ASSOCIATION BETWEEN TARDIVE DYSKINESIA AND DOPAMINE RECEPTOR GENES AMONG PATIENTS WITH CHRONIC SCHIZOPHRENIA

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ABSTRACT

Huei-Ting Tsai: Association between Tardive Dyskinesia and Dopamine Receptor Genes among Patients with Chronic Schizophrenia

(Under the direction of Kari E. North, PhD)

This dissertation aims to study associations between genetic variants and prevalent tardive dyskinesia (TD) among patients with chronic schizophrenia. The etiology of TD is largely unknown but dopamine receptors (DR) have been proposed as the drug target of anti-schizophrenic effects. In addition, the blockade of the dopaminergic pathway from long-term antipsychotic use likely influences the etiology of TD. Therefore, this study interrogated the relationship between DR genes (*DRD* 1, 2, 3, 4 and 5) and the prevalence of TD.

The first study conducted as part of this dissertation was a meta-analysis of 13 association studies between *DRD3* rs6280 and prevalent TD. Results from the meta-analysis implied strong publication bias in the studies on the relationship between rs6280 and TD. Study characteristics moderately associated with heterogeneous effect estimates in the published literature include publication year, criteria of subject's enrollment, TD assessment and diagnosis, age, percent female, and ancestry. In contrast, the summary estimate obtained when assuming a recessive mode of inheritance was not vulnerable to publication bias or heterogeneity

in the published literature and indicated no association between rs6280 and TD (POR= 0.93, 95% C.I.= 0.70, 1.23).

The second study from this dissertation was a cohort study about associations between TD susceptibility and 54 single nucleotide polymorphisms (SNPs) in all DR genes. Study subjects were 711 participants with chronic schizophrenia in the Clinical Antipsychotic Trial of Intervention Effectiveness (CATIE) study. Two hundred and seven participants who ever met the Schooler-Kane criteria in any one of Abnormal Involuntary Movement Scale (AIMS) evaluations in the CATIE were defined as TD. Several *DRD1-3* SNPs demonstrated statistically significant associations with TD. However, after multiple comparison adjustments, no SNPs or haplotypes in DR genes displayed statistically significant association with TD.

In summary, results from a comprehensive meta-analysis of 13 genetic association studies demonstrated no association between polymorphisms of rs6280 and TD. In addition, no association was detected in a cohort study interrogating the relationship between 54 SNPs in DR genes and TD among 711 CATIE participants. These findings suggest that SNPs in DR genes do not exert a strong effect on the pathophysiology of TD.

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LIST OF ABBREVIATIONS

AIMS: Abnormal Involuntary Movement Scale

CATIE: Clinical Antipsychotic Trial of Intervention Effectiveness

CEU: Caucasian in HapMap panel

CHB+ JPT: Asian in HapMap panel

CI: confidence interval

DAG: Directed Acyclic Graph

***DRD1*:** dopamine receptor 1 gene

***DRD2*:** dopamine receptor 2 gene

***DRD3*:** dopamine receptor 3 gene

***DRD4*:** dopamine receptor 4 gene

***DRD5*:** dopamine receptor 5 gene

FWER: family-wise error rate

FDR: false discovery rate

HGI: Human Genetics Initiative

HWE: Hardy-Weinberg Equilibrium

MAF: minor allele frequency

MCMC: Markov chain Monte Carlo

PANSS: Positive and Negative Symptom Scale

PCP: phencyclidine

pFDR: positive false discovery rate

POR: prevalence odds ratio

SNPs: single nucleotide polymorphisms

SCID: Structured Clinical Interview for DSM-IV

TD: tardive dyskinesia

VIF: variance inflation factors

YRI: African in HapMap panel

CHAPTER I.

STATEMENT OF SPECIFIC AIMS

This dissertation aims to study associations between genetic variants and prevalent tardive dyskinesia (TD) among patients with chronic schizophrenia. The etiology of TD is largely unknown but dopamine receptors (DR) have been proposed as the drug target of anti-schizophrenic effects. In addition, the blockade of the dopaminergic pathway from long-term antipsychotic use likely influences the etiology of TD. Therefore, this study interrogated the relationship between DR genes (*DRD* 1, 2, 3, 4 and 5) and the prevalence of TD. Three specific aims include:

Aim 1. Meta-analyses of published studies to evaluate the association between *DRD3* rs6280 and prevalence odds ratio (POR) of TD.

Aim 2. Assess the association between TD and 54 single nucleotide polymorphisms (SNPs) in all DR genes.

Aim 3. Investigate the association between TD and haplotype variations in DR genes.

Chapter II.

BACKGROUND

1. Conceptual framework

The aim of this study is to understand genetic influence on TD, one of most frequent, distressing and persistent side-effects of long-term antipsychotic treatment. Below, I provided a conceptual model to illustrate the hypothesized relationships between the genes of interest, TD and other relevant covariates (Figure. 2.1). Details of TD, genes of interest, and covariates would be further discussed in following text.

2. Background of schizophrenia and TD

2.1. Schizophrenia

2.1.1. Public health significance of schizophrenia

Schizophrenia influences a person's ability of recognizing what is real, managing his or her emotions, thinking clearly, making judgments and communicate with others (1). In the US, schizophrenia is estimated to have a 0.7 % lifetime prevalence (2) and affects approximately 2 million people. A meta-analysis of 188 studies from 46 countries concluded a life-time risk of schizophrenia was 4.0 (95% confidence interval, CI,= 1.6-12.1) (3). Because schizophrenia usually begins during adolescence or early adulthood and has no cure, antipsychotics are prescribed for the duration of most patients' lives. However, the severity of side effects, such as TD, has greatly limited the application of antipsychotic therapies (4, 5). Noncompliance

resulting from intolerable side effect puts patients with schizophrenia at risk of relapse, often requiring hospitalization. Relapse and hospitalization have made schizophrenia a very costly disease. The total economic burden of schizophrenia in the US was estimated at $62.7 billion in 2002 and has likely increase since that time (6).

2.1.2. Suspected risk factors for schizophrenia

Schizophrenia has been recognized as a complex disease with multiple causes and interactions between genetic and environmental factors. Genetic studies, including twin, adoption and family studies, have consistently shown that schizophrenia is a disease with high heritability. Although the inheritance pattern of schizophrenia is not fully understood, studies have reported that concordance rates in monozygotic twins and dizygotic twin are 30-65% and 5-15%, respectively (7-9). A population-based cohort study of 1.75 million in Denmark reported an increased risk of schizophrenia among people with a schizophrenia-affected mother (RR=9.31, 95%C.I. = 7.24-11.96), father (RR= 7.2, 95%C.I. = 5.1-10.6) and sibling (RR= 6.99, 95%C.I. = 5.38-9.09), compared with people without schizophrenia-affected parents or siblings. Several candidate gene regions have been identified, including chromosomal 6p24-22, 1q21-22 and 13q32-34. In addition, several candidate genes have also been suggested in the etiology of schizophrenia, including Neuregulin 1, Dysbindin, G72 protein, 5-HT2A and catechol-*O*-methyltransferase genes (10, 11). All of above evidences supports the role of genetics in schizophrenia development.

Several environmental risk factors have been shown to have a moderate association with schizophrenia (OR~2); these risk factors include prenatal infection,

famine in pregnancy, obstetric complications during pregnancy and delivery, season of birth, disturbance of early development, urbanization and migration in childhood and adolescence (12). Prenatal infections, such as maternal influenza A infection during the first or the second trimester, have been proposed to increase the risk of schizophrenia. For example, Brown et al. conducted a nested case-control study among 64 case and 125 control pregnancies with serological documentation of prenatal exposure to influenza. This study reported an increased risk of schizophrenia among fetuses exposed to maternal influenza A (odds ration, OR=7.0, 95% C.I. = 0.7-75.3). This study also concluded that current evidence about the relationship between prenatal infection and schizophrenia are still controversial and have been criticized because they are frequently vulnerable to recall bias and have small sample sizes (13).

Obstetric complications during pregnancy and delivery and their relationship to schizophrenia have generated a great deal of inquiry. Cannon et al. conducted a meta-analysis to summarize findings from prospective population-based studies and reported significant but modest effects for three types of complications: 1) complications of pregnancy (bleeding, diabetes, rhesus incompatibility, and preeclampsia); 2) abnormal fetal growth and development: (low birth weight, congenital malformations, reduced head circumference), and 3) complications of delivery (uterine atony, asphyxia, emergency Cesarean section). The authors concluded that evidence from studies examining the association between obstetric complications and schizophrenia are limited by insufficient information from the prenatal period and low statistical power to detect interactive effects (14).

Migration, urbanization and season of birth are important risk factors associated with schizophrenia. A meta-analysis of 24 studies reported that the rate of schizophrenia was greater among migrants compared to native-born people (RR= 4.6, 95%C.I.= 1.0-12.8) (15). A population-based cohort of 1.75 million persons in Denmark reported that birth in an urban area (the capital) was associated with increased risk of schizophrenia compared to births in rural areas (RR= 2.4, 95% C.I.= 2.13- 2.7) (16). A review study of over 250 studies have reported an access incidence rate of schizophrenia by 5-8% among birth in spring-winter compared to birth in summer (17). Although these factors have shown to be highly associated with schizophrenia, the complex biological and social factors behind these observations have not been elucidated (18) and have limited their usefulness in developing interventions to prevent schizophrenia.

In summary, schizophrenia is a complex disease with multiple causes, including genetic and environmental factors. Research to understand schizophrenia has been limited by small sample sizes and several methodological shortcomings, such as recall bias and other sources of inaccurate exposure assessment.

2.1.3. Pharmacotherapy of schizophrenia

Before the introduction of antipsychotic pharmacotherapy in the 1950s, schizophrenic patients were commonly committed to custodial institutions(19). The effectiveness of antipsychotic medications allowed patients with schizophrenia to live in the community. These older antipsychotics, now classified as conventional antipsychotic medications (CONV), have been shown to greatly reduced symptoms

such as hallucinations and paranoid thoughts. However, these CONV resulted in many distressing side effects, including sexual dysfunction in males, extrapyramidal symptoms (EPS), and TD (20). TD, in particular, has contributed to a high frequency of noncompliance or discontinued treatment among patients with chronic schizophrenia. As a result, noncompliance is the most frequent cause of relapse and hospitalization among patients with chronic schizophrenia (21).

Beginning in the 1990s, a new series of antipsychotics were introduced for public use, including clozapine in 1990, risperidone in 1993, olanzapine in 1996, quetiapine in 1997, ziprasidone in 2001 and aripiprazole in 2002. These medications were classified as “atypical” because of their different side effect profiles in contrast to CONV. In particular, atypical antipsychotic medications (ATY) result in movement disorders less frequently than CONV (22). With favorable side effect profiles and efficacy equivalent to CONV, ATY have become the first-line drug choices in schizophrenic treatment (23, 24) although there are increasing data that their efficacy is not as good as had been believed (25).

The pharmacological mechanisms of antipsychotics have not been fully explained. Some studies have proposed that the effects of antipsychotics are mediated through the combined effect of dopamine D2 receptor (*DRD2*) and 5-hydroxytryptamine receptor (*HTR-2A*). Compared to CONV, ATY have higher binding affinity to *HTR-2A* and lower binding affinity to *DRD2*. As described in section II-4.2, the difference in binding affinity may also explain a lower rate of side effects, particular the occurrence of movement disorders observed in atypical antipsychotic use compared to conventional antipsychotic use (22, 26-28).

2.2. Tardive dyskinesia (TD)

2.2.1. TD and its impact on schizophrenia treatment

TD is an involuntary movement disorder presenting on the face, extremities and trunk. TD emerges late in the course of long-term antipsychotic therapy and can have profound impacts. In particular, it may cause non-compliance and discontinuation of antipsychotic medications, leading to a high risk of relapse of psychotic symptoms. In the absence of safe and effective therapies, the primary approach to reduce TD symptoms is to discontinue or minimize the use of antipsychotics (29). However, even after discontinuing antipsychotic use, the symptoms of TD can endure for months to years and influence lives of patients with schizophrenia in profound ways (30-32). For example, even though patients with schizophrenia themselves may not sense involuntary movements they present, TD could be quite stressful to individuals around patients with schizophrenia. As a result, TD may contribute to stigma and social segregation of patients with schizophrenia (33).

Currently, there is no safe and effective treatment for TD among those receiving antipsychotic treatment. One main strategy to prevent TD is to prescribe ATY as ATY have less risk of TD than CONV. However, atypical antipsychotic use has recently been challenged because it causes several serious side effects and also is expensive. Specifically, increased risks of serious side effects, such as weight gain and diabetes, have been reported in large-scale clinical trials of ATY(4). In addition, ATY are ten times more expensive than CONV and dramatically increase the economic burden of schizophrenia care. As a result, in developing areas, CONV still play an important

role in schizophrenia treatment, which could lead to a higher risk of TD among disadvantaged populations.

2.2.2. Understanding of TD pathophysiology is limited

Our understanding of TD pathophysiology has not progressed beyond hypotheses (34). A dominant hypothesis is that blockade of dopamine receptors in the nigrostriatal dopamine pathway causes drug-induced movement disorders, such as TD. The rationale is that this pathway, part of the extrapyramidal nervous system, may be responsible for the control of human movement (35). Following the chronic antipsychotic blockade of dopamine receptors, the nigrostraiatal dopamine systems in the brain may increase the sensitivity of dopamine receptors (36, 37).

Some studies suggest that increased dopamine sensitivity may be the result of an increase in dopamine D2 receptors (38, 39). Although hypersensitivity of DR has been a dominant hypothesis for TD pathophysiology since 1970, there are still no direct human data to support this hypothesis of hypersensitivity. Research on rodent models provide some evidence that increased dopaminergic activity results in movement disorders. In rodent studies, following administration of dopamine agonists, rodents exhibited both short- and long-term behavioral responses, including muscular disorders (38). All of this evidence supports the role of dopamine receptors on TD development.

2.2.3. Research diagnosis criteria of TD

TD is diagnosed using standardized examination procedures and rating scales

(40). The Abnormal Involuntary Movement Scale (AIMS) is currently the most widely accepted measurement tool for TD in clinical research (33). AIMS is a 12-item questionnaire. Item 1 to item 7 measure the severity of involuntary movements in several body regions, including mouth and face, extremities, and trunk. Item 8 is an overall judgment on the severity of abnormal movements (41). An AIMS form is attached as Figure 2.2.

Severity of TD was evaluated on a scale ranging from 0 to 4 points with higher scores representing greater severity. AIMS is also used to characterize patients' incapacitation, awareness and overall severity in item 8 to item 10 (33). The popularity of the AIMS has resulted from its convenience and high concordance with other rating scales (42).

AIMS scores may be interpreted using different criteria for TD diagnosis. For example, according to the Glazer-Morgenstern criteria, TD is defined as a total AIMS score from item 1 to item 7 greater than 3 points and at least one AIMS item score greater than 2 points (43). The other criteria, Schooler-Kane criteria, are more restrictive in diagnosing TD, and defines TD as at least one item rated greater than 3 or at least two items rated greater than 2 in item 1 to item 7 (44). This study will use Schooler-Kane criteria because it is more restrictive and also widely accepted.

Since there is no gold-standard in the diagnosis of TD, sensitivity and specificity are less relevant in determining the accuracy of this evaluation tool. Instead, the reliability of this tool is more relevant, particularly when considering the scales performance across raters or at different measurement time points. Previous studies

have assessed the reliability of AIMS (45-47). The reliability of the AIMS instrument is typically evaluated across raters using Pearson Correlation Coefficient (PCC). Estimates of AIMS reliability using PCC range between 0.46 and 0.87 across items for different body regions in AIMS. However, PCC has been criticized because it overestimates the correlations when there are greater than 2 raters. With more than 2 raters, intraclass correlation coefficient (ICC) is the more appropriate statistic (48). One well-done study by Lane et al. used 2 experienced psychiatric faculty members and 2 relatively inexperienced psychiatric residents as examiners to evaluate the reliability of AIMS test among 33 patients with schizophrenia over a 10-month period. They obtained intraclass correlation coefficients, ranging from 0.5 to 0.79 (p <0.001) across items for different body regions in AIMS (45).

2.2.4. Epidemiology of TD

TD, an involuntary movement disorder, emerges late in the course of long-term antipsychotic therapy and has profound effects to patients with schizophrenia. Studies have reported a greater than 20% TD prevalence among patients treated with CONV (49-51). For example, Yassa and Jeste reviewed 76 studies with a total of 39,187 patients and reported an average prevalence of TD was 24.2% (range: 3-62%) among schizophrenic patients treated with CONV (51). The incidence of TD varies by population, depending on age, sex and type of antipsychotic treatment, with a yearly cumulative incidence of 5% reported among adults patients (49) and 25%-30% reported among elderly patients (52).

In addition to antipsychotic exposure, several risk factors have been proposed to

increase the risk of TD. These risk factors include advanced age, female gender, African-American ethnicity, anticholinergic medication use. Psychiatric diagnosis has also been implicated as an independent risk factor for TD, but this association is controversial (see section II-2.2.4.7) (29, 53). However, our current understanding of risk factors for TD is limited because existing studies rarely controlled for important confounders, such as degree of antipsychotic exposure. Details of each of the above risk factors for TD are addressed separately in sections below.

2.2.4.1. Antipsychotics

A. Type of antipsychotics

Antipsychotic exposure has been the most consistent risk factor for TD development, although this risk has been reported to be different for ATY and CONV. ATY have been reported to confer a lower risk for TD than CONV in several recent large-scale clinical trials. A recent systematic review of 2,769 patients from 11 clinical trials investigated the 1-year risk of TD among all ATY, except clozapine. This study reported a summarized annual risk of TD for atypical antipsychotic use in different age groups: 0% in the children, 0.8% (range: 0 - 1.5%) in the adults, and 5.3% (range: 0.0% - 13.4%) in patients aged over 54 years old. Overall, the observed annual risks were lower than that of the control group using the conventional antipsychotic, haloperidol (annual risk= 5.4%, range 4.1% - 7.4%) (22). Studies that report risk of TD due to individual atypical antipsychotic medication use were summarized in Table 2.1.

Several studies have suggested that clozapine, the first atypical antipsychotic,

has a much lower risk of TD development compared to CONV (54-57). For example, Tamminga et al. followed up 32 patients with schizophrenia for 12 months to compare the risk of TD from clozapine with haloperidol. The group treated with clozapine was found to have less motor disorder symptoms than the group treated with haloperidol ($p<0.001$). Povlsen et al. retrospectively investigated 216 patients treated with clozapine for up to 12 years and reported no TD cases.

Risperidone, another atypical antipsychotic, is also reported to have lower risk for TD compared to CONV. Several long-term clinical trials have suggested the yearly risk of TD from risperidone is one-fifth to one-tenth of that from haloperidol (58-62). A very low incident risk (0.23%) among risperidone-treated patients was also supported by a meta-analysis of clinical trials, although this analysis was limited by relatively short follow-up periods among studies (12 months was the longest follow-up across studies) (63). This relationship was also reported among elderly patients with schizophrenia. For example, Jeste et al. reported that risperidone-treated elders had a lower incidence of TD development than haloperidol-treated elders (5% vs. 30%) (64). This finding agreed with an earlier study from Chouinard (65).

Studies have suggested a low risk of TD from the atypical antipsychotic, olanzapine. Beasley et al. conducted a large-scale and double-blind randomized trial of 627 patients with 2.6 years of follow-up to compare the yearly risk of TD among olanzapine-treated subjects to haloperidol-treated subjects. This study reported that the risk of TD observed among olanzapine-treated subjects was much lower than that observed among haloperidol-treated subjects (0.52% vs. 7.45%) (27). This finding has been replicated (66).

Quetiapine use has a similar annual risk of TD as olanzapine. The annual risk of quetiapine use is estimated to be 0.7% in adults (mean age: 36) (67) and 2.7% in an elderly population (mean age: 76) (68). These risks are about one-twelfth of the risk associated with haloperidol. As newly approved ATY, data about ziprasidone's and aripiprazole's risk of TD are limited.

B. Duration of antipsychotic exposure

Longer duration of exposure usually results in a larger accumulation of exposure and confers a higher risk of disease. However, this relationship has not always been observed in medication use because medication exposure can be modified quickly to accommodate intolerable side effects. As a result, a higher incidence rate of adverse events, such as TD, is usually observed among subjects at first exposure to medications compared to chronic users. This phenomenon is called "depletion of susceptibility" in medication-mediated side effects.

Depletion of susceptibility has also been reported in literature dealing with antipsychotic exposure to TD. For example, the Yale Tardive Dyskinesia Study consisted of a cohort of 398 adults who had maintained antipsychotic use for at least 3 months and up to 33 years. This study reported an inverse association between the duration of antipsychotic exposure and TD. Specifically, the TD incidence rate was found to be highest during the first 5 years of antipsychotic treatment and decreased afterward. (43).

In summary, risk of TD increases with time on treatment. However, this association may also diminish with the increase of treatment duration, possibly

because with time, physicians and patients discover treatment regimens with few side-effects.

2.2.4.2. Increased age

Both cross-sectional and longitudinal studies have reported a positive association between age and TD (43, 50, 60, 69-77), but this association was not replicated in other studies (78-80). This positive association has been replicated in studies that investigated associations between genes and TD. In Leon et al's study of 516 patients with schizophrenia, age greater than 45 was identified as a risk factor of TD development (adjusted OR=2.0, 95%C.I.= 1.3-3.0, p=0.002) (81). In Hori et al's study of 200 patients with schizophrenia, advanced age was positively associated with TD (OR= 1.09, [confidence interval not reported], $p<0.01$), after adjusting for antipsychotic exposure (69).

Several explanations for the association between age and TD have been proposed. Age-related neuronal damage, degeneration (82), and reduction of dopamine receptors in the brains (83) may be responsible for the age-TD relationship. But these explanations are speculative. Some investigators have proposed that the increased risk of advanced age on TD may be confounded by a higher baseline prevalence of spontaneous movement disorder among aged participants, i.e. participants aged greater than 65 years. As baseline spontaneous dyskinesia may mimic the development of TD, the TD incidence among elderly may be overestimated. In addition, elderly and chronic patients with schizophrenia are more likely to have a higher cumulative antipsychotic exposure than young patients with schizophrenia.

Therefore, observed associations between age and TD may also be confounded by increasing antipsychotic exposure among elders (84).

2.2.4.3. Female gender

Studies have observed a higher prevalence of TD among females than males. A meta-analysis of 76 selected studies with a total of 39,187 patients reported a higher TD prevalence among female (26.6%) than among male (21.6%) patients with schizophrenia. This study also found female patients with schizophrenia had a higher prevalence of severe TD and spontaneous dyskinesia than male patients with schizophrenia (51).

However, the association between gender and TD has not been conclusive. For example, several prospective studies observed greater prevalence of TD among women compared to men but this relationship was restricted to elder patients with schizophrenia (60, 77, 85, 86). Other studies have found that men have more severe TD than women (51) among younger patients with schizophrenia.

A biological mechanism explaining the effect of gender on TD is still unclear. Some external factors have been proposed to account for the relatively high TD prevalence among females. Compared to male patients with schizophrenia, female patients with schizophrenia have longer hospitalization, larger dosages of antipsychotics (50) and longer duration of antipsychotic treatment (87). All of these factors could confound the association between gender and TD.

2.2.4.4. African-American ethnicity

Race was once thought to be a risk factor to TD. Morgenstern et al. reported that the TD incidence rate among African-Americans was nearly two times that among non-Hispanic Caucasians (43). Lacro et al. also reported a higher TD incidence rate among African-Americans than among Caucasians (88). However, there has been disagreement about whether the observed ethnic effect is confounded by treatment-related factors, such as differences in dosage or types of antipsychotic use across ethnicity(43). A study of 700 patients with schizophrenia found that African-American participants were less likely than White participants to receive first-line antipsychotics, supporting the possible confounding role of medications in the relationship between race and TD (5). In addition, a biological mechanism explaining the association between race and TD has not been established.

2.2.4.5. Substance abuse

Abuse of alcohol and of cigarettes has both been reported to increase the risk of TD. Studies have observed a higher prevalence of TD among subjects with alcohol abuse histories. The association between alcohol abuse and TD has been replicated in several studies (86, 89-93). In the analysis from the CATIE data, substance abuse was associated with baseline TD (adjusted OR= 1.66, 95%C.I.= 1.2~ 2.3, p=0.0032) (94). The mechanism of this association is not understood completely. It is possible that ethanol alters neurotransmitter activity or increases neurological insults after repeated exposure. Yassa et al. reported that smoking was positively associated with TD among antipsychotic-treated patients. This association may be explained by an increase of dopamine released from nigrostriatal neurons after nicotine stimulation

(95).

2.2.4.6. Anticholinergic use

Anticholinergics comprise a class of medication that selectively blocks the binding of the neurotransmitter acetylcholine to its receptors and is used to treat a variety of disorders, including parkinsonism, gastrointestinal cramps, asthma and urinary bladder spasm (96). Anticholinergics are also a major treatment for essential Parkinson's Disease, a slowly progressive neurological disorder characterized by resting tremor, shuffling gait, stooped posture, rolling motion of fingers and drooling (97, 98).

Concomitant use of anticholinergics has been reported to be a risk factor for TD. In the CATIE, concomitant anticholinergic use was 28% and 14% among patients with schizophrenia with and without TD, respectively (94). Some studies also have noted that addition of anticholinergics can exacerbate existing TD (99, 100) and discontinuation of anticholiergics could improve TD symptoms. A biological explanation for the effect of anticholinergics on TD have been suggested by animal models, which show that long-term administration of anticholinergics can induce a supersensitivity of dopamine receptors. This increased sensitivity may in turn cause the symptoms associated with TD.

However, the association between anticholinergics and TD may be confounded by the indication of anticholinergics, particularly parkinsonism (50). A study found that the incidence rate of TD was 40% and 12% among elderly patients with and without parkinsonism, respectively (77). Thus, it is not clear whether the vulnerability to TD

observed among patients taking anticholinergics is confounded by the indication of anticholinergics, i.e. treating patients with higher risk of movement disorder, or if it is anticholinergics which lead to a higher risk of TD.

2.2.4.7. Psychiatric disorders

Psychiatric disorders and in particular, affective disorder and schizophrenia with negative symptoms, have been proposed as risk factors for TD (101-103). This association has been found independent of antipsychotic use. Studies report that approximately 7% of antipsychotic-naïve patients with schizophrenia present with movement disorders at onset of their illness (104, 105). However, other risk factors aside from antipsychotics use may confound the association between TD and psychiatric disorders. For example, in cross-sectional studies, it may be difficult to differentiate between TD symptoms and other spontaneous movement disorders that are concomitant to psychiatric illness (33). As a result, the observed association between psychiatric disorder and TD could be due to misdiagnosis of TD among subjects with other movement disorders.

In addition to the possibility of misdiagnosis of TD, detection bias could occur when TD is not evaluated blindly to medication history. It is widely known that TD occurs more frequently in subjects using CONV than in subjects using ATY or non-antipsychotics. When a patient has a treatment history of CONV, physicians may be predisposed to diagnose any movement disorder as TD. As a result, when evaluation of TD symptoms is not blinded to patients' history of antipsychotic use, a detection bias may occur.

2.2.4.8. Summary of non-genetic risk factors for TD

Overall, several non-genetic risk factors for TD have been proposed, including exposure of CONV, increased age, female gender, African-American race, anticholinergic use, substance abuse, psychiatric disorders. ATY have a lower risk of TD than CONV. However, the relationship between longer duration of antipsychotics exposure and higher risk of TD is not so robust. Increased age also increases the risk of TD but this relationship may be confounded by a higher incidence of other spontaneous movements, rather than TD, or higher cumulative antipsychotic exposure among elder populations than among younger populations. Female gender has a higher risk of TD but this relationship has been inconclusive. African-Americans were suspected to be more susceptible to TD than Caucasians but biological mechanisms for this association have not been established. A history of substance abuse showed an increased risk of TD. Anticholinergic use is positively associated with TD. Although this association has been supported by animal models, the relationship may be confounded by the indication of anticholinergics. Psychiatric disorders also increased the risk of TD. However, more research is needed to eliminate potential biases resulting from difficulties of differentiating TD and other movement disorders in schizophrenia progress.

3. Evidence indicating an association between genetics and TD

A genetic basis for TD has been suggested by the results of both animal and human studies. Evidence from each type of study was addressed below:

3.1. Animal studies

In animal studies in which rats were exposed to antipsychotics, there was significant variation in the onset of vacuous chewing movements and repetitive jaw movements across different genetic strains of rats (106, 107).

3.2. Human studies

In humans, individual variation in the susceptibility to adverse effects, particular TD, is considerable. While previous studies have identified several non-genetic factors associated with an increased risk of TD, these factors can only explain a small proportion of the variance in the occurrence of TD (108).

3.2.1. Family aggregation

Reports of TD aggregated within families indicate that genetic disposition has an important role in TD (109-113). For example, Schulze et al. reported 39 out of 222 schizophrenic or schizoaffective patients with TD had at least one first-degree relative affected TD (113). Yassa et al. surveyed 500 inpatients taking long-term antipsychotics and found a concordance of the presence or absence of TD among eight patients and their first degree relatives (111). Youssef et al. studied 11 relative pairs with chronic schizophrenia. This study reported a complete familial concordance of presence or absence of TD in the following relationships: brother-sister (5 pairs), father-son (3 pairs), brother-brother (2 pairs), and mother-daughter (1 pairs) (112). In addition, other extrapyrimidal disorders, such as

Parkinson's disease (114) and dystonia (115), provide indirect evidence for genetic components in abnormal movement disorder. Together, these studies suggest a role of genetic factors in schizophrenic patients' susceptibility to TD.

