Candidate Genes on Chromosome 6p in Obsessive-Compulsive Disorder and Schizophrenia

by

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Master of Science

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ABSTRACT:

Obsessive-compulsive disorder (OCD) and schizophrenia (SCZ) are serious neuropsychiatric disorders that might involve autoimmune processes. Two genes, mapping to a risk region for OCD/SCZ, the myelin oligodendrocyte glycoprotein (MOG), which mediates the complement cascade, and the gamma-aminobutyric acid type B receptor 1 (GABBR1), which functions in the major inhibitory neurotransmitter system, represent candidates for OCD/SCZ. We investigated the transmission of alleles/haplotypes of four MOG and five GABBR1 polymorphisms for possible association with 191 SCZ case-control pairs, 160 OCD and 111 SCZ families using the transmission disequilibrium test, family-based association test or Kruskal-Wallis Test. Significant associations were observed for MOG-(TAAA)n (P=0.022) with OCD and with OCD severity level (P=0.020). The MOG haplotype-1.2.2.13 (P=0.011) was associated with OCD. Trends of overtransmission of GABBR1–7265A variant (P=0.071) and haplotype-2.1.1.2.1 (P=0.065) were observed in OCD. Although our results suggest that MOG/GABBR1 were not associated with SCZ, both genes might play a role in OCD.

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ABBREVIATIONS:

(In alphabetical order)

5-HT	Serotonin
AAO	Age At Onset
ADEM	Acute Disseminated EncephaloMyelitis
ADHD	Attention-Deficit Hyperactivity Disorder
AF	Attributable Fraction
ANOVA	Analysis of Variance
CI	Confidence Interval
CNPase	Cyclic Nucleotide Phosphodiesterase
CNS	Central Nervous System
CSF	CerebroSpinal Fluid
DIGS	Diagnostic Interview for Genetics Studies
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4^{th} Edition
DTNBP1	Dystrobrevin Binding Protein 1 or Dysbindin
FBAT	Family-Based Association Test
FIGS	Family Interview for Genetic Studies
GABA	Gamma-AminoButyric Acid
$GABA_B$	Gamma-AminoButyric Acid type B
GABBR1	Gamma-AminoButyric Acid type B Receptor 1
GABHS	Group A β-Hemolytic Streptococcal
GAF	Global Assessment of Functioning scale
GPC	Genetic Power Calculator
HLA	Human Leukocyte Antigen
LD	Linkage Disequilibrium
MAG	Myelin-Association Glycoprotein
MBP	Myelin Basic Protein
MHC	Major Histocompatibility Complex
MOG	Myelin Oligodendrocyte Glycoprotein

MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
OC	Obsessive-Compulsive
OCD	Obsessive-Compulsive Disorder
OR	Odd Ratio
PANDAS	Pediatric Autoimmune Neuropsychiatric Disorders Associated with (Group A β-hemolytic) Streptococcal infection
PAWE	Power for Association With Errors
PBAT	Power for Family-Based Association Test
PCR	Polymerase Chain Reaction
RA	Rheumatoid Arthritis
RD	Reading Disabilities
RR	Relative Risk
SC	Sydenham's Chorea
SCID	Structured Clinical Interview for the DSM-IV
SCZ	Schizophrenia
SRIs	Serotonin Reuptake Inhibitors
SSRI	Selective Serotonin Reuptake Inhibitor
TDT	Transmission Disequilibrium Test
TDT/S-TDT	Sib-Transmission Disequilibrium Test
TNF-α	Tumor Necrosis Factor-Alpha
TS	Gilles de la Tourette's Syndrome
Y-BOCS	Yale-Brown Obsessive-Compulsive Scale

CHAPTER 1: General Introduction

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CHAPTER 1: OVERVIEW OF AUTOIMMUNE MECHANISM HYPOTHESIS IN OBSESSIVE-COMPULSIVE DISORDER AND SCHIZOPHRENIA

