeraldi, E., Kennedy, J.L., 1994. Analysis of the D4 dopamine receptor gene variant in an Italian schizophrenia kindred. Arch. Gen. Psychiatry 51, 288–293.

Maddalena, A.S., Fox, M., Hofmann, M., Hock, C., 2004. Esophageal dysfunction on psychotropic medication. A case report and literature review. Pharmacopsychiatry 37, 134–138.

Marcus, M.M., Nomikos, G.G., Svensson, T.H., 2000. Effects of atypical antipsychotic drugs on dopamine output in the shell and core of the nucleus accumbens: role of 5-HT(2A) and alpha(1)-adrenoceptor antagonism. Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol. 10, 245–253.

Martinez, M., Goldin, L.R., Cao, Q., Zhang, J., Sanders, A.R., Nancarrow, D.J., Taylor, J.M., Levinson, D.F., Kirby, A., Crowe, R.R., Andreasen, N.C., Black, D.W., Silverman, J.M., Lennon, D.P., Nertney, D.A., Brown, D.M., Mowry, B.J., Gershon, E.S., Gejman, P.V., 1999. Follow-up study on a susceptibility locus for schizophrenia on chromosome 6q. Am. J. Med. Genet. 88, 337–343.

Martinot, J.L., Paillère-Martinot, M.L., Loc'h, C., Hardy, P., Poirier, M.F., Mazoyer, B., Beaufils, B., Mazière, B., Allilaire, J.F., Syrota, A., 1991. The estimated density of

D2 striatal receptors in schizophrenia. A study with positron emission tomography and 76Br-bromolisuride. Br. J. Psychiatry J. Ment. Sci. 158, 346–350.

Martinot, J.L., Peron-Magnan, P., Huret, J.D., Mazoyer, B., Baron, J.C., Boulenger, J.P., Loc'h, C., Maziere, B., Caillard, V., Loo, H., 1990. Striatal D2 dopaminergic receptors assessed with positron emission tomography and [76Br]bromospiperone in untreated schizophrenic patients. Am. J. Psychiatry 147, 44–50.

Matsumoto, M., Hidaka, K., Tada, S., Tasaki, Y., Yamaguchi, T., 1995. Full-length cDNA cloning and distribution of human dopamine D4 receptor. Brain Res. Mol. Brain Res. 29, 157–162.

McCarthy, R.H., Terkelsen, K.G., 1994. Esophageal dysfunction in two patients after clozapine treatment. J. Clin. Psychopharmacol. 14, 281–283.

McCracken, J.T., Smalley, S.L., McGough, J.J., Crawford, L., Del'Homme, M., Cantor, R.M., Liu, A., Nelson, S.F., 2000a. Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (*DRD4*) with attention deficit hyperactivity disorder (ADHD). Mol. Psychiatry 5, 531–536.

McCracken, J.T., Smalley, S.L., McGough, J.J., Crawford, L., Del'Homme, M., Cantor, R.M., Liu, A., Nelson, S.F., 2000b. Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (*DRD4*) with attention deficit hyperactivity disorder (ADHD). Mol. Psychiatry 5, 531–536.

Miller, D.D., 2000. Review and management of clozapine side effects. J. Clin. Psychiatry 61 Suppl 8, 14–17; discussion 18–19.

Miller, S.C., 1996. Methylphenidate for clozapine sedation. Am. J. Psychiatry 153, 1231–1232.

Mioshi, E., Dawson, K., Mitchell, J., Arnold, R., Hodges, J.R., 2006. The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive test battery for dementia screening. Int. J. Geriatr. Psychiatry 21, 1078–1085.

Mitsuyasu, H., Hirata, N., Sakai, Y., Shibata, H., Takeda, Y., Ninomiya, H., Kawasaki, H., Tashiro, N., Fukumaki, Y., 2001. Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene (*DRD4*) with schizophrenia and personality traits. J. Hum. Genet. 46, 26–31.

Mitsuyasu, H., Ozawa, H., Takeda, Y., Fukumaki, Y., 1999. Novel polymorphisms in the upstream region of the human dopamine D4 receptor (*DRD4*) gene. J. Hum. Genet. 44, 416–418.

Miyamoto, S., Duncan, G.E., Marx, C.E., Lieberman, J.A., 2005. Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. Mol. Psychiatry 10, 79–104.

Miyamoto, S., Miyake, N., Jarskog, L.F., Fleischhacker, W.W., Lieberman, J.A., 2012. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol. Psychiatry 17, 1206–1227.

Moises, H.W., Yang, L., Kristbjarnarson, H., Wiese, C., Byerley, W., Macciardi, F., Arolt, V., Blackwood, D., Liu, X., Sjögren, B., Aschauer, H.N., Hwu, H.-G., Jang, K., Livesley, W.J., Kennedy, J.L., Zoega, T., Ivarsson, O., Bui, M.-T., Yu, M.-H., Havsteen, B., Commenges, D., Weissenbach, J., Schwinger, E., Gottesman, I.I., Pakstis, A.J., Wetterberg, L., Kidd, K.K., Helgason, T., 1995. An international two–stage genome–wide search for schizophrenia susceptibility genes. Nat. Genet. 11, 321–324.

Mosyagin, I., Dettling, M., Roots, I., Mueller-Oerlinghausen, B., Cascorbi, I., 2004. Impact of myeloperoxidase and NADPH-oxidase polymorphisms in drug-induced agranulocytosis. J. Clin. Psychopharmacol. 24, 613–617.

Mowry, B.J., Holmans, P.A., Pulver, A.E., Gejman, P.V., Riley, B., Williams, N.M., Laurent, C., Schwab, S.G., Wildenauer, D.B., Bauché, S., Owen, M.J., Wormley, B., Sanders, A.R., Nestadt, G., Liang, K.Y., Duan, J., Ribble, R., Norton, N., Soubigou, S., Maier, W., Ewen-White, K.R., DeMarchi, N., Carpenter, B., Walsh, D., Williams, H., Jay, M., Albus, M., Nertney, D.A., Papadimitriou, G., O'Neill, A., O'Donovan, M.C., Deleuze, J.-F., Lerer, F.B., Dikeos, D., Kendler, K.S., Mallet, J., Silverman, J.M., Crowe, R.R., Levinson, D.F., 2004. Multicenter linkage study of schizophrenia loci on chromosome 22q. Mol. Psychiatry 9, 784–795.

Mulcrone, J., Kerwin, R.W., 1996. No difference in the expression of the D4 gene in post-mortem frontal cortex from controls and schizophrenics. Neurosci. Lett. 219, 163–166.

Naka, I., Nishida, N., Ohashi, J., 2011. No evidence for strong recent positive selection favoring the 7 repeat allele of VNTR in the *DRD4* gene. PloS One 6, e24410.

Nakajima, M., Hattori, E., Yamada, K., Iwayama, Y., Toyota, T., Iwata, Y., Tsuchiya, K.J., Sugihara, G., Hashimoto, K., Watanabe, H., Iyo, M., Hoshika, A., Yoshikawa, T., 2007. Association and synergistic interaction between promoter variants of the *DRD4* gene in Japanese schizophrenics. J. Hum. Genet. 52, 86–91.

Nemoda, Z., Lyons-Ruth, K., Szekely, A., Bertha, E., Faludi, G., Sasvari-Szekely, M., 2010. Association between dopaminergic polymorphisms and borderline personality traits among at-risk young adults and psychiatric inpatients. Behav. Brain Funct. BBF 6, 4.

Nielsen, J., Damkier, P., Lublin, H., Taylor, D., 2011. Optimizing clozapine treatment. Acta Psychiatr. Scand. 123, 411–422.

Noaín, D., Avale, M.E., Wedemeyer, C., Calvo, D., Peper, M., Rubinstein, M., 2006. Identification of brain neurons expressing the dopamine D4 receptor gene using BAC transgenic mice. Eur. J. Neurosci. 24, 2429–2438.