3.2.2. Twin studies

No twin study conducted on this issue has been found.

3.2.3. Adoption studies

No adoption study conducted on this issue has been found.

3.2.4. Linkage studies

No linkage study conducted on this issue has been found.

4. Dopamine receptor genes as the candidate genes in this study

4.1. Overview

Selection of candidate genes from the large number of possible genes in the human genome has been a fundamental source of difficulty in studies that attempt to identify genetic variants associated with susceptibility to complex phenotypes, such as TD (116). A reasonable approach to select candidate genes associated with TD is to consider the pharmacological mechanisms of antipsychotics as most treatment-related side effects are the result of medications acting upon unintended mechanisms. As a result, the present study aimed to study genes coding for dopamine receptors, most acknowledged drug targets of antipsychotic medications.

All subtypes of dopamine receptor genes were included: dopamine receptors 1 to 5 (*DRD1, DRD2, DRD3, DRD4, DRD5*). Choice of these dopamine receptor genes are informed by our current understanding of the drug targets of antipsychotics.

4.2. Dopamine receptors has been proposed as the drug targets of antipsychotics

Although antipsychotics provide marked reductions in psychotic symptoms, the precise mechanism of action has not been fully understood. The different risks of TD between conventional and ATY has led to a predominant hypothesis: the antipsychotic effect is mediated through the blockade of dopamine D_2 receptor (*DRD2*) in the brain. Since the presence of serotonin can theoretically result in the inhibition of dopamine release in the nigrostriatal pathway and impact on the control of human movement, studies have proposed that the anti-schizophrenic effects of antipsychotics are mediated through the combined effect of dopamine D2 receptor (*DRD2*) and serotonin receptor (*HTR-2A*). However, several investigative trials have reported that several selective serotonin antagonists, such as ritanserin and M100906, are not efficacious for anti-schizophrenic purpose. Thus, dopamine receptors have been dominantly recognized as the drug targets for antipsychotics.

This study consulted the Psychoactive Drug Screening Program (PDSP) K_i database (http://pdsp.cwru.edu/pdsp.php) to obtain the receptor binding affinities (K_i) for the six antipsychotic medications evaluated in CATIE phase 1 and phase 2. It should be noted that $K_i \leq 100$ nM, i.e., $\log_{10}(K_i) \leq 2$, indicates physiologically significant receptor binding between an agent and a target. Consistent with the Food and Drug Administration (FDA)'s clinical trials data

(http://www.accessdata.fda.gov/scripts/cder/drugsatfda/), the genes *DRD2* and *DRD3* demonstrate the greatest potential to mediate the effect of these six drugs. Medication-receptor binding affinities are summarized in Table 2.2.

4.3. Associations between TD and dopamine receptor genes has been inconclusive

Several association studies have been publishe reporting the relationship between dopaminergic receptor genes and TD among schizophrenic patients, but results of these studies are inconclusive. Almost all of these studies used the AIMS scale and followed the Schooler-Kane's criteria for TD. In addition, the study populations were predominantly comprised of patients with chronic schizophrenia with a mean age ranging from 30 to 55 years old. However, the studies also differed in many respects including the number of TD measurements among participants, the genetic variants of dopamine receptor genes selected for study, and the distribution of study populations' demographic characteristics such as gender and ethnicity. The characteristics of studies that investigated association between TD and dopamine receptor genes are summarized in Table 2.3.

4.3.1. Dopamine receptor 1 (*DRD1*)

Literature about the association between *DRD1* and TD are limited but animal models support a role of *DRD1* in oral TD symptoms. For example, one study administered male rats the conventional antipsychotic, fluphenazine, to trigger a syndrome of vacuous chewing movements. These symptoms were successfully suppressed using a selective dopamine D1 receptor antagonist. In addition,

experiments also showed that the chewing disorders can also be triggered by acute administration of a selective dopamine D1 antagonist among drug-naïve animals. This evidence indicates that *DRD1* may play a role in TD development (117).

A 2006 study consisting of 297 patients with schizophrenia (86 with TD, 211 without TD) studied 5 markers of *DRD1* to investigate the associations between polymorphisms in *DRD1* and TD. However, none of investigated variations in *DRD1* gene showed a statistically significant association with TD (118).

4.3.2. Dopamine receptor 2 (*DRD2*)

Many studies (median of the sample sizes: 249) have investigated the effect of several genetic variants on *DRD2* but their associations with TD are inconclusive.

For example, Ser311Cys, the most studied SNP on *DRD2*, has been reported to be both positively and negatively associated with TD across different studies. However, none of the findings from these studies reached statistical significance (69, 71, 80, 118-120). Specifically, one study of 196 Japanese patients with schizophrenia reported an increased risk of TD among those with Ser311Cys genotype (adjusted $OR_{gly/gly+\ ser/gly\ vs.\ ser/ser}$=1.2, p=0.48) (69). In contrast, a study of 419 white and 89 African-American patients with schizophrenia reported an inverse association between TD and Ser311Cys genotype in the univariate analysis ($OR_{ser/gly\ vs.\ ser/ser}$= 0.46, 95% C.I.= 0.13- 1.6, P=0.21). It is important to note that none of study subjects had gly/gly genotype in this study and adjusted $OR_{ser/gly\ vs.\ ser/ser}$ was not presented in the paper (119).

Chen et al reported a marginal association between *Taq*I A genotype and TD

(p=0.03). This same study reported that homogeneous mutant *Taq*IA genotype was associated with TD among females (62% in TD and 24% in non-TD, p=0.001) (121). However, this association was not replicated in later studies with larger sample sizes (69, 80, 118, 120). Studies that investigated associations between other markers on *DRD2* and TD are summarized in Table 2.3.

4.3.3. Dopamine receptor 3 (*DRD3*)

The majority of research to assess the role of dopamine receptor genes in TD has focused on the marker Ser9Gly on *DRD3*, but results from these studies have been inconclusive. Several previous studies reported a positive association between TD and genotypes of Ser9Gly, i.e. Ser/Ser, Ser/Gly, and Gly/Gly (70, 79, 122-124) but this relationship was not replicated in many recent large-scale studies (71, 78, 118, 119, 125-128). Other studies have reported that patients with schizophrenia carrying the Gly/Gly polymorphism have a mild but significant increase for TD risk compared to other Ser9Gly genotypes (70, 108, 122-124). However, Liao et al's study reported that the mean AIMS score among patients with schizophrenia carrying Ser/Gly was 3.6, which is about twice the mean AIMS score among patients with schizophrenia carrying other genotypes in their study (79). In Segman et al's study, the TD group had a larger proportion of Ser/Gly genotypes than the non-TD group (122).

A 2002 "combined-analysis" of eight studies indicated an association between Ser9Gly and both binary TD status and AIMS-measured severity. This study pooled a sample of 780 subjects with schizophrenia or affective disorder (317 with TD and 463 without TD) from six research centers. After controlling for age and gender, two

associations reached statistical significance: TD and genotypes of Ser9Gly (X^2= 7.51, degree of freedom=2, p= 0.002); TD and the allele frequency of Ser9Gly, i.e. Ser and Gly (X^2= 5.02, degree of freedom = 1, p= 0.02). This combined analysis indicated a positive association between Gly/Gly genotype and a higher AIMS score compared to Ser/Gly (p=0.006) or Ser/Ser (p< 0.001) (108).

A 2006 meta-analysis, combining 12 studies with 1610 total subjects (695 patients with TD and 915 without TD), indicated the Gly allele was only mildly associated with TD as compared to the Ser allele (OR=1.17, 95% C.I. = 1.01- 1.37). However, this study reported a publication bias in allele analyses (bias coefficient= -1.82, 95% C.I. = -3.61 - -0.04, p= 0.046). No association was found between genotype and TD and this finding was not confounded by publication bias (129).

4.3.4. Dopamine receptor 4 (*DRD4*)

Two human studies have investigated the association between *DRD4* and TD but the findings on the association from these two studies are inconclusive. An early study done in Israel, consisting of 122 patients with schizophrenia (59 with TD and 63 without TD), reported no association between genetic variants on *DRD4* and TD (80). However, a recent study of 297 North Indian patients with schizophrenia (86 with TD, 211 non-TD) found a statistically significant association between TD and 120bp dup-T-repeat 3, a haplotype composition on *DRD4* (p<0.01) (118).

4.3.5. Dopamine receptor 5 (*DRD5*)

Studies assessing the association between *DRD5* and TD are absent from the

published literature.

5. Other candidate genes for future gene-TD association studies

Although increased dopamine sensitivity has been a dominant hypothesis for TD pathophysiology, this hypothesis can only explains some aspects of TD. Hypotheses regarding pathophysiological models of other neurotransmitters affected by antipsychotics have been proposed, including changes in acetylcholine and γ-aminobutyric acid (GABA). For example, studies suggested a reduced activity of GABA neurons as the basis of TD based on evidence from animals and patients with schizophrenia (130). In addition, a reduction of glutamic acid decarboxylase (GAD), a rate-limiting enzyme in the synthesis process of GABA, has been observed in monkeys following long-term treatment with antipsychotics (131) and among five TD patients with schizophrenia (132).

Another hypothesis of TD pathophysiology is through oxidative stress. The long-term administration of antipsychotic blocks dopamine receptors, leading to an increased dopamine turn over rate and thus to generate free radicals (133). The blockade of dopamine receptors also increase the release of glutamate and aspirate in the striatum, leading to oxidative damage to cellular proteins, cell membrane and DNA. As a result, several oxidative stress-related genes, such as manganese superoxide dismutase (MnSOD) and N-methyl-D-aspartate (NMDA) receptor genes, have been proposed as potential candidate genes for TD (134). Information about potential candidate genes for TD association studies is summarized in Table 2.4. The proposed study will focus on all dopamine receptor genes. Skills and perspectives

obtained from proposed association studies between TD and all dopamine receptor genes can be applied to these other candidate genes in the near future.

6. Essential information about the parent study of study aims 2 and 3

6.1. Overview

Briefly, the CATIE study was a double-blinded randomized clinical trial with 18-month follow-up. The purpose of the CATIE trial was to evaluate the effectiveness of antipsychotics among a heterogeneous group of schizophrenic patients living in the community. The CATIE recruited 1,493 patients with chronic schizophrenia from various sites including public mental health centers, academic hospitals, Veterans' Affairs hospitals, and managed care centers. As opposed to most trials, CATIE included schizophrenic patients with substance abuse and medical comorbidities so that participants in CATIE would more accurately reflect community populations of schizophrenic patients.

6.2. Source of the CATIE population

Detailed inclusion and exclusion criteria have been described in a previous report (135). A summary of the recruitment guidelines in CATIE is described below:

Participants with the following criteria were approached for enrollment:

- age from 18-65 years old
- schizophrenia diagnosed using Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition (DSM-IV).

- appropriate candidate for oral antipsychotic treatment based on participants' judgment in consultation with their physicians.
- decisional capacity to participate in the CATIE program
- informed consent provided

Participants with the following criteria were excluded:

- a DSM-IV diagnosis of schizoaffective disorder, mental retardation, pervasive developmental disorder, delirium, dementia, amnesia or other cognitive disorder.
- well-documented serious adverse reaction, history of failure of response or contradiction to any one of the proposed treatment arms.
- first episode of schizophrenia. Patients who have first begun antipsychotic treatment within the previous 12 months and have had psychotic symptoms for less than 3 years were considered as being in their first episode.
- concomitant use of any investigational drug within 30 days of the baseline visit.
- Women who were pregnant or breastfeeding.
- cardiac comorbidity history, including recent myocardial infarction (<6 months), QTc prolongation, sustained cardiac arrhythmia, uncompensated congestive heart failure, complete left bundle branch block and first-degree heart block with RR interval ≥ 0.22 seconds.

6.3. Design of the CATIE trial

A schematic diagram of CATIE is illustrated in Figure 2.3 of the article by Stroup

et al.(135). In phase I, participants were randomly assigned to one of the investigated antipsychotics. If the assigned antipsychotic treatment on phase I failed, participants would enter phase II to receive another atypical antipsychotic.

The antipsychotic intervention in CATIE consisted of the following ATY: olanzapine, quetiapine, risperidone, ziprasidone, clozapine, and aripiprazole. The control group received perphenazine, a mid-potent conventional antipsychotic treatment. All antipsychotics except clozapine were administered in a double-blind fashion. All CATIE subjects received antipsychotics through the trial. In addition to antipsychotic use, the CATIE study also collected data about treatment responses and adverse events, such as TD, in several regular visits during the trial.

7. Justification for not studying metabolizing enzyme genes in this study

Activities of metabolizing enzymes could affect the duration and concentration of medication in the human body. Thus, metabolizing enzyme genes have been important candidate genes in pharmacogenetic studies in the past several years.

However, it is inappropriate to study drug metabolizing enzyme genes in the CATIE data for two reasons. First, in the CATIE trial, seven antipsychotics were studied across 3 treatment phases to accommodate occurrences of treatment failure in assigned antipsychotics. As the proposed study is limited by inadequate statistical power to investigate genetic effects within individual antipsychotic regimens, grouping antipsychotic exposure into “conventional” and “atypical” groups could increase the statistical power. When studying dopamine receptor genes, this grouping strategy is appropriate because the pharmacological classification of antipsychotics

corresponds to their different binding affinities to dopamine receptors. However, classifying antipsychotic exposure into conventional and atypical classes would be inappropriate in a study that investigates associations between TD and drug metabolizing enzyme genes because each antipsychotic has its own unique metabolizing pathway (Table 2.5), which does not follow the pharmacological classification.

Second, drug metabolizing enzymes compete for drugs and other environmental hazards that need to be metabolized and eliminated from the body, particularly alcohol and cigarette consumption. As the CATIE trial only collects a broad indicator of substance use, i.e. user or non-user of alcohol or cigarettes in the past five years, this indicator is too blunt to be useful in controlling for substance use as a confounder. Thus, in order to improve the validity and statistical power in the proposed study, investigating drug receptor genes are more appropriate than studying metabolizing enzyme genes in exploring genetic influence on TD.

8. Tables

Table 2.1 Comparisons of tardive dyskinesia (TD) risk and pharmacokinetics of antipsychotics studied in the CATIE trial.

Antipsychotic	Risk of TD (%)	Relative potency (mg)	Initial Dose (mg/d)	Dose Range (mg/d)	Max Dose	Freq. of dosing (per day)	Elimination	Major route of Metabolism
Clozapine	~ 0 (54-57)	50	25-50	300-600	900	Once-twice	12	CYP1A2, 3A4, 2E1
Olanzapine	0.52 (27)	4	5-10	15-30	40	Once	30	CYP1A2 Glucuronidation
Quetiapine	0.7- 2.7 (68)	80	25-50	300-800	1000	Twice	6-7	CYP3A4
Risperidone	0.23- 5 (63, 68)	1	2	2-6	8	Once	20	CYP2D6, 3A4
Ziprasidone	---	20	40	80-160	160	Twice	7	CYP3A4 Aldehyde oxidase
Aripiprazole	---	6	10-15	10-15	30	Once	75	CYP2D6, 3A4
Perphenazine	---	8	8-18	8-64	64	Twice	9	CYP2D6

Table 2.2 List of candidate genes for strength of binding affinity to investigated antipsychotics in the CATIE study in the human model

		CATIE Phase 1 & 2 Medications					
Gene	**Description**	C	O	P	Q	R	Z
Receptor Binding Targets		Binding affinity in $\log_{10}(K_i$ in nM)					
DRD1	Dopamine receptor 1	2.2	1.5	--	3.1	2.7	2.2 [a]
DRD2	Dopamine receptor 2	1.7	1.1[b]	-0.8	1.9	0.0	0.7 [b]
DRD3	Dopamine receptor 3	2.5	1.6	-0.9	2.7	1.0	0.8 (0.9 [b])
DRD4	Dopamine receptor 4	1.4	1.0	1.2	3.4	0.5	-0.1
DRD5	Dopamine receptor 5	2.4	1.9	--	3.2	2.8	--

C=clozapine, O=olanzapine, P=perphenazine, Q=quetiapine, R=respiridone, and Z=ziprazidone.
"--"=no data. "a"=no human data. "b" = also documented in FDA approved labeling.

Table 2.3 Association studies between tardive dyskinesia (TD) and genetic variants in dopamine receptor genes

Genetic variants	Rating scale	Repeat rating ?	Mean age (SD) (TD-Y/ TD-N)	Female (%) (TD-Y/ TD-N)	Ethnicity (country)	Sample size TD-Y	Sample size TD-N	Main findings: From categorical analysis	Main findings: From continuous analysis	Ref
I. Studies that investigated dopamine receptor 2 (DRD2)										
-241A>G -141Cins/del *Tag*I B *Tag*I D $Val_{99}Ala$ $Leu_{141}Leu$ $Pro_{310}Ser$ $Ser_{311}Cys$ *Taq*I A	AIMS	2-4 days 12-16 d 26-30d (acute schizophrenics)	38.3 (12) (18~70)	M: 54 F: 46	Caucasian (Germany)	Total n = 584 (2-4 days) = 518 (12-16 days) = 384 (26-30 days)			NO significant association between mean AIMS score and any DRD2 genotype was identified no matter the effect was evaluated before or after adjusting for covariates (i.e. age, gender, chlorpromazine adjusted dose, dose of anticholinergic agents, no. of recurrent exacerbations and smoking). Correlations between AIMS-score and age was 0.3 and 0.2 for AIMS evaluated in 2-4 days and 12-30 days, respectively. Correlations between AIMS-score and Sex are non-significant.	Kaiser *et al.* 2002
-141Cins/del $Ser_{311}Cys$ *Taq*I A	AIMS w S-K criteria	No (chronic schizophrenics)	55 (9.5)	M: 52.5 F: 47.5	Asian (Japan)	44	156	No. of Ser/Ser, Ser/Cys, Cys/Cys = 40, 4, 0 (cases); = 145, 10, 1 (controls)	The association b/w -141C ins/del and total AIMS is significant before adjusting for covariates (p=0.037)	Hori *et al.* 2001

								(Fisher's exact test, p= 0.622). No significant association between allelic and genotypic distribution and TD status. This study provided adjusted OR for each genotype but did not show how genotype is compared within each marker: $Ser_{311}Cys$: OR=1.22 (p= 0.48); -141Cins/del: OR=0.69 (p= 0.28); *Taq*I A: OR= 1.55 (p= 0.43) Age(years): OR= 1.09 (p<0.01)	but not significant after covariates adjustment (p=0.14). Ps. Covariates in this study included age, gender, duration of illness, and antipsychotic use.	
-141Cins/del	AIMS w S-K criteria	Valid by videotype (chronic schizophrenics)	62 (21-82) vs. 55 (28-78)		Asian (Japan)	31	108	No. of Del/Del, Del/Ins, Ins/Ins = 1,12, 18 (case); = 0, 32, 76 (control) (Fisher's exact test: p= 0.121) No associations between TD status and the -141 Del/Ins genotype frequency was found Ps. This study did not adjust for confounding variables.		Inada *et al.* 1999
*Taq*I A	AIMS w S-K criteria	N (chronic schizophrenics)	43 vs. 42	45.2 vs. 48.8	Asian (Taiwan)	93	84	Marginal significance b/w genotype distributions and TD stauts (X^2 = 6.8, p= 0.03). Among female, excess A_2A_2 proportion was associated (62% in TD, 24% in non-TD, p		Chen *et al.* 1997

								= 0.001). Ps. Matched case-control design by age, duration of illness and current antipsychotic dosage.		

II. Studies that investigated Dopamine receptor 3 (DRD3)

Ser9Gly Val66Met	AIMS w S-K criteria	Y: 3 mo later (chronic schizophrenics)	47.5 (9.8) vs. 46.9(9.5)	40.2 vs. 42.1	Asian (Taiwan)	102	114	No. of Ser/Ser, Ser/Gly, Gly/Gly = 51, 41, 10 (cases); = 61, 41, 13 (controls) (Wald = 0.843, *p*= 0.656, df=2) No sig. asso. before and after adjusting for dosage, duration of antipsychotic exposure, smoking. Ps. This study reported some factors are significantly associated with TD, including "Duration of antipsychotic exposure" (p= 0.024); "mean daily drug dosage" (p= <0.001)	Ser9Gly was not significantly associated with total AIMS score (p=0.080), score on orofacial regions (p=0.957), and on limb-trunk regions (p=0.312). Ps. The conclusion was obtained from a ANCOVA analysis adjusting for age but only in TD group?!	Liou *et al.* 2004
Ser9Gly	AIMS w S-K criteria	Y: 4 mo later (chronic schizophrenics)	56.1 (10.4) vs.55.1 (7.3)	0:0 (all are male)	Asian (China)	42	52	No. of Ser/Ser, Ser/Gly, Gly/Gly = 19, 22, 1 (cases); = 30, 17, 5 (controls) (Fishers' test: p= 0.098) No findings reached statistical sig. No regression analysis which adjusted confounding		Zhang *et al.* 2003

								effects.		
Ser9Gly	AIMS w S-K criteria	Not clear (chronic schizophrenics)	42.3 (10.7) vs.38.3 (8.8)	25.4 vs. 22.2	Asian (Korean)	59	54	No. of Ser/Ser, Ser/Gly, Gly/Gly = 25, 28, 6 (cases); = 21, 33, 0 (controls) (X^2= 0.288, Fishers' test: p= 0.028) Gly/Gly was positively associated w TD No regression analysis which adjusted confounding effects. Other significant factors with TD: Age (years) (*p*= 0.038)	The mean (SD) AIMS score in each genotypic group was: 13.8 (9.3) for Ser/Ser, 18.0 (8.9) for Ser/Gly and 9.7 (4.6) for Gly/Gly group. But this study only compared the mean AIMS score among TD group. No significant difference b/w the three classes by ANOVA (p= 0.071, d.f.=2)	Woo *et al.* 2002
Ser9Gly	AIMS w S-K criteria	Not clear (chronic schizophrenics)	57.2 (12.3) vs. 45.6 (10.5)	38.5 vs. 31.8	Asian (Hong Kong)	65	66	No. of Ser/Ser, Ser/Gly, Gly/Gly = 36, 23, 6 (cases); = 42, 18, 6 (controls) (X^2= 1.064, df=2, p= 0.588) Regression analysis was done but no any result was mentioned in the text or shown by tables. Non-genetic risk factors for TD identified in this study include: Age (years) (*p*= <0.0001); Duration of illness (p= 0.047)		Garcia-Barcelo *et al.* 2001
Ser9Gly	AIMS w 6 or	Not clear (chronic	40.7 (9.3)	M: 62.6 F: 37.4	Asian (Taiwan)	21	94	No. of Ser/Ser, Ser/Gly, Gly/Gly	The mean (SD) AIMS score in each genotypic	Liao *et al.*

	above as the cut-off point for TD	schizoph renics)	(18~ 65)					= 6, 14, 1 (cases); = 55, 29, 10 (controls) (X^2= 9.41, df=2, p=0.009) Multiple regression analysis indicated age (p=0.009) and DRD3 genotypes (p=0.01) as risk factors for TD.	group was: 1.9 (6.3) for Ser/Ser, 3.6 (5.8) for Ser/Gly and 1.7 (5.4) for Gly/Gly group. The AIMS score was higher among patients carrying Ser/Gly than other genotype (p= 0.014).	2001
Ser9Gly	TDRS w S-K criteria	Y: 3 mo (chronic schizoph renics)	43.9 (8.7) vs. 42.2 (7.9)	M: 48 F: 52	Caucasian (Germany)	79	78	No. of Ser/Ser, Ser/Gly, Gly/Gly = 39, 37, 3 (cases); = 37, 35, 6 (controls) (OR: 0.47 (95% CI= 0.11- 2.0, p=0.328) Stratification by duration of psychotic illness but no trend observed. Data analyses in this study were not very appropriate. For example: no regression analysis which adjusted for confounding effects.		Rietsch el *et al.* 2000
Ser9Gly	AIMS w 4 or above as the cut-off point for TD	Not clear (chronic schizoph renics)	53 (18) vs. 41 (12)	M: 73 F: 27	Caucasian (UK)	32	39	No. of Ser/Ser, Ser/Gly, Gly/Gly = 11, 14, 7 (cases); = 17, 18, 4 (controls) (Fisher-Freeman-Halton test, *p*= 0.37) Allele frequency: (Gly vs.Ser) = 44% vs. 56% (case) = 33% vs, 67% (control) (OR_{gly} = 1.56, 95% C.I.=		Lovlie *et al.* 2000

								0.74-3.26, *p*= 0.23)		
Ser9Gly	AIMS w S-K criteria	N (chronic schizophrenics)	52.1 (11.6) vs. 49.6 (10.7)	47.2 vs. 46.0	Jewish (Israel)	53	63	No. of Ser/Ser, Ser/Gly, Gly/Gly = 13, 37, 3 (cases); = 29, 29, 5 (controls) (Fisher's exact test: p=0.032) TD was associated with the genotype of Ser9Gly Allele frequency: (Gly vs.Ser) = 41% vs. 59% (case) = 31% vs. 69% (control) (X^2= 2.4, df=1, p> 0.1) Multiple regression showed OR ser/gly+gly/gly was 1.16 (*p*= 0.006) and OR(age at first antipsychotic treatment) = 1.0 (p = 0.01). Overall r^2 of the model is only 0.12. Ps. This is a matched case-control study, matching on age, sex, duration of illness, antipsychotic dosage et al.	Positive association between Association between total AIMS and ser/gly+gly/gly genotypes was identified (p=0.02). When looking at AIMS by body regions, observed positive associations between regional AIMS score and ser/gly+gly/gly genotypes still held. Non-genetic risk factors for higher AIMS score identified: Age at first antipsychotic treatment (*p*= 0.01)	Segman *et al.* 1999
Ser9Gly	AIMS or Simpson Dyskinesia scale	N (chronic schizophrenics)	32.9 (9.6) (16~ 58)	M: 72.4 F: 27.6	Caucasian: 85 (76%) African A: 25 (22%) Asian: 2 (2%) (USA)	N/A	N/A		Mean AIMS score for African Americans (10.7, SD= 12.2) was higher than Caucasians (4.7, SD= 6.6) and Asians (5.4. SD= 8.0). Patients w Gly/Gly genotypes had higher AIMS score in both	Basile *et al.* 1999

								Caucasians (n= 85, F[2, 75]= 3.85, p= 0.026) and African Americans (n= 25, F[1, 23]= 8.10, p= 0.009)	
Ser9Gly	AIMS w S-K criteria	N for cross-section cases Y for longitudinal cases (chronic schizophrenics)	M: 50(14) F: 57 (16)	M: 54 F: 44	(Scotland)	51	49	In cross-sectional TD cases: No. of Ser/Ser, Ser/Gly, Gly/Gly = 23, 17, 11 (cases); = 28, 19, 2 (controls) OR= 6.46 (95%CI=1.28-62.38, p=0.018) Allele frequency: (Gly vs. Ser) = 38% vs. 62% (case) = 23% vs. 77% (control) OR= 2.02 (CI= 1.05- 3.93, p= 0.035). In TD cases identified by longitudinal assessment (3 times): No. of Ser/Ser, Ser/Gly, Gly/Gly = 10, 9,6 (TD-developed/persistent); = 24, 23, 3 (TD-never/fluctuating) OR= 4.95 (CI: 0.92- 32.92, p=0.066) Allele frequency: (Gly vs. Ser) = 42% vs. 58% in TD-developed/persistent group; = 29% vs. 71% (control) OR= 1.77 (CI= 0.82-3.81, p=	Steen, 1997

								0.16).		