1.1 Similarities and Overlap between Obsessive-Compulsive Disorder (OCD) and Schizophrenia (SCZ)

Obsessive-compulsive disorder (OCD) is considered a common and serious neuropsychiatric disorder with a lifetime prevalence of 2 to 3% in the general population (Sasson et al. 1997). The disorder occurs equally between the genders but usually begins during childhood and adolescence, with males having an earlier onset (ages 6-15) than females (ages 20-29) (Zohar 1999). OCD is generally characterized by two major symptom-types, obsessions, which are undesirable intrusive thoughts or images that are recurrent and persistent, and compulsions, which are described as the need to perform excessive rituals or repetitive acts in order to alleviate anxiety (APA 1994). Symptoms (percentage in brackets) (Rasmussen and Tsuang 1986) for obsessions include intrusive distressing thoughts, impulses, or images such as contamination usually through dirt or germs (45%), pathologic doubt (42%), somatic (36%), need for symmetry (31%), aggressive impulse (26%), and sexual impulse (26%), that are not related to real-life problems. OCD patients recognize that their obsessions are a product of their own mind and attempt to ignore, suppress, or neutralize anxiety from obsessions, which eventually resort in the other symptom-type, compulsion. There are two types of compulsions (Rasmussen and Tsuang 1986) in the diagnosis of OCD and they are defined as repetitive behaviours in response to obsessions such as checking (60%), washing (50%), counting (36%), need to ask or confess (31%), symmetry and precision (28%), hoarding (18%), praying, and repeating words silently, and compulsions intended to reduce distress while not connected realistically to preventing

obsession and with excessive measures.

Schizophrenia (SCZ), which is a chronic and disabling brain disease, is not quite as common as OCD, affecting about 0.8 to 1.5% of the general population. Onset of SCZ ranges from 15-30 years of age (Leung and Chue 2000) and there are more males affected with SCZ (male:female ratio was 1.56:1) than females (Castle and Murray 1993). It is usually characterized by diverse and variably expressed symptoms; nonetheless, it can be separated into two symptom-types (IPSS 1992), positive symptoms, which include disorganized thought pattern (50%) and bizarre behaviours, delusional beliefs (persecutory delusions – 64% and delusions of reference – 70%), and sensory hallucinations (auditory – 74% and second person – 65%), and negative symptoms such as blunted emotions (obliviousness to things around oneself – 66%), loss of drive, poverty of thought (52%), cognitive difficulties, apathy, and social withdrawal.

There are many similarities between OCD and SCZ based on their symptoms, comorbidity, pathophysiological and pharmacological studies, which will be discussed briefly below.

1.1.1 Clinical Similarities and Comorbidity between OCD and SCZ

Unlike SCZ, OCD is not considered a psychotic disorder, but both disorders do share similar symptoms such as high levels of anxiety; that is, a sense of fear, nervousness, or danger that does not fit the situation, and tormenting thoughts like obsessive thoughts in OCD and hallucinations in SCZ. These disorders also have similar peak age at onset between the ages of 15 and 30.

The association between OCD and SCZ symptoms has been an issue of debate in the

literature for decades. The etiology of OC phenotype in SCZ remains unclear and *vice versa*. Studies have reported that the frequency of OC symptoms in SCZ ranges from 3.5% to 46% (Rosen 1957; Berman et al. 1995; Fabisch et al. 2001) and that of psychotic symptoms in OCD ranges from 7.8% to 59.2% (Bland et al. 1987; Karno et al. 1988; Eisen et al. 1997; Bermanzohn et al. 2000). High comorbidity rates raise the possibility of a common underlying component in the etiology for both disorders. Patients displaying co-morbid OCD and SCZ have been shown to have significantly higher positive and emotional discomfort symptoms, poorer executive brain function (Lysaker et al. 2000), reduced capacity for global, social and economic functioning (Fenton and McGlashan 1986; Berman et al. 1998; Tibbo et al. 2000), significantly increased negative symptoms, and an increased prevalence of Parkinsonian symptoms (Poyurovsky et al. 1998; Tibbo et al. 2000) than their OCD-free counterparts.

The development of OCD in the SCZ population has recently been of strong interest to clinicians partly due to the publication of a number of case studies detailing apparent psychopharmacologically induced OC symptoms in SCZ patients treated with atypical antipsychotics.

