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The Relationship between Dopamine Beta Hydroxylase Polymorphisms and Attenuated Psychotic Symptoms in Putatively Prodromal Adolescents

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An abstract of the thesis submitted to the Faculty of Emory College

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Abstract

Elevated dopamine in the subcortical regions of the brain such as the striatum is strongly associated with vulnerability to psychotic symptoms. The neural mechanisms of elevated subcortical dopamine remain ambiguous. Dysregulation of dopamine beta hydroxylase (DBH) is one possible contributor to abnormal dopamine levels. One functional single nucleotide polymorphism (SNP) at the DBH gene, -1021C→T, has shown to affect DBH enzyme activity in plasma and cerebrospinal fluid (CSF). The TT genotype of -1021C→T, in particular, has been associated with low DBH enzyme activity. Previous studies suggest that lower DBH activity may contribute to elevated DA levels, which in turn may increase the vulnerability for developing psychotic symptoms. Participants were administered the Scale for Prodromal Symptoms (SOPS) as a measure of prodromal symptom severity. Each participant also provided a saliva sample for DNA extraction. On all four SOPS prodromal symptom scores, there was no significant difference between individuals who are homozygous C and T allele carriers at -1021C→T. Furthermore, there were no significant differences among the three genotypes (CC, CT, and TT) in severity of prodromal symptoms. Though not reaching statistical significance, "T carriers" and TT genotype prodromal individuals showed a trend toward greater positive symptom severity compared to the CC genotype adolescents. Such findings that individuals with the DBH SNP associated with low DBH levels have greater vulnerability for developing psychotic symptoms are consistent with previous literature. Current findings did not reach statistical significance, suggesting that the present sample size was not sufficient to detect effects of the DBH SNP -1021C→T alone on prodromal symptoms in adolescents at high-risk for developing psychosis. It is likely that the young age of the sample at follow-up contributed to the lower conversion rate.

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The Relationship between Dopamine Beta Hydroxylase Polymorphisms and Attenuated Psychotic Symptoms in Putatively Prodromal Adolescents

Numerous studies show that dopamine related genes, such as the dopamine beta hydroxylase (DBH) gene, are implicated in the neurological mechanisms underlying psychosis. A recently proposed model suggests that lower plasma DBH activity is closely associated with vulnerability to psychotic symptoms. The current study proposes to investigate the DBH SNP -1021C→T, which is thought to contribute to lower DBH in the plasma and cerebrospinal fluid, and its association with severity in attenuated positive (psychotic) symptoms in putatively prodromal adolescents. Based on previous literature, the current study predicts that TT genotype of -1021C→T will be associated with more severe prodromal symptoms. Given that DBH SNP -1021C→T, precisely the C to T base change, contributes to lower plasma DBH activity and presumably elevations in the proportion of circulating dopamine, it is of interest to investigate the relationship with this SNP and negative and disorganized prodromal level symptoms, as well as general symptoms of psychopathology. The current study also investigates whether DBH SNP -1021C→T predicts conversion to psychosis.

The current paper begins with a review of the phenomenology and etiology of SZ. The current project's participants include those at high risk for developing a psychotic disorder such as SZ and hence a short description of these prodromal individuals as well as some common diagnostic tools for measuring their subclinical symptoms will be discussed. The paper also provides background information on the dopamine hypothesis of psychosis as well as on dopamine beta hydroxylase and the particular SNP of interest, DBH SNP -1021C→T. Methods and the various data analytic procedures will be discussed. Finally, there will be a brief discussion of the findings and implications for the research literature at the end.

Schizophrenia: Phenomenology and Hypothesized Etiology

Schizophrenia (SZ) and other psychotic disorders are chronic, debilitating psychiatric illnesses, affecting approximately 1~5% of the population across cultures (Jablensky, 1997). Genetic and the phenomenological studies of SZ suggest that SZ is heterogeneous with respect to etiology, course, and comorbidity (MacDonald & Shultz, 2009; Harvey & Bellack, 2009). The clinical onset of schizophrenia and other psychoses is typically in late adolescence/early adulthood. Many children and adolescents who later develop a psychotic disorder show sub-threshold or attenuated psychotic symptoms (Yung et al., 2003). Individuals with SZ typically have a combination of positive and negative symptoms (Schultz, North, & Shields, 2007). Positive symptoms include distortions of thought content such as hallucinations, delusions, and paranoia, disturbances in the language process, as well as the presence of unusual behavior. Negative symptoms include flattened affect, loss of a sense of pleasure, loss of motivation, and social withdrawal (Schultz, North, & Shields, 2007).

Environmental and genetic factors and especially the interactions of the two have been proposed in the etiology of schizophrenia. Environmental risk factors including cannabis use, social stress, isolation, and prenatal complications may trigger the onset of SZ (Walker et al., 2004; Dalman et al., 2008; Compton et al., 2009). A number of genes of small effect have also been reported to be associated with risk for schizophrenia (e.g., COMT, DISC1, DRD2, and DRD4 (Shifman et al., 2002; Kim et al., 2008; Shi, Gershon, and Lius, 2008).

The Neurodevelopmental Hypothesis

Advances in imaging techniques have enhanced the understanding of normal developmental trajectories in the brain, which has improved insight into abnormal patterns of development in various childhood psychiatric disorders (Marsh, Gerber, & Peterson, 2008). It is

speculated that disturbances in early brain development is centrally involved in the pathogenesis of various childhood psychiatric disorders including childhood-onset SZ (Marsh, Gerber, & Peterson, 2008).

Until about 30 years ago, neurodevelopment was assumed to be primarily limited to the prenatal period. Recent brain-imaging studies, however, showed that neurodevelopment extends throughout the lifespan, and the etiological models of SZ broadened to include changes in brain structure and function from the fetal period through young adulthood (Walker et al., 2010). Evidence from genome-wide association studies suggests that there are many genes linked with risk for SZ; acting in additive and interactive ways and may disrupt fetal neurodevelopment (Stefansson et al., 2009). Compared to healthy normal individuals, individuals with SZ show more mutations, such as deletions or duplications of DNA sequences, which may affect gene expression involved in neurodevelopment (St. Clair, 2009). Furthermore, prenatal complications such as exposure to maternal viral infection, nutritional deficiency, and psychosocial stress increase an individual's risk for developing SZ by altering fetal brain development (Charil et al., 2010). While some brain abnormalities originate during fetal development, additional brain abnormalities arise in adolescence (Walker, 2002). During adolescence, around puberty, hormonal changes (i.e. increase in cortisol level) in the body trigger epigenetic processes and affect neurotransmitter function, for example alterations in the GABA, glutamate, and dopamine neurotransmitter systems (Wassef, Baker, & Kochan, 2003; Javitt, 2007; Howes & Kapur, 2009). As a result of these biochemical changes, at-risk youth manifest a decline in hippocampal volume, exaggerated decreases in grey matter, and abnormal dopamine activity in the brain (Walker, 2002). Thus, abnormal neurodevelopmental processes during adolescence may trigger brain dysfunction involved in the development of psychosis.

The Dopamine Hypothesis

Multiple neural systems are implicated in neurodevelopmental models of schizophrenia. In particular, regulation or dysregulation of dopamine (DA) in the mesolimbic pathway of the brain has received much attention as a potential mechanism of psychosis. Dopamine has long been implicated in the etiology of schizophrenia, dating back to the discovery that D2 receptor antagonists were effective in treating positive psychotic symptoms (Creese, Burt, & Snyder, 1976; Laruelle, Abi-Dargham, Gil, Kegeles, & Innis, 1999). Evidence that amphetamines stimulate DA release and subsequently worsen psychotic symptoms (Lieberman, Kinon, & Loebel, 1990) provided additional support for the role of DA in SZ. Numerous risk factors in DA dysregulation; in particular, specific DA-related variants (e.g., COMT, DRD4, and AKT1) are shown to be more prevalent in individuals with psychotic disorders (Arguello & Gogos, 2008).

Recently developed brain imaging techniques have provided methods for observing dopamine synthesis and release and putative synaptic dopamine levels (Moore et al., 2003). Using positron emission tomography (PET) and single photon emission computerized tomography (SPECT), several studies indicated that acutely psychotic patients show elevated presynaptic striatal dopamine activity (Hietala , Syvalahti, & Vuorio, 1995; Howes et al., 2011) as well as greater striatal dopamine release compared to healthy individuals (Abi-Dargham, Gil, & Krystal, 1998; Breier, Su, & Saunders, 1997).

As a result of Human Genome Project, it became clear that there is not a single gene that encodes for SZ, but rather, that there are number of genes each of small effect size that are associated with schizophrenia (Allen, Bagade, & McQueen, 2008). Various gene variants associated with schizophrenia are directly involved in dopaminergic pathways (i.e. COMT,

DISC1, DRD2, and DRD4) (Shifman et al., 2002; Kim et al., 2008; Shi, Gershon, and Lius, 2008). One other noteworthy gene variant is one that affects the vesicular monoamine transporter protein (rs2270641), which contributes to accumulation of dopamine into the striatal vesicles in SZ. Furthermore, other gene variants are speculated to indirectly affect the dopaminergic system (Shi, Gershon, & Liu, 2008). Currently, it may not be possible to integrate all the genes involved in the abnormal dopamine circuitry implicated in schizophrenia because our understanding of the precise number, nature, function, and association among these genes and to schizophrenia is evolving.

Numerous studies have suggested that a vast array of environmental factors such as migration, unemployment, urban upbringing, lack of close friends, and childhood abuse, contribute to the risk for schizophrenia (Cantor-Graae, 2007). Studies in animals in social isolation and subordination indicated that these environmental factors lead to dopaminergic over-activity (Hall, Wilkinson, & Humby, 1998; Hall et al., 1999; Morgan, Grant, & Gage, 2002). There is now substantial evidence from animal models that pre- and perinatal factors can lead to long-term overactivity in mesostriatal dopamine function (Boksa, 2004). Recent PET imaging work has shown that a number of stimulants/psychoactive substances may sensitize the striatal dopamine system and lead to enduring increases in dopamine release in response to amphetamine (Boileau, Dagher, & Leyton, 2006). Also, there is new data indicating that psychoactive drugs acting on other systems may indirectly act on dopaminergic system by potentiating dopamine release previously caused by other factors (Cheer et al., 2004). These discoveries suggest that numerous environmental risk factors of SZ/psychosis may trigger the onset of psychosis via disrupting the dopamine regulating circuitries.