III. Studies that investigated multiple genes including dopamine receptor genes

DRD1 (rs5330, rs5331, rs 13306309, rs686, -48 A>G) DRD2 (-141ins/del C; G>A 1kb upstream from exon 8; Ser311Cys; T>C 10kb downstream from exon 8) DRD3 (rs324026, rs6280, rs1503670, rs905568, intron 3 of ZnF80) DRD4 (120bp duplication, 1.2kb upstream from initiation	AIMS w S-K criteria	N (chronic schizoph renics)	34.5 (12.6) vs. 31.4 (10.2)	38.5 vs. 48.5	Asian (India)	86	211	This study examined 24 markers on DRD1-DRD4, DAT and COMT. However, only markers that showed sig. association were reported. They were 120bp duplication on DRD4, 408 C>G and 472 G>A on COMT. No. of 549/549, 549/429, 429/429 = 35, 44, 7 (cases); = 120, 68, 23 (controls) (X^2= 9.29, df=2, p=0.009) (allele freq: X^2= 2.67, df=1, p=0.1) Among participants who had all the markers on the haplotype been genotyped, the proportion of the haplotype on DRD4 (120 bp dup-T-repeat 3) was 0.31 and 0.36 on TD and non-TD group, respectively (p=0.00, not typo!). When counting the proportion of all individuals genotyped in this study, proportion of the haplotype on DRD4 (120 bp dup-T-repeat 3) was 0.41 and	No any association was found in following analysis by linear and logistic regressions.	Srivast ava *et al.* 2006

codon, -521 C>T, 48bp VNTR in exon 3.)								0.27 on TD and non-TD group, respectively.	
DRD2 (Ser311Cys, -141C del) DRD3 (Ser9Gly) CYP2D6 CYP3A5 PgP GSTM1 GSTT1	AIMS w S-K criteria	Not clear (chronic schizophrenics)	42.4 (12.8)	M: 53 F: 47	White: N= 419 (81.2%) African A: N= 89 (17.2%) (USA)	162	354	No data about the distribution of patients' demographics, allele and genotype. DRD2 and DRD3 were not selected into final models. So, only results from univariate reg. were presented in the paper: Ser311Cys (in DRD2): OR= 0.46, (95%CI=0.13-1.6, p= 0.21) -141 Del (in DRD2): $OR_{wt/wt\ vs.\ others}$= 0.9 (95%CI= 0.6-1.5) Ser9Gly (in DRD3): $OR_{wt/m\ vs.\ wt/wt}$= 1.0 (95%CI=0.68-1.5) $OR_{m/m\ vs\ wt/m}$= 0.81 (95%CI=0.46- 1.4) Other non-genetic risk factors for TD were identified in multivariate regressions: Age> 45: OR= 2.0 (95%CI=1.3-3.0, *p*=0.002) Female sex: OR= 1.5 (95%CI=1.0-2.3, *p*= 0.04) Taking typical anticholinergic	Leon, 2005

							> 5 years: OR= 2.4 (95%CI=1.4- 3.9, *p*=0.001) Taking anticholinergic: OR=2.0 (95%CI=1.2-3.4, *p*= 0.008) No antipsychotic exposure: OR= 0.25 (95%CI=0.07- 0.87, *p*=0.02)		
DRD2 (Ser311Cys) DRD3 (Ser9Gly)	AIMS w S-K criteria	N (not clearly specified. Probably chronic schizophrenics	M: 85 F: 232	Asian (Singapore)	117	200	No. of Ser/Ser, Ser/Gly, Gly/Gly = 60, 46, 11(cases); = 89, 88, 23(controls) (X^2 = 1.409, df= 2, p= 0.495). Allele frequency: (Gly vs. Ser) = 29% vs. 71% (case) = 34% vs. 66% (control) No. of Ser/Ser, Ser/Cys,Cys /Cys = 19, 52, 46(cases); = 42, 92, 66(controls) (X^2 = 1.742, df= 2, p=0.419) Allele frequency: (Cys vs. Ser) = 62% vs. 38% (case) = 56% vs. 44% (control) Risk factors for TD were identified in multivariate regressions: Age (p<0.005) Ser/Ser on DRD3 (p= 0.012)	No significant association between genotypes and total AIMS were found.	Chong et al, 2003

DRD2 (Tap-1 A, -141Cins/del Ser311Cys) DRD4 (exon 3 vntr, promoter 120bp repeat) DAT 5-HT6 5-HTTLPR TPH	AIMS w S-K criteria	N (chronic schizophrenics)	54.3 (13) vs. 50.4 (10)	49.2 vs.47.6	Ashkenazi (57.6% vs. 60.3%) non-Ashkenzai (42.4% vs. 39.7%) (Israel)	59	63	In DRD2: No. of Ser/Ser, Ser/Cys,Cys/Cys = 52, 2, 0 (cases); = 52, 3, 0 (controls) (Fisher's exact test: p=1.000) No association between genotype frequency or allele frequency with TD status was found among all markers investigated in this study. Non-genetic risk factors for TD identified in this study: Cigarette pack years (p= 0.01)		Segman 2003
DRD3 (Ser/Gly) CYP1A2	AIMS	N (chronic schizophrenics)	32.9 (9.6) (16~ 58)	M: 72.4 F: 27.6	Caucasian: 85 (76%) African A: 25 (22%) Asian: 2 (2%) (USA)	N/A	N/A	Nothing here. This study was conducted among same subjects as the article published by Basile et al. in 1999 for the Ser9Gly on DRD3.	Mean AIMS score in each genotype: Ser/Ser: ~ 3.8, n=34 Ser/Gly: ~ 4, n=53 Gly/Gly: ~ 14, n=25 The severity of TD was greater among subjects w Gly/Gly genotype than among subjects with the other two kinds of genotypes.	Ozdemir, 2001
DRD3 (Ser/Gly)	AIMS w S-K criteria	N (chronic schizoph	52.1 (11.6) vs. 49.6	47.2 vs. 46.0	Jewish (Israel)	55	60	The data about DRD3 was published in another article in 1999 by same author. This	This study concluded that $DRD3_{gly}$ and $5\text{-}HT2C_{ser}$ contributed 4.7% and 4.2%,	Segman, 2000

5-HT2C (Cys/Ser)		renics)	(10.7)					study mainly addressed the 5-HT2C$_{ser}$ data added to the same subjects in previous study.	respectively, to the variance in orofacial dyskinesia scores in the AIMS.	
DRD2 (Nco I site) DRD3 (Bal I site)	AIMS w S-K criteria	(chronic schizoph renics)	65 (13) (22~ 89) vs. 57 (10) (34~ 77)	44.9 vs. 55.4	Asian (Japanese)	49	56	No. of A1/A1, A1/A2, A2/A2 on DRD2 = 4, 29, 15 (cases); = 8, 32, 15 (controls) (X^2 = 1.010, df= 2, p=0.604). No. of A1/A1, A1/A2, A2/A2 on DRD3 = 25, 17, 7 (cases); = 33, 19, 4 (controls) (X^2 = 1.573, df= 2, p= 0.455). Non-genetic risk factors for TD identified in this study: Age(years): OR= 1.07 (p<0.01) Sex: OR= 0.43 (p= 0.058) (ps. not clear which gender was the comparison group)		Inada et al. 1997

Table 2.4 Summary of SNP number, pathway and presence of literature of possible candidate genes to TD

Gene name	SNPs no.	Pathway	Medline search*			Chromosome	Product
			Literature	Animal study	Human study		
ACHE	6	acetylcholine	N			7	acetylcholinesterase (Yt blood group)
BCHE	9	acetylcholine	N			3	Butyrylcholinesterase
CHAT	22	acetylcholine	N			10	choline acetyltransferase
CHRM1	10	acetylcholine	N			11	cholinergic receptor, muscarinic 1
CHRM2	31	acetylcholine	N			7	cholinergic receptor, muscarinic 2
CHRM3	55	acetylcholine	N			1	cholinergic receptor, muscarinic 3
CHRM4	2	acetylcholine	N			11	cholinergic receptor, muscarinic 4
CHRM5	11	acetylcholine	N			15	cholinergic receptor, muscarinic 5
CHRNA10	7	acetylcholine	N			11	cholinergic receptor, nicotinic, alpha 10
CHRNA2	16	acetylcholine	N			8	cholinergic receptor, nicotinic, alpha 2 (neuronal)
CHRNA3	4	acetylcholine	N			15	cholinergic receptor, nicotinic, alpha 3
CHRNB3	8	acetylcholine	N			20	cholinergic receptor, nicotinic, alpha 4
CHRNA5	12	acetylcholine	N			15	cholinergic receptor, nicotinic, alpha 5
CHRNA6	4	acetylcholine	N			8	cholinergic receptor, nicotinic, alpha 6
CHRNA7	18	acetylcholine	N			15	cholinergic receptor, nicotinic, alpha 7
CHRNA9	13	acetylcholine	N			4	cholinergic receptor, nicotinic, alpha 9
CHRNB2	10	acetylcholine	N			1	cholinergic receptor, nicotinic, beta 2 (neuronal)
CHRNB3	11	acetylcholine	N			8	cholinergic receptor, nicotinic, beta 3
CHRNB4	8	acetylcholine	N			15	cholinergic receptor, nicotinic, beta 4
SLC18A1	15	acetylcholine	N			8	solute carrier family 18 (vesicular monoamine), member 1
ADORA2A	9	dopamine	N			22	adenosine A2a receptor
DBH	27	dopamine	Y		(136-140)	9	dopamine beta-hydroxylase (dopamine beta-monooxygenase)

DRD1	8	dopamine receptor	Y		(118)	5	dopamine receptor D1
DRD2	25	dopamine receptor	Y		(69, 71, 80, 118-121, 141)	11	dopamine receptor D2
DRD3	17	dopamine receptor	Y		(70, 71, 78, 79, 118, 119, 122-128, 141-143)	3	dopamine receptor D3
DRD4	4	dopamine receptor	Y		(80, 118)	11	dopamine receptor D4
DRD5	3	dopamine receptor	N			4	dopamine receptor D5
RGS9	12	dopamine	Y	(144)		17	regulator of G-protein signalling 9
SLC6A3	19	dopamine	N			5	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3
TH	7	dopamine	Y	(145-147)		11	tyrosine hydroxylase
ACE	19	dopamine Response	Y		(148)	17	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1
COMT	23	dopamine Response	Y		(149-151)	22	catechol-O-methyltransferase
DDC	20	dopamine serotonin	N			7	dopa decarboxylase (aromatic L-amino acid decarboxylase)
MAOA	9	dopamine serotonin	Y		(152, 153)	23	monoamine oxidase A
MAOB	19	dopamine serotonin	Y		(150)	23	monoamine oxidase B
SNAP25	28	dopamine serotonin	N			20	synaptosomal-associated protein, 25kDa
PPP1R1B	3	dopamine serotonin glutamate	Y	(154)		17	protein phosphatase 1, regulatory (inhibitor) subunit 1B (dopamine and cAMP regulated phosphoprotein, DARPP-32)

GAD1	13	GABA glutamate	Y	(131, 155)	(132)	2	glutamate decarboxylase 1 (brain, 67kDa)
GAD2	22	GABA glutamate	N			10	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)
GLS	19	GABA glutamate	Y	(131)		2	glutaminase
GLUL	8	GABA glutamate	N			1	glutamate-ammonia ligase (glutamine synthetase)
CACNG2	47	glutamate	N			22	calcium channel, voltage-dependent, gamma subunit 2
GLUD1	12	glutamate	N			10	glutamate dehydrogenase 1
GLUD2	4	glutamate	N			23	glutamate dehydrogenase 2
GRIA1	48	glutamate	N			5	glutamate receptor, ionotropic, AMPA 1
GRIA2	14	glutamate	N			4	glutamate receptor, ionotropic, AMPA 2
GRIA3	86	glutamate	N			23	glutamate receptor, ionotrophic, AMPA 3
GRIA4	42	glutamate	N			11	glutamate receptor, ionotrophic, AMPA 4
GRIN1	7	glutamate	N			9	glutamate receptor, ionotropic, N-methyl D-aspartate 1
GRIN2A	75	glutamate	N			16	glutamate receptor, ionotropic, N-methyl D-aspartate 2A
GRIN2B	114	glutamate	N			12	glutamate receptor, ionotropic, N-methyl D-aspartate 2B
GRIN2C	8	glutamate	N			17	glutamate receptor, ionotropic, N-methyl D-aspartate 2C
GRIN2D	16	glutamate	N			19	glutamate receptor, ionotropic, N-methyl D-aspartate 2D
GRIN3A	42	glutamate	N			9	glutamate receptor, ionotropic, N-methyl-D-aspartate 3A
GRIN3B	10	glutamate	N			19	glutamate receptor, ionotropic,

							N-methyl-D-aspartate 3B
GRM1	52	glutamate	N			6	glutamate receptor, metabotropic 1
GRM2	4	glutamate	N			3	glutamate receptor, metabotropic 2
GRM3	33	glutamate	N			7	glutamate receptor, metabotropic 3
GRM4	32	glutamate	N			6	glutamate receptor, metabotropic 4
GRM5	76	glutamate	Y	(156)		11	glutamate receptor, metabotropic 5
GRM6	14	glutamate	N			5	glutamate receptor, metabotropic 6
GRM7	200	glutamate	N			3	glutamate receptor, metabotropic 7
GRM8	275	glutamate	N			7	glutamate receptor, metabotropic 8
SLC17A6	15	glutamate	N			11	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6
SLC17A7	9	glutamate	N			19	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7
SLC1A1	51	glutamate	N			9	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1
SLC1A2	37	glutamate	N			11	solute carrier family 1 (glial high affinity glutamate transporter), member 2
SLC1A3	28	glutamate	N			5	solute carrier family 1 (glial high affinity glutamate transporter), member 3
SLC1A6	9	glutamate	N			19	solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6
SLC18A2	17	monoamines, histamine	N			10	solute carrier family 18 (vesicular monoamine), member 2
SOD1	8	SOD1	N			21	superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))
SOD2	7	SOD2	Y		(125, 134,	6	superoxide dismutase 2, mitochondrial

157, 158)

*Medline search was implemented using MeSH term for (tardive dyskinesia OR TD) AND (gene name OR gene) abbreviation in full text

Table 2.5 List of drug metabolizing enzymes with its importance in metabolizing the six antipsychotics in the CAITE in human models

Gene	Drug Metabolizing Enzymes	CATIE Phase 1 & 2 Medications						
		C	O	P	Q	R	Z	A
CYP1A2	Cytochrome P450 1A2	Major	Major	Minor			Minor	
CYP2A6	Cytochrome P450 2A6	Minor						
CYP2C8	Cytochrome P450 2C8	Minor		Minor				
CYP2C9	Cytochrome P450 2C9	Minor		Minor				
CYP2C19	Cytochrome P450 2C19	Minor		Minor				
CYP2D6	Cytochrome P450 2D6	Minor	minor	Major		Major		Major
CYP3A4	Cytochrome P450 3A4	Minor		Minor	Major	Major	Major	Major

C=clozapine, O=olanzapine, P=perphenazine, Q=quetiapine, R=respiridone, and Z=ziprazidone. A=Aripiprazole
"*" = also documented in FDA approved labeling.

9. Figures

Figure 2.1 Conceptual model to illustrate relationships between TD, dopamine receptor genes and covariates

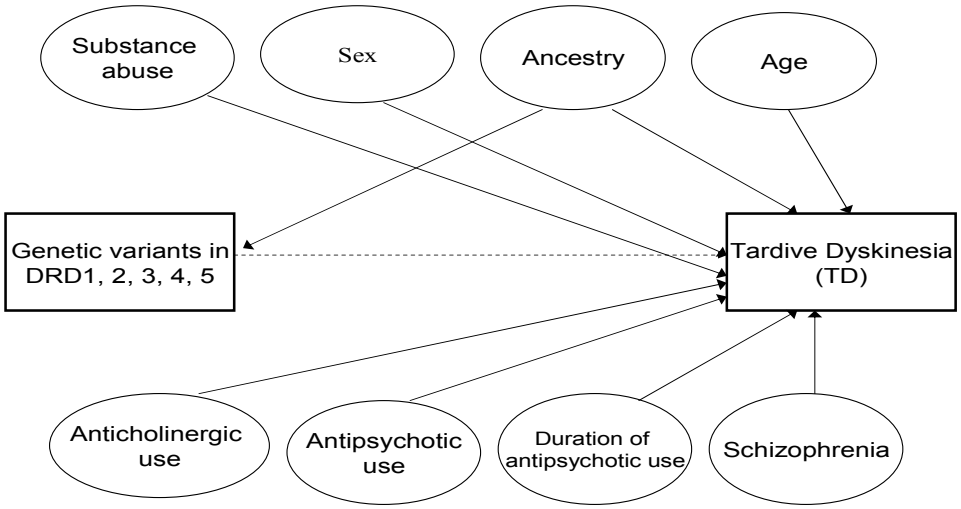


Figure 2.2 Evaluation form of Abnormal Involuntary Movement Scale (AIMS).

NIMH CATIE SCHIZOPHRENIA — **SOURCE DOCUMENT THIS IS NOT A CRF**

Patient initials: | | | **Visit date (mmm dd, yyyy):** | | | |

Patient number: | | | | | | | **Visit:** |

ABNORMAL INVOLUNTARY MOVEMENT SCALE (AIMS)

Examiner Initials: _____

Movement Rati Ratings: Rate highest severity observed.

Facial and Oral Movements

1.	Muscles of Facial Expression (e.g., movements of forehead, eyebrows, periorbital area, cheeks; include frowning, blinking, smiling, grimacing)	None, Normal	= 0	
		Minimal (may be extreme normal)	= 1	
		Mild	= 2	
		Moderate	= 3	
		Severe	= 4	
2.	Lips and Perioral Area (e.g., puckering, pouting, smacking)	None, Normal	= 0	
		Minimal (may be extreme normal)	= 1	
		Mild	= 2	
		Moderate	= 3	
		Severe	= 4	
3.	Jaw (e.g., biting, clenching, chewing, mouth opening, lateral movement)	None, Normal	= 0	
		Minimal (may be extreme normal)	= 1	
		Mild	= 2	
		Moderate	= 3	
		Severe	= 4	
4.	Tongue (rate only increase in movement both in and out of mouth, NOT inability to sustain movement)	None, Normal	= 0	
		Minimal (may be extreme normal)	= 1	
		Mild	= 2	
		Moderate	= 3	
		Severe	= 4	

Patient initials: | | | | Visit date (mmm dd, yyyy): | | | |

Patient number: | | | | | | | Visit: | |

ABNORMAL INVOLUNTARY MOVEMENT SCALE (AIMS) (continued)

Extremity Movements

5.	Upper (arms, wrists, hands, fingers) [Include choreic movements (i.e., rapid, objectively purposeless, irregular, spontaneous); athetoid movements (i.e., slow, irregular, complex, serpentine). Do NOT include tremor (i.e., repetitive, regular, rhythmic)]	None, Normal	= 0	
		Minimal (may be extreme normal)	= 1	
		Mild	= 2	
		Moderate	= 3	
		Severe	= 4	
6.	Lower (legs, knees, ankles, toes) (e.g., lateral knee movement, foot tapping, heel dropping, foot squirming, inversion and eversion of foot)	None, Normal	= 0	
		Minimal (may be extreme normal)	= 1	
		Mild	= 2	
		Moderate	= 3	
		Severe	= 4	

Trunk Movements

7.	Neck, Shoulders, Hips (e.g., rocking, twisting, squirming, pelvic gyrations)	None, Normal	= 0	
		Minimal (may be extreme normal)	= 1	
		Mild	= 2	
		Moderate	= 3	
		Severe	= 4	

Global Judgments

8.	Severity of Abnormal Movements	None, Normal	= 0	
		Minimal (may be extreme normal)	= 1	
		Mild	= 2	
		Moderate	= 3	
		Severe	= 4	

Patient initials: | | | | **Visit date (mmm dd, yyyy):** | | | |

Patient number: | | | | | | | **Visit:** |

ABNORMAL INVOLUNTARY MOVEMENT SCALE (AIMS) (continued)

Global Judgments (continued)

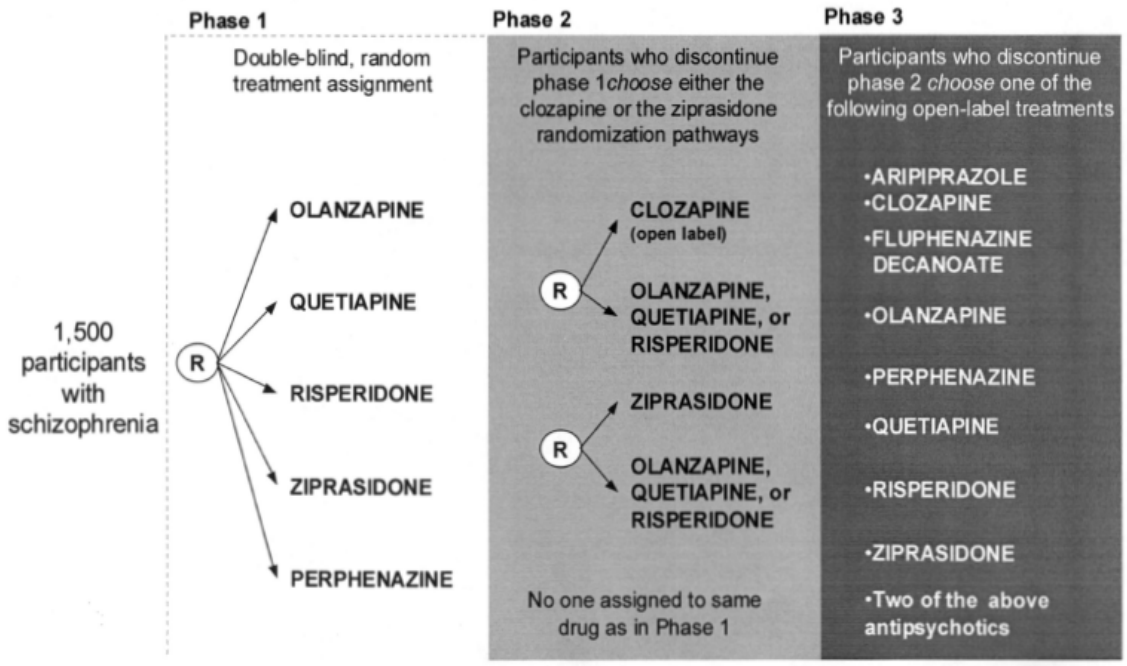
9.	Incapacitation Due to Abnormal Movements	None, Normal = 0	
		Minimal (may be extreme normal) = 1	
		Mild = 2	
		Moderate = 3	
		Severe = 4	
10.	Patient's Awareness of Abnormal Movements (rate only patient's report)	No awareness = 0	
		Aware, no distress = 1	
		Aware, mild distress = 2	
		Aware, moderate distress = 3	
		Aware, severe distress = 4	

Dental Status

11.	Current problems with teeth and/or dentures?	Yes = 1	
		No = 0	
12.	Does patient usually wear dentures?	Yes = 1	
		No = 0	

Comments:

Figure 2.3 Flow diagram of the CATIE study design. (Source: (135))



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Chapter III.

METHODS

1. Meta-analyses of associations between *DRD3* rs6280 and POR of TD

1.1. Overview of the meta-analysis between *DRD3* rs6280 and TD

The purpose of this meta-analysis was to evaluate the evidence for a widely suspected but inconclusive association between prevalence of TD and rs6280 in *DRD3*. Meta-analysis has been recognized as an important tool to summarize scientific knowledge explicitly and objectively (1). However, in contrast to meta-analyses of randomized clinical trials, summary estimates based on meta-analyses of observational studies may be vulnerable to mistaken conclusions if methodological considerations limit study findings (2). Therefore, this meta-analysis of observational studies only reported summary estimates that were not vulnerable to publication bias and heterogeneous findings from the literature.

This meta-analysis included three components: 1) a systematic search of several bibliographic systems; 2) statistical testing for symmetry of funnel plots and homogeneity of effect sizes among studies; and 3) stratified analyses by study characteristic to identify sources of inconclusive findings in the literature. We reported the summarized effect estimate when assuming a recessive model of inheritance, which was not vulnerable to publication bias and heterogeneity across studies, and concluded no association between *DRD3* rs6280 and prevalence of TD.

1.2. Rationale for the meta-analysis study

This meta-analysis study was motivated by several factors. The first reason was to reconcile conflicting results. The rs6280 in *DRD3* is the most widely studied genetic variant that has been associated with TD. However, the reported effect estimates were inconclusive, ranging from OR=0.76 (95%C.I. = 0.48- 1.20) to OR= 3.53 (95%C.I. = 1.26- 9.89). A 2002 combined analysis of 708 patients with chronic schizophrenia in seven groups concluded that the rs6280 polymorphism in *DRD3* significantly contributes to susceptibility of TD (3). However, two large studies published since that time found conflicting results. The heterogeneous findings could be due to methodological limitations inherent in several of these studies, for example small sample sizes (median of total samples: 116 over 13 studies).

Second, heterogeneous findings in the literature may result from differences in study characteristics that may strongly influence the effect size of prevalence TD across studies. However, this important information has not been noted in prior studies. A 2006 meta-analysis of 1,610 patients with chronic schizophrenia reported an increased susceptibility to TD among patients with chronic schizophrenia carrying the Gly allele in comparison to those carrying the Ser allele; however, no association between rs6280 and TD was identified (4). Although notable heterogeneity of effect sizes across the literature was identified in this study, no further analyses were implemented to tackle this concern before relying on the summarized effect estimates. In addition, this study set an alpha value of 0.05 for heterogeneity tests and Egger's tests. As both tests are known to be statistically under-powered, meaningful

heterogeneity and publication bias may have been overlooked in this study.

Lastly, two large studies were published since the publication of the meta-analysis study in 2006. Therefore, this meta-analysis study aimed to examine the association between genotype in rs6280 and TD, while improving an earlier meta-analysis by including recent publications and implementing a more comprehensive evaluation of heterogeneity and publication bias.

1.3. Method of meta-analysis

1.3.1. Literature collection

A systematic search of literature was conducted using several databases, including Pubmed (1966-2006), CINAHL, Web of Science (1955-2006), BIOSIS Previews (1969-2006), and The Cochrane Library, by using keywords: (tardive dyskinesia OR TD) AND (dopamine receptor 3 OR *DRD3*). No language criterion was set. All types of publications were considered in the first search, including original articles with and without full texts, conference proceeding and preliminary reports. Publications were further screened to identify studies which investigated associations between TD and *DRD1*, 2, 3, 4 and 5 genes. A study was included in this meta-analysis if it met the following criteria: 1) the outcome of interest is TD; 2) genetic variants of interest includes rs6280; and 3) in human. A summary of publication on associations between TD and DR genes is tabled in Table 2.3.

1.3.2 Data abstraction

Data abstracted from selected association studies included information of TD

measurement, genotype data, and study characteristics in methodology and study population.

1.3.2.1. Outcome: TD status

As addressed in section II-2.2.3, TD is mainly defined using Schooler-Kane criteria to AIMS evaluation but a few studies applied different criteria. Information abstracted about TD assessment included: 1) whether TD was evaluated using AIMS; 2) whether the Schooler-Kane criterion was adopted as the diagnosis criterion for TD; and 3) whether TD was measured repeatedly.

1.3.2.2. Genotype in *DRD3* rs6280

Counts of rs6280 genotype, including AA, AG and GG, by TD status were abstracted in each study.

1.3.2.3. Study characteristics

Examining study characteristics can help us understand potential reasons for heterogeneous effect sizes across studies. I identified study characteristics from two perspectives: methodological factors and study population factors. Study characteristics identified from each perspective were listed below:

<u>Methodological factors:</u>

1. study design (cohort, matched cohort, case-control, matched case-control);
2. recruiting source (hospital, community, mix);
3. enrollment criteria (only required patients with chronic schizophrenia, only

required on antipsychotic treatment; and require patients with chronic schizophrenia and with history of antipsychotic exposure);

4. year of publication.

Study population factors:

1. ethnicity (European, Asian, African, mixed);
2. age (mean and standard error), which were calculated using total population and control group in crosesstional and case-control studies, respectively;
3. percent female, which were calculated using total population and control group in crosesstional and case-control studies, respectively;
4. Hardy-Weinberg equilibrium p-value in non-TD populations;
5. type of schizophrenia (chronic, acute, mix);
6. type of antipsychotic medication (conventional antipsychotic, atypical antipsychotic, mix).

1.3.2.4. Validation of data abstraction and data entry

Validation of data abstraction and data entry is an important step in meta-analysis because typos in data entries can lead to misleading effect estimates (5, 6). I first abstracted data into a table and then verified the data in the table a few weeks later. The validation work was executed by using another blank working table with the same study characteristics that I abstracted the first time. I performed data abstraction work again and compared the consistency between the new data abstraction results and the prior working table. A few inconsistencies were noted and

I consulted with the initial articles to resolve these discrepancies.

1.3.3. Author contacts

Authors were contacted to obtain information missing in their publications. For example, some studies only provided count data of rs6280 when its association with TD was statistically significant.

I contacted authors systematically by email, making polite requests for further information on their study. When the author did not respond to my first inquiry, I contacted them again a few weeks later to remind them of my request. When no response was obtained from the second email, I worked with Dr. Sullivan to send another request. Up to three author contacts were made.

1.3.4. Analysis plans

1.3.4.1. Overview

I first assessed symmetry of funnel plots among collected publications using symmetry tests and trim-and-fill approaches. I then performed overall heterogeneity tests to determine whether effect estimates across studies were heterogeneous. In order to understand potential sources of heterogeneous findings in the literature, meta-regression and stratified analysis were also performed using 13 study characteristics.

Meta-analysis can be conducted assuming a fixed effect model or random effects model. Both models apply different approaches in estimating a summary estimate and its variance. A fixed effect model computes its summary estimates using

a precision-weighted average of effect sizes in studies. In contrast, random effects model assumes that the true effect estimate is normally distributed with a different mean and variance in each given study. As we think a single summary estimate is appropriate and not an oversimplification of the literature only when heterogeneity of effect estimates does not exist, we used a fixed effect model when estimating summary estimates in this meta-analysis (7).

1.3.4.2. Symmetry tests of funnel plots to detect potential publication bias

Meta-analyses may provide summary effect estimates across published studies. However, summary effects obtained from meta-analyses may not be reliable, particularly when several published studies were not included in the meta-analysis.

Publication bias is caused by multiple sources, including investigators, employers, funding sources, reviewers and also editors. In most situations, study findings in plausible directions with small p-value are highly favored for publication. In contrast, study findings in an implausible direction and with very small p-values are often not published. Therefore, publication bias may be particularly strong when prior knowledge about direction of the association is commonly accepted in the research community.

Three procedures were implemented to examine funnel plot symmetry, an important sign in indicating potential publication bias among articles of interest. First, I graphed a funnel plot, a scatter plot which graphs effect measures by inverse standard error, using the **metabias** command in STATA 8. In a funnel plot, less precise estimates from studies with small sample sizes are expected to spread out

more than scatters from more precise estimates. As a result, if there is no publication bias, the shape of a funnel plot would be close to symmetry. The first assessment of symmetry of funnel plot was made by visually examining graphs

Second, I calculated a p-value for Begg and Mazumdar's log rank test (8) and Egger's regression test (9) using the **metabias** commend in STATA. These two tests provide quantitative assessments of the symmetry of a funnel plot. It is important to note that both Begg's and Egger's tests have low statistical power. As a result, we used a high alpha-value, such as 0.1, in evaluating the evidence of asymmetry of funnel plots in the literature.