1.1.2 Overlap in Pharmacology between OCD and SCZ

Obsessive thinking and compulsive behaviour occur in classic OCD. The etiology of OCD is uncertain but it appears to include a combination of neurological and psychological factors. The dominant neurochemical theory of OCD suggests that the neurotransmitter serotonin plays a central role in the development of the condition (Kaplan and Hollander 2003; Meltzer et al. 2003). Drugs that increase the availability of serotonin in the brain are

effective in ameliorating the symptoms of OCD. Thus, serotonin reuptake inhibitors (SRIs) are the treatments of choice for classic OCD but these alone usually only achieve a partial remission of symptoms. The addition of a neuroleptic for SRI-treatment-resistant patients is common, with reported efficacy in some patients (McDougle 1997). This is particularly the case when OCD occurs in the context of Tourette's tics, where conventional antipsychotics such as haloperidol have often been used. The antidopaminergic properties of antipsychotics may be useful in this disorder because dopamine levels have been found to parallel tic severity in children (Shaywitz et al. 1980). However, frequent adverse motor effects limit the use of conventional antipsychotics.

Alternatively, antipsychotic drugs are the most common medications used to treat patients with SCZ because they decrease most of the major symptoms of SCZ and prevents relapses (Raggi et al. 2004). Currently all effective antipsychotic medications block the dopamine D2 receptor, which appears to be a necessary and sufficient condition for antipsychotic response (Seeman and Van Tol 1994; Kapur and Mamo 2003). Other antipsychotic drugs used to treat SCZ have been reported to alter other brain chemicals and receptors such as serotonin (5HT) 2-receptor, gamma-aminobutyric acid (GABA), and glutamate (Carlsson et al. 1999; Wassef et al. 2003).

The relationship between comorbid OC symptoms in SCZ and atypical antipsychotics remains controversial. Atypical antipsychotics, mainly risperidone and olanzapine, have been used as adjunctive treatment in selective serotonin reuptake inhibitor (SSRI)-refractory cases with obsessive symptoms; however, *de novo* emergence or exacerbation of OC symptoms during treatment with clozapine, risperidone, olanzapine, and quetiapine has been described in the literature (Fountoulakis et al. 2004). Previous reports suggest that some

atypical antipsychotics may have both obsessogenic and anti-obsessional effects (Ramasubbu et al. 2000). Given their higher affinity for 5HT2 receptors than dopamine D2 receptors, it has been speculated that atypical antipsychotics may induce OC symptoms at low doses due to high 5HT2 antagonism, and improve OC symptoms at higher doses due to predominant D2 antagonism.

Medications targeted at the abnormal region of the brain will more likely improve symptom severity due to their specificity to concentrate on a specific area of interest and to limit the amount of adverse effects.

1.1.3 Overlap in Pathophysiology between OCD and SCZ

Magnetic-resonance imaging (MRI) and positron emission tomography (PET) studies have identified the specific neurobiological factors in the onset and persistence of OCD. Most studies have shown that abnormalities linked to OCD lie in the orbitofronto-striatal-pallido-thalamic circuit, the pathway that connects the frontal cerebral cortex with the basal ganglia (see Figure 1) (Modell et al. 1989). In general terms, the frontal lobe is associated with decision-making and judgement, while the basal ganglia act as relay stations where signals for the planning and execution of movements are mediated. Obsessive thinking is thought to be connected to the dysfunction of the basal ganglia's ability to filter messages relayed to the frontal cortex. MRI studies of untreated OCD patients have shown loss of tissue in the caudate nuclei, an important part of the basal ganglia. Significantly increased glucose metabolic rates have been observed in the left orbital gyrus of the frontal lobe in OCD patients (Baxter et al. 1987). Normalization of the hypermetabolism in this area has occurred after administering effective pharmacological or behaviour therapy treatment

(Baxter et al. 1992). Further evidence implicating this pathway in OCD came from surgical evidence as a limbic leucotomy, a procedure that affects the fronto-caudate-thalamic pathway by lesioning the cingulate and orbitomedial frontal areas, has been shown to successfully alleviate OCD symptoms and signs.