Genes and environmental factors do not exist in isolation. There is mounting evidence indicating that both environmental and genetic factors play a role in dopamine dysfunction, independently and interactively (Cannon, van Erp, & Rosso, 2002; Nicodemus, Marenco, & Batten, 2008; Mittal, Ellman, & Cannon, 2008). Furthermore, Howes and Kapur (2009) suggested that interactions between gene variants, including those that affect dopaminergic function, and environmental risk factors could be a possible route to dopaminergic dysfunction. However, it has been suggested that the relative risk for developing schizophrenia due to migration, obstetric complications, and frequent cannabis or amphetamine use is much higher than the risk for any single gene variant (Howes & Kapur, 2009).

SZ individuals have elevated DA activity in the striatum, a subcortical region of the brain, and decreased DA activity in the prefrontal areas of the brain (Howes & Kapur, 2009). Hyperdopaminergia in the striatum is associated with positive psychotic symptoms, and hypodopaminergia in the prefrontal cortex is associated with negative symptoms, which are related to cognitive, social, and emotional impairment (Howes & Kapur, 2009). Interestingly, individuals in the prodromal phase of psychosis also show similar elevations in striatal dopamine (Howes, Montgomery, & Asselin, 2008), brain structural deficits (Wood et al., 2008), longitudinal brain changes (DeLisi, 2008), and neurocognitive impairments (Brewer, Wood, & Phillips, 2006) similar to those seen in SZ patients, although to a lesser degree. Striatal DA appears most elevated in psychotic individuals regardless of the particular form of psychosis (schizophrenia or schizoaffective disorder) compared to individuals with non-psychotic psychiatric disorders (Howes & Kapur, 2009). This suggests then, that hyperdopaminergia is associated with a general psychosis proneness and not specific to psychotic symptoms in schizophrenia.

The Psychosis Prodrome

The psychosis prodrome refers to the period of marked functional decline preceding the onset of clinical psychotic symptoms (Cornblatt, Lencz & Obuchowski, 2002). Individuals in the psychosis prodrome report precursors to delusions, hallucinations, and thought disorder. In individuals who later develop psychosis, functioning typically begins to decline with the onset of puberty. The prodromal period has been noted to last anywhere from a few months to several years (McGorry et al., 1995; Cornblatt et al., 2003). The psychosis prodrome entails attenuated positive, negative, and disorganized symptoms, as well as general psychological dysfunction (Cornblatt et al., 2003). Disorganized symptoms include odd behavior or appearance, bizarre thinking, trouble with focus and attention, and impairment in personal hygiene. Symptoms of general psychological dysfunction include sleep disturbance, dysphoric mood, motor disturbances, and impaired stress tolerance. SZ investigators currently study the prodrome to psychosis to identify mechanisms of conversion to psychosis and to better understand its early course (Cornblatt, Obuchowski, Roberts, Pollack & Erlenmyer-Kimling, 1999; Addington et al., 2007).

Investigators have operationalized heightened risk for psychosis or the prodromal syndrome in a number of different ways. Some investigators have identified individuals at risk for psychosis based on the presence of first-degree relatives (i.e. parent, sibling, or twins) with the disorder. Other approaches define risk status through an individual's clinical status based on standardized behavioral measures linked with subsequent psychosis onset. One diagnostic tool for identifying prodromal individuals is the Scale for Prodromal Symptoms (SOPS). This is one measure that defines four prodromal syndromes that involve subclinical symptoms of psychosis; the current study will focus on two of the SOPS defined prodromal syndromes—Attenuated

Positive Symptom (APS) Syndrome and Schizotypal Personality Disorder (SPD). APS is characterized by presence of at least one sub-threshold positive symptom and no positive symptoms of psychotic level intensity. Schizotypal personality disorder (SPD) is another diagnostic category used to define the prodrome. Similar to APS, SPD individuals are at heightened risk for developing a psychotic disorder compared to healthy individuals and have symptoms that mirror many of the symptoms seen in individuals who meet criteria for APS (Simon et al., 2006; Woods et al., 2009).

In summary, SZ is considered a neurodevelopmental disorder, as it may be deviation from normal neural development that underlies the disorder. SZ investigators now focus on studying the developmental course of psychosis, particularly the prodromal phase of psychosis to better understand the etiology of psychosis. A particular interest in SZ research has been on the neural mechanisms or biological markers that may explain the various symptom presentations in SZ individuals. One biological marker that has been implicated in positive symptom presentation in psychotic individuals is elevated DA level in the subcortical regions of the brain such as the dorsal striatum. Interestingly those who meet clinical criteria for the psychosis prodrome such as the SPD and APS individuals resemble individuals diagnosed with SZ in terms of cognitive deficits, general functional decline, presentation of positive symptoms, as well as subcortical hyperdopaminergia. Therefore, it is predicted that positive symptoms in the prodromal population will continue to exacerbate until DA levels in the dorsal striatum has reached the threshold for conversion to full-blown psychotic disorder.

Dopamine Beta Hydroxylase

Dopamine β-hydroxylase (DBH) is an enzyme that catalyzes the conversion of DA to the neurotransmitter norepinephrine (NE) and is specifically expressed in NE-containing neurons.

NE regulates many functions in the central and peripheral nervous systems, regulating many physiological, cognitive, and behavioral functions (Grace, 2000). In the central nervous system, NE is localized in the hindbrain and midbrain, notably in the locus coeruleus (LC), which projects to the forebrain (Cubells & Zabetian, 2004). Noradrenergic neurons of the LC project widely throughout the brain, including numerous sub-cortical brain regions and the neocortex (Foote et al., 1983). DBH is contained within NE storage vesicles in the presynaptic nerve terminals and with nerve stimulation may be released together with NE during synaptic transmission (Weinshilboum et al., 1971). DBH activity, derived largely from sympathetic nerves, can be measured in both the serum/plasma and in the cerebrospinal fluid (CSF) (Kaufman & Friedman, 1965). Although plasma activity levels vary widely among different individuals, they are stable within individuals over time (Weinshilboum et al. 1973; Fahndrich et al. 1982). Family and twin studies indicated that plasma DBH is under strong genetic control (Ross, Wetterberg, & Myrhed, 1973). It was suggested that DBH originating from the central noradrenergic neurons terminate in the CSF (Lerner et al., 1978). CSF and plasma levels of DBH are weakly but significantly related in individual subjects; much evidence suggests that this correlation does not result from plasma DBH contaminating CSF (Lerner et al., 1978). Rather, CSF DBH levels appear to be determined by the same genetic factors that determine serum DBH levels (Sternberg et al., 1983).

DBH Deficiency

DBH deficiency is a rare disorder in humans, characterized by pronounced decreases in blood pressure upon standing, and a variety of other signs and symptoms of sympathetic nervous system failure (Robertson et al., 1991). Individuals with DBH deficiency lack detectable peripheral NE or NE metabolites, and manifest elevated plasma dopamine and dopamine

metabolites (Robertson et al., 1986). Elevated DA and decreased NE are highly correlated with presentation of psychotic symptoms; however, no psychiatric condition has been over-reported in individuals with DBH deficiency. One might speculate that this is because these DBH-deficient individuals may still have central DBH and NE in the presynaptic terminals.

A study by Kim and colleagues (2002) and then later by Zabetian and colleagues (2003) showed that certain sequence variants at DBH associate with DBH deficiency. Kim and colleagues (2002) noted prevalence of a particular single nucleotide polymorphism (SNP), IVSI+2T→C, at the DBH locus, and attributed it to the etiology of DBH deficiency. The homozygous C genotype of IVSI+2T→C was highly correlated with low expression of DBH. Furthermore, another gene variant at the DBH locus -1021C→T was prevalent in DBH deficient individuals (Zabetian et al., 2003). Zabetian and colleagues (2003) demonstrated that the T allele of this particular variant was associated with low expression of DBH as individuals with the TT genotype manifested the lowest DBH levels (Zabetian et al. 2003).

DBH and Psychosis

Numerous studies have investigated plasma and CSF DBH levels in patients with psychiatric and neurological disorders. Nearly all of these studies were conducted prior to discovery of the DBH gene as a major locus affecting plasma and CSF DBH levels. Historically, many plasma and CSF studies yielded inconclusive and sometimes conflicting results. More recently, researchers have conducted genotype-based studies. To date, evidence suggests that neither plasma DBH levels nor genotypes at the DBH locus distinguish SZ patients from healthy individuals (Meszaros et al., 1996; Williams et al., 1999). Although neither DBH in the plasma and CSF nor sequence variants at the DBH locus appear to be directly associated with the disease itself, evidence suggests that plasma and CSF DBH levels, and specific alleles at the DBH locus,

associate with clinically important characteristics of SZ (Sternberg et al., 1982, 1983; Bartko et al., 1990; Cubells et al., 2000; Yamamoto et al., 2003).

Plasma DBH and Psychosis

Early serum/plasma DBH activity studies found no difference between individuals with SZ and healthy control subjects (Wetterberg, et al., 1972; Dunner et al., 1973; DeLisi et al., 1980). However, Fujita and associates (1978) observed serum DBH in schizophrenia patients and reported that serum DBH activity was significantly lower in individuals with schizophrenia compared to normal healthy individuals. Similarly, Meltzer et al. (1980) reported that individuals with chronic paranoid schizophrenia had significantly lower serum DBH compared to normal healthy individuals. SZ Individuals with low serum DBH activity demonstrated better response to antipsychotic medication compared to other SZ individuals with relatively higher serum DBH (Bartko et al. 1990). In essence, individuals with more severe psychotic symptoms showed lower plasma DBH activity and responded better to antipsychotic medications.

CSF DBH and Psychosis

Studies comparing SZ patients with patients with other psychiatric disorders or normal controls reported no significant differences in CSF DBH activity (Lerner et al., 1978; Okada et al., 1976). Sternberg and colleagues (1983) measured CSF DBH activities in thirty patients meeting Research Diagnostic Criteria for SZ or schizoaffective disorder and reported that DBH activity was significantly lower in the patients who responded to antipsychotic medications (Sternberg et al. 1983). They also reported that patients with lower DBH activity tended to exhibit better premorbid adjustment. These findings suggested that low CSF DBH activity predicted more severe positive symptoms, but better overall clinical outcome with treatment with

antipsychotic. Furthermore, Sternberg and associates (1983) suggested that lower DBH activity may elevate DA levels, which in turn may contribute to psychotic symptoms.