Third, I used Duval and Tweedie's trim-and-fill imputation (10) procedure as an additional analysis of funnel plot symmetry. The trimmed-and-filled procedure imputes effect estimates in three steps: 1) remove estimates that made the funnel asymmetry, forming a trimmed dataset; 2) use the trimmed dataset to compute a presumptively less biased summary effect and standard error; and 3) return trimmed estimates into the dataset and fill the datasets with estimates that had the same standard error as the summary effect obtained from the trimmed dataset but an opposite sign of the effect from the trimmed estimates. Summary estimates from the final trimmed-and-filled dataset was more valid than summary estimates computed from existing publications.

1.3.4.3. Overall Heterogeneity

After examining the degree of publication bias in the literature, I assessed the heterogeneity of effect estimates among published studies. The rationale of

heterogeneity assessment is to assure observed study-specific estimates were not too inconsistent or heterogeneous to be over-simplified as one summary estimate. Heterogeneity assessment was implemented by computing a p-value of Cochran's Q statistics in a homogeneity test (11) using the **metan** command in STATA. As homogeneity testing is known to have low statistical power, a higher than usual alpha value of 0.1 was applied.

1.3.4.4. Meta-regression

Meta-regression analyses were performed in order to explore potential sources of heterogeneous estimates in literature. In meta-regression analyses, the outcome was the magnitude of the effect estimate in each study and the independent variables were the study characteristics of interest. Therefore, the meta-regression of study characteristics provided us information regarding the strength of each study characteristic for explaining potential sources of heterogeneity among studies. Meta-regression was implemented using the **metareg** command in STATA.

It is important to note that the unit of analysis for the meta-regression was the collection of all the studies examined in the meta-analysis. Therefore, the sample size of the meta-regression was up to 13 studies, depending on the study characteristic investigated. As a result, each meta-regression was performed to examine one study characteristic at a time. Study characteristics identified from meta-regression were factors that may have contributed to heterogeneity of effect sizes in the literature.

1.3.4.5. Stratified analysis

We performed stratified analysis when a study characteristic was suspected to have an important influence on the observed heterogeneity or when the stratum-specific summary estimates were of interest. As long as a suspected study characteristic was presented in at least two studies for each of its categories, stratified analyses were performed, including 1) examination of heterogeneity of effect estimates in a subgroup; 2) assessment of Begg's and Egger's tests for symmetry of funnel plots, and 3) comparison between imputed effect estimates with summary estimates of published studies.

2. Association study between single nucleotide polymorphisms (SNPs) in dopamine receptor genes and POR of TD

2.1. Overview

This study aimed to investigate SNPs in DR genes and the prevalence of TD using 711 CATIE subjects. Fifty four SNPs in *DRD1*-5 genes were selected to implement both SNP-based and haplotype-based analysis. An illustration of the relationship between TD, DR genes, and several important risk factors for TD was presented Figure 2.1. Associations were assessed applying a minimum-adjusted model, in which adjustment was made for ancestry only (Figure 3.1) and a final model, which adjusted for all covariates with significant effects on TD in the CATIE dataset (Figure 3.2).

2.2. Study design

The closest description of the study design is a cohort study of prevalent TD. The

TD group was composed by all individuals with TD, either those observed at baseline or those that identified over the course of the CATIE trial period. The non-TD group consisted of participants who never met TD criteria in any of their AIMS evaluations. The measure of effect was POR of TD across different genotypes of selected SNPs in DR genes.

The rationale of including TD detected at any time point during the CATIE study as TD group was to accommodate the complicated detecting force for the presence of TD. The presence of TD can be masked or revealed by change of antipsychotic use, including both type and dosage. For example, TD symptoms can be temporarily suppressed when increasing the dosage of typical antipsychotics or starting an antipsychotic treatment. However, TD symptoms could also be revealed shortly after patients discontinued antipsychotic medications and be mistaken as an incident TD. Moreover, TD symptoms could also be transient without changes of antipsychotic therapy. Therefore, this study included all TD at any time point to assure we capture all participants genetically predisposed to TD.

2.3. Outcome Definition

This study utilized the Schooler-Kane's criteria for probable TD, which required at least one item in the AIMS evaluation rated greater than 3 (moderate) or at least two items are rated greater than 2 (mild). Participants who ever showed an AIMS evaluation that met Schooler-Kane's criteria were classified with TD. Participants were classified as non-TDs if none of their AIMS evaluations throughout the CATIE study met the Schooler-Kane's criteria.

2.4. Selection of genetic markers

Given the large number of genetic variants on the human genome and the high degree of redundancy involved in densely spaced genotyping, SNP tagging has been proposed as an effective strategy to reduce the cost of genotyping (12). Several selection methods for tagging SNPs have been proposed, each using different criteria for evaluation. These methods can be broadly split into two types: capturing the diversity of original haplotypes present in the known SNP set; and demonstrating a strong association between proposed SNPs s (13). Among these two types of selection criteria, the second method measured the direct relevance to association between tag SNPs and with the original SNP sets and has been accepted as the more appropriate selection strategy in population association studies.

Our study used the multiple-marker haplotype r^2 statistic to select tag SNPs on *DRD1-DRD5*. Haplotype r^2 is equivalent to the one-way analysis of variance of locus *i* among the SNP-defined groups and has been widely used to measure association between a reduced tag SNPs set and the known SNPs set K. A minimum r^2 of $\geqq 0.85$ between the SNPs set and the known SNP set K was required. The minimum r^2 of $\geqq 0.85$ criteria assure only a modest loss of power when genotyping tag SNPs exclusively. In addition, the tag SNPs were selected using the HapMap data, which includes European and African populations. The tag SNPs identified from HapMap should be representative of the tag SNPs in the proposed study population, given the predominant white and African-American ancestry of participants from the CATIE study. SNP selection was implemented using TagIT software (13).

In addition to tag SNPs, several functional SNPs were also interrogated in the proposed study. Functional SNPs are genetic variants that could potentially change protein characteristics such as physical properties, stability, and folding kinetics, leading to an altered protein. A total of 54 tag and functional SNPs in *DRD1-DRD5* were selected for the second part of this dissertation work. These SNPs are listed in Table 3.1.

2.5. Genotyping method and quality control

Genotyping was conducted using Illumina Golden Gate technology (http://www.illumina.com). This choice was dictated by high genotype call rates (>99.6%), high reproducibility (>99.59%) and competitive pricing (14). All genotyping was conducted according to protocol at the Duke University core facility directed by Dr. Kevin Shianna (15). Illumina Bead Studio software (version 2.0) was used for genotype calling.

2.5.1. Genotyping method

These assays are based on an array of wells (usually in 96 well format) patterned into an optical imaging fiber bundle (14). The optical imaging fiber bundles used by Illumina consist of ~50,000 individual fibers fused into a hexagonally packed matrix that can hold up to ~50,000 beads. Each bead has a distinct oligonucleotide capture probe. Since the assembly of beads into wells is a random process, the location and identity of beads in the array must be decoded post-assembly (16). Highly multiplexed genotyping (up to 1,536 SNPs per well) is based on allele-specific

extension with read-out on random arrays of universal capture probes. There are three probes per SNP (two allele-specific oligos and one locus-specific oligo). Allele-specific extension followed by ligation joins the allele-specific and locus-specific oligos to create a PCR template that can be amplified with universal primers. The extension reaction provides allele selectivity. The fluorescently labeled PCR products are hybridized to capture probes on beads in the array. The signal ratio from the two allele-specific extension products indicates the genotype.

2.5.2. Quality control

As all CATIE participants were unrelated, genotyping error proceeded as follows: First, I performed Hardy-Weinberg Equilibrium (HWE) tests in the whole study population separately by ancestry. Second, I referred to the resources listed below as external sources to compare allele frequencies among CATIE samples and existing datasets to detect potential signals for genotyping error. [dbSNP (http://www.ncbi.nlm.nih.gob/SNP); NHLBI/SeattleSNPs (http://pga.mbt.washington.edu); NIEHS/geneSNPs (http://www.genome.utah.edu); NCI/SNP500Cancer (http://snp500cancer.nci.nih.gov)].

2.6. Measurement of potential confounding factors

2.6.1. Ancestry

In the CATIE study, self-reported race was collected by a closed-ended questionnaire. Respondents could select one (or maybe more than 1) of the following five categories: White, Black or African-American, American-Indian or Alaska Native,

Asian, Native Hawaiian or other Pacific Islander. Among these five categories, White and Black categories are the two largest groups, counting for 85% of the total study population (Table 3.2). However, validity of self-reported ancestry might be a concern in most studies. Therefore, this study performed Structure Analysis, using software *Structure* (http://pritch.bsd.uchicago.edu/structure.html) (17) to obtain Structure-allocated proportion for ancestry. The computing process generated a set of estimated proportions for each participant's ancestry in each of three main ancestries: Europe, Africa and Asia, rather than categorized ancestry origins. This study adjusted for Structure-allocated ancestry proportion in regression models to more precisely control population stratification and also to obtain better statistical power than stratified analyses by ancestry. The population stratification issue is further addressed in section III-2.8.6.1.

2.6.2. Anticholinergic use at baseline

"ANTICHOL", a variable indicating participants' anticholinergic use within 14 days prior to randomization, was the only available information about anticholinergic use in the CATIE study. Therefore, this study used "ANTICHOL" in evaluating confounding by anticholinergic use.

2.6.3. Substance use

Substance use implies alcohol and/or illicit drug use. Several indicators were used to dichotomize substance use into categories characterized by abuse and/or dependence on substances. These indicators included: (1) clinicians' rating using the

Structured Clinical Interview for DSM-IV (SCID) in the screening step. Participants' alcohol or drug abuse/ dependence presented in the past month are indicated as "Current substance abuse or dependence". (2) hair assay for illicit drug use including cocaine, opiates, phencyclidine (PCP), Methamphetamine, and marijuana at screening, every 6 months, and at the end of each phase of the trial; (3) urine assay for illicit drug use (cocaine, cannabinoids, ethanol, dextroamphetamine, methamphetamine, hydrocodone, morphine, codeine, hydromorphone, propoxyphene, heroin) at baseline screening and every three months during the trial. Participants with substance abuse records on the SCID form or testing positive for any of the above illicit substances were classified as having a substance abuse or dependence disorder.

2.6.4. Duration of schizophrenia illness and antipsychotic treatment

Lifetime antipsychotic exposure is very difficult to measure due to the lack of long-term follow-up data and also the low reliability of patients' self-reported exposure of antipsychotic medications. This study explored the use of a variable, "yrspres0", which indicated "year since first prescribed antipsychotic" to approximate accumulated duration of schizophrenia illness and prior antipsychotic use.

We understand that "year since first prescribed antipsychotic" may not approximate lifelong treatment duration well as it assumed all antipsychotics are comparable in the same duration of use and also assumed discontinuation of antipsychotics was not of great concern. The first assumption may be acceptable as atypical antipsychotics constitute over 90% of antipsychotic prescriptions, and current

data indicate that all ATY have equivalent efficacy in schizophrenic treatment and remission maintenance after a psychotic episode (18). The second assumption may be of concern because TD may affect participants' willingness for continued use of antipsychotic, i.e. depletion of susceptibility in long term medication users. As a result, this study explored the control of "years since first antipsychotic use" with caution.

2.7. Assessment of confounders

Principally, confounders would be identified using the following criteria: 1) the variable is a risk factor of TD development; 2) the variable is differentially distributed across different genotypes on most SNPs; 3) after adjusting for the varible of interest there is a 10% or greater change in the effect of the main exposure variable, measured by |ln(crude OR)-ln(adjusted OR)|, and 4) clinical plausibility. To ease the interpretation of genetic effects of 54 SNPs studies in this study, we identified a set of confounders by considering biological plausibility, forward model selection (entry level=0.2) and expert opinions.

When a covariate is a continuous variable, such as baseline age and years since first antipsychotic prescription, I compared their group means using student's *t* test and analysis of variance. When the covariate is a categorical variable, such as sex, anticholinergic use and substance use, I used Person's X^2 test to estimate a potential confounders' relationship with SNP distribution and TD. More details about the analysis strategies were addressed in sections III-2.8.3 and III-2.8.4.

2.8. Statistical analysis

2.8.1. Overview

The present study estimated genotype-phenotype associations among 711 unrelated CATIE participants. We implemented analysis of SNPs and haplotype in DR genes to assess their associations with TD. Details of these analyses are addressed in section III-2.8. Specifically, we used *STRUCTURE*-inferred ancestry to address the concern of population stratification in a genotype-phenotype study. Details of *STRUCTURE* allocated ancestry would be addressed in sections III-2.8.6.1.

Although specific antipsychotic use may modify the association between TD and genetic variants in DR genes, literature about existence and strength of the interaction is missing. This study, therefore, decided not to implement stratified analyses by 5 specific antipsychotic in a concern of limited statistical power to detect genotype-antipsychotic interactions and preference to reduce unnecessarily for multiple comparisons. In addition, cluster effects among clinical sites was not a concern in the present study as participants were recruited from many clinical sites but were randomized by individual, not by sites.

2.8.2. Data exploration and quality control

Before analyzing the data, the following steps were implemented for quality control of the dataset:

1ST: remove CATIE participants whose genetic data were missing for more than 10% of all genetic markers.

2nd: remove those genetic markers that have an allele frequency of less than 1% so

that all strata are sufficiently large to produce stable estimates

3rd: apply Fisher's exact test to examine HWE separately by European-only and African-only participants (19). We examined HWE in the total population in this crossectional study. When tests for HWE were not rejected, the possibility of genotyping errors was small. Otherwise, an inquiry to the lab was sent to verify the validity of the genetic data.

4th: check the range of continuous covariates such as age, duration of prior antipsychotic use in the total population to detect any outliers. For data outside the plausible range of values, I verified the value with assistance from the CATIE data coordinating center.

5th: check the distribution of categorical variables such as sex, ancestry, baseline anticholinergic use, status of substance abuse, in TD and non-TD group.

6th: compare prevalence of missing data in each variable by TD status. This comparison aimed to examine whether missing data is related to participants' outcome status.

2.8.3. Single marker analysis

2.8.3.1. Overview

Single marker analysis was implemented to estimate the association between each tSNP and POR of TD. Several main steps included contingency test, regression analysis only adjusting for ancestry and regression analysis adjusting for all meaningful confounders identified.

2.8.3.2. Rationale

Given the extensive genetic variation in the human genome, the probability of any single marker being the cause of a disease, including TD, is very low. However, it is still important to begin the analytic process by estimating the effect of each selected SNP. The purpose of this analysis is to determine whether any tSNP is a disease-causing locus or whether there is strong linkage disequilibrium with the real casual allele. This step provides us an overview of effect sizes of associations between TD and each tSNP with ancestry adjustment.

2.8.3.3. Contingency testing between a SNP and TD

I first performed contingency tests to compare the distribution of three genotypes (e.g. AA, Aa, aa) across TD status using Fisher's exact tests. The contingency test is valuable because it does not set any strong assumptions in testing the proportionality of genotype distribution across disease groups. Findings from contingency tests provided me a crude overview of all investigated SNPs-TD associations.

2.8.3.4. Estimating effects of SNPs using univariate models

The univariate model contained three components: a) outcome: TD status; b) genotype information; and c) *Structure*-inferred proportion of ancestry in Europe and Asia. Thus, the univariate model presented as $\ln(\pi_{ij}/(1-\pi_{ij}) = \beta_0 + \beta_1 (g=1,1) + \beta_2(g=1,0) + \beta_3$ (% of European ancestry)$+ \beta_4$ (% of Asian ancestry). As described in section III-2.6.1., Structure computed and allocated each participant's ancestry into admixture

proportions of European, African and Asian ancestry. As proportion of European and African ancestry showed a strong inverse correlation (correlation coefficient< -0.6), we selected proportions of European and Asian ancestry in the regression adjustment.

Among four genetic models of inheritance (dominant, additive, recessive and general model), we implemented general model as it does not assume any relationship between any two of three genotypes, e.g. AA, Aa, aa. I assumed the most common genotype, i.e. the wide type, as the reference group, thereby maximizing statistical efficiency. When the genotype count of a SNP was smaller than or equal to 5, I implemented Fisher's exact test between homozygous and heterozygous variants by TD status to examine if the genotypic distribution by TD were similar in both genotypes. When the Fisher's exact test was not rejected, I used the dominant model to assess their associations with TD. By assuming the dominant model of inheritance, I pooled the heterozygous variant and homozygous variant together to obtain more informative estimates of SNP effect on the PORs of TD than effect estimates when assessing the genetic effects in the general model of inheritance.

2.8.3.5. Estimating SNPs effects using covariates-adjusted model

A covariate-adjusted model was used to control confounding effects when estimating the SNP-TD association (Figure 3.2). As a result, each regression model contained three components: a) outcome: TD status; b) exposure: genetic

polymorphisms; c) potential confounders, including age, sex, ancestry, year since first antipsychotic prescription, baseline antipsychotic use, substance use and baseline PANSS. The full regression model before model selection processes was parameterized as below: $\ln(\pi_{ij}/(1-\pi_{ij}) = \beta_0 + \beta_1(g=1,1) + \beta_2(g=1,0) + \beta_3$ (baseline age)+ β_4 (sex)+ β_5 (year since first antipsychotic use) +β_6 (only use atypical antipsychotic medications)+ β_7 (use conventional antipsychotic medications)+ β_8 (baseline PANSS)+ β_9 (% of inferred European ancestry)+ β_{10} (% of inferred Asian ancestry) +β_{11} (anticholinergic use)+ β_{12} (substance use)+ their interaction terms.

The model building processes involved several steps: 1) using forward model selection strategy; 2) exploring different formats of covariates in the model, and 3) referring psychiatrists' suggestions. A forward model selection process in the initial parameterized model identified four important covariates: participants' baseline age, ancestry, total PANSS at baseline, and anticholinergic use.

The investigator then explored the model building process by excluding the "years since first antipsychotic prescription" covariate in a concern of poor approximation of this measurement to lifelong antipsychotic exposure and also its incompleteness with 4% missing data. After excluding "year since first antipsychotic prescription" from the initially parameterized model, forward model selection procedure was performed again. The model selection process at this step only identified participants' baseline age, ancestry, and baseline total PANSS as important covariates for the odds of TD.

I discussed the model selection results with psychiatrists, statisticians and epidemiologists. As anticholinergic medications have wide indications, including

controlling movement disorders such as Parkinsonism, anticholinergic use may be reflecting a treatment purpose in the early onset of the TD symptoms. Therefore, we decided to exclude this variable from the final model.

In addition, antipsychotic use was included in the final model because of biological plausibility. The model selection process did not identify status of antipsychotic use as an important factor for TD. However, we decided to include antipsychotic status (2 dummy variables for the 3 levels of the covariate) in the final model because previous studies have sbowed a higher rate of TD among patients using conventional antipsychotic medications than using atypical antipsychotics.

As a result, covariates included in the final model were participants' baseline age, ancestry (proportion in European and Asian ancestry), baseline total PANSS, sex and type of antipsychotic use (3 levels).

2.8.4. Haplotype-based analysis

2.8.4.1. Overview

It has been argued that evidence from single-SNP-association studies is inadequate because of the growing belief that most clinical outcomes are mediated through complex genetic traits. Haplotypes are a specific combination of nucleotides on the same chromosome. In contrast to SNP-based analysis, haplotype-based analyses investigate effects of multiple linked-SNPs on TD.

2.8.4.2. Rationale

Haplotype-based analysis can be informative for several reasons (20). First,

haplotypes reflect multilocus mutations on a chromosome. The multiple mutations may be required in order to change proteins' physical properties, stability and folding kinetics, leading to functional disorders. As a result, variations of haplotypes could have a stronger impact on a phenotype than a single variant. This hypothesis has been supported in many studies. For example, a combination of multiple mutations have been shown to influence the function of various genes including lipoprotein lipase (21), actions of catecholamines which influence bronchodilation (22), intestinal lactase activity (23), and prostate cancer(24).

Second, haplotypes consider the dependence among SNPs on the same chromosome rather than viewing each SNP independent of one another. By considering haplotype effects, multiple association testing may be reduced, resulting in a gain of statistical power (20). Third, studies have found the numbers of haplotypes are much smaller than all possible allele combinations, suggesting that variations among population genetics are intrinsically organized in haplotype format. For example, Drysdale et al. found 13 SNPs were organized into 12 haplotypes out of 8,192 possible combinations among 13 SNPs (22), supporting haplotype structure to genetic variations.

2.8.4.3. Strategies for haplotype analysis

This study used score test methods developed by Schaid et al (25) and Lake et al (26) for haplotype-based analysis. Schaid et al's method has been widely used through the operation of haplo.stat software in R. This method implements generalized lineal models (GLMs) to adjust for environmental factors when estimating

genetic effects.

Haplo.stat applies the score test to examine associations between disease traits and haplotypes, regardless of whether the phase of the haplotype is confirmed or ambiguous. In contrast to other methods, such as EM algorithm method, this method provides a global score statistic and also haplotype-specific score statistics, which enable me to compare haplotype-specific effects. In addition, the score statistics are more efficient in the computing process than the conventional EM algorithm method. This haplotype-based analysis includes two main steps:

1st: use haplo.em to estimate haplotype frequencies and obtain posterior probabilities of haplotype pairs for each subject, conditional on observed genotype data in the CATIE. In this step, I set a command to exclude haplotype less or equal to 1% as no informative inference can be drawn in rare haplotype frequency. The haplotype with the highest frequency was set as the baseline group in subsequent analyses.

2nd: use haplo.glm program to run regressions for TD on simple haplotype-specific effects and covariate-adjusted haplotype effects. For haplotypes with a low frequency, we set 5 as the minimum expected count in TD and non-TD group for haplo.glem analysis. In this step, I obtained a global score statistic for loci that is composed of haplotypes and haplotype-specific score statistics. I used empirical p-values obtained from simulation for a reliable p-value in significance testing.

2.8.5. Examinations of statistical assumptions for logistic regression models

2.8.5.1. Overview

We also examined the statistical assumptions of the logistic regression model,

particularly the assumptions of adequate responses across discrete variable levels and no multicollinearity between independent variables.

2.8.5.2. Ratio of cases to discrete variables

Adequate responses across levels of discrete variables in a logistic regression model are important in order to obtain valid effect estimates and standard errors. Discrete variables in our models were genotype, sex, baseline substance abuse/ dependence, baseline antipsychotic use, and baseline anticholinergic use. By referring to Table 4.2.1, we knew case number in every given category of the discrete covariates were not small. Regarding small cell count in a given genotype category, we combined that with the heterogeneous variant after Fisher's exact tests. Therefore, this study met this assumption for logistic regression analyses.

2.8.5.3. Collinearity between markers and covariates

Collinearity between independent variables in a regression could result in biased estimates of regression coefficients, inflated coefficients of variance, and p-value. I examined the collineraity between genetic markers and the covariates age, year since first antipsychotic prescription, baseline PANSS, percentage of European ancestry, percentage of African ancestry, percentage of Asian ancestry, sex, substance use and anticholineargic use. I first checked the correlation matrix between markers and covariates. When a strong (i.e. >=0.6) correlation between a marker and a covariate was identified, I examined the variance inflation factors (VIF) of the marker and the covariate. A VIF greater than 10 was further investigated and

the covariate was removed from the multiple covariates-adjusted models.

2.8.6. Special considerations in genetic analysis

2.8.6.1. Adjusting for empirical ancestry to reduce confounding by population stratification

Population stratification could confound findings of genetic association studies when subpopulations have different risk to the disease and also when the allele frequencies are fairly different across the subpopulations (27). In order to control confounding from population substructure, 75 ancestry informative markers selected using HapMap panels were included in the Illumina genotyping runs and genotyped in CATIE participants. HapMap samples were then used as the prototypes for continental ancestry to which CATIE subjects can be compared. We then used the *Structure* program, (http://pritch.bsd.uchicago.edu/structure.html) (17), which use a Bayesian approach and Markov chain Monte Carlo (MCMC) method, to determine the posterior probability for each study subject being classified into one of three main sources of human ancestry (African, East Asian, and European). These three probabilities sum to 1.0 and subjects could have had substantial ancestry from each source. Detailed steps to generate Structured-allocated admixture fraction and the results are listed below:

Step 1: Identify SNPs with high Fst values for use with STRUCTURE

- Considered Caucasian (CEU), African (YRI), and Asian (CHB+JPT) HapMap panels. Used ALL SNPS genotyped in HapMap.
- Selected SNPs with allele frequencies in the [0.05 - 0.95] range in all panels

- Calculated Fst values
- Formulas from Weir and Hill (28), three pairwise combinations of HapMap populations
- Ranked each of the three pairwise comparisons
- Dropped SNPs that were within 50 kb of each other
- Selected 100 SNPs with high pairwise Fst values (CEU-YRI & CEU-ASI given priority given the demographics of CATIE).

Step 2: genotype these SNPs in all of CATIE.

- Done at Duke core facility
- 75 of 100 SNPs requested were successfully genotyped.
- Genotyping was successful in only N=719 (of 745)
- No evidence of the “allele flip problem” in HapMap1
- Pretty divergent – the minimum difference between allele frequencies in “Black” versus “White” was 0.49

Step 3: use STRUCTURE

Step 3a: use HapMap populations as a guide

- All SNPs in Step 2 were genotyped in HapMap
- Use the HapMap samples as “exemplars”, as the prototypes for continental ancestry to which CATIE subjects can be compared.
- HapMap data (N=270)

- used Hapmap sets (CEU 30x3, JPT+CHB 45x2, YRI 30x3)
- kept founders (210=60 CEU, 90 JPT and 60 YRI)
- dropped NA19012 who had missing for 43/75 (other missings 0-4 range)
- Ran STRUCTURE 3 times (settings – burnin 25K, run length 200K, use pop info, correlated allele frequency, all others defaults)
- Some people were not well classified based on these SNP data and were dropped
- Final numbers: 60 YRI, 80 ASI, and 58 EUR

Step 3b: use STRUCTURE in a supervised way. I want to determine posterior probability for each CATIE subject being classified into one of three human continental ancestries using HapMap data as exemplars.

- Goal is to classify CATIE into groups defined by HapMap exemplar groupings
- NOT to discover new classifications (number of SNPs insufficient for this task)
- Checks – allele calls very similar in CATIE and HapMap
- N=917 individuals (HapMap=198 and CATIE=719) and 75 SNPs
- Details:
    - K=3.
    - For HapMap, popID (or popdata)=1-2-3 for pop of origin & popflag=1. This tells STRUCTURE to use this person for pop learning
    - For CATIE, popID=0 & popflag=0
    - Burnin 25K, run length 200K, use pop info (advanced, use defaults admixture model, gensback=2 & migrprior=0.05), correlated allele freqs

    - advanced - I turned on update allele freqs with POPFLAG=1 data (PFOMPOPFLAGONLY), manual p19
    - all others defaults
- Ran STRUCTURE four times. Results highly similar across runs.

Preliminary data generated by Dr. Sullivan have suggested that misclassifications of ancestry based on self-reported ancestry in the CATIE study population are unusual (Table 3.3). In addition, the preliminary data also demonstrate that the posterior probabilities inferred from Structure are sensible, particularly among CATIE participants who reported more than one ancestry. A summary of CATIE subjects by their self-reported race and the inferred posterior probabilities from Structure is listed in Table 3.4. These findings were important because they demonstrate the validity of using Structure-referred ancestry admixture proportion to represent population substructure. Therefore, I used Structure-allocated admixture fraction to assess confounding and control for population stratification. The distribution of Structure-allocated ancestry proportions are shown in Figure 3.3.

2.8.6.2. Controlling positive false discovery rate (pFDR) in multiple testing

Multiple comparisons are an unavoidable issue in genetic association studies, particularly in studies investigating a large number of genetic variants. This is a problem because multiple testing may lead to increased type I error and generate a certain amount of false-positive findings. Two strategies have been commonly used to adjust for multiple testing: controlling family-wise error rate (FWER) and controlling the false discovery rate (FDR). Using methods for FWER control, such as a

Bonferroni correction, assures the probability of any single false positive testing is less than 0.05 in all loci testing. However, this strategy has been criticized to be too conservative in genotype-phenotype association testing because it is reasonable to expect a sizeable proportion of genetic markers could be truly significant findings when examining a large amount of genetic markers.

Instead of using Bonferroni method and setting a very restrictive p-value for all tests, this study applied Storey et al's method to control positive false discovery rate (pFDR) in multiple tests (29). pFDR is defined as minimum expected proportion of errors among rejected hypothesis. Controlling pFDR method enables proposed study to balance the opportunistic cost between generating false positive findings and missing truly positive findings.

In operating the control of pFDR, I first performed statistical tests for each variant to obtain a variant-specific p-value. Second, I ordered the p-values from each testing in the same model in ascending fashion. Third, I entered all the p-values into the QVALUE software (http://faculty.washington.edu/~jstorey/qvalue/) to calculate the q-value. I set a q-value of 0.05 as a tolerable pFDR, which means this study accepts 5% erroneous rejections among all rejected hypotheses from individual testing. So, only statistical testing that obtained a q-value less than 0.05 would be interpreted SNP with statistically significant association with TD after adjustment of multiple comparisons.

2.9. Power calculation

In order to obtain an overview of statistical power in this genotype-phenotype association study, we performed power calculation across a range of effect sizes and minor allele frequencies in SNPs in this study. We set 15% of TD prevalence, alpha-value equal to 0.001, and additive model of genetic inheritance in power calculation using software Quanto version 1.1.1 (30).

2.10. Human Subject

2.10.1. Type of subjects

The present study was involved with 711 CATIE participants who agreed to provide their DNA sample for genetic studies. To enter the trial, a subject must be a patient with schizophrenia, aged between 18 to 65 years old, non-pregnant, non-breastfeeding, and with decisional capacity in study participation. In addition, subjects who were in their first episode of schizophrenia, with contraindication or history of treatment failure to any proposed antipsychotic treatment were not recruited in this study.