Nyberg, S., Farde, L., 2000. Non-equipotent doses partly explain differences among antipsychotics - implications of PET studies. Psychopharmacology (Berl.) 148, 22–23.

O'Carroll, R., 2000. Cognitive impairment in schizophrenia. Adv. Psychiatr. Treat. 6, 161–168.

Oades, R.D., Lasky-Su, J., Christiansen, H., Faraone, S.V., Sonuga-Barke, E.J., Banaschewski, T., Chen, W., Anney, R.J., Buitelaar, J.K., Ebstein, R.P., Franke, B., Gill, M., Miranda, A., Roeyers, H., Rothenberger, A., Sergeant, J.A., Steinhausen, H.-C., Taylor, E.A., Thompson, M., Asherson, P., 2008. The influence of serotonin- and other genes on impulsive behavioral aggression and cognitive impulsivity in children with attention-deficit/hyperactivity disorder (ADHD): Findings from a family-based association test (FBAT) analysis. Behav. Brain Funct. BBF 4, 48.

Oak, J.N., Oldenhof, J., Van Tol, H.H., 2000a. The dopamine D(4) receptor: one decade of research. Eur. J. Pharmacol. 405, 303–327.

Oak, J.N., Oldenhof, J., Van Tol, H.H., 2000b. The dopamine D(4) receptor: one decade of research. Eur. J. Pharmacol. 405, 303–327.

Oak, J.N., Oldenhof, J., Van Tol, H.H., 2000c. The dopamine D(4) receptor: one decade of research. Eur. J. Pharmacol. 405, 303–327.

Okuyama, Y., Ishiguro, H., Nankai, M., Shibuya, H., Watanabe, A., Arinami, T., 2000. Identification of a polymorphism in the promoter region of *DRD4* associated with the human novelty seeking personality trait. Mol. Psychiatry 5, 64–69.

Okuyama, Y., Ishiguro, H., Toru, M., Arinami, T., 1999a. A genetic polymorphism in the promoter region of *DRD4* associated with expression and schizophrenia. Biochem. Biophys. Res. Commun. 258, 292–295.

Okuyama, Y., Ishiguro, H., Toru, M., Arinami, T., 1999b. A genetic polymorphism in the promoter region of *DRD4* associated with expression and schizophrenia. Biochem. Biophys. Res. Commun. 258, 292–295.

Olney, J.W., Farber, N.B., 1995. Glutamate receptor dysfunction and schizophrenia. Arch. Gen. Psychiatry 52, 998–1007.

Opolka, J.L., Rascati, K.L., Brown, C.M., Gibson, P.J., 2003. Role of Ethnicity in Predicting Antipsychotic Medication Adherence. Ann. Pharmacother. 37, 625–630.

Overall, J.E., Gorham, D.R., 1962. THE BRIEF PSYCHIATRIC RATING SCALE. Psychol. Rep. 10, 799–812.

Owen, M.J., Cardno, A.G., O'Donovan, M.C., 2000. Psychiatric genetics: back to the future. Mol. Psychiatry 5, 22–31.

Owen, M.J., Williams, N.M., O'Donovan, M.C., 2004. The molecular genetics of schizophrenia: new findings promise new insights. Mol. Psychiatry 9, 14–27.

Pal, P., Mihanović, M., Molnar, S., Xi, H., Sun, G., Guha, S., Jeran, N., Tomljenović, A., Malnar, A., Missoni, S., Deka, R., Rudan, P., 2009. Association of tagging single nucleotide polymorphisms on 8 candidate genes in dopaminergic pathway with schizophrenia in Croatian population. Croat. Med. J. 50, 361–369.

Paredes, U.M., Quinn, J.P., D'Souza, U.M., 2013. Allele-specific transcriptional activity of the variable number of tandem repeats in 5' region of the *DRD4* gene is stimulus specific in human neuronal cells. Genes Brain Behav. 12, 282–287.

Patel, S., Freedman, S., Chapman, K.L., Emms, F., Fletcher, A.E., Knowles, M., Marwood, R., Mcallister, G., Myers, J., Curtis, N., Kulagowski, J.J., Leeson, P.D., Ridgill, M., Graham, M., Matheson, S., Rathbone, D., Watt, A.P., Bristow, L.J., Rupniak, N.M., Baskin, E., Lynch, J.J., Ragan, C.I., 1997. Biological profile of L-745,870, a selective antagonist with high affinity for the dopamine D4 receptor. J. Pharmacol. Exp. Ther. 283, 636–647.

Paunio, T., Ekelund, J., Varilo, T., Parker, A., Hovatta, I., Turunen, J.A., Rinard, K., Foti, A., Terwilliger, J.D., Juvonen, H., Suvisaari, J., Arajärvi, R., Suokas, J., Partonen, T., Lönnqvist, J., Meyer, J., Peltonen, L., 2001. Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. Hum. Mol. Genet. 10, 3037–3048.

Pearlman, C., 1994. Clozapine, nocturnal sialorrhea, and choking. J. Clin. Psychopharmacol. 14, 283.

Pennebaker, J.W., Susman, J.R., 1988. Disclosure of traumas and psychosomatic processes. Soc. Sci. Med. 1982 26, 327–332.

Pereira, A., Dean, B., 2006. Clozapine bioactivation induces dose-dependent, drug-specific toxicity of human bone marrow stromal cells: a potential in vitro system for the study of agranulocytosis. Biochem. Pharmacol. 72, 783–793.

Quinn, B.P., 1999. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Primary Care Version. Prim. Care Companion J. Clin. Psychiatry 1, 54–55.

R Development Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Raaska, K., Neuvonen, P.J., 1998. Serum concentrations of clozapine and N-desmethylclozapine are unaffected by the potent CYP3A4 inhibitor itraconazole. Eur. J. Clin. Pharmacol. 54, 167–170.

Rabinowitz, J., Levine, S.Z., Brill, N., Bromet, E.J., 2007. The premorbid adjustment scale structured interview (PAS-SI): preliminary findings. Schizophr. Res. 90, 255–257.

Raedler, T.J., Bymaster, F.P., Tandon, R., Copolov, D., Dean, B., 2007. Towards a muscarinic hypothesis of schizophrenia. Mol. Psychiatry 12, 232–246.

Rao, P.A., Pickar, D., Gejman, P.V., Ram, A., Gershon, E.S., Gelernter, J., 1994. Allelic variation in the D4 dopamine receptor (*DRD4*) gene does not predict response to clozapine. Arch. Gen. Psychiatry 51, 912–917.

Remington, G., Kapur, S., 1999. D2 and 5-HT2 receptor effects of antipsychotics: bridging basic and clinical findings using PET. J. Clin. Psychiatry 60 Suppl 10, 15–19.

Riley, B., Kendler, K.S., 2006. Molecular genetic studies of schizophrenia. Eur. J. Hum. Genet. EJHG 14, 669–680.

Roberts, D.A., Balderson, D., Pickering-Brown, S.M., Deakin, J.F., Owen, F., 1996. The relative abundance of dopamine D4 receptor mRNA in post mortem brains of schizophrenics and controls. Schizophr. Res. 20, 171–174.

Ronai, Z., Szekely, A., Nemoda, Z., Lakatos, K., Gervai, J., Staub, M., Sasvari-Szekely, M., 2001. Association between Novelty Seeking and the -521 C/T polymorphism in the promoter region of the *DRD4* gene. Mol. Psychiatry 6, 35–38.

Safferman, A., Lieberman, J.A., Kane, J.M., Szymanski, S., Kinon, B., 1991. Update on the clinical efficacy and side effects of clozapine. Schizophr. Bull. 17, 247–261.