The DBH Gene

The structural locus of DBH that encodes for plasma DBH activity is located at chromosome 9q34 (Cubells, 2000). A single nucleotide polymorphism, -1021C→T (rs1611115), accounts for a majority of the variation in DBH enzyme activity levels. It has been reported that the C→T transition at 1021 base pairs upstream to the ATG translational start codon (-1021C→T) accounts 35% - 52% of the variation in pDBH activity in African American, Caucasian, and ethnic Japanese populations (Zabetian et al., 2001). In all three populations, individuals homozygous T (TT) at -1021C→T have very low DBH activity, heterozygotes (CT) have intermediate enzyme activity, and individuals homozygous C (CC) have the highest DBH activity. The strong association of TT genotype with very low plasma DBH suggests that the T allele at -1021 contributes to lower expression of DBH protein. The fact that DBH SNP -1021C→T is located in the 5' region of the DBH gene and that individuals with low level of DBH activity have lower circulating levels of DBH protein suggest that -1021T lowers expression of DBH relative to -1021C by attenuating DBH gene transcription (Cubells & Zabetian, 2004).

Gene Variants and Psychosis

Since plasma DBH levels are under strong genetic control by variation at DBH, several studies attempted to determine the specific genetic variants underlying lower plasma DBH levels and psychotic symptoms. Several genetic association studies, examining a variety of polymorphisms at DBH, found no evidence of association with schizophrenia (Meszaros et al. 1996; Wei et al. 1997; Arrufat et al. 2000). After realizing that DBH polymorphisms 444A→G

& -4784—4803del are associated with variation in plasma DBH, Cubells et al. (2000) sought to find any relationship between haplotypes of the two variants to cocaine-induced paranoia in a European American sample dependent on cocaine. Their results suggested that the del-A (haplotype associated with low DBH activity) was significantly more prevalent in those with cocaine-induced psychosis than healthy, normal individuals, which is consistent with the idea that low-DBH variants associate with psychosis. Yamamoto et al. (2003) also examined the relationship of 444A→G and -4784—4803del haplotypes to treatment outcomes in European American SZ patients. As expected from prior studies, they showed that there was no difference in plasma DBH activity between SZ patients and normal individuals. However, they found that SZ patients whose symptoms are not controlled by antipsychotics have a significantly larger proportion of the low DBH activity del-A haplotype compared to SZ patients whose symptoms are controlled by antipsychotics. Yamamoto et al. also showed that individuals with at least one del-A haplotype (low pDBH activity) had significantly higher scores on the brief psychiatric rating scale (BPRS) than individuals who do not carry this haplotype. Such findings were consistent with associations between more pronounced psychotic symptoms and lower pDBH activity observed by Sternberg et al. (1983). Taken together, the studies of Cubells et al. (2000) and Yamamoto et al. (2003) suggest that variants at DBH that associate with low DBH activity also associate with vulnerability to positive psychotic symptoms in idiopathic and psychostimulant-induced psychoses. It should be noted that both the Cubells et al. (2000) and Yamamoto et al. (2003) studies were conducted prior to the discovery of DBH SNP -1021C→T—the functional SNP that is shown to regulate the expression of plasma DBH up to 52 percent. As it turns out, both 444A→G and -4784-4803del are in strong linkage disequilibrium (LD) with -1021C→T, which explains their association with low DBH activity. In a more recent

study, Park et al. (2007) sought to assess the role of the DBH gene in modifying the vulnerability to psychotic symptoms. Park and colleagues investigated three polymorphisms of the DBH gene known to be associated with pDBH levels in Korean SZ patients and healthy controls: DBH5'ins/del, -1021C→T, and DBH444G/A. Park et al. examined the association of these three polymorphisms with vulnerability to auditory hallucinations and persecutory delusions in their sample of 140 Korean patients with SZ and 161 control participants. In this particular study, the SZ patients were subdivided according to psychotic symptoms: auditory hallucinations and persecutory delusions. Results of this study indicated no difference in genotype distributions and allele frequencies between the SZ patients and healthy controls as well as no significant difference between each subgroup of SZ patients and the controls. In line with previous studies of DBH, results from the Kim et al. study suggested that DBH gene alone does not explain risk for SZ (Jonsson et al. 2003). However, a significant difference in the genotype distribution of the DBH444G/A polymorphism between SZ patients with both auditory hallucinations and persecutory delusions and the control group was noted. In addition, the GG genotype of DBH SNP 444G/A was significantly more frequent in the SZ patients with auditory hallucinations and persecutory delusions compared to the control group. In summary, Kim et al. suggested that genetic variants of the DBH gene may be related to a risk for the development of psychotic symptoms.

Current Study

Findings from a variety of studies suggested relationships between lower plasma and CSF DBH and vulnerability to psychotic symptoms in several idiopathic and drug-induced psychoses. Based on this literature, it is predicted that the TT genotype of -1021C→T will be associated with more severe positive prodromal symptoms. Several research questions were also

investigated in the current study, 1) What is the relationship between -1021C→T and negative symptoms, 2) what is the relationship between -1021C→T and disorganized symptoms, 3) what is the relationship between -1021C→T and general prodromal symptoms, and 4) Does -1021C→T predict conversion to an Axis I psychotic disorder.

Method

Participants

Participants were selected from the Emory University Adolescent Development Project (Total N=130, genetic data N=64). Participants ranged in age from 11-18 (M=14.5, SD=1.88). Three main racial groups were represented, Caucasian (51.6%), African American (42.2%), and Asian American (4.70%). Nearly 2% of individuals fell outside of these groups. Approximately, fifty-three percent of the sample was male. Diagnostic groups based on symptom presentation were: individuals meeting criteria for a prodromal syndrome (SPD or APS; N=25), individuals with no personality disorder (No PD; N=19), and individuals with other personality disorders (OPD; N=20). Parental education (number of years in school) was used as a measure of socioeconomic status. All demographic characteristics are described in Table 1.

High-risk Group

Participants were recruited as part of the Emory University Adolescent Development Project. They were recruited through announcements seeking adolescents with diagnostic symptoms of SPD (stated in lay terms on recruitment materials). All potential participants were first screened over the phone to exclude those with possible Axis I diagnoses, mental retardation, or current substance abuse or addiction. Respondents who were deemed good candidates and likely to meet criteria for SPD, were invited to participate in a four-hour research assessment. Once in the research lab, individuals were included in the SPD group on the basis of their SIDP-

IV interviews, even if they also met criteria for other axis II disorder(s). In total, 15 participants met diagnostic criteria for SPD. This recruitment strategy also yielded a group of participants meeting criteria for the APS syndrome.

Individuals who scored in the prodromal range (rating of 3-5) for one or more of the 5 SOPS positive symptom domains were placed in the APS group. In total, 16 participants met diagnostic criteria for the APS syndrome. In this study, the prodromal group (N=31) included participants who met criteria for either APS syndrome (N=31) or SPD.

Adolescent Controls

Individuals in the control group were recruited through the Emory University registry of children and adolescents in the Atlanta area. Potential participants were screened over the phone and selected for the control group because of the likely absence of any axis I or II diagnoses. In addition, some individuals in the control group were originally invited to participate due to the possibility of a clinical high-risk status. These individuals were also screened over the phone, but were subsequently invited to participate based on the possibility that they would meet criteria for SPD or other high risk groups, but did not meet criteria for any axis I or II diagnoses on any measure when they came in for their baseline assessment. In total, 25 participants were placed in the healthy control group (No PD).

Other Personality Disorder Adolescents

Some participants invited to participate due to their suspected high-risk status did not meet high risk criteria, but instead were diagnosed with a personality disorder other than SPD. These participants were placed in the other personality disorder (OPD) group. In total, 24 participants met criteria for a personality disorder other than SPD. The OPD served as a psychiatric comparison group.

Procedures

Participants were recruited from clinical referrals and through newspaper announcements aimed at the parents of children with adjustment problems. The newspaper announcement consisted of a lay description of key diagnostic criteria present in SPD. Healthy controls were recruited through the Emory University registry of children and adolescents in the Atlanta area. All prospective participants underwent telephone-screening interviews, and those who met study inclusion criteria were invited for a baseline research assessment. Exclusion criteria were neurological disorder, mental retardation, Axis I disorder, and current substance abuse/addiction. Written consent was obtained from all participants and a parent, in accordance with the guidelines of the Emory University Human Subjects Review Committee. Participants included in the present study completed a battery of diagnostic interviews administered by trained graduate-level clinicians. Each participant provided a saliva sample for DNA collection.

Clinical Measures

Three diagnostic interviews were administered to each participant to determine the three diagnostic groups: No PD (those without any personality disorders and do not meet criteria for the Prodrome), Prodrome (those who meet criteria for either SPD or APS), and OPD (those who meet criteria for a personality disorder other than SPD).

Structured Interview for DSM-IV Personality Disorder (SIDP-IV; Pfohl et al., 1986). The SIDP-IV is used to evaluate DSM-IV Axis II personality disorder. The criteria for various personality disorders are imbedded in a series of questions about relationships, interests, activities, and emotions. Personality disorder criteria are rated on a scale from 0 (not present) to 3 (strongly present). The instrument also includes ratings of behavioral observations of each participant.

Structured Clinical Interview for Axis I Disorders (SCID-I; Spitzer et al., 1992). The SCID-I is used to diagnose DSM-IV Axis I disorders. It was administered to participants to confirm any suspected Axis I psychopathology.

Scale for Prodromal Symptoms (SOPS; Miller et al., 2002). Noteworthy among measures of the prodrome is the Scale of Prodromal Symptoms (SOPS) (Miller et al. 1999) and its companion interview, the Structured Interview for Prodromal Syndromes (SIPS) (Miller et al. 2002). The SIPS is a structured diagnostic interview used to diagnose the three prodromal syndromes on the SOPS: Brief Intermittent Psychotic Symptom syndrome (BIPS), Attenuated Positive Symptom syndrome (APS), and Genetic Risk and Deterioration syndrome (GRD). The SOPS also includes a Schizotypal Personality Disorder Checklist. Using the SOPS, studies have indicated that the rate of conversion to Axis I psychosis in individuals meeting clinical risk characteristics (APS and GRD) is about 30~40% within two years, though some studies have reported rates up to 50% (Miller et al., 2003; Yung et al., 2003; Cannon et al., 2008). The Miller et al. group provided data suggesting that excellent inter-rater reliability could be established for diagnosis through the use of SIPS companion interview as a diagnostic tool (Miller et al., 2003).

The Scale for Prodromal Symptoms was used to assess and diagnose the severity of prodromal symptoms. It is composed of 19 symptom-items, each rated on a 0-6 scale. Scores of 0 indicate the absence of a symptom while scores of 1-2 indicate the non-prodromal presence of a symptom. Scores between 3 and 5 are considered to be within the prodromal range and a score of 6 is in the psychotic range. The 19 symptom-items are grouped into four symptom scales: positive, negative, disorganized, and general symptoms. The positive symptom scale includes items that assess unusual thought content and delusional ideas, suspiciousness and persecutory ideas, grandiosity, perceptual abnormalities and hallucinations, and disorganized communication.