2.10.2. Method of recruitment

Participants were enrolled from various recruitment sites, including managed care centers, public mental health, and Veteran's Affairs, regardless of their race/ethnicity, sex and disease severity. This study did not enroll patients under 18 years old as the development of chronic schizophrenia is less common among persons under 18 years of age. Only participants who consented with DNA samples when entering the trial were eligible in the study about DR genes and TD.

2.10.3. Informed consent

Participants must have the decisional capacity in the participation of the CATIE and would like to sign the informed consent to be recruited. Participants consented DNA samples through an additional informed consent for CATIE HGI (Human Genetics Initiative) study with an agreement for research purpose to improve etiological understanding of schizophrenia and its treatment.

2.10.4. Risk to participants

The present study was involved with genotyping work of existing DNA samples and linking the genetic data to the parent study. No additional physical damage would cause to participants due to this study.

2.10.5. Confidentiality of data

The genotyping work was blinded to subjects, investigators and health care providers. In order to reduce the risk of disclosure of participants' confidentiality, all datasets were processed and stored without coding of personal identification, such as name. Each participant was assigned a pseudo unique identifier by the CATIE study and be traced by the psueo-ID for data link purpose. Password was configured. Therefore, access to the datasets was available to limited study personnel. In addition, participants' names and the name of clinical sites from which participants were recruited were also excluded from future publications. When the study is completed, I would return the data to the CATIE committee.

3. Tables

Table 3.1 List of tag single neucleotide polymorphisms (SNPs), functional and structural SNPs in dopamine receptor genes.

Gene	Location Chromosome no. (Start- End position)	Length (base pair)	tagSNP no.	SNPs
DRD1	chr5 (174,828,959-174,872,086)	43,128	8	rs2453737, rs265973, rs265974, rs265976, rs686, rs5326 [b], rs2168631, rs267418
DRD2	chr11 (112,797,968-112,903,544	105,577	23	rs1079594 [b], rs1079596 [b], rs12364283, rs17115461 [b], rs1799978[a], rs1800497 [b], rs1800498 [b], rs2234690 [b], rs2587548 [b], rs2734836 [b], rs2734848[a b], rs4581480 [b], rs4586205 [b], rs4648317 [b], rs4648318 [b], rs4986918[a b], rs6275[a b], rs6277[a b], rs6279 [b], rs6589377, rs7103679 [b], rs7109897 [b], rs7125415 [b]
DRD3	chr3 (115,148,457-115,238,657)	90,201	17	rs6808291, rs1486012, rs2399496, rs9824856[b], rs2134655 [b], rs2251177[a b], rs963468 [b], rs3773678 [b], rs2630349 [a b], rs167771 [b], rs167770 [b], rs324029 [b], rs10934256 [b], rs1486009 [b], rs3732783 [b], rs6280 [b], rs9825563
DRD4	chr11 (607,536-650,933)	43,398	4	rs3758653, rs11246226, rs936465, rs1800443[a b]
DRD5	chr4 (9,514,485-9,556,515)	42,031	2	rs2867383, rs4516717[a]

a: SNPs predicted in silico to be functional (i.e. functional SNPs)

b: SNPs in basic structural elements (i.e. structural SNPs)

Table 3.2 Distribution of self-reported ancestry by tardive dyskinesia (TD) classification in 711 participants in the present study.

	Anytime TD		
Self-reported race	*Non-TD*	*TD*	**Total (%)**
Africa only	140 (28%)	69 (33%)	209 (29%)
Europe only	287 (57%)	112 (54%)	399 (56%)
Other	77 (15%)	26 (13%)	103 (15%)
Total	504	207	711

Table 3.3 Consistency comparison between self-reported race and Structured-inferred ancestry with inconsistent data marked in bold.

Self-reported ancestry	Inferred best class of continental ancestry by Structure			Total
	AFR	ASI	EUR	
Africa only	210 (98.59%)	0 (0%)	**3 (1.41%)**	213
Europe only	**1 (0.25%)**	**1 (0.25%)**	400 (99.5%)	402
Other	11 (10.58%)	20 (19.23%)	73 (70.19%)	104
Total	222	21	476	719

Table 3.4 Summary of CATIE subjects by their self-reported race and the inferred posterior probabilities from *Structure*.

Self-reported race among CATIE participants						*Structured*-allocated admixture fraction			No. of subjects
WHITE	BLACK	NATIVE AMERICAN	ASIAN	PACIFIC ISLANDER	HISPANIC LATINO	P-AFR	P-ASI	P-EUR	N
1	0	0	0	0	0	0.01	0.03	**0.95**	402
0	**1**	0	0	0	0	**0.80**	0.06	0.10	213
1	0	0	0	0	**1**	0.10	0.14	**0.74**	69
0	0	0	**1**	0	0	0.02	**0.93**	0.05	15
0	**1**	**1**	0	0	0	**0.68**	0.09	0.18	6
0	0	**1**	0	0	**1**	0.25	0.04	**0.71**	3
0	**1**	0	0	0	**1**	**0.61**	0.07	0.32	3
0	0	0	0	0	1	0.18	0.05	0.78	1
0	0	0	0	1	0	0.03	0.92	0.05	1
0	0	1	0	0	0	0.18	0.05	0.77	1
1	0	0	1	0	0	0.04	0.38	0.58	1
1	0	1	0	0	0	0.02	0.02	0.96	1
1	0	1	0	0	1	0.02	0.04	0.94	1
1	0	1	0	1	1	0.01	0.03	0.96	1
1	1	1	0	0	0	0.43	0.06	0.51	1

* P-AFR: posterior probability of African origin; P-ASI: probability of East Asian origin, P-EUR: probability of European continental ancestry.

4. Figures

Figure 3.1 A Directed Acyclic Graph (DAG) that models genetic effect to prevalent tardive dyskinesia (TD), adjusting for ancestry.

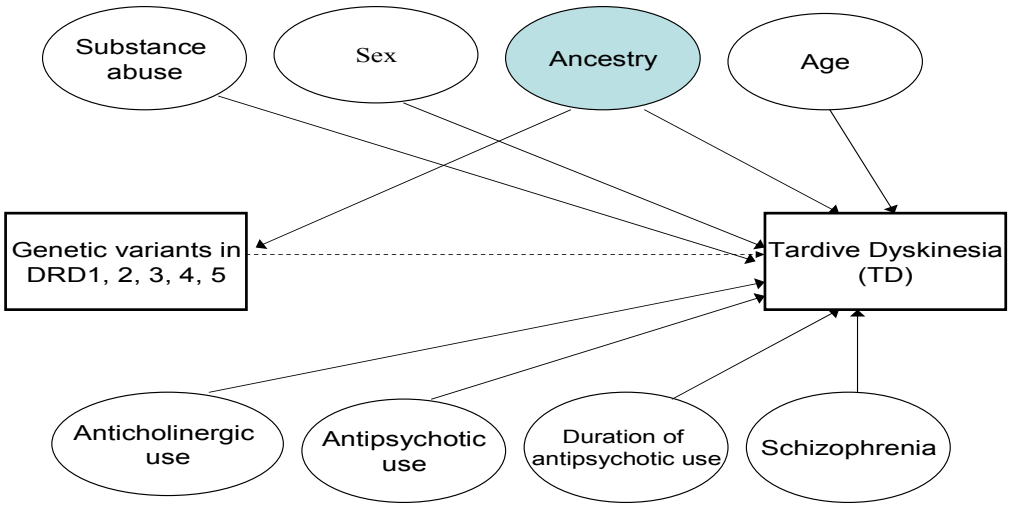


Figure 3.2 A Directed Acyclic Graph (DAG) that models genetic effect to TD among prevalent TD, adjusting for multiple covariates. Covariates filled with blue color were covariates identified as confounders in final model.

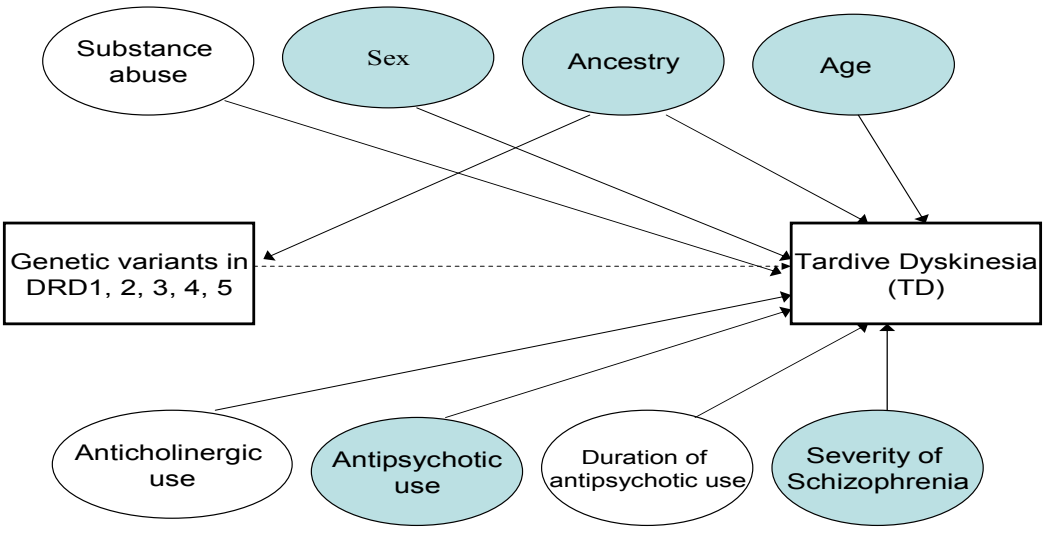
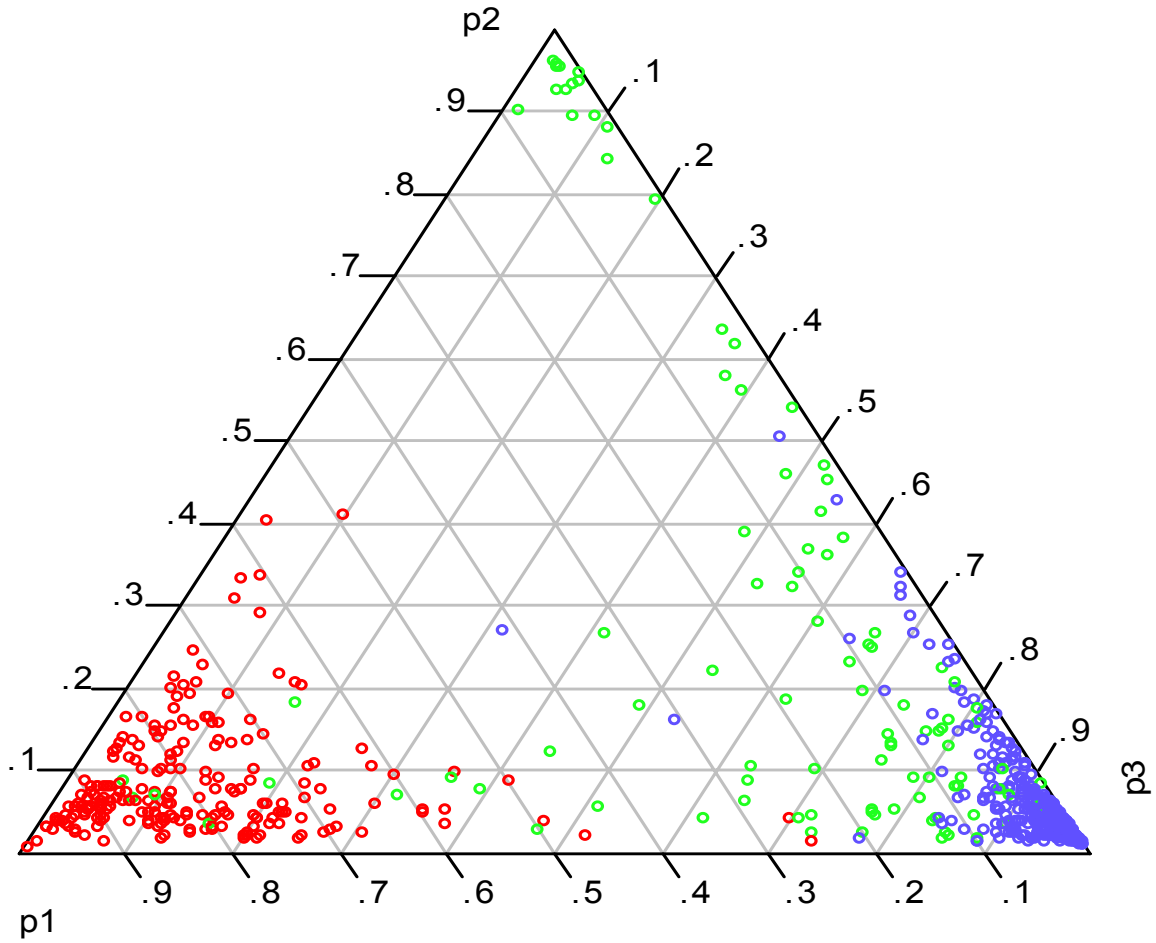


Figure 3.3 Ternary plot to present Structured-inferred proportion of African ancestry (P1), Asian ancestry (P2) and European ancestry (P3) in the CATIE study participants. Every dot represents self-report ancestry of each participant as “African-American” (red dot), "White" (blue dot), or “Other" (green dot).



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Chapter IV.
RESULTS

1. Paper I: The DRD3/Ser9Gly polymorphism and prevalence of tardive dyskinesia: A meta-analysis

1.1 Abstract

To elucidate a widely suspected but inconclusive association between rs6280 in the dopamine receptor 3 gene (*DRD3*) and prevalence of tardive dyskinesia (TD), we conducted a meta-analysis of results obtained in a systematic search of several bibliographic systems. We conducted several analyses of funnel plot asymmetry, overall heterogeneity, and study characteristics in analyses analogous to general, dominant and recessive inheritance models with the prevalence odds ratio (POR) as the measure of association. Thirteen eligible studies were identified with publication dates between 1997 and 2007. Evidence of reporting bias was discerned from funnel plot asymmetry in the dominant and general model analyses, but not in the recessive model analysis. Stratified analyses indicated that publication year, TD assessment method (Schooler-Kane criteria or other) and TD assessment frequency (single or repeated) were moderately associated with average PORs in the literature. Study population factors, such as average age, gender (percent female) and ancestry (Asian or European) also presented a moderate influence in the average PORs in the literature. Summary effect estimates under the dominant and general inheritance models were not warranted due to funnel plot asymmetry and heterogeneity. These

contraindications were not present under the recessive model, for which the summary estimate was POR= 0.93 (95% CI 0.70, 1.23). We conclude that there is no association between *DRD3* rs6280 polymorphisms and prevalence of TD.

1.2 Introduction

Tardive dyskinesia (TD), an involuntary movement disorder affecting the face, extremities and trunk, is a frequent, distressing, and potentially persistent side effect of long-term antipsychotic therapy (1). In the absence of safe and effective therapies for TD, understanding risk factors for prevalent TD is important for TD prevention in long-term schizophrenia care. Several risk factors have been proposed for TD, including antipsychotic exposure (particularly conventional agents), advanced age, female sex, African-American ancestry, substance abuse and anticholinergic use (2). However, these risk factors explain only a small portion of differential susceptibility to TD among patients with schizophrenia exposed to antipsychotics. Strong aggregate genetic effects on TD have been recognized across multiple populations (3-7), although the identification of specific and highly replicated sequence variation has thus far been lacking.

Biological plausibility has motivated studies to investigate the association between TD and rs6280, a polymorphic site in the dopamine receptor 3 gene (*DRD3*). The *DRD3* gene is positioned at chromosome 3q13.3 and has been hypothesized as a strong candidate gene for TD because DRD3 receptors densely distribute in the human ventral striatum and DRD3 mRNA is widely expressed in regions that are responsible for motor function (8). The single nucleotide polymorphism (SNP) rs6280

is located 25 base pairs downstream from the starting ATG codon in *DRD3*. A transition from adenine (A) to guanine (G) in rs6280 results in a serine to glycine substitution at position 9 in the extracellular N-terminal part of the receptor (9). Studies have demonstrated that replacement of the A allele (serine) with the G allele (glycine) increases the binding affinity of dopamine, which may result in differential susceptibility to TD (10).

However, literature on the association between rs6280 and TD has been inconclusive with prevalence odds ratios (PORs) ranging from 0.76 (95% confidence interval (C.I.)= 0.48- 1.20) (11) to 3.53 (95%C.I.= 1.26- 9.89) (12) when assuming a dominant model of inheritance. In 2002, a combined analysis of 780 patients with schizophrenia in seven groups reported an increased susceptibility to TD among subjects carrying at least one Gly allele in comparison to those carrying Ser/ Ser in rs6280 (OR= 1.33, 95%C.I.= 1.04, 1.70) (13). In 2006, a meta-analysis of 1,610 patients with schizophrenia indicated a slightly elevated risk of TD among those carrying the Gly allele in comparison to those with the Ser allele (OR= 1.17, 95%C.I.= 1.01- 1.37) but no association between rs6280 genotype and TD was identified (14).

Inconclusive findings in the literature may be due to small sample sizes in individual studies (median sample size= 116 of 13 studies) or differences in study characteristics. The 2006 meta-analysis study identified heterogeneity of effect sizes (14) but did not explore factors associated with the heterogeneous findings. Therefore, this study aimed to examine the association between genotypes in rs6280 and TD, while improving upon the earlier meta-analysis by including recent publications and implementing a more comprehensive evaluation of heterogeneity

and funnel plot asymmetry.

1.3 Methods

A systematic literature search was conducted in several bibliographic systems, including PubMed (1966-2007), CINAHL, Web of Science (1955-2007), BIOSIS Previews (1969-2007), and the Cochrane Library, using keywords: (tardive dyskinesia OR TD) AND (dopamine receptor 3 OR DRD3). No language criterion was set. All publications that met the following criteria were included: 1) TD as an outcome; 2) data on rs6280; 3) in human, and 4) not an abstract. We contacted authors up to three times by email in an attempt to acquire missing information.

TD outcome, genotype data and study characteristics were abstracted from all studies. The study characteristics were: 1) study design (cohort, matched cohort, case-control, matched case-control); 2) whether the Abnormal Involuntary Movement Scale (AIMS) (15) was implemented for TD assessment; 3) whether the Schooler-Kane criteria were employed that defined a subject as a probably TD if he/ she showed at least one 3 or 4 point item or at least two 2 point items among AIMS items 1 to 7 (16); 4) whether TD was evaluated repeatedly; 5) enrollment source (hospital, community, mix); and 6) publication year; 7) enrollment criteria of subjects' diagnosis (only schizophrenia, schizophrenia and other mental disorders); 8) average age; 9) sex (percent female); 10) ancestry (European, Asian, African, mixed); 11) Hardy-Weinberg equilibrium (HWE) p-value; 12) type of schizophrenia (chronic, acute, mix); 13) history of antipsychotic use (Yes, No); 14) current or past conventional antipsychotic use (Yes, No).

For cell counts of 2 or fewer persons, we conducted a sensitivity analysis of the six sparse-data smoothing or continuity correction methods described by Sweeting et al. (17). Statistical analyses included a standard heterogeneity test (18), and the funnel plot symmetry tests of Begg and Mazumdar (19) and Egger et al. (20). Duval and Tweedie's trim-and-fill imputation procedure was used as an additional analysis of funnel plot symmetry (21).

Stratified and random-effects meta-regression analyses (5) were conducted to identify study characteristics associated with effect measure estimates. A restricted maximum likelihood method was used to estimate the among-population variance and, for each study characteristic, the stratum with the largest number of studies was used as the referent. Continuous study characteristics were grouped as below in stratified analyses: average age (≤ 50 and > 50 years), percent female (< 0.4 and ≥ 0.4), HWE p-value (< 0.1 and ≧0.1), and publication year (1997-2001; 2002-2007). All statistical analyses were implemented in three genetic models of inheritance: general, dominant, and recessive model, using STATA 8.0 (Stata Corporation, College Station, TX, USA.). In the general model, the three groups (Gly/Gly, heterozygotes, and Ser/Ser) are treated as three distinct groups, two of which are contrasted with a single referent (Ser/Ser). In the dominant model, the heterozygotes are grouped with those who are homozygous Gly/Gly and contrasted with those who are homozygous Ser/Ser. In the recessive model, those who are homozygous Gly/Gly are compared with the union of the heterozygotes and those who are homozygous Ser/Ser.

1.4 Results

A total of 13 studies met inclusion criteria from 120 PubMed, 97 ISI, 183 BIOSIS and 7 The Cochrane Library citations identified as of Jun 2007. There were 16 citations that assessed the association between TD and *DRD3* Ser9Gly. One study was excluded because the TD outcome was only examined continuously using the AIMS score (22). Another study was excluded because it was a repeat analysis from a prior study (23). Two conference abstracts (24, 25) were excluded because the majority of contextual information needed for the stratified analyses was missing. An additional study was identified when reviewing the references of the original studies (26). The study information from the 13 studies included in this meta-analysis is summarized in tables 4.1.1 and 4.1.2.

All studies assessed were cross-sectional investigations of prevalent TD among chronic patients with schizophrenia. Only one study did not use AIMS in TD assessment (27). Two studies used AIMS but did not adopt Schooler-Kane criteria for TD diagnosis (12, 28). Ten studies reported experience of typical antipsychotic use in their study populations, while 3 studies did not specify types of antipsychotic use in their study populations. All studies were conducted among patients with a history of antipsychotic medications. Four studies had a cell count of 2 or smaller in the cross-classification of TD in the homozygous genotype cell (12, 29-31). In the sensitivity analysis, the meta-analytic results were very similar across the different approaches to smoothing or continuity correction (17). Therefore, we followed convention by allowing the “metan” macro in STATA to add 0.5 to all cell counts for each study with a zero cell count.

In the analysis of these 13 studies, small p-values of symmetry tests were noted mainly when implementing a dominant model of inheritance (Table 4.1.3). The funnel plot shows that after including 5 imputed estimates obtained from the trim-and-fill procedure, the summarized effect was reduced from 1.16 to 1.02 (Figure 4.1.1). Heterogeneity of POR estimates was moderately indicated when comparing those participants with the Ser/Gly genotype to those with the Ser/Ser genotype and while assuming a general model of inheritance. The POR of each study, assuming a recessive model and a general model are presented in Figures 4.1.2 and 4.1.3, respectively. No significant relationship between rs6280 genotypes and TD was noted. The summarized POR in a recessive inheritance model was the only estimate for which heterogeneity and asymmetry of funnel plots were not detected.

Several study characteristics showed an association with PORs across these 13 studies (Table 4.1.4). Methodological factors associated with TD PORs were publication year, TD diagnostic criteria, and requirement of repeated TD evaluations. Studies published between 1997-2001 reported a stronger association than studies published between 2002-2007. Two studies that did not apply the Schooler-Kane criteria for TD diagnosis reported ~ 2 times stronger PORs than studies using the Schooler-Kane criteria. Studies that required repeated TD evaluations reported smaller PORs than studies that identified TD based on one AIMS evaluation.

Percent female, average age and ancestry also showed an association with PORs. Studies with fewer female participants or with older subjects had stronger PORs than studies with higher numbers of female participants or who with younger subjects. In contrast to those studies that included Asian populations, studies with

European subjects reported a consistent increase of PORs for all genetic models examined. The association between ancestry and PORs of TD was particularly strong when contrasting Gly/Gly genotypes with other genotypes. Prevalence odds ratio reported in the literature were not associated with either the HWE p-value or the inclusion of subjects with mental disorders other than schizophrenia. Most small p-values in the symmetry tests occurred when implementing a dominant model of inheritance. After trim-and-fill imputation, estimates with small p-values in the symmetry test were almost reduced to the null (Appendix 1).

1.5 Discussion

Overall, the results from this study do not support an association between rs6280 and TD. This conclusion of no association was most convincing when applying the recessive model of inheritance because no evidence for heterogeneity or asymmetry of funnel plots was noted. However, the null results extended to the dominant and general models.

Symmetry tests of funnel plots in overall and stratified analyses indicated that the PORs obtained when implementing a dominant model of inheritance were more likely to be inflated than estimates obtained when implementing other inheritance models. Moreover, when using the trim-and-fill imputation, the majority of summary estimates decreased to near the null. The observed asymmetry of funnel plots when implementing a dominant model (Figure 4.1.1.) could be due to publication bias, to important study characteristics that are associated with study size, or both and also chance (32). As the frequency of homozygous genotypes was small in the majority of

studies, researchers tended to examine the relationship between genotype and TD using a dominant model of inheritance to increase their statistical power. Therefore, the number of possible unpublished studies that would have implemented a dominant model of inheritance was probably higher that the number that would have implemented some other model of inheritance. This may partially explain why an asymmetric funnel plot was more obvious when implementing the dominant model rather than either the general or recessive models. We also found a strong association between publication year and strength of the TD POR, indicating that "statistically significant estimates" found in earlier studies were not supported in later publications, a common occurrence in genetic epidemiology studies (33).

A moderate to strong association between rs6280 and TD was noted among studies applying the Schooler-Kane criteria for TD diagnosis or in studies that did not require repeated TD evaluations. However, the elevated association diminished in the contrast group, implying that different TD diagnosis criteria may partially explain heterogeneous estimates across studies. Although these observations were consistently noted when applying different models of inheritance, informative confidence intervals of the PORs were not obtained due to the small number of studies that were available for consideration. In addition, the association between rs6280 and prevalent TD may be modified by age, sex and ancestry as a moderate rs6280 TD association was observed in studies with fewer female, aged, and European subjects, but not in their contrasting groups.

As TD is a common outcome and we were obliged by the design of the case-control studies to use the POR as the measure of association, it would lend

context to translate even some of the higher summary PORs in our analysis into absolute differences in prevalence (34). With typical baseline TD prevalences on the order of 40% to 50% in the available cohort studies (11, 12, 26-28, 30, 35-39), a POR of 1.2 (e.g., the Ser/Gly vs. Ser/Ser summary estimate from all studies without imputation, Table 4.1.3) would correspond to a prevalence difference in the range of 2% to 5%. A POR of 1.8 (e.g., the Gly/Gly vs. Ser/Ser summary estimate in European patient populations, Table 4.1.4) would correspond to a prevalence difference of about 15%.

Some limitations of this meta-analysis should be noted. First, we were unable to adjust our PORs for the effects of confounders because information on many covariates was missing in the majority of earlier studies. However, the degree of confounding effect by environmental factors may not be of great concern as literature has not supported an association between the rs6280 polymorphisms and environmental factors (40). Second, this meta-analysis did not include two recent conference abstracts, which may affect the completeness of the literature we assessed. However, as our conclusions were consistent with study findings in both abstracts, excluding the abstracts should not strongly affect results of this meta-analysis. Third, symmetry and heterogeneity tests in this study may only have moderate statistical power due to the small number of studies included in this meta-analysis.

This meta-analysis was strengthened by an extensive search of the literature in several bibliographic systems and also by the use of secondary references to supplement the initial search. Particularly, two recent large studies were added in this

updated meta-analysis. Second, we refrained from using summary estimates when asymmetry of funnel plots was present. This avoided misleading conclusions for the rs6280 prevalent TD association because of a biased sample of publications. Third, this study implemented stratified analysis of many study characteristics to explore sources of heterogeneity in studies. We suggested important methodological factors and population features which may have affected the strength of the association between rs6280 and TD.

Study findings in this meta-analysis indicated some directions for future studies. First, the association between rs6280 polymorphisms and prevalence of TD may be subtle. Large studies that carefully consider environmental factors and that comprehensively explore the relationship between TD and other genetic variations are needed to elucidate the role of genetics in TD etiology. In addition, the effect of genetic variants on TD may differ by criteria for TD assessment and diagnosis, age, sex ratio and ancestry of a study population. Information on these study characteristics should be clearly described in a TD genetic association study. Lastly, reporting bias was indicated in this meta-analysis, particularly when we examined the association when assuming a dominant model of inheritance. Mechanisms to minimize the underreporting of studies with “no statistically significant findings” must be encouraged.

1.6 Tables

Table 4.1.1 Summary of association studies between DRD3 rs6280 and tardive dyskinesia (TD)

First author (publication year)	Ethnicity (country)	TD (N=928)				non-TD (N=1098)			
		Total	Ser/Ser	Ser/Gly	Gly/Gly	Total	Ser/Ser	Ser/Gly	Gly/Gly
Steen (1997)	European (Scotland)	**51**	45%	33%	22%	**49**	57%	39%	4%
Inada (1997)	Asian (Japan)	**49**	51%	35%	14%	**56**	59%	34%	7%
Segman (1999)	European (Israel)	**53**	24%	70%	6%	**63**	46%	47%	8%
Lovlie (2000)	European (UK)	**32**	34%	44%	22%	**39**	44%	46%	10%
Rietschel (2000)	European (Germany)	**79**	49%	47%	4%	**78**	47%	45%	8%
Liao (2001)	Asian (Taiwan)	**21**	28%	67%	5%	**94**	58%	31%	11%
Garcia (2001)	Asian (Hong Kong)	**65**	55%	35%	10%	**66**	64%	27%	9%
Woo (2002)	Asian (Korean)	**59**	42%	48%	10%	**54**	39%	61%	0%
Chong (2003)	Asian(Singapore)	**117**	51%	39%	10%	**200**	45%	44%	11%
Zhang (2003)	Asian (China)	**42**	45%	53%	2%	**52**	58%	33%	9%
Liou (2004)	Asian (Taiwan)	**102**	50%	40%	10%	**115**	53%	36%	11%
Leon (2005)	Mixed (US)*	**162**	43%	43%	14%	**354**	42%	42%	16%
Srivastava (2006)	Asian (India)	**96**	28%	57%	15%	**238**	33%	49%	18%

*Study population in the study was a mix of European and African-ancestry.