Imaging techniques have also revealed abnormal activity in the basal ganglia (Mehler and Warnke 2002) in addition to the prefrontal cortex (Weinberger et al. 2001), and the temporal lobes in SCZ patients. Reduction in volume of the prefrontal cortex, which affects verbal memory, attention, reasoning, aggression, and meaningful speech, has been observed in many SCZ patients. Overactivity in the specific parts of the frontal and the right temporal lobes has been associated with auditory hallucinations (e.g., hearing voices). Loss of volume in the temporal lobe affects the limbic areas encompassing the hypothalamus, amygdala, and hippocampus. Activity in this area is related to emotions and memory, and abnormalities are associated with positive symptoms, including delusions and hallucinations, and also with disordered thinking. Dysfunction of the basal ganglia have been found to be related to SCZ symptoms in that findings suggest a role in monitoring intention, its consequences, and actions. This is because the basal ganglia are part of a circuit that participates in the development of motor and cognitive patterns. The basal ganglia's association with other areas of the brain plays a large role in the development of the diverse symptoms of SCZ.

There are significant overlaps between OCD and SCZ on clinical and neurobiological bases, which include defects common to OCD and SCZ such as the basal ganglia, frontal cortex, thalamus (Andreasen et al. 1994a; Andreasen et al. 1994b; Kim et al. 2001), and the proposed functional circuits [frontal-striatal circuits (Baxter et al. 1988; Cummings 1993; Kim et al. 2000), and hippocampus-amygdala complex (Morgan and LeDoux 1995; Rauch et

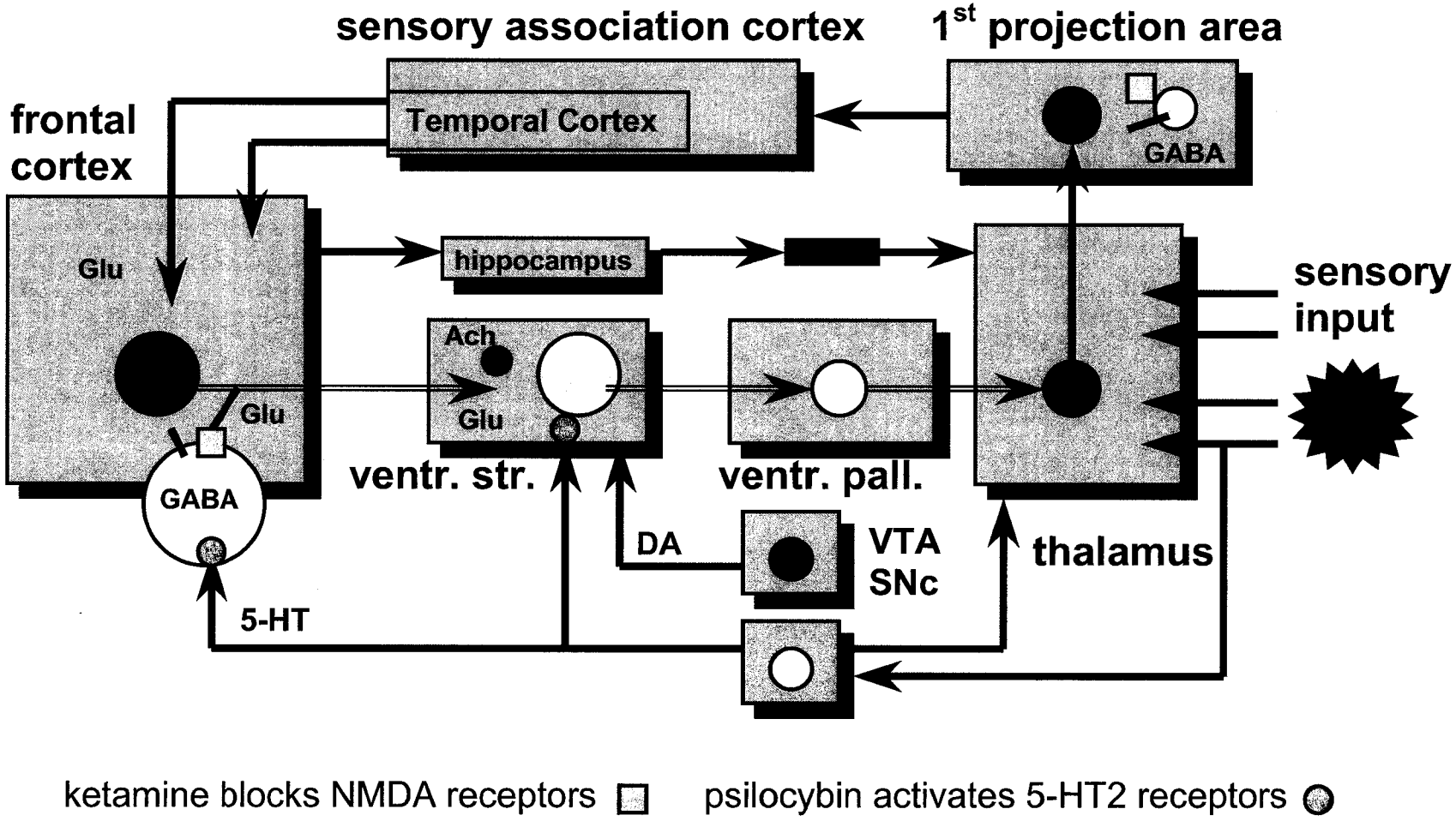
al. 1998)] (see Figure 1 for diagram of circuit) and dysfunction at the neurotransmitter level that might lead to co-expression of symptoms.