Sánchez-Mora, C., Ribasés, M., Casas, M., Bayés, M., Bosch, R., Fernàndez-Castillo, N., Brunso, L., Jacobsen, K.K., Landaas, E.T., Lundervold, A.J., Gross-Lesch, S., Kreiker, S., Jacob, C.P., Lesch, K.-P., Buitelaar, J.K., Hoogman, M., Kiemeney, L.A.L.M., Kooij, J.J.S., Mick, E., Asherson, P., Faraone, S.V., Franke, B., Reif, A., Johansson, S., Haavik, J., Ramos-Quiroga, J.A., Cormand, B., 2011. Exploring *DRD4* and its interaction with SLC6A3 as possible risk factors for adult ADHD: a meta-analysis in four European populations. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 156B, 600–612.

Schinka, J.A., Letsch, E.A., Crawford, F.C., 2002. *DRD4* and novelty seeking: results of meta-analyses. Am. J. Med. Genet. 114, 643–648.

Schmauss, M., Wolff, R., Erfurth, A., Rüther, E., 1989. Tolerability of long term clozapine treatment. Psychopharmacology (Berl.) 99 Suppl, S105–108.

Schultz, S.H., North, S.W., Shields, C.G., 2007. Schizophrenia: a review. Am. Fam. Physician 75, 1821–1829.

Schwab, S.G., Hallmayer, J., Albus, M., Lerer, B., Eckstein, G.N., Borrmann, M., Segman, R.H., Hanses, C., Freymann, J., Yakir, A., Trixler, M., Falkai, P., Rietschel, M., Maier, W., Wildenauer, D.B., 2000. A genome-wide autosomal screen for schizophrenia susceptibility loci in 71 families with affected siblings: support for loci on chromosome 10p and 6. Mol. Psychiatry 5, 638–649.

Schwab, S.G., Hallmayer, J., Albus, M., Lerer, B., Hanses, C., Kanyas, K., Segman, R., Borrman, M., Dreikorn, B., Lichtermann, D., Rietschel, M., Trixler, M., Maier, W., Wildenauer, D.B., 1998. Further evidence for a susceptibility locus on chromosome 10p14-p11 in 72 families with schizophrenia by nonparametric linkage analysis. Am. J. Med. Genet. 81, 302–307.

Seaman, M.I., Fisher, J.B., Chang, F., Kidd, K.K., 1999. Tandem duplication polymorphism upstream of the dopamine D4 receptor gene (*DRD4*). Am. J. Med. Genet. 88, 705–709.

Seeman, P., 2004. Atypical Antipsychotics: Mechanism of Action. Focus 2, 48–58.

Seeman, P., Guan, H.C., Van Tol, H.H., 1995. Schizophrenia: elevation of dopamine D4-like sites, using [3H]nemonapride and [125I]epidepride. Eur. J. Pharmacol. 286, R3–5.

Selvam, B., Porter, S.L., Tikhonova, I.G., 2013. Addressing selective polypharmacology of antipsychotic drugs targeting the bioaminergic receptors through receptor dynamic conformational ensembles. J. Chem. Inf. Model. 53, 1761–1774.

Serretti, A., Macciardi, F., Catalano, M., Bellodi, L., Smeraldi, E., 1999. Genetic Variants of Dopamine Receptor D4 and Psychopathology. Schizophr. Bull. 25, 609 –618.

Shaikh, S., Collier, D., Kerwin, R.W., Pilowsky, L.S., Gill, M., Xu, W.M., Thornton, A., 1993. Dopamine D4 receptor subtypes and response to clozapine. Lancet 341, 116.

Shaikh, S., Collier, D.A., Sham, P., Pilowsky, L., Sharma, T., Lin, L.K., Crocq, M.A., Gill, M., Kerwin, R., 1995. Analysis of clozapine response and polymorphisms of the dopamine D4 receptor gene (*DRD4*) in schizophrenic patients. Am. J. Med. Genet. 60, 541–545.

Simpson, J., Vetuz, G., Wilson, M., Brookes, K.J., Kent, L., 2010. The *DRD4* receptor Exon 3 VNTR and 5' SNP variants and mRNA expression in human post-mortem brain tissue. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 153B, 1228–1233.

Small, J.G., Milstein, V., Marhenke, J.D., Hall, D.D., Kellams, J.J., 1987. Treatment outcome with clozapine in tardive dyskinesia, neuroleptic sensitivity, and treatment-resistant psychosis. J. Clin. Psychiatry 48, 263–267.

Smythies, J., 2002. The adrenochrome hypothesis of schizophrenia revisited. Neurotox. Res. 4, 147–150.

Srivastava, V., Varma, P.G., Prasad, S., Semwal, P., Nimgaonkar, V.L., Lerer, B., Deshpande, S.N., BK, T., 2006. Genetic susceptibility to tardive dyskinesia among schizophrenia subjects: IV. Role of dopaminergic pathway gene polymorphisms. Pharmacogenet. Genomics 16, 111–117.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., Brynjolfsson, J., Gunnarsdottir, S., Ivarsson, O., Chou, T.T., Hjaltason,

O., Birgisdottir, B., Jonsson, H., Gudnadottir, V.G., Gudmundsdottir, E., Bjornsson, A., Ingvarsson, B., Ingason, A., Sigfusson, S., Hardardottir, H., Harvey, R.P., Lai, D., Zhou, M., Brunner, D., Mutel, V., Gonzalo, A., Lemke, G., Sainz, J., Johannesson, G., Andresson, T., Gudbjartsson, D., Manolescu, A., Frigge, M.L., Gurney, M.E., Kong, A., Gulcher, J.R., Petursson, H., Stefansson, K., 2002. Neuregulin 1 and susceptibility to schizophrenia. Am. J. Hum. Genet. 71, 877–892.

Straub, R.E., Jiang, Y., MacLean, C.J., Ma, Y., Webb, B.T., Myakishev, M.V., Harris-Kerr, C., Wormley, B., Sadek, H., Kadambi, B., Cesare, A.J., Gibberman, A., Wang, X., O'Neill, F.A., Walsh, D., Kendler, K.S., 2002. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am. J. Hum. Genet. 71, 337–348.

Straub, R.E., MacLean, C.J., Martin, R.B., Ma, Y., Myakishev, M.V., Harris-Kerr, C., Webb, B.T., O'Neill, F.A., Walsh, D., Kendler, K.S., 1998. A schizophrenia locus may be located in region 10p15-p11. Am. J. Med. Genet. 81, 296–301.

Straub, R.E., MacLean, C.J., O'Neill, F.A., Burke, J., Murphy, B., Duke, F., Shinkwin, R., Webb, B.T., Zhang, J., Walsh, D., 1995. A potential vulnerability locus for schizophrenia on chromosome 6p24-22: evidence for genetic heterogeneity. Nat. Genet. 11, 287–293.

Svensson, T.H., 2003. Alpha-adrenoceptor modulation hypothesis of antipsychotic atypicality. Prog. Neuropsychopharmacol. Biol. Psychiatry 27, 1145–1158.

Tabarés-Seisdedos, R., Rubenstein, J.L.R., 2009. Chromosome 8p as a potential hub for developmental neuropsychiatric disorders: implications for schizophrenia, autism and cancer. Mol. Psychiatry 14, 563–589.

Takahashi, S., Cui, Y.-H., Kojima, T., Han, Y.-H., Zhou, R.-L., Kamioka, M., Yu, S.-Y., Matsuura, M., Matsushima, E., Wilcox, M., Arinami, T., Shen, Y.-C., Faraone, S.V., Tsuang, M.T., 2003a. Family-based association study of markers on chromosome 22 in schizophrenia using African-American, European-American, and Chinese families. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 120B, 11–17.

Takahashi, S., Ohtsuki, T., Yu, S.-Y., Tanabe, E., Yara, K., Kamioka, M., Matsushima, E., Matsuura, M., Ishikawa, K., Minowa, Y., Noguchi, E., Nakayama, J., Yamakawa-Kobayashi, K., Arinami, T., Kojima, T., 2003b. Significant linkage to chromosome 22q for exploratory eye movement dysfunction in schizophrenia. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 123B, 27–32.

Tamminga, C.A., Holcomb, H.H., 2005. Phenotype of schizophrenia: a review and formulation. Mol. Psychiatry 10, 27–39.