Table 4.1.2 Characteristics of 13 studies of DRD3 rs6280 and tardive dyskinesia (TD) prevalence

First author (publication year)	Methodological factors*						Study population factors				
	Design	AIMS use	Repeated TD evaluation	TD classification in S-K criteria	Enrollment source	Enrollment criteria	Ancestry	Average age	HWE* p-value	Percent female	Chronic schizophrenia
Steen (1997)	cohort	Yes	No	Yes	community	SCZ	EUR	52.1	1	0.44	Yes
Inada (1997)	cohort	Yes	Yes (12 months)	Yes	hospital	Rx	ASI	60.7	0.7	0.50	Yes
Segman (1999)**	cs-cn	Yes	No	Yes	hospital	SCZ+ Rx	EUR	49.6	0.8	0.47	Yes
Lovlie (2000)	cohort	Yes	No	No	hospital	SCZ+ Rx	EUR	46.4	1	0.27	Yes
Rietschel (2000)	cohort	No	Yes (3 months)	Yes	hospital	Rx	EUR	43.1	0.8	0.52	Yes
Liao (2001)	cohort	Yes	No	No	hospital	SCZ+ Rx	ASI	40.7	0.06	0.37	Yes
Garcia (2001)	cohort	Yes	No	Yes	hospital	SCZ	ASI	51.3	0.08	0.35	Yes
Woo (2002)	cohort	Yes	No	Yes	hospital	SCZ+ Rx	ASI	40.4	0.001	0.24	Yes
Chong (2003)	cohort	Yes	Yes (3 months)	Yes	hospital	SCZ	ASI	65.9	0.9	0.73	Yes
Zhang (2003)**	cs-cn	Yes	Yes (4 months)	Yes	hospital	SCZ+ Rx	ASI	55.1	0.3	0.00	Yes
Liou (2004)	cohort	Yes	Yes (3 months)	Yes	hospital	SCZ+ Rx	ASI	47.2	0.2	0.41	Yes
Leon (2005)	cohort	Yes	No	Yes	Mix	Rx	EurAA	42.4	0.05	0.47	Yes
Srivastava (2006)	cohort	Yes	No	Yes	hospital	SCZ	Asian	32.3	1	0.46	Yes

*Design: cs-cn= matched case-control study

AIMS= Abnormal Involuntary Movement Scale

S-K criteria: Schooler-Kane criteria

Underlying condition: SCZ+ Rx=patients with chronic schizophrenia with history of antipsychotic use; SCZ= only required schizophrenia as a comorbidity; = as long as on antipsychotic use.

Ancestry: EUR= European; ASI= Asian

HWE= Hardy-Weinberg equilibrium

**: Average age and percent female were abstracted from the control group

Table 4.1.3 Homogeneity test p-values, funnel plot symmetry test p-values, and summary prevalence odds ratio (POR) estimates and 95% confidence intervals (CI) with and without trim and fill imputation, by inheritance model, from 13 studies of DRD3 rs6280 and tardive dyskinesia (TD).

Model and contrast	Homogeneity test p-value	Summary POR (95% CI) without imputation	Symmetry test p-value		No. of results Imputed by trim and fill	Summary POR (95% CI) with imputation
			Begg	Egger		
General model						
Gly/Gly vs. Ser/Ser	0.3	1.02 (0.76, .37)	0.2	0.1	1	0.99 (0.74, 1.34)
Ser/Gly vs. Ser/Ser	0.1	1.19 (0.99, 1.42)	0.1	0.05	4	1.03 (0.87, 1.21)
Dominant model						
Gly+ vs. Gly-	0.2	1.16 (0.98, 1.38)	0.003	0.004	5	1.02 (0.87, 1.19)
Recessive model						
Gly/Gly vs. others	0.2	0.93 (0.70, 1.23)	0.5	0.3	0	0.93 (0.70, 1.23)

Table 4.1.4 Stratified and meta-regression analyses of methodological and population study characteristics in 13 studies of DRD3 rs6280 and summary prevalence odds ratio (POR) of tardive dyskinesia (TD).

Characteristic	Contrast	Component	Studies	Homogeneity p-value	Meta-regression OR (95% CI)	Summary OR (95% CI)
Enrollment criteria	Gly/Gly vs. Ser/Ser	Schizophrenia	4	0.1	0.85 (0.39, 1.84)	1.03 (0.64, 1.66)
	Ser/Gly vs. Ser/Ser			0.4	0.70 (0.42, 1.17)	1.08 (0.79, 1.46)
	Gly+ vs. Gly-			0.3	0.74 (0.47, 1.16)	1.09 (0.81, 1.45)
	Gly/Gly vs. others			0.09	0.97 (0.47, 2.02)	0.95 (0.61, 1.47)
	Gly/Gly vs. Ser/Ser	Antipsychotics	3	0.3	0.73 (0.33, 1.61)	0.89 (0.55, 1.46)
	Ser/Gly vs. Ser/Ser			0.9	0.67 (0.39, 1.14)	1.04 (0.76, 1.42)
	Gly+ vs. Gly-			0.7	0.68 (0.43, 1.09)	1.01 (0.75, 1.36)
	Gly/Gly vs. others			0.3	0.90 (0.42, 1.89)	0.88 (0.55, 1.39)
	Gly/Gly vs. Ser/Ser	Schizophrenia & Antipsychotics	6	0.4	1.0	1.22 (0.66, 2.26)
	Ser/Gly vs. Ser/Ser			0.05	1.0	1.53 (1.11, 2.12)
	Gly+ vs. Gly-			0.2	1.0	1.48 (1.08, 2.02)
	Gly/Gly vs. others			0.2	1.0	0.98 (0.54, 1.76)
Study design	Gly/Gly vs. Ser/Ser	Matched case-control	2	0.3	0.80 (0.21, 3.01)	0.83 (0.23, 2.99)
	Ser/Gly vs. Ser/Ser			0.6	2.21 (1.19, 4.10)	2.43 (1.35, 4.38)
	Gly+ vs. Gly-			0.4	1.91 (1.05, 3.48)	2.09 (1.18, 3.71)
	Gly/Gly vs. others			0.4	0.51 (0.15, 1.80)	0.49 (0.14, 1.67)
	Gly/Gly vs. Ser/Ser	Cohort	11	0.2	1.0	1.03 (0.76, 1.40)
	Ser/Gly vs. Ser/Ser			0.3	1.0	1.10 (0.92, 1.33)
	Gly+ vs. Gly-			0.4	1.0	1.10 (0.92, 1.31)
	Gly/Gly vs. others			0.1	1.0	0.96 (0.72, 1.28)
TD	Gly/Gly vs. Ser/Ser	Non-S-K criteri	2	0.4	2.01 (0.58, 7.02)	1.96 (0.59, 6.58)

classification		a				
	Ser/Gly vs. Ser/Ser			0.08	1.96 (0.89, 4.35)	2.27 (1.09, 4.75)
	Gly+ vs. Gly-			0.2	1.96 (0.93, 4.13)	2.22 (1.10, 4.49)
	Gly/Gly vs. others			0.2	1.65 (0.52, 5.28)	1.48 (0.48, 4.58)
	Gly/Gly vs. Ser/Ser	S-K criteria	11	0.2	1.0	0.98 (0.72, 1.33)
	Ser/Gly vs. Ser/Ser			0.3	1.0	1.14 (0.94, 1.37)
	Gly+ vs. Gly-			0.4	1.0	1.12 (0.94, 1.33)
	Gly/Gly vs. others			0.2	1.0	0.90 (0.67, 1.20)
TD evaluation	Gly/Gly vs. Ser/Ser	Repeated	5	0.4	0.72 (0.39, 1.35)	0.83 (0.50, 1.36)
	Ser/Gly vs. Ser/Ser			0.4	0.81 (0.52, 1.27)	1.06 (0.80, 1.40)
	Gly+ vs. Gly-			0.4	0.78 (0.52, 1.16)	1.02 (0.78, 1.33)
	Gly/Gly vs. others			0.4	0.84 (0.47, 1.52)	0.83 (0.51, 1.34)
	Gly/Gly vs. Ser/Ser	Non-repeated	8	0.2	1.0	1.14 (0.79, 1.66)
	Ser/Gly vs. Ser/Ser			0.08	1.0	1.29 (1.02, 1.64)
	Gly+ vs. Gly-			0.2	1.0	1.28 (1.02, 1.61)
	Gly/Gly vs. others			0.09	1.0	0.98 (0.69, 1.39)
Publication year	Gly/Gly vs. Ser/Ser	1997- 2001	7	0.3	1.92 (0.99, 3.72)	1.62 (0.93, 2.84)
	Ser/Gly vs. Ser/Ser			0.2	1.42 (0.95, 2.12)	1.50 (1.09, 2.04)
	Gly+ vs. Gly-			0.3	1.53 (1.06, 2.19)	1.54 (1.15, 2.07)
	Gly/Gly vs. others			0.2	1.66 (0.88, 3.12)	1.34 (0.78, 2.30)
	Gly/Gly vs. Ser/Ser	2002- 2007	6	0.6	1.0	0.85 (0.60, 1.20)
	Ser/Gly vs. Ser/Ser			0.3	1.0	1.06 (0.84- 1.32)
	Gly+ vs. Gly-			0.6	1.0	1.01 (0.82, 1.25)
	Gly/Gly vs. others			0.4	1.0	0.81 (0.58, 1.12)
Average age	Gly/Gly vs. Ser/Ser	< 45	5	0.4	0.75 (0.41, 1.36)	0.89 (0.60, 1.33)
	Ser/Gly vs. Ser/Ser			0.6	0.79 (0.49, 1.25)	1.09 (0.84, 1.41)

	Gly+ vs. Gly-			0.8	0.77 (0.51, 1.17)	1.05 (0.82, 1.33)
	Gly/Gly vs. others			0.4	0.76 (0.43, 1.33)	0.81 (0.57, 1.19)
	Gly/Gly vs. Ser/Ser	≧ 45	8	0.2	1.0	1.20 (0.77, 1.86)
	Ser/Gly vs. Ser/Ser			0.04	1.0	1.30 (1.00, 1.68)
	Gly+ vs. Gly-			0.08	1.0	1.30 (1.01, 1.66)
	Gly/Gly vs. others			0.1	1.0	1.09 (0.71, 1.66)
Percent female	Gly/Gly vs. Ser/Ser	< 40%	5	0.3	1.50 (0.65, 2.47)	1.44 (0.66, 3.11)
	Ser/Gly vs. Ser/Ser			0.08	1.31 (0.80, 2.15)	1.48 (1.00, 2.18)
	Gly+ vs. Gly-			0.3	1.33 (0.86, 2.06)	1.47 (1.01, 2.12)
	Gly/Gly vs. others			0.1	1.34 (0.60, 2.99)	1.19 (0.57, 2.50)
	Gly/Gly vs. Ser/Ser	≧ 40%	8	0.2	1.0	0.96 (0.69, 1.32)
	Ser/Gly vs. Ser/Ser			0.3	1.0	1.12 (0.91, 1.37)
	Gly+ vs. Gly-			0.2	1.0	1.09 (0.90, 1.33)
	Gly/Gly vs. others			0.2	1.0	0.89 (0.66, 1.20)
Ancestry	Gly/Gly vs. Ser/Ser	Europeans	4	0.1	1.97 (0.83, 4.68)	1.76 (0.82, 3.75)
	Ser/Gly vs. Ser/Ser			0.2	1.08 (0.58, 1.98)	1.35 (0.91, 2.02)
	Gly+ vs. Gly-			0.2	1.25 (0.75,2.09)	1.45 (0.99,2.12)
	Gly/Gly vs. others			0.06	1.82 (0.80, 4.13)	1.46 (0.71, 3.01)
	Gly/Gly vs. Ser/Ser	Asians	8	0.5	1.0	0.97 (0.65, 1.44)
	Ser/Gly vs. Ser/Ser			0.08	1.0	1.20 (0.94, 1.52)
	Gly+ vs. Gly-			0.2	1.0	1.15 (0.92,1.44)
	Gly/Gly vs. others			0.4	1.0	0.87 (0.60,1.27)
		Mix	1	N/A	N/A	N/A
HWE p value	Gly/Gly vs. Ser/Ser	< 0.1	4	0.4	0.89 (0.48, 1.66)	0.95 (0.58, 1.55)
	Ser/Gly vs. Ser/Ser			0.04	1.00 (0.60, 1.65)	1.16 (0.85, 1.58)

Gly+ vs. Gly-			0.1	0.97 (0.62, 1.51)	1.12 (0.84, 1.50)
Gly/Gly vs. others			0.3	0.92 (0.51, 1.65)	0.88 (0.55, 1.40)
Gly/Gly vs. Ser/Ser	≧ 0.1	9	0.2	1.0	1.06 (0.73, 1.54)
Ser/Gly vs. Ser/Ser			0.3	1.0	1.20 (0.96, 1.50)
Gly+ vs. Gly-			0.3	1.0	1.19 (0.96, 1.47)
Gly/Gly vs. others			0.1	1.0	0.96 (0.67, 1.36)

1.7 Figures

Figure 4.1.1 Funnel plot of prevalence odds ratios (solid circles) from 13 studies of DRD3 rs6280 and tardive dyskinesia (TD) under the dominant model (Gly/Gly and Ser/Gly vs. Ser/Ser). Five estimates imputed by the trim and fill procedure are shown as hollow circles.

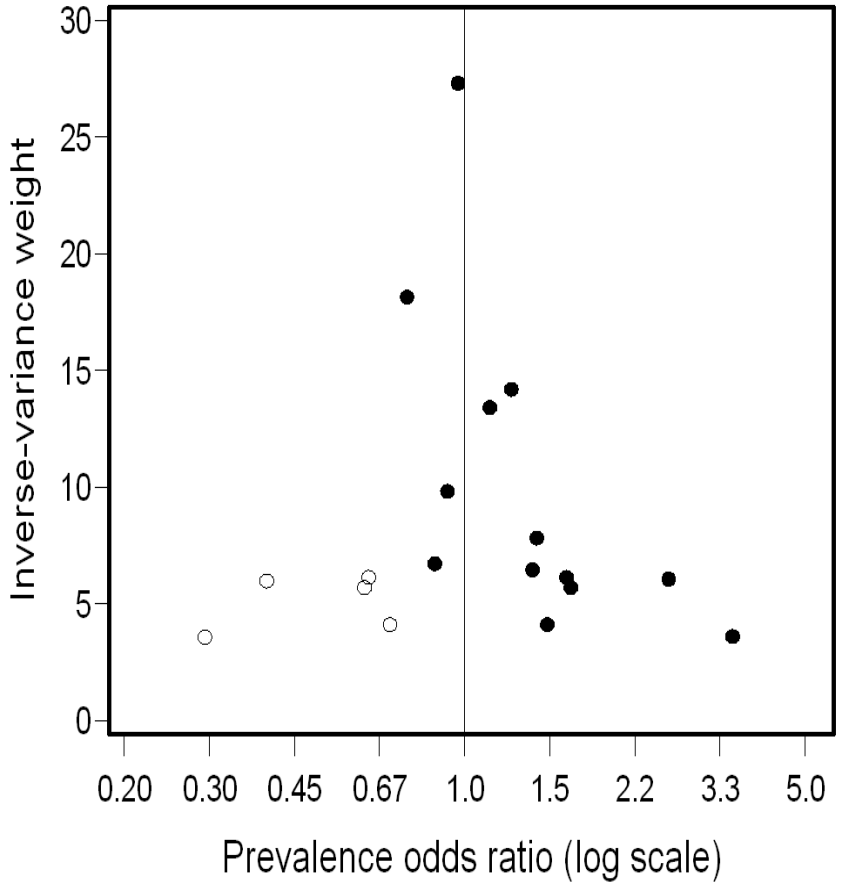


Figure 4.1.2 Prevalence odds ratios and 95% confidence intervals from 13 studies of TD and rs6280 when comparing Gly/Gly to SerGly+ Ser/Ser polymorphism under the recessive model of inheritance.

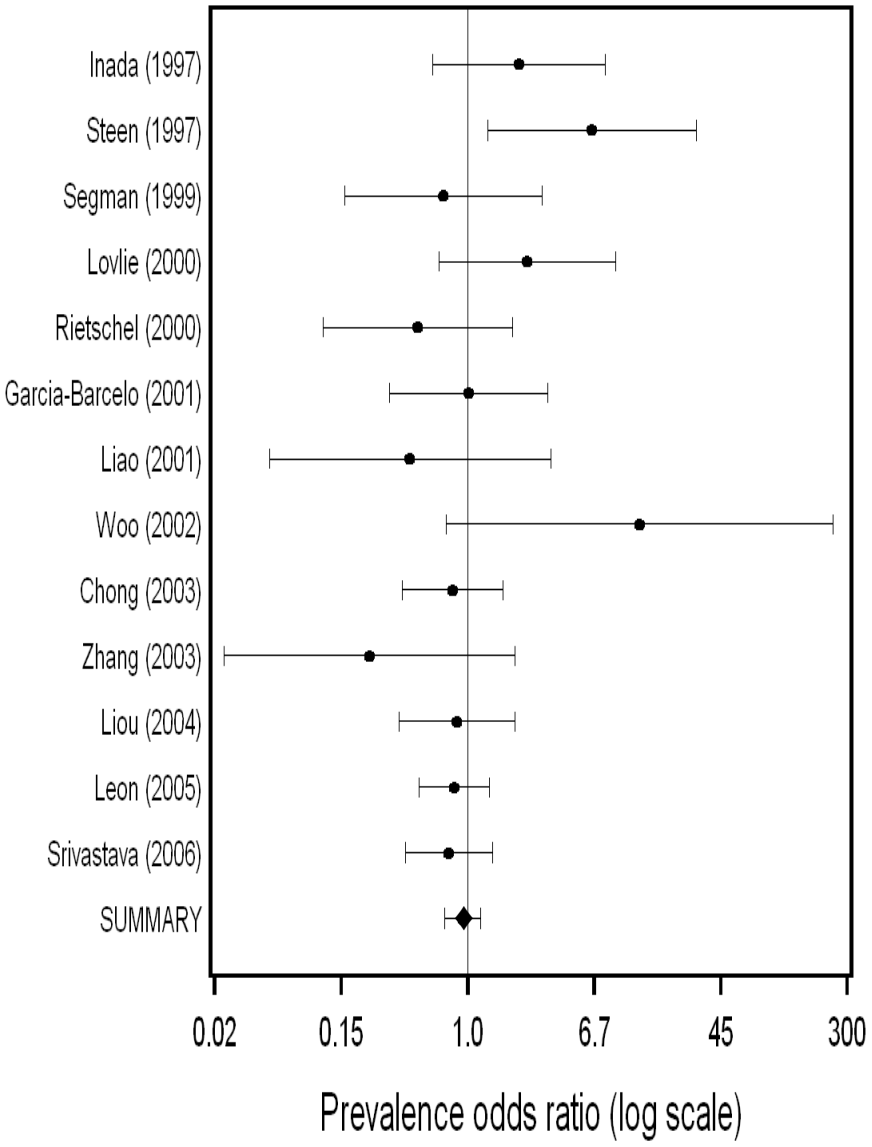
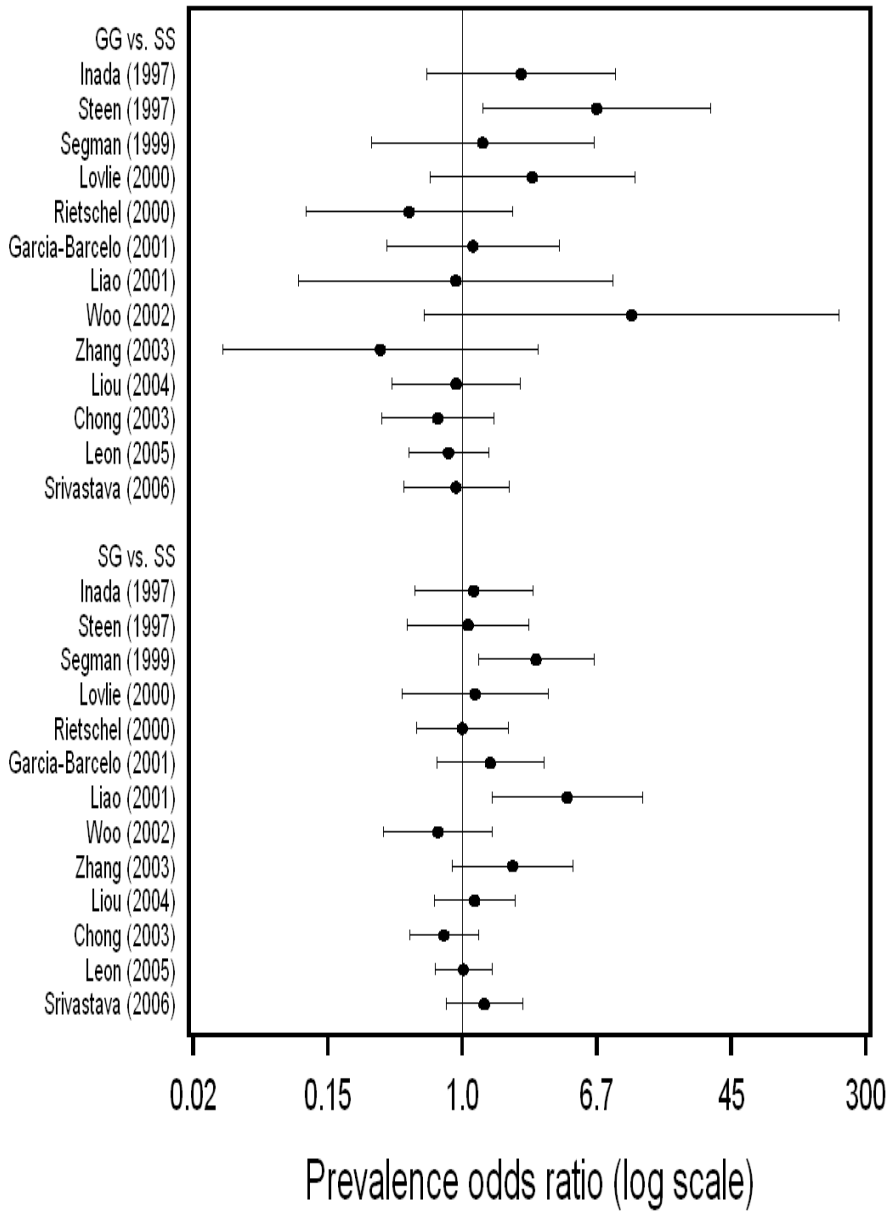


Figure 4.1.3 Prevalence odds ratios and 95% confidence intervals from 13 studies of TD and rs6280 under the general inheritance model. The top part of the figure contrasts Gly/Gly with Ser/Ser and the bottom part contrasts Ser/Gly with Ser/Ser.



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2. Paper II: Association between tardive dyskinesia and dopamine receptor genes among patients with chronic schizophrenia: an ancillary study to the CATIE trial

2.1 Abstract

Tardive dyskinesia (TD), an involuntary movement disorder, is a serious and potentially irreversible adverse effect in the course of long-term antipsychotic therapy. Current understanding about TD pathophysiology is limited. This study investigated associations between TD and 54 single nucleotide polymorphisms (SNPs) in dopamine receptor genes (*DRD1, DRD2, DRD3, DRD4,* and *DRD5*) among 711 patients with chronic schizophrenia. While several SNPs demonstrated nominal associations with TD, after multiple comparison adjustments, no SNPs or haplotype in these five dopamine receptor genes showed a statistically significant association with TD.

2.2 Introduction

Tardive dyskinesia (TD), an involuntary movement disorder, is a frequent and potentially irreversible side effect of long term antipsychotic treatment. Studies have reported a greater than 20% prevalence of TD among patients treated with conventional antipsychotic medications (1-3). No effective treatment for TD is available so far (4). Fortunately, the introduction of atypical antipsychotic medications since 1990s have greatly reduced the risk of TD in long-term antipsychotic treatment (5). However, atypical antipsychotics also incur several serious side effects, such as

weight gain (6) and changes in glucose and lipid metabolism (7, 8). In addition, atypical antipsychotic therapy is, on average, ten times more expensive than conventional antipsychotic therapy, greatly increasing the financial burden of long-term antipsychotic therapy. Therefore, understanding TD is an important task for optimal long-term schizophrenia care.

Several risk factors for TD have been proposed, including advanced age, conventional antipsychotic use, African-American ancestry, anticholinergic medication use, female gender, psychiatric diagnosis, and substance abuse (9). However, the data on these associations are still inconclusive and only explain a small portion of the considerable individual variation in the risk of TD. It has been suggested that genetic factors contribute to the pathogenesis of TD. Animal studies have reported significant variation in the onset of vacuous chewing movement and repetitive jaw movement, similar orofacial symptoms of TD across different genetic strains of rats (10, 11). Strong aggregate genetic effects on TD have been recognized across multiple populations (12-16), although the identification of specific variants has thus far been lacking.

Several lines of evidence support the evaluation of dopamine receptor genes as candidate genes for TD. First, dopamine receptors, particularly *DRD2* and *DRD3* (17, 18), have been widely suspected as drug targets for antipsychotic medications. Second, TD has been widely suspected to be caused by blockade of dopamine D2 receptors in the basal ganglia, resulting in hypersensitivity of nigrostriatal dopamine pathway in the brain, a system particularly involved in production of movement (19). In addition, animal and human studies have demonstrated an association between

alternations in gene expression in both *DRD1* and *DRD2* and the pathogenesis of neurological toxicity in long-term antipsychotic use (20, 21).

However, the current literature on the association between dopamine receptor genes and TD has been largely contradictory, which could be due to many factors, including inadequate statistical power in most studies (22, 23), absence of confounding adjustment (23, 24), reliance on one or a few genetic markers, and differences across studies in important study characteristics. This study aimed to evaluate the relationship between single nucleotide polymorphisms (SNPs) in five dopamine receptor genes (*DRD1, DRD2, DRD3, DRD4,* and *DRD5*) and risk of TD, while improving upon earlier work, as no study has yet to perform such a comprehensive analysis in terms of the coverage of these five genes, the large size of the study population, and the careful consideration of multiple confounders.

2.3 Methods

The study population consisted of 711 subjects who participated in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) funded by National Institute of Health (NIH) and agreed to provide a sample of their DNA. Inclusion and exclusion criteria for CATIE have been detailed previously (25). Briefly, participants in CATIE were 18-65 years old, met diagnostic criteria for chronic schizophrenia defined by Diagnostic and Statistical Manual of Mental Disorder-Fourth Edition (DSM-IV) (25) and had decisional capacity to participate in the study.

TD was diagnosed using standardized examination procedures and rating scales (26). The Abnormal Involuntary Movement Scale (AIMS) is currently the most widely

accepted measurement tool for TD in clinical research (27). The AIMS is a 12-item questionnaire that measures the severity of involuntary movements in several body regions, including: mouth, face, extremities, and trunk. Severity of TD is evaluated on a scale ranging from 0 to 4 points with higher scores representing greater severity. AIMS scores may be interpreted using different criteria for TD diagnosis; in this study we implemented the Schooler-Kane criteria, which defines TD as at least one item rated greater than 3 or at least two items rated grater than 2 in item 1 to item 7 (28). We did not implement the criterion of at least three months of prior antipsychotic exposure.

AIMS evaluation was repeatedly measured in CATIE, including at baseline, every three months during the follow-up, and at the end of each phase of the trial (29). This study investigated the association between SNPs in dopamine receptor genes and having TD at any time in the CATIE study. TD was considered present if a subject met probable TD criteria at least once, either at the baseline evaluation or at any time during the 18-months follow-up of the CATIE trial. The reference group was composed of participants who never met the Schooler-Kane criteria for probable TD at any study assessment.

Fifty four SNPs for five dopamine receptor genes were selected using TAMAL (30) and multiple-marker haplotype r^2 statistics (31) based on the HapMap Phase 1 data (32) were selected using TagIT (31). A minimum r^2 of ≥ 0.85 was required. Genotyping was conducted using Illumina GoldenGate technology (http://www.illumina.com) according to protocol at the Duke University core facility directed by Dr. Kevin Shianna.

In order to control confounding from population substructure, 75 ancestry informative markers selected using HapMap panels were included in the Illumina genotyping runs and genotyped in CATIE study subjects. HapMap samples were then used as the prototypes for continental ancestry to which CATIE subjects can be compared. We then used the *Structure* program (33), which uses a Bayesian approach and Markov chain Monte Carlo (MCMC) method to determine the posterior probability for each study subject being classified into one of three main sources of human ancestry (African, East Asian, and European). These three probabilities sum to 1.0 and subjects could have had substantial ancestry from each source. The probabilities of European and East Asian ancestry were used as covariates as their intercorrelation was the lowest.

Several other covariates were measured in this study, including age, sex, antipsychotic use, Years since first antipsychotic use, commitment anticholinergic use, and substance use at baseline. Type of antipsychotic use at baseline was classified into three categories: no use, only atypical antipsychotic use and conventional antipsychotic use. As participants without TD at baseline were randomly assigned to all treatment arms in CATIE, only baseline antipsychotic use was considered in confounding adjustment (25). Years since first antipsychotic use was also included as an approximate measure of age of onset. Substance use, including alcohol and illicit drug use, was measured as a dichotomous variable at baseline, using information from several indicators, including clinician's ratings using the Structured Clinical Interview for DSM-IV (SCID) (34), and toxological assays of participants' hair and urine. Participants meeting DSM-IV criteria for substance abuse or dependence

(excluding nicotine and caffeine) via the SCID or testing positive for any illicit drug were classified as having clinically significant substance use.