Similar regions have been identified to be functionally or morphologically abnormal in both illnesses but they tend to occur in opposite directions. For example the fronto-striatal system is hyperfunctional in OCD (Baxter et al. 1988) but hypofunctional in SCZ (Kim et al. 2000), and the thalamus is enlarged in OCD (Kim et al. 2001) but atrophic in SCZ (Andreasen et al. 1994a; Andreasen et al. 1994b). Moreover, while both disorders have impairments in the prefrontal cortex, subregions of interest are different (Behar et al. 1984; Cavedini et al. 1998; Saxena et al. 1998; Bunney and Bunney 2000), that is, a core dorsolateral prefrontal cortex dysfunction is observed in SCZ (Weinberger et al. 2001) and a core ventromedial prefrontal cortex dysfunction is observed in OCD (Saxena et al. 1998).

In addition to the similar brain regions that are affected, similar dysfunctions of common neurotransmitter systems, which include the dopaminergic (van Rossum 1966; Seeman 1993; Baron 2001; Kaplan and Hollander 2003) and serotonergic (Kaplan and Hollander 2003; Meltzer et al. 2003) systems, have been reported. These similar neuroanatomical and functional interactions between OCD and SCZ are multiple and complex. However, the dopamine and serotonin hypotheses have not yielded any definitive answers regarding the etiology of OCD and SCZ. Other different but equally important systems such as the immune and the (gamma-aminobutyric acid) GABAergic systems have not yet been thoroughly investigated for their roles in OCD and SCZ.

Figure 1. Schematic diagram of the cortico-striatal-thalamic cortical feedback loops (CSTC)

Legend: Glu=glutamate; Ach= acetylcholine; 5-HT=serotonin; DA=dopamine; GABA=gamma-aminobutyric acid; Ventr. Str.=ventral striatum; Ventr. Pall.=ventral pallidus; VTA=ventral tegmental area; SNc=substantia nigra pars compacta