Thomas, P., Srivastava, V., Singh, A., Mathur, P., Nimgaonkar, V.L., Lerer, B., Thelma, B.K., Deshpande, S.N., 2008. Correlates of response to Olanzapine in a North Indian Schizophrenia sample. Psychiatry Res. 161, 275–283.

Tovo-Rodrigues, L., Rohde, L.A., Roman, T., Schmitz, M., Polanczyk, G., Zeni, C., Marques, F.Z.C., Contini, V., Grevet, E.H., Belmonte-de-Abreu, P., Bau, C.H.D., Hutz, M.H., 2012. Is there a role for rare variants in *DRD4* gene in the susceptibility for ADHD? Searching for an effect of allelic heterogeneity. Mol. Psychiatry 17, 520–526.

Tsutsumi, A., Kanazawa, T., Kikuyama, H., Okugawa, G., Uenishi, H., Miyamoto, T., Matsumoto, N., Koh, J., Shinosaki, K., Kishimoto, T., Yoneda, H., Kinoshita, T., 2009. Genetic polymorphisms in dopamine- and serotonin-related genes and treatment responses to risperidone and perospirone. Psychiatry Investig. 6, 222–225.

Valevski, A., Klein, T., Gazit, E., Meged, S., Stein, D., Elizur, A., Narinsky, E.R., Kutzuk, D., Weizman, A., 1998. HLA-B38 and clozapine-induced agranulocytosis in Israeli Jewish schizophrenic patients. Eur. J. Immunogenetics Off. J. Br. Soc. Histocompat. Immunogenetics 25, 11–13.

Van den Wildenberg, E., Janssen, R.G.J.H., Hutchison, K.E., van Breukelen, G.J.P., Wiers, R.W., 2007. Polymorphisms of the dopamine D4 receptor gene (*DRD4* VNTR) and cannabinoid CB1 receptor gene (CNR1) are not strongly related to cue-reactivity after alcohol exposure. Addict. Biol. 12, 210–220.

Van Os, J., Kapur, S., 2009. Schizophrenia. Lancet 374, 635–645.

Van Tol, H.H., Bunzow, J.R., Guan, H.C., Sunahara, R.K., Seeman, P., Niznik, H.B., Civelli, O., 1991. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 350, 610–614.

Vasic, N., Plichta, M.M., Wolf, R.C., Fallgatter, A.J., Sosic-Vasic, Z., Grön, G., 2012. Reduced Neural Error Signaling in Left Inferior Prefrontal Cortex in Young Adults With ADHD. J. Atten. Disord.

WHO, n.d. WHODAS II Disability Assessment Schedule: 12- Item interviewer administered version. Geneva World Health Organ. 2001.

Wildenauer, D.B., Schwab, S.G., Albus, M., Hallmayer, J., Lerer, B., Maier, W., Blackwood, D., Muir, W., St Clair, D., Morris, S., Moises, H.W., Yang, L., Kristbjarnarson, H., Helgason, T., Wiese, C., Collier, D.A., Holmans, P., Daniels, J., Rees, M., Asherson, P., Roberts, Q., Cardno, A., Arranz, M.J., Vallada, H., Ball, D., Kunugi, H., Murray, R.M., Powell, J.F., Nanko, S., Sham, P., Gill, M., McGuffin, P., Owen, M.J., Pulver, A.E., Antonarakis, S.E., Babb, R., Blouin, J.-L., DeMarchi, N., Dombroski, B., Housman, D., Karayiorgou, M., Ott, J., Kasch, L., Kazazian, H., Lasseter, V.K., Loetscher, E., Luebbert, H., Nestadt, G., Ton, C., Wolyniec, P.S., Laurent, C., de Chaldee, M., Thibaut, F., Jay, M., Samolyk, D.,

Petit, M., Campion, D., Mallet, J., Straub, R.E., MacLean, C.J., Easter, S.M., O'Neill, F.A., Walsh, D., Kendler, K.S., Gejman, P.V., Cao, Q., Gershon, E., Badner, J., Beshah, E., Zhang, J., Riley, B.P., Rajagopalan, S., Mogudi-Carter, M., Jenkins, T., Williamson, R., DeLisi, L.E., Garner, C., Kelly, M., LeDuc, C., Cardon, L., Lichter, J., Harris, T., Loftus, J., Shields, G., Comasi, M., Vita, A., Smith, A., Dann, J., Joslyn, G., Gurling, H., Kalsi, G., Brynjolfsson, J., Curtis, D., Sigmundsson, T., Butler, R., Read, T., Murphy, P., Chen, A.C.-H., Petursson, H., Byerley, B., Hoff, M., Holik, J., Coon, H., Levinson, D.F., Nancarrow, D.J., Crowe, R.R., Andreasen, N., Silverman, J.M., Mohs, R.C., Siever, L.J., Endicott, J., Sharpe, L., Walters, M.K., Lennon, D.P., Hayward, N.K., Sandkuijl, L.A., Mowry, B.J., Aschauer, H.N., Meszaros, K., Lenzinger, E., Fuchs, K., Yang, L., Heiden, A.M., Moises, H.W., Kruglyak, L., Daly, M.J., Matise, T.C., 1996. Additional support for schizophrenia linkage on chromosomes 6 and 8: A multicenter study. Am. J. Med. Genet. 67, 580–594.

Williams, H.J., Owen, M.J., O'Donovan, M.C., 2007. Is COMT a susceptibility gene for schizophrenia? Schizophr. Bull. 33, 635–641.

Wong, A.H.C., Van Tol, H.H.M., 2003. Schizophrenia: from phenomenology to neurobiology. Neurosci. Biobehav. Rev. 27, 269–306.

Xing, Q., Wu, S., Lin, Z., Li, H., Yang, J., Feng, G., Wang, M., Yang, W., He, L., 2003. Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene in schizophrenia. Schizophr. Res. 65, 9–14.

Youngren, K.D., Inglis, F.M., Pivirotto, P.J., Jedema, H.P., Bradberry, C.W., Goldman-Rakic, P.S., Roth, R.H., Moghaddam, B., 1999. Clozapine Preferentially Increases Dopamine Release in the Rhesus Monkey Prefrontal Cortex Compared with the Caudate Nucleus. Neuropsychopharmacology 20, 403–412.

Yunis, J.J., Corzo, D., Salazar, M., Lieberman, J.A., Howard, A., Yunis, E.J., 1995. HLA associations in clozapine-induced agranulocytosis. Blood 86, 1177–1183.