Analytical Methods

We first implemented contingency testing using Fisher's exact test and assuming a general inheritance model (2-degree of freedom test) to obtain an overview of unadjusted associations between each SNP and TD. For SNPs with cell counts less than or equal to 5, we examined the distributions of genotype across TD status using Fisher's exact test to determine whether statistical differences between homozygote variant and heterozygote variant were noted. When the Fisher's exact test was not rejected, we grouped the rare homozygote variant and the heterozygote genotypes together to examine SNP-TD associations, assuming a dominant model of inheritance. These analyses were implemented using SAS 9.0 (SAS Institute Inc., Cary, North Carolina).

Next, we implemented logistic regression analysis. Missing covariate data were imputed using the multiple imputation procedure in SAS. To maximize our power to detect genetic effects, we considered two different models of covariate adjustment. In Model 1, adjustments were made for ancestry only. In Model 2, we screened several variables, including baseline age, sex, ancestry, antipsychotic use, substance abuse/ dependence, years since first antipsychotic prescription and baseline total Positive and Negative Symptom Scale (PANSS) (35) for comprehensive covariate adjustment. By using forward model selection procedures ($p < 0.10$), covariates selected for adjustment in model 2 were baseline age, sex, structured-inferred proportion of

European and Asian ancestry, antipsychotic use (2df), and baseline total PANSS.

In addition, to adjust for the multiple testing, we use the false discovery rate (FDR) controlling procedure of Storey (36). We set a FDR threshold at 5% to assure that on average, up to 5% of the total positive discoveries are false. We then estimated the q-value of each test, which reflects the expected proportion of false positives occurred when rejecting a particular test and those test whose p-values are less than this test.

The FDR calculation was implemented using the Q-value 1.0 software (36)

Following genotype-based analyses, we implemented haplotype analyses. Haplotype blocks were defined using Gabriel et al's method (37) as implemented in the Haploview program (38). As the structure of linkage disequilibrium differs greatly by ancestry (32), we implemented haplotype analyses separately by self-reported ancestry as "European-only" or as "African-only". Haplotype analyses was implemented using haplo.stat in R by Schaid et al. (39).

When the minor allele frequency (MAF) of a SNP varied from 10 - 50%, the power to detect a genetic effect for TD with an effect size of 1.75 ranged from 0.43 – 0.99, respectively. When the effect size was greater than 2 and the MAF of a SNP varied from 10 - 50%, the statistical power varied from 0.73 – 0.99, respectively.

2.4 Results

A total of 765 out of 1410 participants in CATIE provided DNA samples. Fifty four participants were excluded because they were missing over 10% of their genotypic data (N =33) and because of concerns over site integrity in the CATIE study (N =21).

We compared subjects who did and did not provide a DNA sample and found that subjects who provided a DNA sample had lower average total PANSS score (74 versus 77) and lower proportion of African ancestry (29% versus 40%) (Appendix 2A). Importantly, however, the participation rate was not associated with TD status – either the presence/absence of TD, total AIMS score, or the region-specific AIMS components.

A total of 207 TD cases were identified among 711 participants in this study (Table 4.2.1). CATIE subjects with TD were older, had higher total PANSS scores, and had a higher prevalence of conventional antipsychotic use and commitment anticholinergic use at baseline. In addition, TD participants, on average, had 5-year longer history since first antipsychotic prescription and 5% higher proportion of African-ancestry.

In the analyses of individual SNPs under a general model (2 df), 2 SNPs displayed nominal associations with TD. However, no statistically significant associations were noted after adjustment for multiple comparisons (Table 4.2.2). SNPs that showed a moderate association with TD before multiple comparison adjustment included *DRD1* rs265973 and *DRD2* rs4648317. Full results for all 54 SNPs investigated in this study can be found in Appendix 2B.

To assess the feasibility of implementing a dominant model for SNPs with small MAFs, we tested for significant deviations in the frequency of TD between individuals homozygous and heterozygous for the infrequent minor alleles using Fisher's exact test. No statistical deviations were detected. Therefore, we assessed the association between TD and SNPs with small genotype frequency using a dominant model

(Appendix 2C). No association between TD and SNPs with small MAFs in DRD genes was identified when using a dominant model of inheritance.

Finally, we conducted multi-marker analyses separately in subjects with exclusively European and African ancestry. Of the statistical analyses of 7 and 11 haplotype compositions in DRD genes in European and African ancestry populations, respectively, the global p-values were significant in 1 analysis. Results of haplotype analyses showed that subjects with A alleles for *DRD3* rs167770 and *DRD3* rs324029 were at increased risk of having TD (Table 4.2.3). However, this association was observed only among those participants with African ancestry and was from rare haplotype frequency. No other significant haplotype effects were noted (Appendix 2D and 2E).

2.5 Discussion

This study aimed to understand associations between 54 SNPs in DR genes and TD in 711 participants of the CATIE trial. Several SNPs showed suggestive associations with TD, including *DRD1* rs265973 and *DRD2* rs4648317. However, after adjustment for multiple comparisons, no significant associations with TD were noted. The haplotype composition of the *DRD3* gene tagged by the minor alleles of rs167770-rs324029 presented a potential association with TD among African-ancestry participants, but this association should be interpreted with caution due to small sample sizes.

SNPs that demonstrated suggestive associations with TD, including *DRD1* rs265973, *DRD2* rs4648317 and *DRD3* rs167770-rs324029, are not located in

conventionally recognized genomic positions with functional roles (transcript factor binding site, enhancer, promoter, coding SNP, or splice site). Instead, these SNPs are located in a region predicted to contain a regulatory element (30, 40-42).

To our best knowledge, associations between TD and *DRD1* rs265973 or *DRD2* rs12364283 have not been reported in the literature. In contrast, consistent with our study, no association between TD and *DRD2* rs4648317 was found in 202 European Caucasians (43). Also consistent with our study, no association between *DRD1* rs686 and TD was identified in a recent Indian study of 297 subjects (86 TD and 211 non-TD) (44). *DRD3* rs6280 (Ser9Gly), is the most widely studied SNP for TD although results have been inconsistent. A recent meta-analysis of 11 studies of this variant concluded that there is no association between *DRD3* rs6280 variants and TD (45), which is also consistent with findings from this study. Association between *DRD2* rs1801020 (Ser311Cys) has also been assessed in several studies although results have been contradictory. As this study did not include rs1801020 or other SNP in high linkage disequilibrium with rs1801020, no further evidence was contributed.

Non-significant associations between dopamine receptor genes and medication-mediated side effect, such as TD, can be explained by a lack of statistical power for detection, errors in methodology and truly no effect between investigated SNPs and TD. As we indicated earlier, we had at least 80% power to detect an effect of 1.75 when the minor allele frequency of a given SNP was over 20%. With an effect size $\geqq$ 2, we had at least 80% power with a MAF as low as 10% (Appendix 2F). Therefore, negative findings across all 54 SNPs might be mainly due to a small genetic effect on TD as 80% and 50%of the SNPs we investigated had a MAF over

10% or 20%, respectively. However, for some SNPs, the power to detect genetic effects was less than adequate and may explain some of the null associations.

Methodological shortcomings in investigating risk factors for prevalent disease status may also have had the potential to bias study findings toward the null, leading to non-significant associations. Commonly observed shortcomings include selection bias in participants' recruitment and inappropriate control of confounding factors. As indicated in Appendix 2A, this genetic study only enrolled about 50% of initial CATIE participants. In a comparison of characteristics between participants and non-participants, African-ancestry patients with schizophrenia were under-represented in this study. In addition, participants in this genetic study had less severe symptoms of schizophrenia at study baseline than non-participants. Nevertheless, providing a DNA sample was not associated with exposure or outcome investigated in this study as the distribution of AIMS scores were almost identical regardless of participation status. Therefore, potential selection bias resulting from the participation process may not be of great concern in this study.

Mistakenly controlling intermediate factors in the causal pathway of an exposure to an outcome could also bias study findings toward the null. This study considered biological plausibility and also statistical efficiency in choosing covariates included in the fully adjusted model (Model 2). Among the five factors chosen as confounders (baseline age, sex, ancestry, type of antipsychotic use, and baseline PANSS score for severity of schizophrenia) in Model 2, none of them has been proposed as a potential mediator in the pathway of dopamine receptor genes and TD. Finally, it may be that these five dopamine receptor genes have no effect on TD.

This study has several strengths. First, this study included 711 subjects, which is a study sample that is 3-fold larger than any prior study of its kind. Second, this study investigated SNP-based and also haplotype-based relationship with TD while assessing confounding and while controlling for multiple comparisons. Third, in contrast with prior studies, participants in this study were from various clinical sites in the US and were not excluded due to their comorbidity of substance abuse or other medical illness, except those with life-threatening cardiovascular symptoms. Thus, findings from this study should be more applicable to the general population of schizophrenic patients than prior studies.

Some limitations in this study need to be recognized. First, misclassification of TD is possible but would occur non-differentially across genotypes, which may bias results toward the null. Misclassification of non-TD as TD may occur when other clinical conditions produced involuntary movement disorder and was mistaken for TD (46). In addition, misclassifying TD as non-TD is also possible as TD symptoms could be suppressed or masked when increasing antipsychotic dosage or reinstituting other kinds of antipsychotic medications (28). However, as this study classified participants with TD as long as they had one AIMS evaluation that met TD criteria, degree of misclassifying TD as non-TD should not be of great concern.

Second, discontinuation of treatment occurred commonly in the CATIE trial due to following reasons: inefficacy of antipsychotic treatment (15~28% across all treatment arms), occurrence of intolerable side effects (10~19%) and patient's decision (24~34%) (47). However, anticipating its impact on the direction of bias is difficult. Third, we had limited ability to account for accumulated antipsychotic

exposure, making it difficult to control confounding factors of TD completely. Fourth, as our case group was defined as prevalent TD at baseline but also as all those participants that developed TD during the CATIE trial, the effect sizes could have been attenuated if each sub-group displayed an association that was in opposite directions. Finally, as in most other epidemiological studies, competing risk could have removed participants from the study prior to the TD onset.

In summary, this study did not support an association between DR genes and TD. Some important implications for future research are suggested below. First, the effect of dopamine receptors genes on TD may be very subtle and studies with large sample sizes are needed. Second, our current understanding of TD pathophysiology and antipsychotic mechanisms may not be adequate for strong candidate gene selection. The implementation of a genome-wide association approach should be considered in order to efficiently identify promising loci for TD. Lastly, other measures of genetic composition, such as copy number variation and gene expression, should also be explored to better understand the role of genetic predisposition to TD.

2.6 Tables

Table 4.2.1 Distribution of demographic and clinical characteristics of participants in the CATIE study stratified by tardive dyskinesia (TD) status across all TD assessments in CATIE study.

	TD status		
Characteristics	Non-TD (n=504)	TD (N=207)	p-value
Baseline age (sd in years)	39.15 (10.97)	45.16 (10.06)	<.0001
Gender (% male)	365 (72%)	159 (77%)	0.2270
Age by gender			
Female (sd in years)	41.07 (10.52)	46.38 (9.26)	0.0022
Male (sd in years)	38.42 (11.08)	44.80 (10.29)	<.0001
Self-reported ancestry			0.2866
European ancestry	287 (57%)	112 (54%)	
African ancestry	140 (28%)	69 (33%)	
Other	77 (15%)	26 (13%)	
Baseline total PANSS	72.60 (17.41)	76.55 (17.38)	0.0063
Baseline clinician rated CGI severity score	3.87 (0.98)	4.01 (0.88)	0.0633
Year since first prescribed antipsychotic (sd)	12.85 (10.40)	17.88 (11.27)	<.0001
Baseline AIMS score			
total (sd)	0.46 (0.99)	4.46 (4.15)	<.0001
facial (sd)	0.28 (0.70)	2.94 (2.98)	<.0001
extremity (sd)	0.16 (0.49)	1.28 (1.56)	<.0001
trunk (sd)	0.02 (0.18)	0.24 (0.59)	<.0001
Baseline antipsychotic use			
% no antipsychotic	127 (25%)	43 (21%)	0.0565
% taking atypical only	301 (60%)	118 (57%)	
% taking conventional	76 (15%)	46 (22%)	
Baseline substance abuse/ dependence	191 (38%)	84 (41%)	0.5046
Baseline anticholinergic use	85 (17%)	52 (25%)	0.0112

Table 4.2.2 Dopamine receptor tagSNPs demonstrating a significant association with tardive dyskinesia (TD) when implementing in general model of inheritance: effect estimates, p-values and q-values in ancestry-adjusted and full model adjustment models.

				Ancestry-adjusted effect (Model 1)			Covariates-adjusted effect* (Model 2)			
Gene/ SNP	**Genotype**	**Non-TD**	**TD**	Global-p	OR (95% C.I.)	p-value	Global-p	q-value	OR (95% C.I.)	p-value
DRD1	*CC*	170 (34%)	76 (37%)	0.0478	1		0.0171	0.1413	1	
rs265973	*CT*	234 (46%)	106 (51%)		1.04 (0.72, 1.48)	0.8496			1.01 (0.69, 1.47)	0.9548
	TT	100 (20%)	25 (12%)		0.56 (0.33, 0.95)	0.0299			0.49 (0.28, 0.84)	0.0097
rs686	*AA*	163 (32%)	83 (40%)	0.0268	1		0.1069	0.1413	1	
	AG	240 (48%)	78 (38%)		0.60 (0.41, 0.87)	0.0072			0.65 (0.44, 0.97)	0.0346
	GG	101 (20%)	46 (22%)		0.77 (0.49, 1.22)	0.2600			0.80 (0.49, 1.29)	0.3525
DRD2	*CC*	356 (71%)	145 (71%)	0.0171	1		0.0260	0.1413	1	
rs4648317	*CT*	137 (27%)	49 (24%)		0.95 (0.65, 1.39)	0.7813			1.10 (0.73, 1.65)	0.6475
	TT	9 (2%)	9 (5%)		4.78 (1.59, 4.39)	0.0054			4.82 (1.54, 15.11)	0.0069
	missing	2	4							

*Covariate-adjusted model adjusted for age at baseline, sex, baseline antipsychotic use (3 levels), and proportion of European and Asian ancestry.

Table 4.2.3 Haplotypes shown statistically significant association with tardive dyskinesia (TD) among participants of this ancillary study to the CATIE trial.

Gene	Haplotype name and loci		Haplotype frequency		Global p-value*	OR (95%C.I.)**
DRD3	rs167770	rs324029	non-TD (n=140)	TD (n=69)		
	G	A	0.66	0.62	0.0002	1
	A	G	0.33	0.30		1.15 (0.69, 1.92)
	A	A	0.01	0.08		24.77 (4.44, 138.19)

Note: This haplotype was identified only among African-ancestry population

* after 10000 times of permutation

** OR was obtained in additive model to approximate the effect in general model

2.7 Reference

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CHAPTER V.

SIGNIFICANCE OF THIS STUDY

1. Improving medication care of schizophrenia

TD is a serious side effect of long-term antipsychotic therapy leading to therapeutic intolerability and discontinuation. Although the wide usage of ATY has greatly reduced TD prevalence among patients with chronic schizophrenia, ATY use has several serious side effects, such as weight gain and changes in glucose and lipid metabolism. In addition, ATY is on average, ten times more expensive than conventional ATY.Thus, understanding TD is an important task for optimal long-term schizophrenia care.

2. Advancing knowledge about factors associated with TD prevalence

The current understanding about the factors associated with TD prevalence is limited. Published studies assessing the association between dopamine receptor genes and TD have been inconclusive. Such conflicting findings may be due to small sample sizes in individual studies or differences in key study characteristics across stuides. Finding form first part of this work identified several study characteristics, which may explain factors leading to heterogeneous POR estimates of TD across studies.

In addition, such conflicting findings may also be related to methodolgical inadequacecies such as lack of adjustment for confounding or multiple comparisions

in individual study. The work conducted for second part of this dissertation project improves upon previous studies by 1) using a relatively large sample size (n=711) with less restriction of comorbidity of substance and other medical illness and 2) assessing a comprehensive SNPs-based and also haplotype-based relationship between SNPs in DR genes and TD while assessing confounding and while controlling for multiple comparisions. Thus, findings from this study should be more applicable to the general population of schizophrenic patients than prior studies.

CHAPTER VI.

CONCLUSIONS

TD is one of most frequent, distressing and potentially persistent side effects emerging from the course of long-term antipsychotic use. Since effective and safe reatment for TD is unavailable, understanding factors associated with its prevalence is crucial in order to reduce the disease burden from TD. This study investigated the relationship between TD and SNPs in DR genes and concluded no apparent relationship between these factors. Future research should consider other measures of genetic composition, such as copy number variation and gene expression, when selecting dopamine receptor genes as candidate genes for TD. It is also important to recognize current understaning of TD pathophysiology and antipsychotic mechanisms may not be adequate for strong candidate gene selections. The implementation of genome-wide association approach should be considered in order to efficiently identify promising loci for genotype-TD associationi studies.

APPENDICES

Appendix 1. Analyses of symmetry of funnel plots by study characteristics from 13 studies of DRD3 rs6280 and summary prevalence odds ratio (POR) of tardive dyskinesia (TD).

Characteristic	Contrast	Component	Symmetry test p-values Begg	Egger	No. of results imputed	Imputed effect estimates: OR (95% CI)	Summary OR (95% CI)
Enrollment Criteria	Gly/Gly vs. Ser/Ser	Schizophrenia	0.3	0.1	2	0.81 (0.53, 1.25)	1.03 (0.64, 1.66)
	Ser/Gly vs. Ser/Ser		1.0	0.5	2	0.84 (0.66, 1.08)	1.08 (0.79, 1.46)
	Gly+ vs. Gly-		0.09	0.1	2	0.94 (0.73, 1.21)	1.09 (0.81, 1.45)
	Gly/Gly vs. others		0.09	0.1	0	0.95 (0.61, 1.47)	0.95 (0.61, 1.47)
	Gly/Gly vs. Ser/Ser	Antipsychotics	1.0	0.8	0	0.89 (0.55, 1.46)	0.89 (0.55, 1.46)
	Ser/Gly vs. Ser/Ser		0.3	0.5	0	1.04 (0.76, 1.42)	1.04 (0.76, 1.42)
	Gly+ vs. Gly-		0.3	0.5	0	1.01 (0.75, 1.36)	1.01 (0.75, 1.36)
	Gly/Gly vs. others		1.0	0.9	0	0.88 (0.55, 1.39)	0.88 (0.55, 1.39)
	Gly/Gly vs. Ser/Ser	Schizophrenia & Antipsychotics	1.0	0.6	0	1.22 (0.66, 2.26)	1.22 (0.66, 2.26)
	Ser/Gly vs. Ser/Ser		0.3	0.3	1	1.37 (1.00, 1.87)	1.53 (1.11, 2.12)
	Gly+ vs. Gly-		0.3	0.2	2	1.19 (0.90, 1.57)	1.48 (1.08, 2.02)
	Gly/Gly vs. others		0.7	0.8	0	0.98 (0.54, 1.76)	0.98 (0.54, 1.76)
Study design	Gly/Gly vs. Ser/Ser	Matched case-control	1.0	N/A	0	0.83 (0.23, 2.99)	0.83 (0.23, 2.99)
	Ser/Gly vs. Ser/Ser		1.0	N/A	0	2.43 (1.35, 4.38)	2.43 (1.35, 4.38)
	Gly+ vs. Gly-		1.0	N/A	0	2.09 (1.18, 3.71)	2.09 (1.18, 3.71)
	Gly/Gly vs. others		1.0	N/A	0	0.49 (0.14, 1.67)	0.49 (0.14, 1.67)
	Gly/Gly vs. Ser/Ser	Cohort	0.04	0.04	2	0.93 (0.69, 1.26)	1.03 (0.76, 1.40)
	Ser/Gly vs. Ser/Ser		0.3	0.1	3	0.98 (0.82, 1.16)	1.10 (0.92, 1.33)
	Gly+ vs. Gly-		0.01	0.02	4	0.99 (0.84, 1.17)	1.10 (0.92, 1.31)

	Gly/Gly vs. others		0.09	0.08	1	0.94 (0.70, 1.25)	0.96 (0.72, 1.28)
TD classification	Gly/Gly vs. Ser/Ser	Non-S-K	1.0	N/A	0	1.96 (0.59, 6.58)	1.96 (0.59, 6.58)
	Ser/Gly vs. Ser/Ser	criteria	1.0	N/A	1	1.20 (0.66, 2.20)	2.27 (1.09, 4.75)
	Gly+ vs. Gly-		1.0	N/A	1	1.48 (0.82, 2.64)	2.22 (1.10, 4.49)
	Gly/Gly vs. others		1.0	N/A	0	1.48 (0.48, 4.58)	1.48 (0.48, 4.58)
	Gly/Gly vs. Ser/Ser	S-K criteria	0.2	0.1	1	0.95 (0.70, 1.29)	0.98 (0.72, 1.33)
	Ser/Gly vs. Ser/Ser		0.3	0.1	2	1.05 (0.87, 1.25)	1.14 (0.94, 1.37)
	Gly+ vs. Gly-		0.008	0.03	4	0.99 (0.84, 1.17)	1.12 (0.94, 1.33)
	Gly/Gly vs. others		0.4	0.2	0	0.90 (0.67, 1.20)	0.90 (0.67, 1.20)
TD evaluation	Gly/Gly vs. Ser/Ser	Repeated	0.5	0.8	0	0.83 (0.50, 1.36)	0.83 (0.50, 1.36)
	Ser/Gly vs. Ser/Ser		0.2	0.1	2	0.90 (0.71, 1.15)	1.06 (0.80, 1.40)
	Gly+ vs. Gly-		0.09	0.06	2	0.91 (0.71, 1.15)	1.02 (0.78, 1.33)
	Gly/Gly vs. others		0.5	0.6	0	0.83 (0.51, 1.34)	0.83 (0.51, 1.34)
	Gly/Gly vs. Ser/Ser	Non-repeated	0.06	0.03	4	0.89 (0.64, 1.26)	1.14 (0.79, 1.66)
	Ser/Gly vs. Ser/Ser		0.3	0.2	0	1.29 (1.02, 1.64)	1.29 (1.02, 1.64)
	Gly+ vs. Gly-		0.02	0.04	4	1.07 (0.88, 1.31)	1.28 (1.02, 1.61)
	Gly/Gly vs. others		0.2	0.1	1	0.95 (0.67, 1.34)	0.98 (0.69, 1.39)
Publication year	Gly/Gly vs. Ser/Ser	1997- 2001	0.8	1.0	0	1.62 (0.93, 2.84)	1.62 (0.93, 2.84)
	Ser/Gly vs. Ser/Ser		0.4	0.2	0	1.50 (1.09, 2.04)	1.50 (1.09, 2.04)
	Gly+ vs. Gly-		0.04	0.05	0	1.54 (1.15, 2.07)	1.54 (1.15, 2.07)
	Gly/Gly vs. others		1	0.7	0	1.34 (0.78, 2.30)	1.34 (0.78, 2.30)
	Gly/Gly vs. Ser/Ser	2002- 2007	1.0	0.5	0	0.85 (0.60, 1.20)	0.85 (0.60, 1.20)
	Ser/Gly vs. Ser/Ser		0.5	0.5	0	1.06 (0.84, 1.32)	1.06 (0.84- 1.32)
	Gly+ vs. Gly-		0.5	0.4	1	0.97 (0.79, 1.20)	1.01 (0.82, 1.25)

	Gly/Gly vs. others		0.7	0.6	0	0.81 (0.58, 1.22)	0.81 (0.58, 1.12)
Average age	Gly/Gly vs. Ser/Ser	< 45	0.5	0.3	1	0.85 (0.57, 1.27)	0.89 (0.60, 1.33)
	Ser/Gly vs. Ser/Ser		0.8	1.0	0	1.09 (0.84, 1.41)	1.09 (0.84, 1.41)
	Gly+ vs. Gly-		0.8	0.7	0	1.05 (0.82, 1.34)	1.05 (0.82, 1.34)
	Gly/Gly vs. others		0.8	0.3	0	0.82 (0.57, 1.19)	0.82 (0.57, 1.19)
	Gly/Gly vs. Ser/Ser	≧ 45	0.7	0.4	1	1.04 (0.68, 1.59)	1.20 (0.77, 1.86)
	Ser/Gly vs. Ser/Ser		0.2	0.05	1	1.20 (0.93, 1.54)	1.30 (1.00, 1.68)
	Gly+ vs. Gly-		0.02	0.004	4	1.01 (0.82, 1.25)	1.30 (1.01, 1.66)
	Gly/Gly vs. others		1.0	0.9	0	1.09 (0.71, 1.66)	1.09 (0.71, 1.66)
Percent female	Gly/Gly vs. Ser/Ser	< 40%	0.8	0.9	0	1.44 (0.66, 2.11)	1.44 (0.66, 3.11)
	Ser/Gly vs. Ser/Ser		0.5	0.3	1	1.25 (0.87, 1.80)	1.48 (1.00, 2.18)
	Gly+ vs. Gly-		0.2	0.2	2	1.19 (0.87, 1.64)	1.47 (1.01, 2.12)
	Gly/Gly vs. others		1.0	1.0	0	1.19 (0.57, 2.50)	1.19 (0.57, 2.50)
	Gly/Gly vs. Ser/Ser	≧ 40%	0.2	0.2	1	0.88 (0.64, 1.21)	0.96 (0.69, 1.32)
	Ser/Gly vs. Ser/Ser		0.5	0.2	0	1.12 (0.91, 1.37)	1.12 (0.91, 1.37)
	Gly+ vs. Gly-		0.02	0.04	3	0.98 (0.82, 1.17)	1.09 (0.90, 1.33)
	Gly/Gly vs. others		0.3	0.3	0	0.89 (0.66, 1.20)	0.89 (0.66, 1.20)
Ancestry	Gly/Gly vs. Ser/Ser	Europeans	0.7	0.5	1	1.20 (0.60, 2.38)	1.76 (0.82, 3.75)
	Ser/Gly vs. Ser/Ser		0.7	0.8	0	1.35 (0.91, 2.02)	1.35 (0.91, 2.02)
	Gly+ vs. Gly-		0.7	0.4	2	1.06 (0.77, 1.46)	1.45 (0.99,2.12)
	Gly/Gly vs. others		0.7	0.8	0	1.46 (0.71, 3.01)	1.46 (0.71, 3.01)
	Gly/Gly vs. Ser/Ser	Asians	0.3	0.3	0	0.97 (0.65, 1.44)	0.97 (0.65, 1.44)
	Ser/Gly vs. Ser/Ser		0.1	0.08	2	1.06 (0.85, 1.32)	1.20 (0.94, 1.52)
	Gly+ vs. Gly-		0.03	0.04	2	1.05 (0.85, 1.30)	1.15 (0.92,1.44)

	Gly/Gly vs. others		0.5	0.5	0	0.87 (0.60,1.27)	0.87 (0.60,1.27)
		Mix	N/A	N/A	N/A	N/A	N/A
HWE p value	Gly/Gly vs. Ser/Ser	< 0.1	0.3	0.2	2	0.83 (0.53, 1.31)	0.95 (0.58, 1.55)
	Ser/Gly vs. Ser/Ser		0.7	0.4	1	1.03 (0.76, 1.38)	1.16 (0.85, 1.58)
	Gly+ vs. Gly-		0.3	0.3	1	1.02 (0.77, 1.35)	1.12 (0.84, 1.50)
	Gly/Gly vs. others		0.7	0.5	1	0.82 (0.52, 1.30)	0.88 (0.55, 1.40)
	Gly/Gly vs. Ser/Ser	≧ 0.1	0.3	0.4	0	1.06 (0.73, 1.54)	1.06 (0.73, 1.54)
	Ser/Gly vs. Ser/Ser		0.6	0.1	0	1.20 (0.96, 1.50)	1.20 (0.96, 1.50)
	Gly+ vs. Gly-		0.03	0.02	4	1.02 (0.84, 1.23)	1.19 (0.96, 1.47)
	Gly/Gly vs. others		0.8	0.5	0	0.96 (0.67, 1.36)	0.96 (0.67, 1.36)

Appendix 2A. Comparisons of population characteristics and clinical condition between participants and non-participants of CATIE subjects in this study.

Characteristics	CATIE subjects (n= 1410)	Participants (n= 711)	Excluded participants (n= 54)	Non-participants (n=695)	Global p-value
Baseline age (sd in years)	40.6 (11.1)	40.9 (11.1)	41.3 (10.8)	40.1 (11.2)	0.3900
Gender (% male)	1079 (74%)	559 (73%)	34 (63%)	521 (75%)	0.1500
Self-reported ancestry					<0.0001
European ancestry only	722 (49%)	399 (56%)	31 (57%)	292 (42%)	
African ancestry only	506 (35%)	209 (29%)	17 (32%)	280 (40%)	
Others	232 (16%)	103 (15%)	6 (11%)	123 (18%)	
Baseline total PANSS	75.7 (17.6)	73.8 (17.5)	81.4 (16.6)	77.2 (17.5)	<0.0001
Year since first antipsychotic use (sd)	14.6 (10.7)	14.5 (10.8)	13.7 (9.9)	14.7 (10.6)	0.7582
Baseline AIMS score					
total score (sd)	1.6 (3.1)	1.6 (3.0)	1.4 (2.5)	1.6 (3.2)	0.8587
facial (sd)	1.1 (2.1)	1.1 (2.1)	1.0 (2.0)	1.1 (2.2)	0.9085
extremity (sd)	0.5 (1.1)	0.5 (1.1)	0.3 (0.9)	0.4 (1.1)	0.5038
trunk (sd)	0.1 (0.4)	0.1 (0.4)	0.1 (0.3)	0.1 (0.5)	0.6837

CATIE: Clinical Antipsychotic Trial of Intervention Effectiveness; sd= standard deviation

Appendix 2B. Relationship between tardive dyskinesia (TD) and single nucleotide polymorphisms (SNPs) in dopamine receptors genes (DRD) among participants of this ancillary study to the CATIE trial.