1.2 Overview of Possible Neuroimmunological Processes in Psychiatric Disorders

1.2.1 Overview of the Major Histocompatibility Complex in the Immune System

The HLA region contains a number of closely linked genes with many contributing to the immune response, responsible for the recognition of foreign antigens and regulating the complement cascade. The function of major histocompatibility complex (MHC) molecules is to bind peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T lymphocytes. The MHC complex extends over approximately 4 cM, or about 4×10^6 bp, and contains more than 200 genes in humans (Janeway et al. 1999). The genes encoding the α chains of MHC class I molecules and the α and β chains of MHC class II molecules are linked within the complex. All the MHC class I and class II molecules are capable of presenting antigens to T cells and, because each protein binds a different range of peptides, the presence of several loci means that any one individual is equipped to present a much broader range of different peptides than if only one MHC protein of each class were expressed at the cell surface. Nucleated cells within the brain have a moderate level of MHC class I molecules expression and only the microglia within the brain, which are related to macrophages, are MHC class II-positive. Although the most important known function of the gene products of the MHC is the processing and presentation of antigens to T cells, many other genes map within this region with other roles in the immune system or with other uncharacterized functions.

1.2.2 Overview of Possible Neuroimmunological Processes in Psychiatric Disorders

Recent advances in neuroimmunology have markedly enhanced our understanding of the specific factors mediating interactions between the brain, infectious agents, and the immune system (Ader 2000). Since the first report of the existence of anti-brain antibodies in sera and cerebrospinal fluid (CSF) in psychiatric patients (Skalickova et al. 1962; Detlav 1976), immune mechanisms have been implicated in the pathogenesis of psychiatric disorders (Shapiro 1961; Fessel 1962). Several decades later, studies have demonstrated serum antibodies against human brain tissue in SCZ (Heath et al. 1989; Henneberg et al. 1994) and more specifically brain-membrane proteins in patients with SCZ (Sundin and Thelander 1989) and autism (Todd et al. 1988). Immunoglobulin levels have been reportedly increased in blood and CSF of psychiatric patients (Ahokas et al. 1985; Ramchand et al. 1994; Muller and Ackenheil 1995). More interestingly, the blood-brain barrier does not always seem to prevent autoantibodies from reaching their targets in the brain (Schwarz et al. 1998; Fetissov et al. 2002) (Whitney et al. 1999). Immune mediators are able to enter and leave the CNS under normal resting conditions (Archelos and Hartung 2000) as studies have found elevated antibody titers against unspecific antigens, such as heat shock proteins (Schwarz et al. 1998; Schwarz et al. 1999) or enolase (Deckmann et al. 2002).

Emerging research has also implicated cytokines in the pathogenesis of a variety of neuropsychiatric disorders, including SCZ (Muller et al. 2000; Gaughran 2002; Skurkovich et al. 2003; Toyooka et al. 2003), OCD (Brambilla et al. 1997; Monteleone et al. 1998), posttraumatic stress disorder (Maes et al. 1999), depression (Connor and Leonard 1998; Rothermundt et al. 2001), and dementia (Leonard 2001).

The action of cytokines has been of particular interest because these polypeptides are

known to play a central role within the brain and systemically in mediating inflammatory and immune responses (Kronfol and Remick 2000). Growing evidence suggests that in addition to providing communication between immune cells, specific cytokines play a role in signaling the brain to produce neurochemical, neuroendocrine, neuroimmune, and behavioral changes in order to maintain homeostasis and to face physical and/or psychological stress (Kronfol and Remick 2000).

Many other connections have been found between autoimmune mechanisms and psychiatric symptoms such as high rates of autoimmune thyroiditis in bipolar disorder patients (Kupka et al. 2002).

There is increasing evidence that the etiology of many neuropsychiatric disorders are autoimmune-related and are mediated by antibodies that cause dysfunction within the central nervous system (CNS). More recently other post-streptococcal clinical phenotypes have been described, notably inflammatory autoimmune encephalitis associated with dystonia and emotional lability (Dale et al. 2001). Peterson et al. (Peterson et al. 2000) have proposed that streptococcal infection might cause neuropsychiatric phenotypes, in particular, an attention-deficit hyperactivity disorder (ADHD)-like syndrome. Other reports to date have suggested group A β-hemolytic streptococcal (GABHS) infection is associated with clinical phenotypes including extrapyramidal movement (chorea, tics, dystonia) and psychiatric (OCD, ADHD, anxiety, depression) disorders. The classic example of GABHS induced neuropsychiatric disorder is Sydenham's Chorea (SC; also known as St Vitus dance).