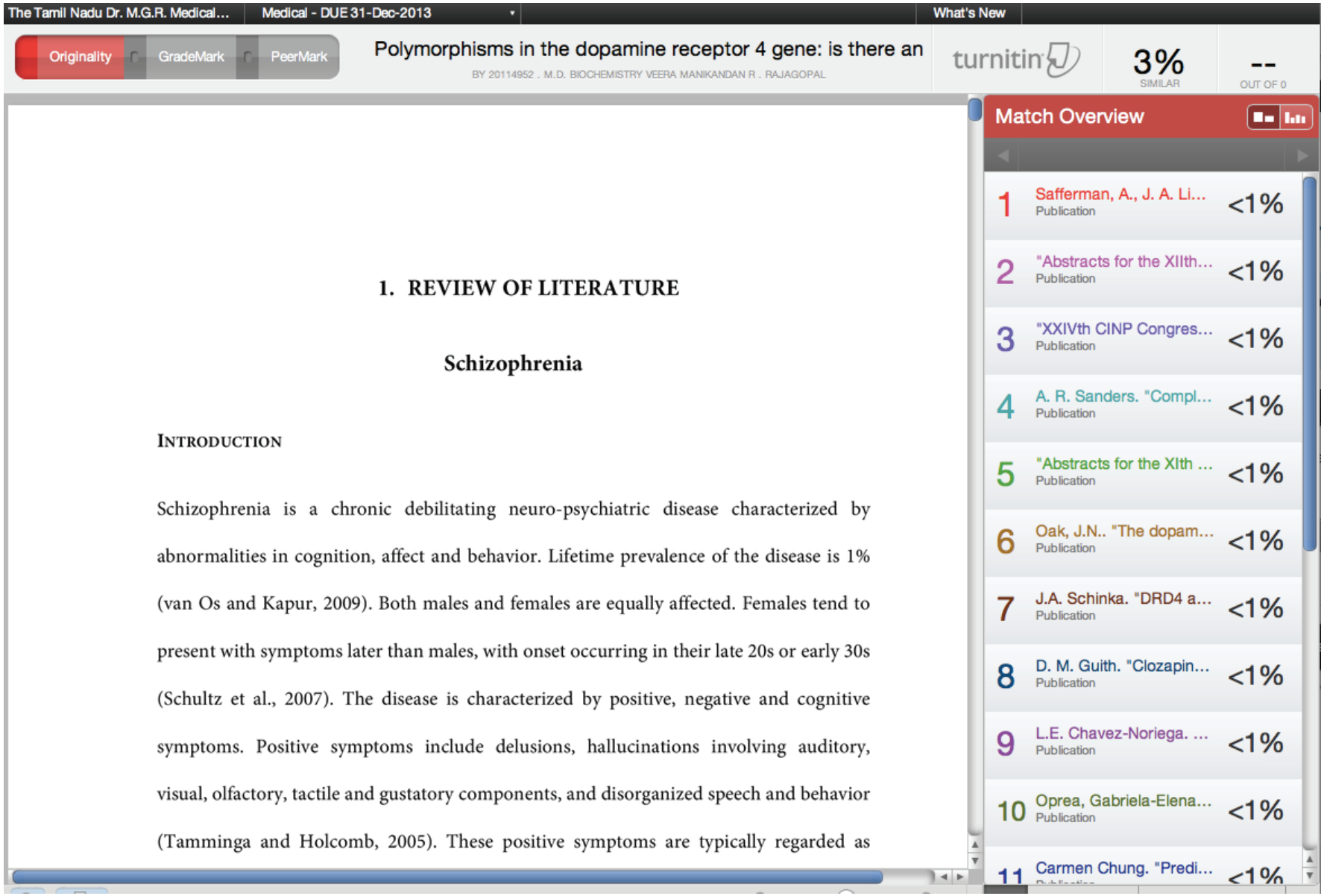
Zhang, K., Zhang, M., He, H., Zhang, J., Chi, W., Guo, Y., Huang, S., Zheng, Z., Zhang, F., Gao, X., 2012. A family-based association study of dopamine receptor D4 and mental retardation in Qinba region of China. Neurosci. Lett. 516, 1–4.

Zheng, C., Shen, Y., Xu, Q., 2012. Rs1076560, a functional variant of the dopamine D2 receptor gene, confers risk of schizophrenia in Han Chinese. Neurosci. Lett. 518, 41–44.

Zorn, S.H., Jones, S.B., Ward, K.M., Liston, D.R., 1994. Clozapine is a potent and selective muscarinic M4 receptor agonist. Eur. J. Pharmacol. 269, R1–2.

9. APPENDIX

1) Certificate of plagiarism check.
2) Informed consent.
3) Structured questionnaire for clinical assessment
4) Brief Psychiatric Rating scale (BPRS)
5) Abnormal Involuntary Movements Scale (AIMS)
6) World Health Organization Disability Assessment Schedule –II (WHODAS-II)
7) Childhood Traumatic Events Scale (CTES)
8) Recent Traumatic Events Scale (RTES)



INFORMED CONSENT DOCUMENT

Title of study:

Pharmachogenetics of Clozapine Drug Response in Treatment Resistant Schizophrenia.

Institution:

Christian Medical College & Hospital, Vellore (CMCH)

Nature and purpose of the study:

You are invited to take part in a new research. Genetic variations in human beings may determine their response and adverse effects to certain drugs. This study aims to investigate the association between genetic polymorphisms and response to clozapine.

Explanation of procedure to be followed:

Doctors and two research fellows from the department of psychiatry will conduct this study. You will undergo assessment of your psychiatric symptoms, abnormal movements, cognition, childhood experiences, previous functioning and current disability status. You will also be asked provide 10ml blood sample for assessing genetic polymorphisms. Approximately 100 participants will be recruited in this study

Expected duration of involvement:

The assessment will be done in one session of 2 hours duration, at baseline,6 months to 12 months.

Possible benefits of the study:

You will not be charged for this assessment. The information we obtain will help us to assess your psychological problems and genetic polymorphisms. Others may also benefit from the overall conclusions at the end of the study. The investigators are unable to foresee any risks involved in this study.

Confidentiality

The records and all details obtained in this study will remain strictly confidential at all times, but will need to be available to the investigators conducting the study. Your identity will not otherwise be revealed. Your personal data will be collected and processed only for the research purposes in connection with the study. You will not be referred to by name or identified in any report or publication.

Right to withdraw from the study

You are free to leave the study at any time. Your decision for not participating in this study will not cause any loss of benefits or affect your future medical or psychiatric care. For further queries, you may contact,

Dr. K.S.Jacob
Dr. Anju kuruvilla
Dr. Rajesh
Dr. Thangadurai
Department of psychiatry,
Christian Medical College, Vellore- 632002.
Phone: 0416 228 4516
Email: psych1@cmcvellore.ac.in

I/We have read/_____had read out to us, the above information before signing this consent form.

Signature (or Thumb impression) of the participant /Legally Acceptable

Signature of the Participant: _____Date: _____/_____/_____

Name of the participants: _____

Signature of the Investigator: _____Date: _____/_____/_____

Investigator's Name: _____

Signature of the Witness: _____Date: _____/_____/_____

Name of the Witness: _____

10.3. Structured questionnaire for clinical assessment:

I. Socio Demographic Data:

1. Participant Name:
2. Participant ID:
3. Interview Date:
4. Father/ Husband's name:
5. Gender: Male / Female
6. Age (in years):
7. Marital status: Single/ Married /Widowed/ Separated
8. Residence: Rural/ Urban
9. Accommodation: Own/Rented
10. Education: No formal education/ Primary/Middle/Secondary/ Higher secondary/ Graduate/ Professional
11. Education (Number of years):
12. Occupation: Unemployed/ Labourer/ Skilled/ Professional/Others
13. Total family income per month (In INR):
14. Total number of family members:
15. Average number of cups of coffee/ day:
16. Average number of cups of tea/ day:
17. Average number of cups of grape juice/ day:
18. History of migration: Yes/ No If yes, provide details:

19. Past history of obstetric complications: Yes/ No

20. History of developmental delay during childhood: Yes/ No

II. Clinical profile:

21. Hospital number:

22. ICD-10 diagnostic codes:

23. Family history: Dementia/ psychosis/ mood / seizure/ nil/

Other neuropsychiatry morbidity, specify _____.