The negative symptom scale includes items that assess social anhedonia, avolition, reduced expression of emotion, decreased experience of emotion and self, ideational richness, and deterioration of role functioning. Items on the disorganized symptom scale assess odd behavior or appearance, bizarre thinking, trouble with focus and attention, and impairment in personal hygiene. The general symptom scale contains items that assess sleep disturbance, dysphoric mood, motor disturbances, and impaired tolerance to normal stress.

DNA Collection and Extraction

Saliva samples were collected from each participant using Oragene DNA Self-Collection kits from DNAGenotek (cat. No. OG-100). Participants deposited their saliva into an Oragene solution inside the kid designed to preserve DNA. A proprietary buffer rapidly sterilized the DNA and it was then stabilized within the buccal cells that shed and accumulated in the saliva. The Pure Link genomic DNA mini-kit (Invitrogen, cat. No. K1820-01) was used to extract DNA from 800μL of saliva collected in the Oragene kit. Between 80μL and 100μL of DNA was collected. DNA was then quantified at the Emory Center for Medical Genomics using pico-green. The DNA was then diluted to a working concentration of 1ng/μL and plated, using the Biomek FX, onto a 384-well microplate. For each sample, 5μL of working DNA was added to an individual well (5ng total) and allowed to dry down overnight. Negative controls and duplicates were incorporated into the DNA plate and the sample order was documented on an excel spreadsheet. The following day, 5μL of assay mix was added to each DNA well on the 384-well plate. This was done using the Biomek FX. The assay mix contained Universal PCR Master Mix no amperase UNG (Applied Biosystems cat. No. 4326614), SNP genotyping assay (specific to each SNP, Applied Biosystems), and dH20. Real time polymerase chain reaction (PCR) was performed using the Taqman 7900HT and analyzed using the Sequence Detection

Systems (SDS) Automation Controller software, version 2.3 from Applied Biosystems. Genotypes were acquired using the allelic discrimination program (within SDS, version 2.3) for samples with quality values at 95% or greater (call rate). The absolute quantification multi-component plots were used to make manual calls for the genotypes as needed. Because of the limited number of samples, genotyping was repeated twice for accuracy. The genotypes from both experiments were compared using a quality check program. The duplicates concordant rate is ≥ 98%. Any discordant data were reanalyzed using the real time PCR data. When the reaction signal was not robust, genotypes were considered indeterminable.

Results

Sixty-four participants with data on DBH SNP -1021C→T were drawn from an original sample of 130 participants. For statistical reasons described below, the TT genotype and the CT genotype were combined into one group denoted "T carriers." To test for differences in SOPS scores between homozygous C and "T carriers" Independent Sample Student's t-tests were performed. To test for diagnostic group differences in SOPS scores, One-way ANOVAs were performed using diagnostic status (No PD, Prodrome, and OPD) as the independent variable. Logistic Regression (LR) procedures were performed to determine whether the -1021C→T polymorphism (Homozygous C vs. T carriers) predicts conversion to psychosis. Lastly, because of the possibility of a continuous "dose-response" relation of the T allele with the risk for positive symptoms, logistic regression analyses were repeated, treating the three genotypes as a continuum, with CT heterozygotes being intermediate between the CC and TT.

Preliminary Analyses

Analysis of the distributions of each dependent variable revealed that all SOPS prodromal symptom scores were significantly positively skewed and did not resemble a normal distribution.

SOPS symptom domain scores that were greater than 2 standard deviations beyond the mean were considered outliers and were removed for each dependent variable. Three participants were removed for the positive symptom domain; four participants were removed for the negative symptom domain; five participants were removed for the disorganized symptom domain; three participants were removed for the general symptom domain. After removing outliers, the SOPS symptom domains remained positively skewed. A One-Sample Kolmogorov-Smirnov test was conducted to determine if the apparent positive skew resulted in a distribution that was significantly different from a normal distribution. For the positive and general symptom domains, SOPS symptom domains were not significantly different from the normal distribution ($z=1.29$, $p=0.07$ and $z=1.29$, $p=0.07$ respectively). This indicated that removing outliers 2 standard deviations beyond the mean normalized the distributions of positive and general SOPS domains. For the negative and disorganized symptom domains, SOPS score distributions were significantly different from the normal distribution ($z=1.58$, $p=0.01$ and $z=2.02$, $p=0.001$). Removing outliers did not successfully normalize the SOPS score distributions of the negative and disorganized domains; therefore, square root and log transformations were conducted in an attempt to normalize these dependent variable distributions. These transformations were seen as preferable to non-parametric tests because the latter are less powerful at detecting group differences. Neither a square root transformation nor a logarithmic transformation successfully normalized distributions.

The initial exploration of the data also revealed a limited sample of individuals with the TT genotype (N=6) (Table 2). Thus, individuals with the CT and TT genotypes were combined into one group denoted "T-carriers." Given this, analyses were conducted to compare the mean SOPS scores of the four symptom domains (positive, negative, disorganized, and general)

between the homozygous C genotype and individuals with at least one T allele on -1021C→T (Table 3).

Data Analyses

The prediction that T carriers at DBH-1021C→T would be associated with more severe positive prodromal symptoms was tested using a t-test. The t-test was repeated to explore research questions related to the general symptom domain. Mann-Whitney test was used to explore research questions related to the negative and disorganized symptom domains. Analysis of Variance (ANOVA) was also conducted to compare mean scores of the positive and general symptom scales among the diagnostic groups. Kruskal-Wallis test was conducted to compare the mean scores of the negative and disorganized symptom scales among the diagnostic groups. Logistic regression was used to determine whether -1021C→T polymorphism predicts conversion to a psychotic disorder. Previous studies suggest that there is a continuous "dose-response" relation of the T allele with the risk for positive symptoms (Cubells & Zabetian, 2004; Zabetian et al., 2001); therefore, regression analyses were conducted, treating the genotypes as a continuum with CT heterozygotes being intermediate between the CC and TT, to test the relationship between the -1021C→T polymorphism and prodromal symptoms.

As stated earlier, outliers above and beyond 2 standard deviations from the mean were identified and removed to normalize data. This only normalized distribution for positive and general SOPS scores. Therefore SOPS data for the negative and disorganized symptom dimensions were transformed however, neither a square root transformation nor logarithmic transformation successfully normalized the distribution of negative and disorganized symptom SOPS scores. Therefore nonparametric tests were used for further evaluation of negative and disorganized SOPS scores. To determine the difference in positive and general symptom

severity between the homozygous C and T carriers of DBH SNP -1021C→T, Independent Samples Student's t-tests were performed. To determine the difference in positive and general symptom severity among the three diagnostic groups (No PD, Prodrome, and OPD), ANOVA analyses were performed. To determine the difference in negative and disorganized symptom severity between the CC genotype and T carriers of DBH SNP -1021C→T, Mann-Whitney tests were performed. The Mann-Whitney test is the non-parametric version of the t-test that can be used even if data violate parametric test assumptions (normal distribution and homogeneity of variance). To determine the difference in negative and disorganized symptom severity among the three diagnostic groups (No PD, Prodrome, and OPD), Kruskal-Wallis analyses were performed. The Kruskal-Wallis test is the non-parametric version of the ANOVA that can be used even if data violate assumptions of parametric tests (normal distribution and homogeneity of variance).

Genotyping Quality Control Analyses

As stated in the above section describing DNA *Collection and Extraction*, duplicate genotyping data was used to evaluate genotype concordance/discordance (concordance ≥ 98%). Allele frequencies were also examined in the current sample to determine if they conformed to Hardy-Weinberg expectation (HWE) in order to determine if allele frequencies were constant across generations. Most human populations conform to HWE, and deviations from HWE often indicate genotyping error or the presence of population stratification (Wigginton, Cutler, & Abecasis, 2005). DBH rs1611115 (-1021C→T) conformed to HWE (p allele freq = 0.74; q allele freq = 0.26). Genotype frequencies in the sample and genotype frequencies by diagnostic groups can be found in Table 4 and 5.

Symptom Severity by Diagnostic Groups

A one-way ANOVA revealed that the prodromal group had higher positive symptom ($F(2, 57)=30.0$, $p<0.001$) and higher general symptoms ($F(2, 57)=10.5$, $p<0.001$). Levene's Test indicated unequal variances for positive and general symptom domains. However, follow-up contrasts correcting for unequal variances indicated significant difference between the No PD and Prodromal groups as well as between OPD and Prodromal groups ($t(2)=6.26$, $p<0.001$; $t(2)=6.12$, $p<0.001$ respectively) for positive symptom domain. There was no significant difference between No PD and OPD groups for positive symptoms ($t(2)=0.31$, $p=0.76$). For general symptoms, follow-up contrasts indicated a significant difference between all three groups (No PD vs. Prodromal: $t(2)=4.74$, $p<0.001$; OPD vs. Prodromal: $t(2)= 2.21$, $p=0.03$; OPD vs. No PD: $t(2)=2.68$, $p=0.01$ respectively). The Kruskal-Wallis test revealed a significant difference between the groups in negative ($\chi2(2, N=58)) =20.4$, $p<0.001$) and disorganized symptoms ($\chi2(2, N=58)=14.8$, $p=0.001$). Mann-Whitney U tests were conducted to follow-up on the significant differences between the different diagnostic groups in negative and disorganized symptom domains. Follow-up analyses revealed significant difference between No PD and Prodromal as well as between OPD and Prodromal groups for negative symptoms ($z= -4.01$, $p<0.001$; $z= -3.60$, $p<0.001$ respectively). There was no significant difference between the No PD and OPD groups ($z= -0.87$, $p=0.39$). Follow-up analyses revealed significant difference between No PD and Prodromal groups as well as between Prodromal and OPD groups for disorganized symptoms ($z= -3.37$, $p=0.001$; $z= -3.16$, $p=0.002$ respectively). There was no significant difference between No PD and OPD groups ($z= -0.51$, $p=0.61$).

Symptom Severity by Genotype (T carrier vs. Homozygous C)

The DBH SNP (-1021C→T) T-carrier group was not significantly different from the homozygous C group when using the t-test on the positive symptoms ($t(58)=-0.48$, $p=0.64$) or

general symptom factor (t(58)=-0.31, p=0.76). T-carrier group was not significantly different from the homozygous C group when using the Mann-Whitney Test for negative symptoms (z= -0.89, p=0.37) or disorganized symptoms (z= -0.35, p=0.73). With the exception of the disorganized symptom domain, findings were generally in the expected direction; T-carrier individuals showed slightly greater symptom severity than the homozygous C individuals (Figure 1, Figure 2, Figure 3, and Figure 4).