				Ancestry-adjusted effect(Model 1)			Covariates-adjusted effect* (Model 2)			
Gene/ SNP	**Genotype**	**Non-TD**	**TD**	Global-p	OR (95% C.I.)	p-value	Global-p	q value	OR (95% C.I.)	p-value
DRD1	CC	104 (21%)	48 (23%)	0.5914	1.20 (0.75, 1.92)	0.4368	0.3483	0.1363	1.36 (0.83, 2.22)	0.2265
rs2453737	CT	237 (47%)	102 (49%)		1.21 (0.83, 1,78)	0.3283			1.31 (0.88, 1.97)	0.1864
	TT	162 (32%)	57 (28%)		1				1	
	missing	1	0							
rs265973	CC	170 (34%)	76 (37%)	0.0478	1		0.0171	0.1413	1	
	CT	234 (46%)	106 (51%)		1.04 (0.72, 1.48)	0.8496			1.01 (0.69, 1.47)	0.9548
	TT	100 (20%)	25 (12%)		0.56 (0.33, 0.95)	0.0299			0.49 (0.28, 0.84)	0.0097
rs265974	AA	170 (34%)	59 (28%)		1				1	
	AG	208 (41%)	86 (42%)	0.6755	1.15 (0.77, 1,72)	0.4854	0.8345	0.1676	1.01 (0.67, 1.55)	0.9469
	GG	126 (25%)	62 (30%)		1.24 (0.75, 2.05)	0.3990			1.15 (0.68, 1.95)	0.5948
rs265976	GG	275 (55%)	108 (52%)	0.8662	1		0.6119	0.1654	1	
	GT	178 (35%)	73 (35%)		0.93 (0.64, 1.34)	0.6877			0.85 (0.58, 1.26)	0.4162
	TT	51 (10%)	26 (13%)		1.06 (0.60, 1.86)	0.8485			1.08 (0.60, 1.95)	0.8046
rs686	AA	163 (32%)	83 (40%)	0.0268	1		0.1069	0.1413	1	
	AG	240 (48%)	78 (38%)		0.60 (0.41, 0.87)	0.0072			0.65 (0.44, 0.97)	0.0346
	GG	101 (20%)	46 (22%)		0.77 (0.49, 1.22)	0.2600			0.80 (0.49, 1.29)	0.3525
rs5326*	AA	13 (3%)	5 (2%)	0.8277	*See appendix 2C*		0.8082	0.1676	*See appendix 2C*	
	AG	112 (22%)	41 (20%)							
	GG	379 (75%)	161 (78%)							
rs2168631	AA	27 (5%)	12 (6%)	0.4440	1.20 (0.58, 2.47)	0.6294	0.6013	0.1654	1.16 (0.54, 2.49)	0.7060
	AG	159 (32%)	74 (36%)		1.25 (0.88, 1.78)	0.2116			1.21 (0.83, 1.74)	0.3227
	GG	318 (63%)	121 (58%)		1				1	
rs267418	CC	68 (14%)	31 (15%)	0.5275	1.06 (0.63, 1.79)	0.8199	0.7179	0.1675	0.97 (0.56, 1.69)	0.9247

	CG	224 (44%)	79 (38%)		0.84 (0.57, 1.23)	0.3642		0.85 (0.57, 1.28) 0.4399
	GG	212 (42%)	97 (47%)		1			1
DRD2	AA	304 (60%)	109 (53%)	0.0375	1	0.1007	0.1363	1
rs2734848	AG	181 (36%)	82 (40%)		1.18 (0.83, 1.68)	0.3648		1.18 (0.81, 1.71) 0.3855
	GG	19 (4%)	16 (8%)		2.11 (1.02, 4.38)	0.0451		2.31 (1.06, 5.01) 0.0343
rs17115461*	AA	442 (88%)	173 (84%)					
	AG	56 (11%)	30 (14%)	0.7479	*See appendix 2C*	0.6978	0.1675	*See appendix 2C*
	GG	6 (1%)	4 (2%)					
rs1800497	CC	264 (52%)	106 (51%)	0.4728	1	0.3481	0.1363	1
	CT	191 (38%)	87 (42%)		1.13 (0.80, 1.59)	0.5016		1.21 (0.84, 1.74) 0.2977
	TT	48 (10%)	14 (7%)		0.76 (0.40, 1.46)	0.4093		0.78 (0.40, 1.53) 0.4692
	missing	1	0					
rs6279	CC	87 (17%)	35 (17%)	0.1750	1.09 (0.63, 1.87)	0.7689	0.1550	0.1363 1.10 (0.62, 1.93) 0.7475
	CG	228 (45%)	110 (53%)		1.40 (0.96, 2.04)	0.0815		1.44 (0.97, 2.15) 0.070
	GG	189 (38%)	62 (30%)		1			1
rs1079594*	GG	27 (5%)	5 (2%)	0.4292	*See appendix 2C*	0.3016	0.1676	*See appendix 2C*
	GT	129 (26%)	54 (26%)					
	TT	348 (69%)	148 (72%)					
rs6277	CC	211 (42%)	85 (41%)	0.3555	1	0.3805	0.1363	1
	CT	208 (41%)	94 (46%)		1.20 (0.81, 1.77)	0.3715		1.18 (0.78, 1.79) 0.4351
	TT	85 (17%)	27 (13%)		0.85 (0.49, 1.50)	0.5807		0.83 (0.46, 1.50) 0.5315
	missing	0	1					
rs6275	CC	197 (39%)	68 (33%)	0.3050	1	0.2314	0.1363	1
	CT	219 (44%)	104 (50%)		1.31 (0.90, 1.90)	0.1629		1.38 (0.93, 2.04) 0.1119
	TT	87 (17%)	35 (17%)		1.03 (0.60, 1.76)	0.9084		1.07 (0.61, 1.87) 0.8241
	missing	1	0					

rs2734836	AA	24 (5%)	6 (3%)	0.7089	0.72 (0.28, 1.82)	0.4822	0.5764	0.1654	0.60 (0.23, 1.57) 0.2940
	AG	141 (28%)	52 (25%)		0.90 (0.62, 1.32)	0.5979			0.97 (0.65, 1.44) 0.8729
	GG	339 (67%)	149 (72%)		1				1
rs1800498	CC	184 (37%)	67 (33%)	0.2154	1		0.1355	0.1363	1
	CT	214 (42%)	102 (49%)		1.40 (0.94, 2.10)	0.1024			1.51 (0.98, 2.33) 0.0595
	TT	106 (21%)	38 (18%)		1.12 (0.66, 1.89)	0.685			1.16 (0.66, 2.04) 0.6014
rs2234690	AA	184 (37%)	67 (32%)	0.2154	1		0.1355	0.1363	1
	AT	214 (42%)	102 (49%)		1.40 (0.94, 2.10)	0.1024			1.51 (0.98, 2.33) 0.0595
	TT	106 (21%)	38 (18%)		1.12 (0.66, 1.89)	0.685			1.16 (0.66, 2.04) 0.6014
rs2587548	CC	184 (37%)	68 (33%)	0.2662	1		0.1631	0.1363	1
	CG	214 (42%)	101 (49%)		1.36 (0.91, 2.05)	0.1338			1.48 (0.96, 2.28) 0.0744
	GG	106 (21%)	38 (18%)		1.09 (0.64, 1.85)	0.7417			1.14 (0.65, 2.00) 0.6427
rs4986918	CC	488 (97%)	199 (96%)	0.9778	1		0.8097	0.1676	1
	CT	16 (3%)	8 (4%)		1.01 (0.41, 2.49)	0.9777			1.13 (0.42, 3.03) 0.8097
rs1079596	AA	29 (6%)	6 (3%)	0.3762	0.59 (0.24, 1.47)	0.2582	0.1685	0.1363	0.49 (0.19, 1.26) 0.1365
	AG	148 (29%)	66 (32%)		1.13 (0.79, 1.61)	0.5082			1.20 (0.82, 1.75) 0.3456
	GG	327 (65%)	135 (65%)		1				1
rs7103679*	CC	341 (68%)	154 (74%)						
	CT	154 (30%)	50 (24%)		*See appendix 2C*		0.5875	0.1654	*See appendix 2C*
	TT	9 (2%)	3 (2%)						
rs4586205	GG	84 (17%)	38 (18%)	0.6805	1.13 (0.66, 1.96)	0.6532	0.6572	0.1675	1.01 (0.57, 1.80) 0.9745
	GT	217 (43%)	97 (47%)		1.18 (0.81, 1.72)	0.3801			1.18 (0.79, 1.75) 0.4161
	TT	203 (40%)	72 (35%)		1				1
rs7125415*	CC	367 (73%)	162 (78%)	0.1797	*See appendix 2C*		0.1822	0.1363	*See appendix 2C*
	CT	128 (25%)	44 (21%)						
	TT	9 (2%)	1 (1%)						

rs4648318	AA	226 (45%)	86 (41%)	0.4674	1		0.5267	0.1654	1	
	AG	211 (42%)	97 (47%)		1.14 (0.80, 1.62)	0.4727			1.13 (0.78, 1.64)	0.5094
	GG	67 (13%)	24 (12%)		0.82 (0.46, 1.46)	0.5035			0.83 (0.46, 1.51)	0.5431
rs7109897	CT	32 (6%)	13 (6%)	0.4168	0.74 (0.36, 1.53)	0.417	0.3434	0.1363	0.69 (0.32, 1.48)	0.3434
	TT	472 (94%)	194 (94%)		1				1	
rs4581480	CC	39 (8%)	17 (8%)	0.7901	0.74 (0.36, 1.53)	0.533	0.7205	0.1675	0.75 (0.36, 1.55)	0.4368
	CT	155 (31%)	67 (32%)		0.80 (0.40, 1.61)	0.6154			0.98 (0.64, 1.50)	0.9339
	TT	310 (61%)	123 (59%)		1				1	
rs4648317	CC	356 (71%)	145 (71%)	0.0171	1		0.0260	0.1161	1	
	CT	137 (27%)	49 (24%)		0.95 (0.65, 1.39)	0.7813			1.10 (0.73, 1.65)	0.6475
	TT	9 (2%)	9 (5%)		4.78(1.59,14.39)	0.0054			4.82 (1.54, 15.11)	0.0069
	missing	2	4							
rs1799978*	AA	424 (84%)	173 (84%)	0.9688	*See appendix 2C*		0.8678	0.1676	*See appendix 2C*	
	AG	75 (15%)	32 (15%)							
	GG	4 (1%)	2 (1%)							
	missing	1	0							
rs12364283*	AA	457 (91%)	181 (87%)	0.3488	*See appendix 2C*		0.2018	0.1363	*See appendix 2C*	
	AG	45 (9%)	25 (12%)							
	GG	2 (0%)	1 (1%)							
rs6589377	AA	265 (53%)	97 (47%)	0.2419	1		0.3083	0.1363	1	
	AG	195 (39%)	86 (41%)		1.23 (0.87, 1.76)	0.2429			1.26 (0.86, 1.82)	0.2345
	GG	44 (8%)	24 (12%)		1.55 (0.88, 2.73)	0.1282			1.49 (0.82, 2.72)	0.1920
DRD3	AA	427 (85%)	166 (80%)	0.7684	*See appendix 2C*		0.7371	0.1676	*See appendix 2C*	
rs6808291*	AT	68 (13%)	36 (17%)							
	TT	9 (2%)	5 (3%)							
rs1486012	AA	102 (20%)	41 (20%)	0.7799	0.86 (0.53, 1.39)	0.5351	0.7116	0.1676	0.84 (0.50, 1.39)	0.4889

	AT	272 (54%)	107 (52%)		0.89 (0.60, 1.30)	0.5398			0.86 (0.57, 1.29)	0.4530
	TT	130 (26%)	58 (28%)		1				1	
	missing	0	1							
rs2399496	AA	104 (21%)	38 (18%)	0.3517	1.11 (0.68,1.82)	0.6711	0.3675	0.1363	1.17 (0.69, 1.97)	0.5580
	AT	245 (48%)	113 (55%)		1.31 (0.90, 1.93)	0.1609			1.33 (0.89, 1.99)	0.1599
	TT	155 (31%)	56 (27%)		1				1	
rs9824856	AA	416 (83%)	157 (76%)	0.4421	1		0.2750	0.1363	1	
	AC	69 (14%)	41 (20%)		1.37 (0.84, 2.25)	0.2083			1.51 (0.90, 2.53)	0.1224
	CC	17 (3%)	8 (4%)		1.05 (0.42, 2.66)	0.9140			0.98 (0.37, 2.61)	0.9674
	missing	2	1							
rs2134655	AA	20 (4%)	12 (6%)	0.2478	1.78 (0.83, 2.82)	0.1394	0.5799	0.1654	1.32 (0.59, 2.95)	0.4992
	AG	163 (32%)	70 (34%)		1.22 (0.85, 1.77)	0.2799			1.2 (0.82, 1.77)	0.3560
	GG	321 (64%)	125 (60%)		1				1	
rs2251177*	CC	2 (1%)	1 (1%)	0.0932	*See appendix 2C*		0.1852	0.1363	*See appendix 2C*	
	CT	24 (4%)	22 (11%)							
	TT	478 (95%)	184 (89%)							
rs963468	AA	54 (11%)	18 (9%)	0.6990	0.89 (0.48, 1.65)	0.7099	0.8132	0.1676	0.89 (0.47, 1.70)	0.7317
	AG	195 (39%)	80 (39%)		1.12 (0.76, 1.65)	0.5637			1.08 (0.72, 1.63)	0.7022
	GG	255 (51%)	109 (53%)		1				1	
rs3773678	CC	292 (58%)	114 (55%)	0.7624	1		0.7641	0.1676	1	
	CT	152 (30%)	62 (30%)		0.86 (0.55, 1.34)	0.5091			0.89 (0.56, 1.41)	0.6155
	TT	59 (12%)	31 (15%)		0.98 (0.52, 1.85)	0.9445			0.78 (0.40, 1.54)	0.4726
	missing	1	0							
rs2630349	AA	14 (3%)	7 (4%)	0.9305	1.05 (0.40, 2.76)	0.9262	0.8881	0.1675	0.78 (0.28, 2.17)	0.6364
	AG	99 (20%)	44 (21%)		0.92 (0.59, 1.45)	0.7322			1.01 (0.63, 1.61)	0.9845
	GG	391 (77%)	156 (75%)		1				1	
rs167771	AA	244 (48%)	102 (49%)	0.3408	1		0.2533	0.1363	1	

	AG	156 (31%)	56 (27%)		0.74 (0.49, 1.12)	0.1564			0.82 (0.53, 1.26)	0.3641
	GG	104 (21%)	49 (24%)		0.73 (0.40, 1.34)	0.3097			0.59 (0.31, 1.11)	0.0987
rs167770	AA	179 (35%)	75 (36%)	0.4964	1		0.3213	0.1363	1	
	AG	226 (45%)	94 (45%)		0.91 (0.62, 1.32)	0.6049			1.06 (0.71, 1.58)	0.7716
	GG	99 (20%)	38 (18%)		0.73 (0.43, 1.23)	0.2379			0.73 (0.42, 1.26)	0.2558
rs324029	AA	101 (20%)	48 (23%)	0.7944	0.98 (0.59, 1.63)	0.9256	0.9744	0.1804	1.01 (0.59, 1.73)	0.9773
	AG	224 (44%)	86 (42%)		0.89 (0.60, 1.30)	0.5330			1.04 (0.70, 1.57)	0.8349
	GG	179 (36%)	73 (35%)		1				1	
rs10934256	AA	22 (4%)	8 (4%)	0.9158	1.01 (0.43, 2.34)	0.9860	0.4024	0.1388	1.16 (0.48, 2.80)	0.7441
	AC	135 (27%)	56 (27%)		1.08 (0.75, 1.57)	0.6769			1.31 (0.88, 1.95)	0.1795
	CC	347 (69%)	143 (69%)		1				1	
rs1486009*	AA	435 (86%)	188 (91%)	0.3435	*See appendix 2C*		0.3816	0.1363	*See appendix 2C*	
	AG	67 (13%)	19 (9%)							
	GG	2 (1%)	0 (0%)							
rs3732783*	AA	425 (84%)	181 (87%)	0.4866	*See appendix 2C*		0.6069	0.1654	*See appendix 2C*	
	AG	76 (15%)	25 (12%)							
	GG	3 (1%)	1 (1%)							
rs6280	CC	119 (24%)	53 (26%)	0.6937	0.84 (0.51, 1.41)	0.5157	0.8843	0.1676	0.93 (0.54, 1.59)	0.7777
	CT	223 (44%)	86 (41%)		0.85 (0.57, 1.26)	0.4120			1.04 (0.69, 1.58)	0.8492
	TT	162 (32%)	68 (33%)		1				1	
rs9825563	AA	222 (44%)	91 (44%)	0.9362	1		0.8633	0.1676	1	
	AG	222 (44%)	89 (43%)		0.94 (0.66, 1.34)	0.7497			1.03 (0.71, 1.49)	0.8800
	GG	60 (12%)	27 (13%)		1.01 (0.60, 1.72)	0.9609			1.17 (0.67, 2.05)	0.5886
DRD4	CC	15 (3%)	7 (3%)	0.4125	1.19 (0.47, 3.01)	0.7212	0.3078	0.1363	1.04 (0.39, 2.78)	0.9420
rs3758653	CT	166 (33%)	57 (28%)		0.80 (0.56, 1.14)	0.2185			0.75 (0.51, 1.09)	0.1303
	TT	323 (64%)	143 (69%)		1				1	

rs1800443**	GT	16 (3%)	1 (0.5%)	0.0417	0.12 (0.02, 0.92)		0.0693	0.1363	0.15 (0.02, 1.17)	0.0693
	TT	488 (97%)	206 (99.5%)		1				1	
rs11246226	AA	103 (20%)	48 (23%)	0.4326	1.21 (0.76, 1.93)	0.4127	0.5792	0.1654	1.21 (0.74, 1.98)	0.4410
	AC	250 (50%)	94 (46%)		0.92 (0.63, 1.35)	0.6645			0.98 (0.64, 1.43)	0.8342
	CC	151 (30%)	65 (31%)		1				1	
rs936465	CC	135 (27%)	56 (27%)	0.3644	1		0.3585	0.1363	1	
	CG	254 (50%)	96 (46%)		0.93 (0.63, 1.38)	0.7121			0.95 (0.62, 1.43)	0.7874
	GG	115 (23%)	55 (27%)		1.24 (0.79, 1.96)	0.3527			1.28 (0.79, 2.07)	0.3097
DRD5	AA	455 (91%)	183 (88%)	0.9875	*See appendix 2C*		0.6939	0.1675	*See appendix 2C*	
rs4516717*	AG	40 (8%)	21 (10%)							
	GG	6 (1%)	3 (2%)							
	missing	2	0							
rs2867383	AA	86 (17%)	29 (14%)	0.2482	0.90 (0.54, 1.49)	0.6727	0.2074	0.1363	0.90 (0.53, 1.52)	0.6853
	AG	213 (42%)	102 (49%)		1.27 (0.89, 1.82)	0.1911			1.31 (0.90, 1.92)	0.1569
	GG	204 (41%)	76 (37%)		1				1	
	missing	1	0							

Appendix 2C. Relationship between TD and single nucleotide polymorphisms (SNPs) in dopamine receptor genes 1, 2, 3, 4, and 5 (DRD1, DRD2, DRD3, DRD4, DRD5) with genotype count less than or equal to 5 in dominant model of inheritance.

					Ancestry-adjusted effect		Covariates-adjusted effect*	
Gene	**SNP**	**Genotype**	**Non-TD**	**TD**	OR (95% C.I.)	p-value	OR (95% C.I.)	p-value
DRD1	rs5326	AA+ AG	125 (25%)	46 (22%)	0.89 (0.60, 1.31)	0.5546	0.88 (0.58, 1.32)	0.5259
		GG	379 (%)	161 (78%)	1		1	
DRD2	rs17115461	AA	442 (88%)	173 (84%)	1		1	
		AG+ GG	62 (12%)	34 (16%)	1.22 (0.70, 2.11)	0.4801	1.23 (0.69, 2.20)	0.4843
	rs1079594	GG+ GT	156 (31%)	59 (29%)	1.00 (0.69, 1.44)	0.9874	1.01 (0.68, 1.48)	0.9774
		TT	348 (69%)	148 (72%)	1		1	
	rs2734836	AA+ AG	165 (33%)	58 (28%)	0.88 (0.61, 1.27)	0.4921	0.91 (0.62, 1.34)	0.6420
		GG	339 (67%)	149 (72%)	1		1	
	rs1079596	AA+ AG	177 (35%)	72 (35%)	1.05 (0.74, 1.48)	0.7814	1.08 (0.75, 1.55)	0.6885
		GG	327 (65%)	135 (65%)	1		1	
	rs7103679	CC	341 (68%)	154 (74%)	1		1	
		CT+ TT	163 (32%)	53 (26%)	0.79 (0.54, 1.14)	0.2117	0.81 (0.55, 1.20)	0.3039
	rs7125415	CC	367 (73%)	162 (78%)	1		1	
		CT+ TT	137 (27%)	45 (22%)	0.72 (0.49, 1.07)	0.1003	0.74 (0.49, 1.12)	0.1488
	rs4648317	CC	356 (71%)	145 (70%)	1		1	
		CT+ TT	146 (29%)	58 (28%)	1.07 (0.74, 1.55)	0.7141	1.23 (0.83, 1.81)	0.3008
		missing	2	4				
	rs1799978	AA	424 (84%)	173 (84%)	1		1	
		AG+ GG	79 (16%)	34 (16%)	1.0 (0.63, 1.57)	0.9871	0.88 (0.55, 1.42)	0.6034
		missing	1	0				

	rs12364283	AA	457 (91%)	181 (87%)	1		1	
		AG+ GG	47 (9%)	26 (13%)	1.47 (0.87, 2.45)	0.1469	1.64 (0.95, 2.84)	0.0755
DRD3	rs6808291	AA	427 (85%)	166 (80%)	1		1	
		AT+ TT	77 (15%)	41 (20%)	1.19 (0.74, 1.92)	0.4682	1.22 (0.74, 2.00)	0.4371
	rs9824856	AA	416 (83%)	157 (76%)	1		1	
		AC+ CC	86 (17%)	49 (24%)	1.32 (0.82, 2.12)	0.2539	1.41 (0.86, 2.32)	0.1778
	rs2251177	CC+ CT	26 (5%)	23 (11%)	2.01 (1.05, 3.84)	0.0349	1.78 (0.90, 3.52)	0.0988
		TT	478 (95%)	184 (89%)	1		1	
	rs2630349	AA+ AG	113 (22%)	51 (25%)	0.94 (0.61, 1.45)	0.7725	0.97 (0.62, 1.54)	0.9119
		GG	391 (78%)	156 (75%)	1		1	
	rs10934256	AA+ AC	157 (31%)	64 (31%)	1.07 (0.75, 1.53)	0.6992	1.29 (0.88, 1.89)	0.1865
		CC	347 (69%)	143 (69%)	1		1	
	rs1486009	AA	435 (86%)	188 (91%)	1		1	
		AG+ GG	69 (14%)	19 (9%)	0.65 (0.38, 1.11)	0.1145	0.66 (0.38, 1.16)	0.1490
	rs3732783	AA	425 (84%)	181 (87%)	1		1	
		AG+ GG	79 (16%)	26 (13%)	0.75 (0.46, 1.21)	0.2321	0.78 (0.47, 1.28)	0.3190
DRD4	rs3758653	CC+ CT	181 (36%)	64 (31%)	0.83 (0.58, 1.17)	0.2844	0.77 (0.53, 1.11)	0.1603
		TT	323 (64%)	143 (69%)	1		1	
DRD5	rs4516717	AA	455 (91%)	183 (88%)	1		1	
		AG+ GG	46 (9%)	24 (12%)	1.03 (0.57, 1.86)	0.9300	1.16 (0.62, 2.17)	0.6426
		missing	3	0				

*Covariate-adjusted model adjusted for baseage, sex, baseline antipsycohtic use (3 levels), proportion of European and Asian ancestry

Appendix 2D. Association between TD and haplotypes in dopamine receptor genes 1, 2, 3, 4, and 5 (DRD1, DRD2, DRD3, DRD4, DRD5) in European ancestry population.

		Haplotype frequency		
Gene	**Haplotype name and loci**	non-TD (n=287)	TD (n=112)	**Global p-value***
DRD1	G G	0.38	0.37	0.7753
	A A	0.15	0.15	
	A G	0.46	0.48	
	*SNP order: rs686 ,rs5326			
	A G	0.17	0.16	0.8082
	G G	0.38	0.38	
	G C	0.45	0.46	
	* SNP order: rs2168631 , rs267418			
DRD2	G T T C	0.51	0.50	0.9142
	G G C C	0.18	0.18	
	C T C T	0.29	0.30	
	C T C C	0.01	0.02	
	* SNP order: rs6279 , rs1079594 , rs6277 , rs6275			
	G T T G C G C G C G	0.02	NA	0.4360
	G C A C C G C G T G	0.11	0.09	
	A C A C C A T T C A	0.17	0.16	
	A C A C C A C T C A	0.01	0.01	
	G T T G C G C T C A	0.54	0.57	
	G C A C C G C G C G	0.13	0.16	
	* SNP order: rs2734836, rs1800498, rs2234690, rs2587548, rs4986918, rs1079596, rs7103679, rs4586205, rs7125415, rs4648318			
DRD3	A C G T T C		0.46	0.46
	A T G T T C		0.05	0.04
	A T G G C C		0.18	0.18
	A C C T C T		0.29	0.30
	A C C T C C		0.01	0.02
	* SNP order: rs1486012, rs2399496, rs9824856, rs2134655, rs2251177, rs963468			

	G C G C G C	0.02	NA
	C C G C G T	0.11	0.09
	C C A C T C	0.01	0.01
	C C A T T C	0.17	0.16
	G C G C T C	0.55	0.57
	C C G C G C	0.14	0.17

* SNP order: rs167770, rs324029, rs10934256, rs1486009, rs3732783, rs6280

DRD4	C A	0.81	0.79
	C G	0.05	0.05
	T A	0.14	0.15
	C A	0.81	0.79

* SNP order: rs11246226, rs936465

Appendix 2E. Association between TD and haplotypes in dopamine receptor genes 1, 2, 3, 4, and 5 (DRD1, DRD2, DRD3, DRD4, DRD5) in African ancestry population.

Gene	Haplotype name and loci	Haplotype frequency non-TD (n=140)	Haplotype frequency TD (n=69)	global p-value*
DRD1	G G	0.28	0.24	0.5528
	A G	0.25	0.25	
	G T	0.46	0.51	
	SNP order: rs265974, rs265976			
	G G	0.61	0.51	0.1261
	A A	0.10	0.07	
	A G	0.29	0.41	
	SNP order: rs686, rs5326			
DRD2	G G	0.11	0.09	0.4039
	C T	0.63	0.62	
	G T	0.26	0.30	
	SNP order: rs6279, rs1079594			
	C T	0.25	0.23	0.6185
	C C	0.63	0.62	
	T C	0.12	0.15	
	SNP order: rs6277, rs6275			
	C A C	0.83	0.78	0.2064
	T T G	0.17	0.22	
	SNP order: rs1800498, rs2234690, rs2587548			
	C G	0.81	0.80	0.8717
	C A	0.14	0.14	
	T A	0.06	0.06	
	SNP order: rs4986918, rs1079596			
	T G	0.19	0.15	0.4533
	C G	0.33	0.34	
	C A	0.47	0.51	

	SNP order: rs7125415, rs4648318			
	C C	0.52	0.49	0.2726
	T C	0.36	0.36	
	T T	0.12	0.15	
	SNP order: rs4581480, rs4648317			
DRD3	T G G	0.34	0.31	0.7651
	C G G	0.15	0.12	
	C G A	0.24	0.22	
	T A G	0.27	0.35	
	SNP order: rs3773678, rs2630349, rs167771			
DRD4	C C	0.59	0.57	0.3272
	A G	0.34	0.35	
	C G	0.07	0.09	
	SNP order: rs11246226, rs936465			
DRD5	A A	0.47	0.43	0.5586
	G G	0.17	0.18	
	A G	0.36	0.39	
	SNP order: rs4516717, rs2867383			

*The global-p value were obtained from 10,000 times of permutation

Appendix 2F. Power calculation on aditive model among 207 TD and 504 non-TD across different minor allele frequency of single nucleotide polymorphisms (SNPs) in dopamine receptor genes 1, 2, 3, 4, and 5 (DRD1, DRD2, DRD3, DRD4, DRD5).

MAF*	Effect Size (OR), at alpha-level= 0.001					% of MAF in total 711 participants	
	1.25	1.50	1.75	2.00	2.25	% range of MAF	% in CATIE
0.01	0.2%	0.6%	1%	3%	5%	<0.01	---
0.05	0.8%	5%	16%	34%	55%	0.01~ <0.05	7%
0.1	2%	14%	43%	73%	90%	0.05~ <0.1	13%
0.2	4%	34%	77%	96%	99%	0.1~ <0.2	20%
0.3	7%	48%	89%	99%	100%	0.2~ <0.3	17%
0.4	8%	55%	92%	99%	100%	0.2~ <0.4	13%
0.5	8%	56%	92%	99%	100%	0.4~ <0.5	30%

*MAF: Minor allele frequency