24. Suicidal risk: Present/ Absent

25. Past history of catatonic symptoms: Present/ Absent

26. Duration of current episode:

27. Duration of illness:

28. Age at onset of illness:

29. Duration of untreated psychosis:

30. Course of Illness: Continuous/ Episodic

31. If episodic, number of past episodes:

32. Axis I Co-morbidity: Present/ Absent. If present, specify,_____

33. Axis II diagnosis: Present/ Absent. If present, specify,_____

34. Axis III diagnosis: Present/ Absent. If present, specify,_____

35. Height:

36. Weight:

37. Smoking status: [Current smoker/ Past smoker/ Non-smoker]

If yes, pack years:

III. Treatment profile:

DRUG	DURATION	DOSE	ADVERSE EVENTS
Typical Antipsychotics (Specify)_____			
Atypical Antipsychotics (Specify)_____			
Depot Antipsychotics (Specify)_____			
Clozapine			
Lithium (recent level)			
Anti-convulsants (level) (Specify)_____			
Antidepressants (Specify)_____			
Anticholinergics (Specify)_____			
Benzodiazepines (Specify)_____			
Others, (Specify)_____			

38. Total duration of drug treatment:

39. Psychotherapy: Present/ Absent, If present, duration_____

40. Occupational therapy: Present/ Absent; If present, Regular/ Irregular

41. Electro Convulsive Therapy: Yes/ No

42. If yes, How many ECT:

43. Oral contraceptive pills: Yes/ No

44. Number of adequate trials of antipsychotics in the past:

Scoring Procedure

Please enter the score for the term which best describes the patient's condition 0 = not assessed, 1 = not present, 2 = very mild, 3 = mild, 4 = moderate, 5 = moderately severe, 6 = severe, 7 = extremely severe

1. SOMATIC CONCERN	
Degree of concern over present bodily health. Rate the degree to which physical health is perceived as a problem by the patient, whether complaints have a realistic basis or not.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

2. ANXETY	
Worry, fear, or over-concern for present or future. Rate solely on the basis ot verbal report of patient's own subjective experiences. Do not infer anxiety from physical signs or from neurotic defense mechanisms.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

3. EMOTIONAL WITHDRAWAL	
Deficiency in relating to the interviewer and to the Interviewer situation. Rate only the degree to which the patient gives the impression of tailing to be in emotional contact with other people in the interview situation.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

4. CONCEPTUAL DISORGANIZATION	
Degree to which the thought processes are confused, disconnected, or disorganized. Rate on the basis of integration of the verbal products of the patient; do not rate on the basis of patient's subjective impression of his own level of functioning	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

5. GUILT FEELINGS	
Over-concern or remorse for past behavior. Rate on the basis of the patient's subjective experiences of guilt as evidenced by verbal report with appropriate affect; do not inter guilt feelings from depression, anxiety or neurotic defenses.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

6. TENSION	
Physical and motor manifestations of tension "nervousness', and heightened activation level. Tension should be rated solely on the basis of physical signs and motor behavior and not on the basis of subjective experiences of tension reported by the patient.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

7. MANNERISMS AND POSTURING	
Unusual and unnatural motor behavior, the type of motor behavior which causes certain mental patients to stand out in a crowd of normal people. Rate only abnormality of movements; do not rate simple heightened motor activity here.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

8. GRANDIOSITY	
Exaggerated self-opinion, conviction of unusual ability or powers. Rate only on the basis of patient’s statements about himself or self-in-relation-to-others, not on the basis of his demeanor in the interview situation.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

9. DEPRESSIVE MOOD	
Despondency in mood. sadness. Rate only degree of despondency; do not rate on the basis 0 inferences concerning depression based upon general retardation and somatic complaints.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

10. HOSTILITY	
Animosity, contempt, belligerence, disdain for other people outside the interview situation. Pate solely on the basis of the verbal report of feelings and actions of the patient toward others; do not infer hostility from neurotic defenses, anxiety, nor somatic complaints. (Rate attitude toward interviewer under uncooporativeness").☐	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

11. SUSPICIOUSNESS	
Belief (delusional or otherwise) that others have now, w have had in the past, malicious or discriminatory intent toward the patient. On the basis of verbal report, rate only those suspicions which are currently held whether they concern past or present circumstanes.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

12. HALLUCINATORY BEHAVIOR	
Perceptions without normal external stimulus correspondence. Rate only those experiences which are reported to have occurred within the last week and which are described as distinctly different from the thought and imagery processes of normal people.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

13. MOTOR RETARDATION	
Reduction in energy level evidenced in slowed movements. Rate on the basis of observed behavior of the patient only; do not rate on the basis of patient's subjective impression of own energy level.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

14. UNCOOPERATIVENESS	
Evidence d resistance, unfriendliness, resentment and lack of readiness to cooperate with the interviewer. Rate only on the basis of the patients attitude and responses to the interviewer and the interview situation; do not rate on basis of reported resentment or uncooperativeness outside the interview situation.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

15. UNUSUAL THOUGHT CONTENT	
Unusual, odd, strange or bizarre thought content. Rate here the degree of unusualness, not the degree of disorganizations of thought processes.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

16. BLUNTED AFFECT	
Reduced emotional tone, apparent lack of normal feeling or involvement	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

17. EXCITEMENT	
Heightened emotional tone, agitation. increased reactivity.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

18. DISORIENTATION	
Contusion or lack of proper association for person, place or time.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7

Examination Procedure

Either before or after completing the examination procedure, observe the patient unobtrusively at rest (e.g., in the waiting room).
The chair to be used in this examination should be a hard, firm one without arms.

1. Ask the patient whether there is anything in his or her mouth (such as gum or candy) and, if so, to remove it.
2. Ask about the *current* condition of the patient's teeth. Ask if he or she wears dentures. Ask whether teeth or dentures bother the patient *now*.
3. Ask whether the patient notices any movements in his or her mouth, face, hands, or feet. If yes, ask the patient to describe them and to indicate to what extent they *currently* bother the patient or interfere with activities.
4. Have the patient sit in chair with hands on knees, legs slightly apart, and feet flat on floor. (Look at the entire body for movements while the patient is in this position.)
5. Ask the patient to sit with hands hanging unsupported -- if male, between his legs, if female and wearing a dress, hanging over her knees. (Observe hands and other body areas).
6. Ask the patient to open his or her mouth. (Observe the tongue at rest within the mouth.) Do this twice.
7. Ask the patient to protrude his or her tongue. (Observe abnormalities of tongue movement.) Do this twice.
8. Ask the patient to tap his or her thumb with each finger as rapidly as possible for 10 to 15 seconds, first with right hand, then with left hand. (Observe facial and leg movements.)
9. Flex and extend the patient's left and right arms, one at a time.
10. Ask the patient to stand up. (Observe the patient in profile. Observe all body areas again, hips included.)
11. Ask the patient to extend both arms out in front, palms down. (Observe trunk, legs, and mouth.)
12. Have the patient walk a few paces, turn, and walk back to the chair. (Observe hands and gait.) Do this twice.

Patient Information									
Patient		Date	Day	Mth.	Year	Time	Hour	Min
Personal notes									

Scoring Procedure

Complete the examination procedure before making ratings.
For the movement ratings (the first three categories below), rate the highest severity observed.
0 = none, 1 = minimal (may be extreme normal), 2 = mild, 3 = moderate, 4 = severe.
According to the original AIMS instructions, one point is subtracted if movements are seen **only on activation**, but not all investigators follow that convention.

Facial and Oral Movements	
1. Muscles of facial expression, e.g., movements of forehead, eyebrows, periorbital area, cheeks. Include frowning, blinking, grimacing of upper face.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4
2. Lips and perioral area, e.g., puckering, pouting, smacking.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4
3. Jaw, e.g., biting, clenching, chewing, mouth opening, lateral movement.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4
4. Tongue. Rate only increase in movement both in and out of mouth, not inability to sustain movement.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Abnormal Involuntary Movement Scale (AIMS)

Extremity Movements	
5. Upper (arms, wrists, hands, fingers). Include movements that are choreic (rapid, objectively purposeless, irregular, spontaneous) or athetoid (slow, irregular, complex, serpentine). Do not include tremor (repetitive, regular, rhythmic movements).	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4
6. Lower (legs, knees, ankles, toes), e.g., lateral knee movement, foot tapping, heel dropping, foot squirming, inversion and eversion of foot.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Trunk Movements	
7. Neck, shoulders, hips, e.g., rocking, twisting, squirming, pelvic gyrations. Include diaphragmatic movements.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Global Judgements	
8. Severity of abnormal movements. Based on the highest single score on the above items.	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4
9. Incapacitation due to abnormal movements.	☐ none, normal ☐ minimal ☐ mild ☐ moderate ☐ severe
10. Patient's awareness of abnormal movements.	☐ no awareness ☐ aware, no distress ☐ aware, mild distress ☐ aware, moderate distress ☐ aware, severe distress