Conversion Status by Genotype (T carrier vs. Homozygous C)

Thirty-six percent of individuals (N=9) in the prodromal group converted to psychosis; no participant in the No PD or OPD group developed a psychotic illness in the current sample (Table 6). Approximately 13.5% of the Homozygous C at -1021C→T individuals (N=5) converted to an Axis I psychotic disorder, while nearly 14.8% of the T carriers at -1021C→T (N=4) converted to an Axis I psychotic disorder (Table 7 and Figure 5). According to logistic regression analysis, having at least one T allele at -1021C→T does not significantly predict conversion to psychosis (b=-1.07, p=0.88).

Symptom Severity by Genotypes (Genotype as a continuum)

Studies on the DBH SNP -1021C→T (Cubells & Zabetian, 2004; Zabetian et al., 2001) suggest a continuous "dose-response" relation of the T allele with the risk for positive symptoms, thus it was deemed optimal to use an analytic approach, regression analysis, that treated the genotypes as a continuous predictor variable, with CT heterozygotes being intermediate between the CC and TT. When only looking within the prodromal group (N=25), genotype was not a significant predictor of prodromal symptom severity; that is, the "dose of the T allele" was not a significant predictor of prodromal symptoms; however, the pattern was for individuals with the TT genotype to have greater positive prodromal symptoms as originally hypothesized (b = -0.06,

t(22)= -0.15, p = 0.88) (Figure 7). Additionally, genotype was not a significant predictor of negative, disorganized, or general symptom severity; but there was a pattern for individuals with the TT genotype to have greater negative, disorganized, and general symptoms (b=0.12, t(21)=0.27, p=0.79; b= -0.38, t(22)= -1.01, p=0.32; b=0.05, t(22)=0.13, p=0.90 respectively) (Figure 8, Figure 9, and Figure 10).

*Conversion Status by Genotype (Genotype as a Continuum)_*

Within the prodromal group, approximately 13.5% of the CC genotype individuals (N=5) converted to an Axis I psychotic disorder, 14.3% of the CT (N=3) converted to an Axis I psychotic disorder, and 16.7% of the TT (N=1) converted to an Axis I psychotic disorder (Table 8 and Figure 6). Logistic regression analyses revealed that the TT genotype at -1021C→T was not a significant predictor of conversion to psychosis (b=-0.10, p=0.85).

Discussion

The Current Study

The present study examined the relationship between DBH SNP -1021C→T and prodromal symptoms in putatively prodromal adolescents. The TT genotype at -1021C→T has been associated with significantly decreased pDBH activity across various studies; therefore, it was predicted that DBH SNP -1021C→T may be associated with more severe positive prodromal symptoms via subcortical hyperdopaminergia. It was hypothesized that carrying at least one T allele at -1021C→T would be associated with more severe prodromal symptoms—particularly the positive symptoms. The data analyses comparing the "T carriers" and the Homozygous C genotype revealed no significant differences between the two groups across all symptom domains; however findings were generally in the expected direction where the T carrier group showed slightly greater prodromal symptom severity. Furthermore, analyses

comparing symptom severity across all three genotypes (CC, CT, and TT) in the prodromal adolescent group also revealed no significant differences in prodromal symptom severities; however, the TT genotype showed more severe positive prodromal symptoms than the CT or CC genotypes.

Although results comparing either T carrier group to Homozygous C group as well as results comparing the three genotypes separately did not reach statistical significance, they were consistent with previous genetic studies demonstrating that certain DBH polymorphisms contributing to low DBH activity are more prevalent in psychotic individuals (Cubells et al., 2000; Yamamoto et al., 2003; Park et al., 2007).

Dominant Inheritance Model vs. Dose-Response Model

A dominant inheritance model argues that a particular trait is monogenetically inherited by an autosomal dominant mechanism. Using DBH SNP -1021C→T as an example, a dominant inheritance model would suggest that if the T allele were dominant over the C allele, then individuals with both the CT and TT genotypes would exhibit almost the same trait – possibly lower pDBH activity – and hence, be linked with a more severe positive prodromal symptom presentation. Interestingly, the analyses conducted in the current study supported the model of dominant inheritance as T carrier group, which included both the TT and CT genotypes, showed more severe positive prodromal symptoms than the homozygous C genotype. However, such finding is not consistent with previous literature on DBH SNP -1021C→T demonstrating intermediate effects of the CT genotype. Cubells and Zabetian (2004) and Zabetian et al. (2001) indicated that across three different racial groups (Asian, African American, and Caucasian), individuals who were homozygous T at -1021C→T showed very low DBH activity, heterozygotes showed intermediate levels of DBH, and individuals who were homozygous C

showed higher mean levels of DBH. Such findings support a dose-response model rather than a dominant inheritance model.

In the current study, individuals with the CT and the TT genotypes were combined in one group to improve statistical power because of the small number of individuals with the TT genotype. Making such a manipulation assumes that the -1021C→T SNP is an autosomal dominant trait; however, current literature on the DBH -1021C→T SNP does not support a dominant inheritance model. Therefore additional analyses that treated the three genotypes as a continuum, with CT heterozygotes being intermediate between the CC and TT, were conducted.

Risk for Psychosis in Putatively Prodromal Adolescents

Researchers using the SOPS have reported conversion rates of about 30-40% to an Axis I psychotic disorder within two years; some studies report rates up to 50% (Miller et al., 2002; Yung et al., 2003). In the current study 36% percent of prodromal adolescents developed a psychotic disorder at 2-year follow-up. This is consistent with previous literature, though at the lower end of the range. The slightly low conversion rate in the current study is most likely due to the effect of age. Conversion status was ascertained in the current study when participants ranged from 17 to 19. The modal age of onset of psychosis is typically in young adulthood (Schultz et al., 2007), hence at the time of follow-up participants had not yet passed through the heightened risk period for developing a psychotic disorder. Thus, it is hypothesized that the conversion rate in the current sample (36%) was lower than it would be if prodromal participants had been followed through their early 20's (40-50%) (Yung et al., 2003).

False Positive Rates in Risk for Psychosis

While a greater conversion rate with an extended follow-up period is expected to yield increased rates of conversion, not all adolescents thought to be in the prodromal phase of

psychosis will ultimately develop a psychotic disorder. Only a subgroup of the prodromal participants will progress to a psychotic disorder. Thus, a percentage of putatively prodromal individuals may be false positives; and hence would not be expected to manifest the most severe levels of prodromal symptoms.

DBH -1021C-T and Risk for Psychosis

The -1021C→T polymorphism is thought to be a functional SNP at the DBH locus that has shown significant associations with plasma and CSF DBH activity across many studies. The TT genotype at -1021C→T has been strongly associated with very low pDBH. Hence, it has been proposed that low pDBH leads to hyperdopaminergia in the subcortical regions of the brain by affecting the conversion of DA to NE in the noradrenergic synthetic pathway. It was then hypothesized that prodromal adolescents with more severe positive symptoms would have at least one T allele at -1021C→T, and that these individuals would show higher rates of conversion to psychosis compared to those who are homozygous C at -1021C→T.

In the current study, the presence of at least one T allele at DBH SNP -1021C→T did not indicate greater conversion to psychosis. Individuals in the homozygous C and T carrier groups showed similar conversion rates to a psychotic disorder. This was not expected because the T allele at the DBH SNP -1021C→T is thought to contribute to decreased DBH expression, which would in turn contribute to subcortical hyperdopaminergia, which has been associated with psychotic symptoms. Regression analyses were conducted using the -1021C→T polymorphisms as a continuous variable, mainly to test whether separating the TT and the CT genotype would significantly change the outcome when predicting positive symptom severity. Genotype, investigated as a continuous variable did not significantly predict symptom severity for any of the symptom domains; however, the TT genotype individuals showed a pattern for more severe

positive symptoms than both the CT and the CC genotypes. Lastly, logistic regression analysis demonstrated that genotype as a continuous variable was not a significant predictor of conversion to psychosis. Though findings did not reach statistical significance, they are consistent with previous DBH literature that find the TT genotype to be most strongly associated with low DBH expression and that low DBH expression may contribute to increased vulnerability for development of psychotic symptoms.

Study Limitations

The total sample size (N = 64) was small for most genetic studies. Due to the small sample size, few participants had the TT genotype. Therefore, the CT and TT genotypes were collapsed into one group. This manipulation presupposes a certain theory of how the T allele behaves, through in an autosomal dominant manner, however, data thus far suggest an alternative mechanism for DBH action: a dose-response manner. This is an inherent weakness in the analyses conducted that collapsed across the CT and TT genotypes. This manipulation may have washed out the effect of the T allele because the TT genotype was not well represented within the T carrier group. To ensure that the effect of T allele was not masked by the effect of C allele in the T carrier group, regression analyses were conducted using all three genotypes; however, the results were not significant. It is not clear whether results did not reach statistical significance because there were relatively fewer -individuals with the TT genotype than CT individuals in the T-carrier group. Increasing the sample size will answer this question and also reveal more pronounced differences between the three genotypes.

Study Implications

Due to limited sample size, the role of the TT genotype at DBH -1021C→T remains to be elucidated. Results did not reach statistical significance to support the hypothesis that genetic

variants on DBH are a risk factor for the development of psychotic symptoms. Epigenetic and environmental factors also contribute to the expression of DBH, and as previous literature suggests, these factors may account for a larger proportion of dopamine dysregulation implicated in the development of psychotic symptoms than any polymorphic changes on DBH (Howes & Kapur, 2009).

Future Directions

The question whether DBH deficiency leads to elevated levels of DA in the striatum has yet to be answered. The current study, while making the assumption that elevated striatal DA in prodromal individual increases the probability of conversion to psychosis, did not directly test this hypothesis. Future studies should address such questions. Future research investigating the assumptions of the current study may begin to explain one possible etiological mechanism of SZ.

Conclusion

In conclusion, with the provision that current findings must be interpreted with the usual caution for association studies, the present study provides evidence that the DBH gene may play a role in increasing the vulnerability to psychotic symptoms. Furthermore, results of the current study add to the current thinking that genetic susceptibilities alone cannot explain the etiology of psychosis or in this case the precursor to psychosis. Again, while a number of genetic associations have been identified, none of them account for a significant portion of the dysfunction in psychosis. The current study sheds light on the current view of SZ genetics and reemphasizes the critical role for the additive and interactive effects of other risk factors – environmental – for schizophrenia.