Dental status	
11. Current problems with teeth and/or dentures.	☐ no ☐ yes
12. Does patient usually wear dentures?	☐ no ☐ yes

WORLD HEALTH ORGANIZATION

DISABILITY ASSESSMENT SCHEDULE

WHODAS II

Phase 2 Field Trials – Health Services Research
12-Item Interviewer Administered Version
February 2000

SECTION 1. Face Sheet

ITEMS F1- F6 ARE TO BE COMPLETED BY INTERVIEWERS PRIOR TO STARTING EACH INTERVIEW

F1 RESPONDENT I.D . #

☐☐☐ – ☐☐☐ – ☐

CENTRE # - SUBJECT #

F2 INTERVIEWER I.D. #

☐☐☐ – ☐☐☐ – ☐

CENTRE # - INTERVIEWER #

F3 ASSESSMENT TIME POINT (1, 2, ETC.) ☐

F4 INTERVIEW DATE ___ ___/___ ___/___ ___

month day year

F5 LIVING SITUATION AT TIME OF INTERVIEW (CIRCLE ONLY ONE)

Independent in Community	1
Assisted Living	2
Hospitalized	3

F6 SAMPLE *(CIRCLE ONLY ONE)*

General population	1
Drug related problems	2
Alcohol related problems	3
Mental health problems	4
Physical problems	5
Other (*specify*)	6

_____

SECTION 4. CORE QUESTIONS

H1	How do you rate your overall health in the past 30 days? *Read choices to respondent.*	Very good	Good	Moderate	Bad	Very Bad

SHOW FLASHCARD #2 to participant

In the last 30 days how much difficulty did you have in:

		None	Mild	Moderate	Severe	Extreme /Cannot Do
S1	Standing for long periods such as 30 minutes?	1	2	3	4	5
S2	Taking care of your household responsibilities?	1	2	3	4	5
S3	Learning a new task, for example, learning how to get to a new place?	1	2	3	4	5
S4	How much of a problem did you have joining in community activities (for example, festivities, religious or other activities) in the same way as anyone else can?	1	2	3	4	5
S5	How much have you been emotionally affected by your health problems?	1	2	3	4	5

Continue to next page…

In the last 30 days how much difficulty did you have in:						
		None	Mild	Moderate	Severe	Extreme /Cannot Do
S6	Concentrating on doing something for ten minutes?	1	2	3	4	5
S7	Walking a long distance such as a kilometre [or equivalent]?	1	2	3	4	5
S8	Washing your whole body?	1	2	3	4	5
S9	Getting dressed?	1	2	3	4	5
S10	Dealing with people you do not know?	1	2	3	4	5
S11	Maintaining a friendship?	1	2	3	4	5
S12	Your day to day work?	1	2	3	4	5

		None	Mild	Moderate	Severe	Extreme /Cannot Do
H2	Overall, how much did these difficulties interfere with your life? *Read choices to respondent.*	1	2	3	4	5
H3	Overall, in the past 30 days, how many days were these difficulties present?	RECORD NUMBER OF DAYS ___/___				
H4	In the past 30 days, for how many days were you totally unable to carry out your usual activities or work because of any health condition?	RECORD NUMBER OF DAYS __/__				
H5	In the past 30 days, not counting the days that you were totally unable, for how many days did you cut back or reduce your usual activities or work because of any health condition?	RECORD NUMBER OF DAYS __/__				

This concludes our interview, thank you for participating.

10.4.4. Addenbrooke's Cognitive Examination – ACE-R:

Orientation:

- **Ask what is the :** Day/ Date/ Month/ Year/ Season **(Score 0-5)**
- **Ask which is the** : Building/ Floor/ Town/ state/ country **(Score 0-5)**

Registration:

Tell: "I am going to give three words and I'd like you to repeat me: Lemon, Key, and Ball". After subject repeats, say "Try to remember them because I am going to ask you later". Score only the first trial (repeat three times if necessary)

Register number of trials_____ **(Score 0-3)**

Attention and concentration:

Ask the subject: "Could you take seven away from 100? After the subject responds, ask him/her to take away another seven to a total of five subtractions. If subject makes a mistake carry on and check the subsequent answer (i.e., 93, 84, 77, 70, and 63-score-4)

Stop after five subtractions (93, 86, 79, 72, 65)……. (**Score 0-5)**

Ask: 'could you please spell the word **WORLD** for me? Then ask him/her to spell in backwards……. **(Score 0-5)**

Memory – Recall:

Ask: 'Which three words did I ask you to repeat and remember?"

_____, _____,_____ **(Score 0-3)**

Memory – Anterograde memory:

Tell: I am going to give you a name and an address and I'd like you to repeat after me.

	1st Trial	2nd Trial	3rd Trial
Selvakumar,			
42, Nehru street,			
Gandhi Nagar,			
Vellore.			

We'll be doing that three times, so you have a chance to learn it. I'll be asking you later'. Score only the third trial **(Score 0-7)**

Memory – Retrograde Memory:

- Name of the current chief minister of Tamil Nadu……
- Name of the woman who was the prime Minister of India…….
- Name of the Indian Prime minister assassinated in 1991……
- Name of the current Indian Prime Minister…….....

Verbal Fluency – Letter 'P' and animals

Letters:

Say: I am going to give you an alphabet and I'd like you to generate as many words you can beginning with that letter, but not names of people or places. Are you ready? You've got a minute and the letter is p' **(Score 0-7)**

Animals

Say: Can you name as many animals as possible, beginning with any letter? **(Score 0-7)**

>21= 7; 17-21= 6; 14-16= 5; 11-13= 4; 9-10= 3; 7-8= 2; 5-6= 1; <5=0

Language - Comprehension:

- **Show written command** **(Score 0-1)**

Close Your Eyes

- **Three stage command** **(Score 0-3)**

Take a paper in your hand / Fold the paper / put the paper on the table

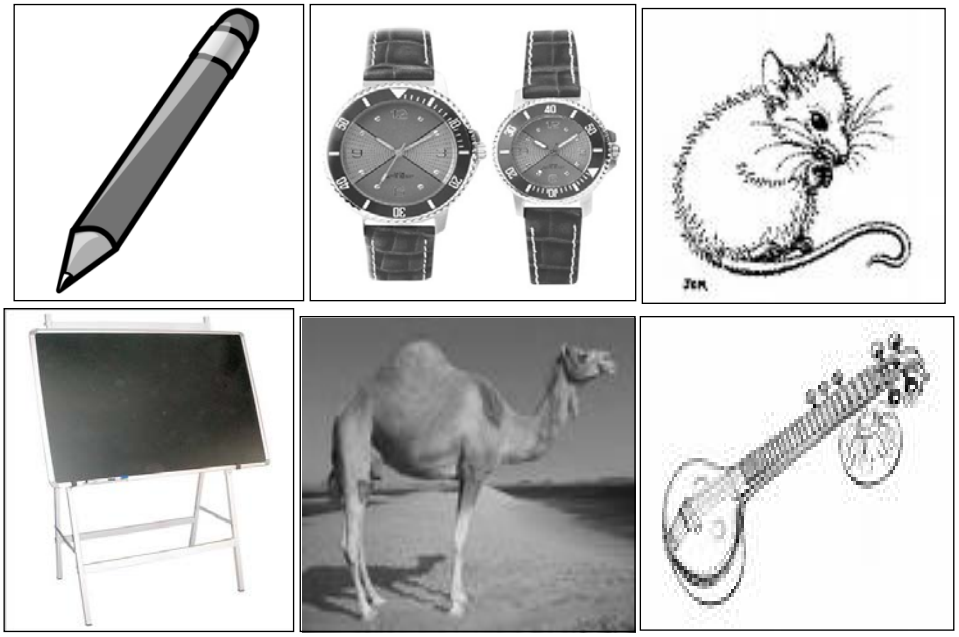
- **Writing**

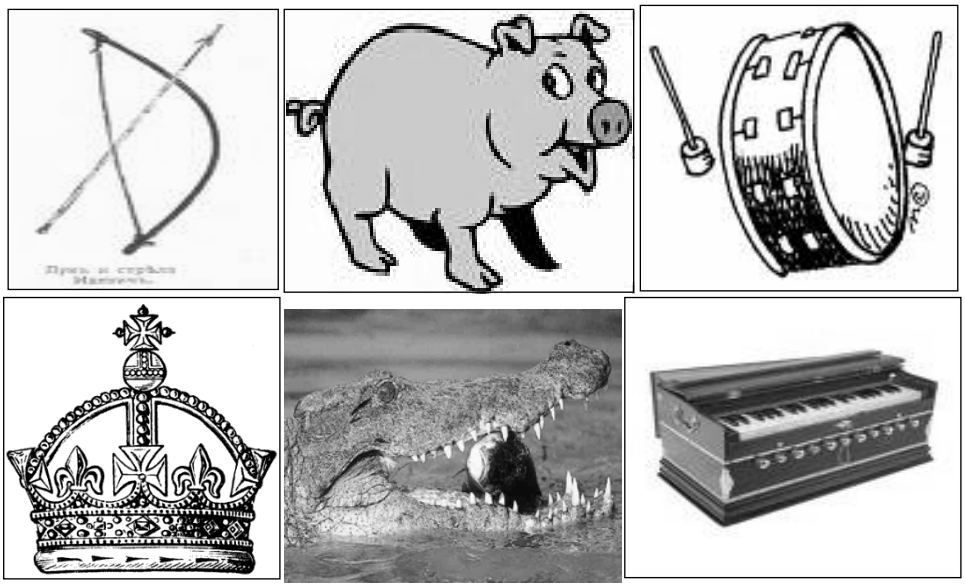
Ask the subject to make up a sentence and write it in the space below: score 1 if sentence contains a subject and a verb. **(Score 0-1)**

Language – Repetition:

- Ask the subject to repeat 'hippopotamus', 'eccentricity', 'unintelligible', and 'statistician'. Score: 2 if all correct: 1 if 3 correct: 0 if 2 or less
- Ask the subject to repeat: 'above beyond and below' **(Score 0-1)**
- Ask the subject to repeat: 'No ifs, ands or buts' **(Score0-1)**

Language – Naming:





Language: comprehension:

Using the pictures above, ask the subjects to: **(Score 0-4)**

Point to the one which is associated with the monarchy _____

Point to the one which is a rodent _____

Point to the one which is found in the desert _____

Point to the one which is associated with warriors _____

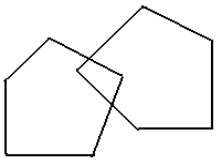
Language: reading:

Ask the subject to read the following words: [score 1 only if all correct] **(Score 0-1)**

SEW	**PINT**	**SOOT**	**DOUGH**	**HEIGHT**

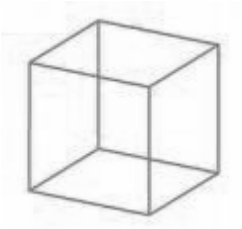
Visuo-spatial Abilities:

- **Overlapping pentagons:** ask the subject to copy this diagram **(Score 0-1)**



- **Wire cube:** ask the subject to copy this drawing

(Score 0-2)



- **Clock:** Ask the subject to draw a clock face with numbers and the hands at ten past five. (For scoring, circle= 1, numbers = 2, hands = 2 if all correct) **(Score 0-5)**

Perceptual abilities:

- Ask the subject to count the dots without pointing them. **(Score 0-4)**
- Ask the subject to identity the letters **(Score 0-4)**

Recall:

Ask "Now tell me what you remember of that name and address we were repeating at the beginning" **(Score 0-7)**

Selvakumar,	
42, Nehru street,	
Gandhi Nagar,	
Vellore.	

Recognition:

This test should be done if subject failed to recall one or more items. If all items were recalled, skip the test and score 5. If only part is recalled start by ticking items recalled in the shadowed column on the right hand side. Then test not recalled items by telling" ok, I'll give you some hints; was the name X, Y or Z?" and so on. Each recognized item scores one point, which is added to the point gained by recalling.

Nallakumar		Selvakumar		Selvakrishnan		Recalled	
24		42		49			
Nehru Road		Patel street		Nehru street			
Chrompet		Gandhi nagar		Saidapet			
Vellore		Nellore		Nagpur			

General Scores	
MMSE	**/30**
ACE-R	**/100**
Sub scores	
Attention and Orientation	**/18**
Memory	**/26**
Fluency	**/14**
Language	**/26**
Visuo-spatial	**/16**

Childhood Traumatic Events Scale

For the following questions, answer each item that is relevant. Be as honest as you can. Each question refers to any event that you may have experienced **prior to the age of 17**.

1. Prior to the age of 17, did you experience a death of a very close friend or family member?_____ If yes, how old were you?_____

   If yes, how traumatic was this? (using a 7-point scale, where 1 = not at all traumatic, 4 = somewhat traumatic, 7 = extremely traumatic)_____

   If yes, how much did you confide in others about this traumatic experience at the time? (1 = not at all, 7 = a great deal)_____

2. Prior to the age of 17, was there a major upheaval between your parents (such as divorce, separation)?_____ If yes, how old were you?_____

   If yes, how traumatic was this? (where 7 = extremely traumatic)_____

   If yes, how much did you confide in others? (7 = a great deal)_____

3. Prior to the age of 17, did you have a traumatic sexual experience (raped, molested, etc.)?_____ If yes, how old were you?_____

   If yes, how traumatic was this? (7 = extremely traumatic)_____

   If yes, how much did you confide in others? (7 = a great deal)_____

4. Prior to the age of 17, were you the victim of violence (child abuse, mugged or assaulted -- other than sexual)?_____ If yes, how old were you?_____

   If yes, how traumatic was this? (7 = extremely traumatic)_____

   If yes, how much did you confide in others? (7 = a great deal)_____

5. Prior to the age of 17, were you extremely ill or injured?_____ If yes, how old were you?_____

   If yes, how traumatic was this? (7 = extremely traumatic)_____

   If yes, how much did you confide in others? (7 = a great deal)_____

6. Prior to the age of 17, did you experience any other major upheaval that you think may have shaped your life or personality significantly?_____ If yes, how old were you?_____

   If yes, what was the event?_____

   If yes, how traumatic was this? (7 = extremely traumatic)_____

   If yes, how much did you confide in others? (7 = a great deal)_____

Recent Traumatic Events Scale

For the following questions, again answer each item that is relevant and again be as honest as you can. Each question refers to any event that you may have experienced **within the last 3 years**.

1. Within the last 3 years, did you experience a death of a very close friend or family member? _____

   If yes, how traumatic was this? (1 = not at all traumatic, 7 = extremely traumatic)_____

   If yes, how much did you confide in others about the experience at the time? (1 = not at all, 7 = a great deal)_____

2. Within the last 3 years, was there a major upheaval between you and your spouse (such as divorce, separation)?_____

   If yes, how traumatic was this?_____

   If yes, how much did you confide in others?_____

3. Within the last 3 years, did you have a traumatic sexual experience (raped, molested, etc.)?_____

   If yes, how traumatic was this?_____

   If yes, how much did you confide in others?_____

4. Within the last 3 years, were you the victim of violence (other than sexual)?_____

   If yes, how traumatic was this?_____

   If yes, how much did you confide in others?_____

5. Within the last 3 years, were you extremely ill or injured?_____

   If yes, how traumatic was this?_____

   If yes, how much did you confide in others?_____

6. Within the last 3 years, has there been a major change in the kind of work you do (e.g., a new job, promotion, demotion, lateral transfer)?_____

   If yes, how traumatic was this?_____

   If yes, how much did you confide in others?_____

7. Within the last 3 years, did you experience any other major upheaval that you think may have shaped your life or personality significantly?_____

   If yes, what was the event?_____

   If yes, how traumatic was this?_____

   If yes, how much did you confide in others?_____