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Table 1: Clinical and Demographic Characteristics

	Diagnostic Groups (N=64)			
	No PD (N=19)	Prodromal (N=25)	OPD (N=20)	Significance
Age, Mean (SD)	14.4 (1.95)	14.28 (1.79)	14.85 (1.95)	F=1.52 ; p=0.22
Male, n (%)	9 (47.4)	17 (68.0)	8 (40.0)	$\chi2$=3.86 ; p=0.15
CAU, n (%)	9 (47.4)	15 (60.0)	9 (45.0)	$\chi2$=5.40 ; p=0.49
AA, n (%)	10 (52.6)	8 (32.0)	9 (45.0)	$\chi2$=5.40 ; p=0.49
Asian American, n (%)	-------	2 (8.0)	1 (5.0)	$\chi2$=5.40 ; p=0.49
Other	-------	-------	1 (5.0)	$\chi2$=5.40 ; p=0.49
Stimulants, n (%)	1 (5.3)	6 (24.0)	3 (15.0)	$\chi2$=2.88 ; p=0.24
Antipsychotics, n (%)	1 (5.3)	3 (12.0)	1 (5.0)	$\chi2$=1.00 ; p=0.61
Mood Stabilizer, n (%)	-------	2 (8.0)	1 (5.0)	$\chi2$=1.55 ; p=0.46
SSRI, n (%)	1 (5.3)	6 (24.0)	-------	$\chi2$=7.46 ; p=0.02
Other antidepress, n (%)	-------	-------	1 (5.0)	$\chi2$=2.24 ; p=0.33
Mother education, Mean (SD)	13.5 (4.33)	15.7 (4.70)	13.4 (5.60)	F=1.51; p=0.23
Father education, Mean (SD)	13.0 (5.61)	13.8 (4.83)	12.0 (6.70)	F=0.52; p=0.02

Prodromal Group Includes: individuals meeting criteria for SPD or APS

Table 2: Clinical and Demographic Characteristics

	1021C/T Genotypes			
	CC (N=37)	CT (N=21)	TT (N=6)	Significance
Age, Mean (SD)	14.8 (1.82)	14.0 (1.83)	14.7 (2.34)	F=1.27; p=0.29
Male, n (%)	19 (51.4)	13 (61.9)	2 (33.3)	$\chi2$=1.64; p=0.44
CAU, n (%)	15 (40.5)	14 (66.7)	4 (66.7)	$\chi2$=7.46; p=0.28
AA, n (%)	20 (54.1)	5 (23.8)	2 (33.3)	$\chi2$=7.46; p=0.28
Asian American, n (%)	1 (2.7)	2 (9.5)	-------	$\chi2$=7.46; p=0.28
Other	1 (2.7)	-------	-------	$\chi2$=7.46; p=0.28
Stimulants, n (%)	3 (8.1)	5 (23.8)	2 (33.3)	$\chi2$=4.08; p=0.13
Antipsychotics, n (%)	3 (8.1)	1 (4.8)	1 (16.7)	$\chi2$=0.93; p=0.63
Mood Stabilizer, n (%)	1 (2.7)	2 (9.5)	-------	$\chi2$=1.72; p=0.42
SSRI, n (%)	4 (10.8)	3 (14.3)	-------	$\chi2$=0.99; p=0.61
Other antidepress, n (%)	1 (2.7)	-------	-------	$\chi2$=0.74; p=0.69
Mother education, Mean (SD)	14.7 (3.72)	14.1 (6.17)	13.3 (6.53)	F=0.23; p=0.80
Father education, Mean (SD)	13.2 (4.80)	12.3 (6.66)	14.2 (7.06)	F=0.33; p=0.72

Table 3: Clinical and Demographic Characteristics

	Homozygous C and T Carriers		
	CC (N=37)	T Carriers (N=27)	Significance
Age, Mean (SD)	14.8 (1.82)	14.1 (1.93)	F =1.87; p =0.18
Male, n (%)	19 (51.4)	15 (55.6)	$\chi2$=0.11; p=0.74
CAU, n (%)	15 (40.5)	18 (66.7)	$\chi2$=6.46; p=0.09
AA, n (%)	20 (54.1)	7 (25.9)	$\chi2$=6.46; p=0.09
Asian American, n (%)	1 (2.7)	2 (7.4)	$\chi2$=6.46; p=0.09
Other	1 (2.7)	-------	$\chi2$=6.46; p=0.09
Stimulants, n (%)	3 (8.1)	7 (25.9)	$\chi2$=3.76; p=0.05
Antipsychotics, n (%)	3 (8.1)	2 (7.4)	$\chi2$=0.01; p=0.92
Mood Stabilizer, n (%)	1 (2.7)	2 (7.4)	$\chi2$=0.77; p=0.38
SSRI, n (%)	4 (10.8)	3 (11.1)	$\chi2$=0.001; p=0.97
Other antidepress, n (%)	1 (2.7)	-------	$\chi2$=0.74; p=0.39
Mother educaiton, Mean (SD)	14.7 (3.72)	14.0 (6.13)	F=0.34; p=0.56
Father educaiton, Mean (SD)	13.2 (4.80)	12.7 (6.66)	F=0.14; p=0.71

Table 4: Genotype frequencies (1021C/T) in the sample

	Frequency	Percent (%)
CC	37	57.8
CT	21	32.8
TT	6	9.4
Total	64	100

Table 5: Genotype frequencies by diagnostic groups

1021C/T	CC	CT	TT
No PD	11	5	3
Prodromal	14	10	1
OPD	12	6	2
Total	37	21	6

Prodromal Group Includes: individuals meeting criteria for SPD or APS

Table 6: Conversion Diagnoses by Diagnostic Groups

	No PD	Prodromal	OPD
Schizoaffective	0 (0%)	3 (12.0%)	0 (0%)
Undiff Type SZ	0 (0%)	3 (12.0%)	0 (0%)
Biploar	0 (0%)	3 (12.0%)	0 (0%)
Total	0 (0%)	9 (36%)	0 (0%)

Schizoaffective Disorder
Undifferentiated Type of Schizophrenia
Bipolar disorder (most recent episode mixed, severe with psychotic features)

Table 7: Conversion Diagnoses by Genotype (collapsed)

	Homozygous C	T Carriers
Schizoaffective	2 (5.4%)	1 (3.7%)
Undiff Type SZ	2 (5.4%)	1 (3.7%)
Biploar	1 (2.7%)	2 (7.4%)
Total	5 (13.5%)	4 (14.8%)

Schizoaffective Disorder
Undifferentiated Type of Schizophrenia
Bipolar disorder (most recent episode mixed, severe with psychotic features)

Table 8: Conversion Diagnoses by Genotype (not collapsed)

	CC	CT	TT
Schizoaffective	2 (5.4%)	1 (4.8%)	1 (16.7%)
Undiff Type SZ	2 (5.4%)	2 (9.5%)	0 (0%)
Biploar	1 (2.7%)	0 (0%)	0 (0%)
Total	5 (13.5%)	3 (14.3%)	1 (16.7%)

Schizoaffective Disorder
Undifferentiated Type of Schizophrenia
Bipolar disorder (most recent episode mixed, severe with psychotic features)

Figure Captions:

1. Mean positive symptoms by DBH SNP groups (CC vs. T Carrier)

2. Mean negative symptoms by DBH SNP groups (CC vs. T Carrier)

3. Mean disorganized symptoms by DBH SNP groups (CC vs. T Carrier)

4. Mean general symptoms by DBH SNP groups (CC vs. T Carrier)

5. Conversion diagnoses by DBH SNP groups (CC vs. T Carrier)

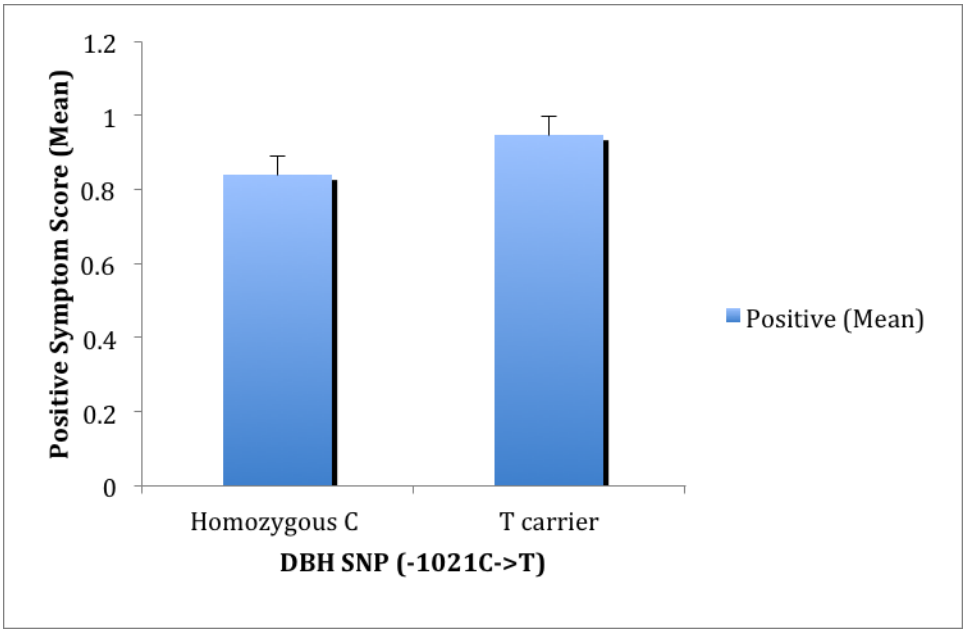
6. Conversion diagnoses by DBH SNP groups (CC vs. CT vs. TT)

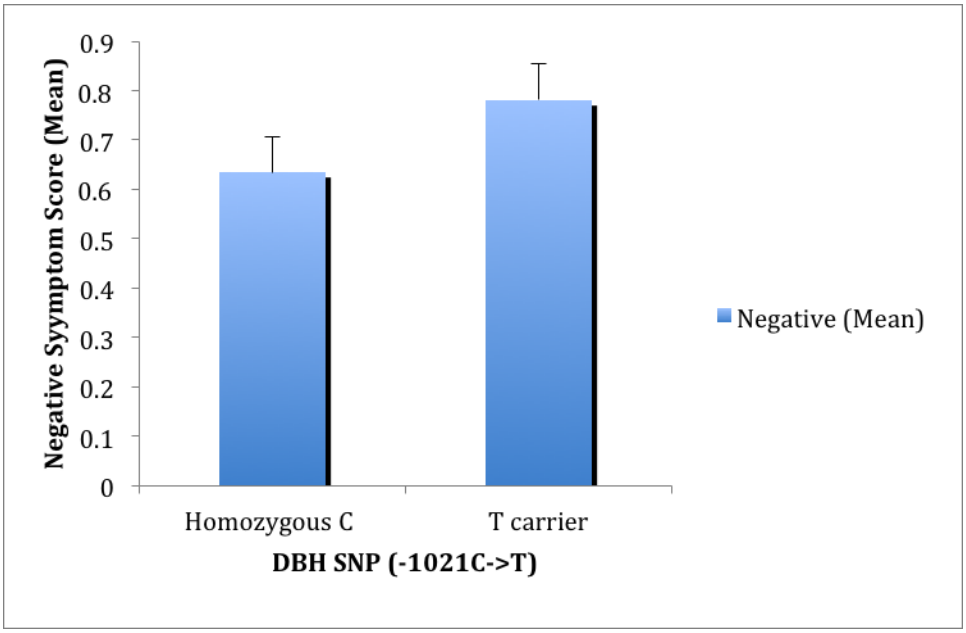
7. Mean positive symptoms by DBH SNP genotypes

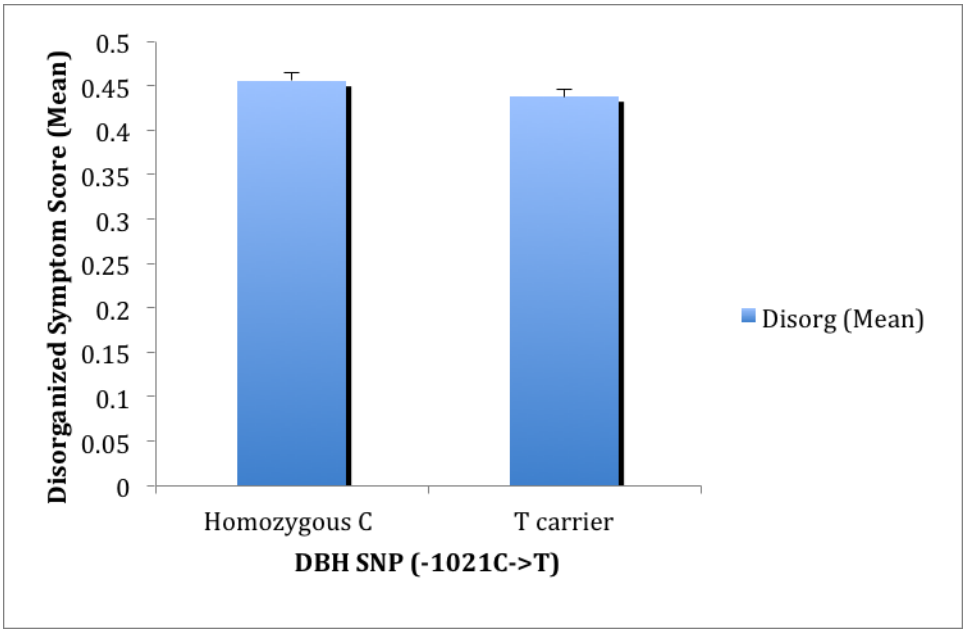
8. Mean positive symptoms by DBH SNP genotypes

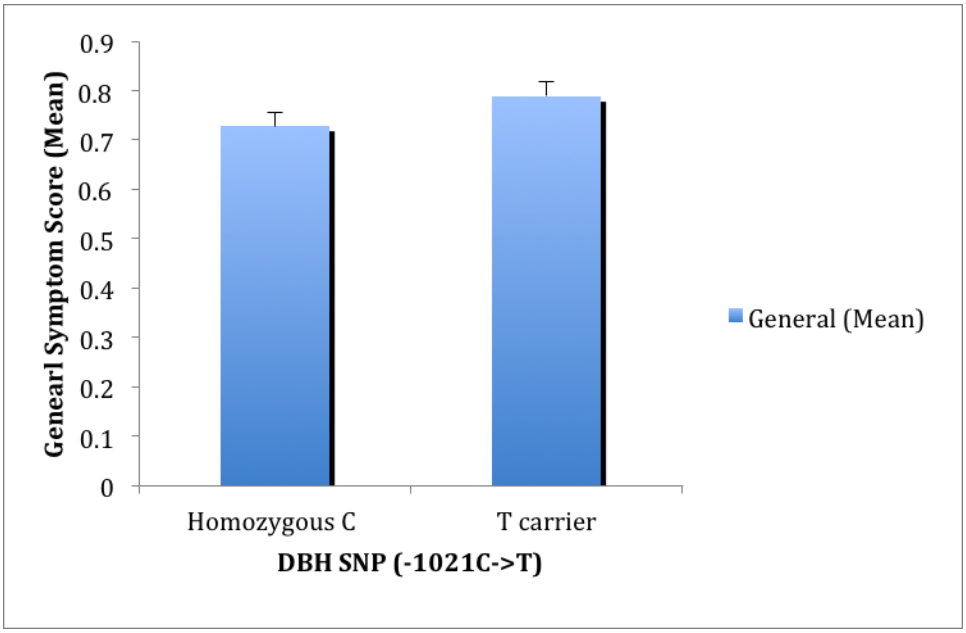
9. Mean positive symptoms by DBH SNP genotypes

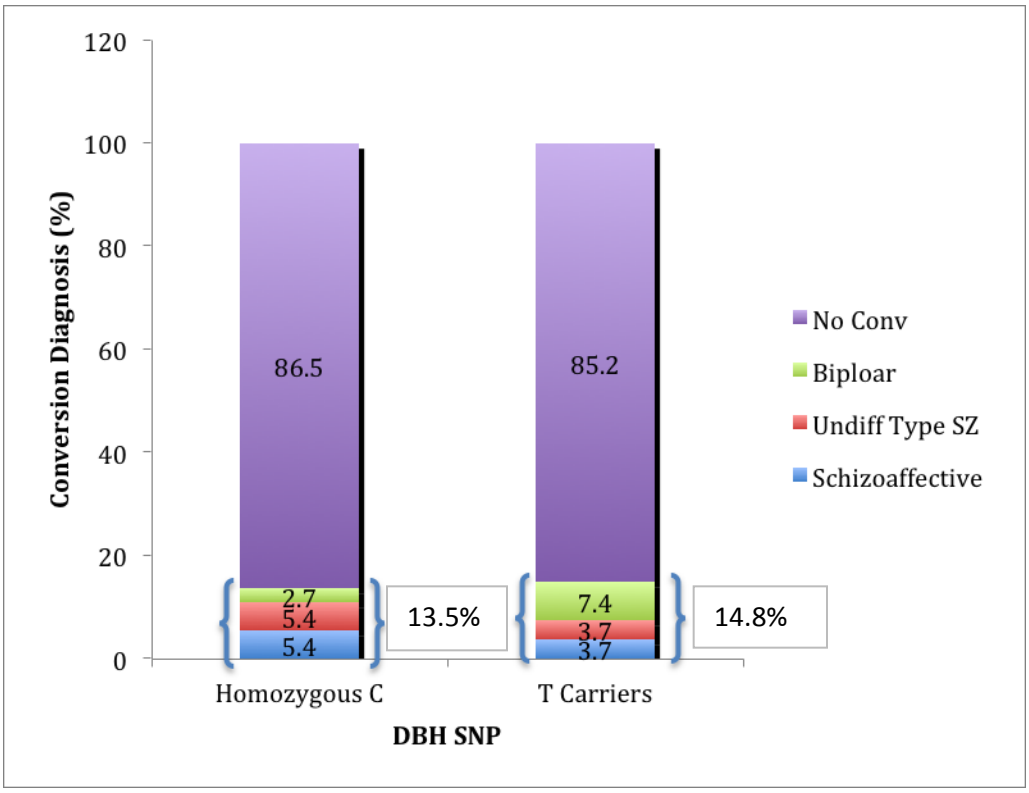
10. Mean positive symptoms by DBH SNP genotypes

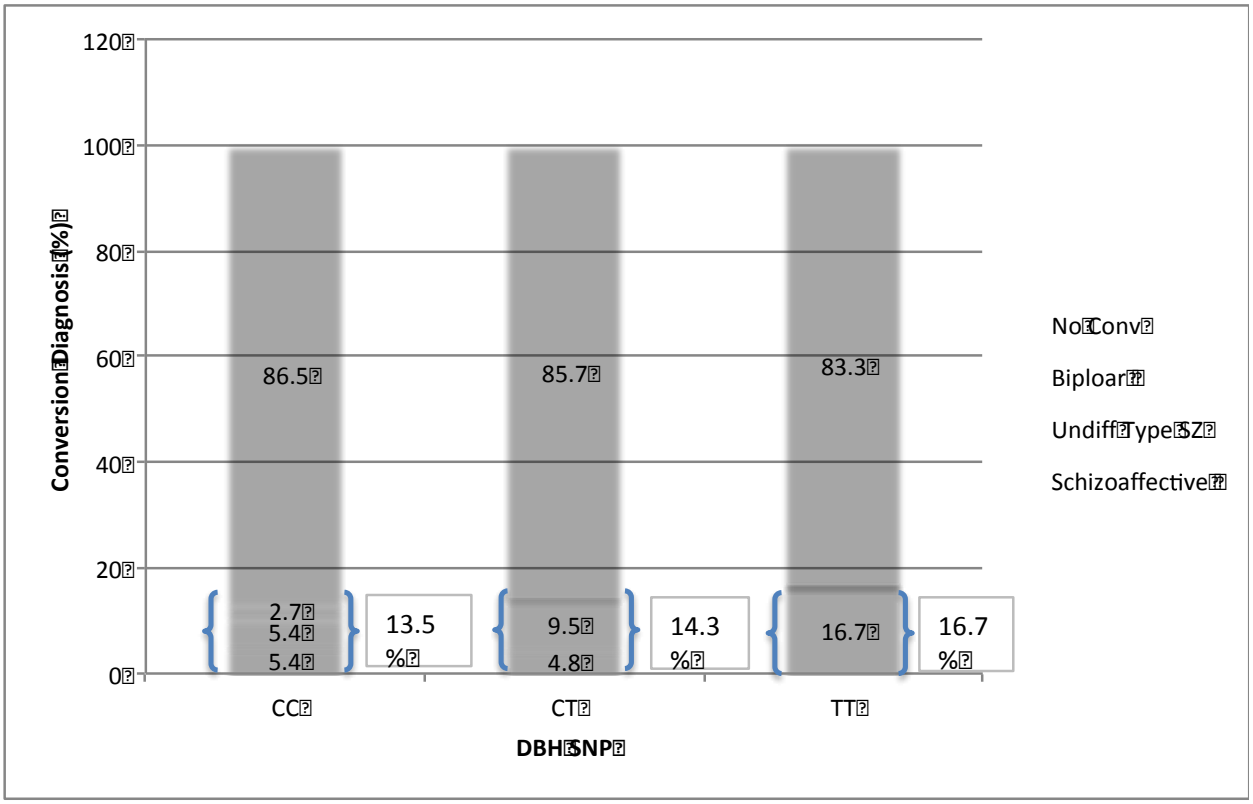

1.2
1
0.8
0.6
0.4
0.2
0
Positive Symptom Score (Mean)
Homozygous C
T carrier
DBH SNP (-1021C->T)
Positive (Mean)

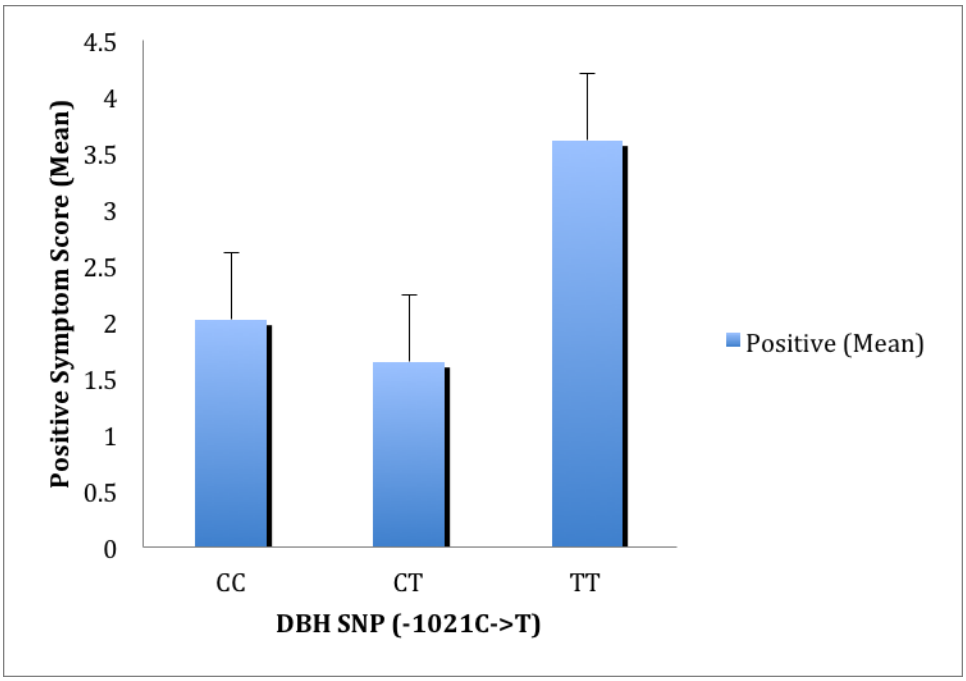

Negative Symptom Score (Mean)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Homozygous C
T carrier
DBH SNP (-1021C->T)
Negative (Mean)

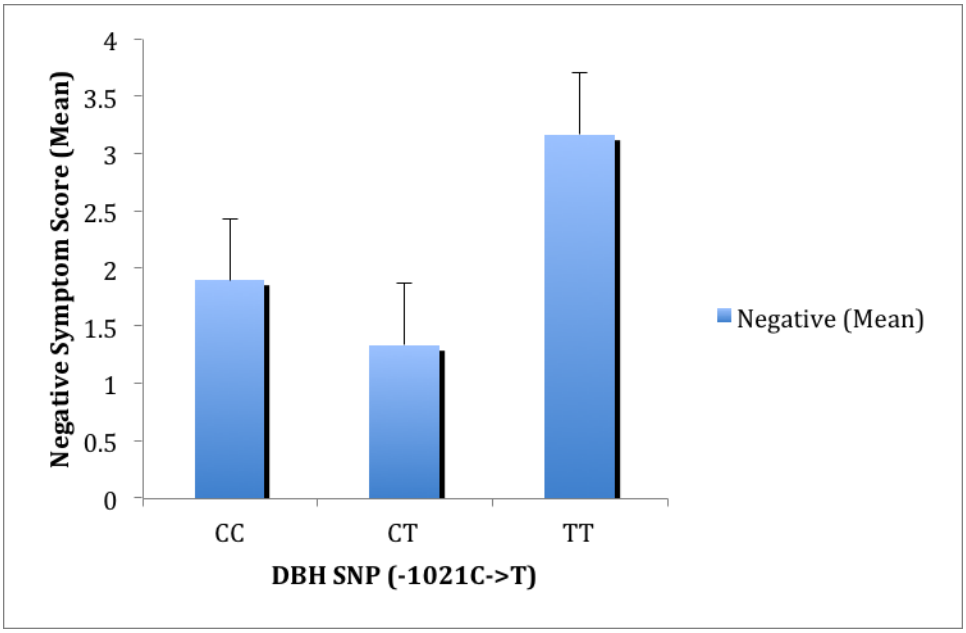

Disorganized Symptom Score (Mean)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Homozygous C
T carrier
DBH SNP (-1021C->T)
Disorg (Mean)

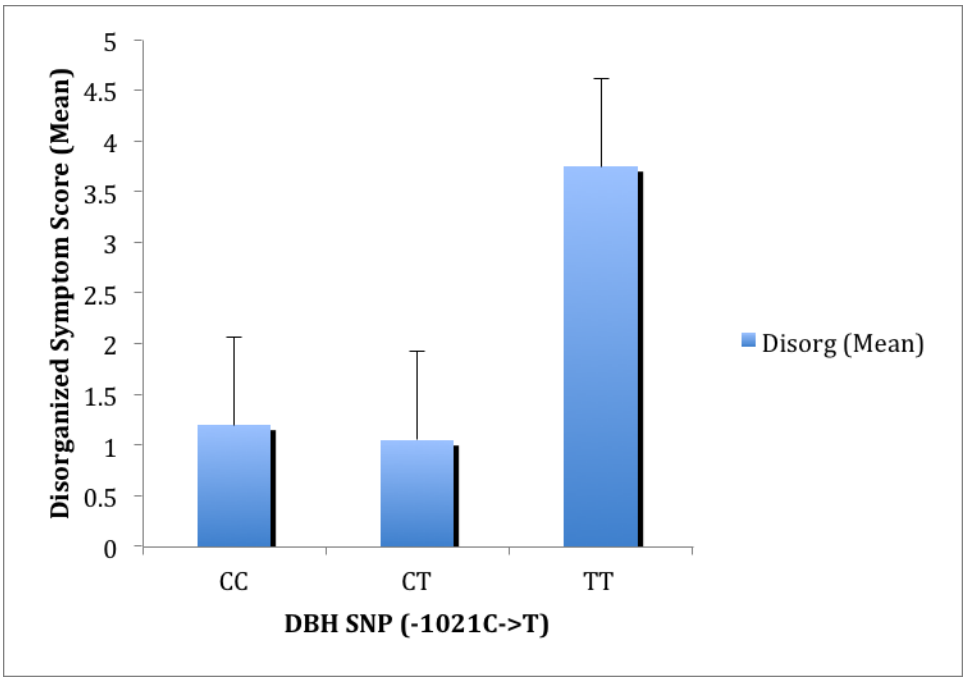

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Genearl Symptom Score (Mean)
Homozygous C
T carrier
DBH SNP (-1021C->T)
General (Mean)

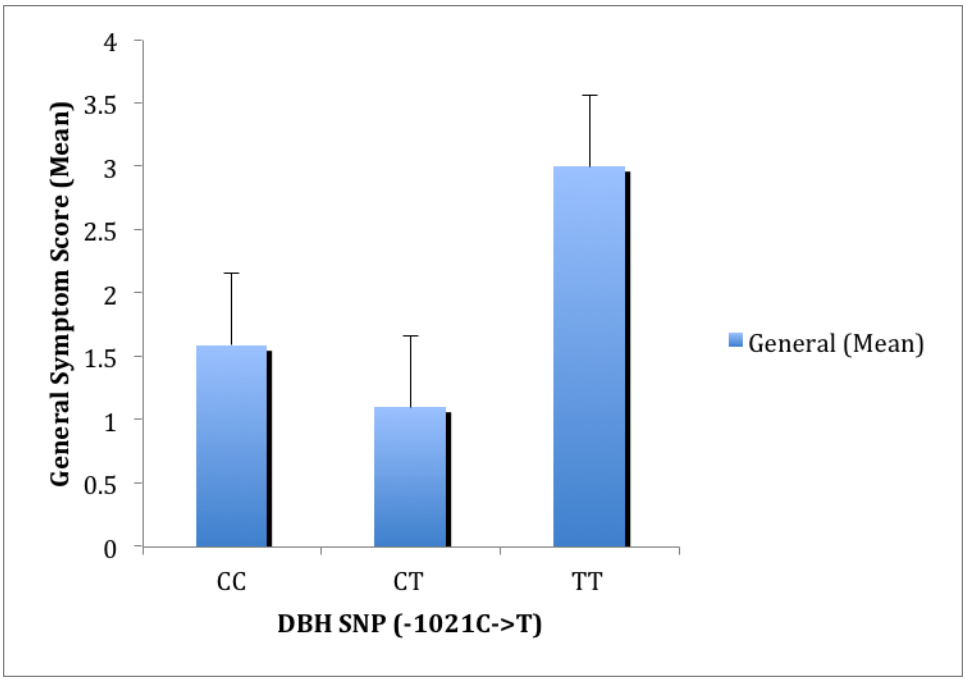

120
100
80
60
40
20
0
Conversion Diagnosis (%)
86.5
85.2
2.7
5.4
5.4
7.4
3.7
3.7
13.5%
14.8%
No Conv
Biploar
Undiff Type SZ
Schizoaffective
Homozygous C
T Carriers
DBH SNP


120
100
80
60
40
20
0
Conversion to Diagnosis (%)
86.5
85.7
83.3
2.7
5.4
5.4
9.5
4.8
16.7
13.5 %
14.3 %
16.7 %
CC
CT
TT
DBH SNP
No Conv
Biploar
Undiff Type SZ
Schizoaffective


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Positive Symptom Score (Mean)
CC
CT
TT
DBH SNP (-1021C->T)
Positive (Mean)


Negative Symptom Score (Mean)
4
3.5
3
2.5
2
1.5
1
0.5
0
CC
CT
TT
DBH SNP (-1021C->T)
Negative (Mean)


Disorganized Symptom Score (Mean)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
CC
CT
TT
DBH SNP (-1021C->T)
Disorg (Mean)


General Symptom Score (Mean)
4
3.5
3
2.5
2
1.5
1
0.5
0
CC
CT
TT
DBH SNP (-1021C->T)
General (Mean)