Although evidence for the involvement of the immune system is not always consistent, a pattern emerges suggesting aspects of immune activity being involved in the pathology of neuronal development that characterizes OCD and SCZ.

1.3 Support of the Involvement of Immunological Processes in the Etiology of OCD and SCZ

1.3.1 Support for Immune Mechanisms in OCD

Cytokine abnormalities have been reported by Mittleman et al. (Mittleman et al. 1997) who suggested that there is a relative skewing of CSF level profiles toward type 1 cytokines in patients with OCD. IL-1 β and tumor necrosis factor-alpha (TNF-α) concentrations were significantly lower in OCD patients than in controls (Brambilla et al. 1997; Monteleone et al. 1998).

However, the strongest evidence of autoimmune involvement in OCD came from the secondary effects of neurotoxicity associated with streptococcal infections (Swedo et al. 1998). OCD onset can occur following damage to the basal ganglia resulting from carbon monoxide poisoning, allergic reaction, bee sting, or certain infections (Rapoport and Fiske 1998). SC, a movement disorder linked to rheumatic fever, is believed to result from an autoimmune response to GABHS infection, which affects parts of the basal ganglia. Several studies have since then explored various aspects of the autoimmune hypothesis of OCD (Leonard and Swedo 2001). Seventy percent of children develop OCD after GABHS infection, which further supports the hypothesis that an autoimmune mechanism is involved in OCD. The term, pediatric autoimmune neuropsychiatric disorder associated with GABHS infection (PANDAS), has been used to describe children who have acute onset of OC symptoms with or without tics after streptococcal infection. Swedo (Swedo 1994) has proposed a model for PANDAS cases, in which autoimmunity is triggered via a mechanism known as molecular mimicry, in which antibodies or T cells generated in the response to an

infectious agent (GABHS in the case of PANDAS) cross-react with self-antigens (Figure 2; for examples, see Table 1). Another study found that an antigen on peripheral blood mononuclear cells (D8/17) marks a genetic tendency for production of abnormal antibodies to GABHS, in the presence or even absence of SC, making it possible that OCD is a secondary effect of a faulty autoimmune response (Rapoport and Fiske 1998). Furthermore, their OC and tic symptoms respond to immunomodulatory interventions such as plasma exchange and intravenous immunoglobulin (Perlmutter et al. 1999). Long-term follow-up showed continued improvement of symptoms for most patients, especially when antibiotic prophylaxis had been effective in prevention of recurrent streptococcal infections.

A neurological disorder is recognized as autoimmune based on five experimental features: presence of autoantibody, immunoglobulin at target structure, response to plasma exchange, passive transfer of disease to animals, and disease induction with autoantigen (Archelos and Hartung 2000). For SC/PANDAS, three of five criteria have been met to date, including amelioration of symptoms when antibody is removed. Furthermore, a controlled treatment trial in patients with PANDAS demonstrated symptom remediation with plasma exchange and intravenous immunoglobulin (Perlmutter et al. 1999). Support for this immune hypothesis includes the presence of serum antibodies that bind to basal ganglia proteins found in both SC (Husby et al. 1976) and PANDAS (Kiessling et al. 1993; Singer et al. 1998). New findings demonstrate that these antibodies bind specifically to basal ganglia proteins, and are universal in acute SC and post-streptococcal dystonia (Dale et al. 2001; Church et al. 2002). Anti-basal ganglia antibodies are rarely found in children with uncomplicated streptococcal infection or in neurological controls, suggesting that this is both a sensitive and specific marker, and promises to be a useful diagnostic tool (Dale et al. 2001). However, not all

investigators have shown such specific autoimmunity; one recent report found autoantibodies against a variety of neural components without any clear common antigen binding (Morshed et al. 2001). Two recent studies with an animal model for Gilles de la Tourette's syndrome (TS), a tics disorder, have reported the induction of stereotypies in rats after intrastriatal infusions of serum from TS patients (Hallett et al. 2000; Taylor et al. 2002). However, no animal models for OCD or PANDAS have been published for studing passive transfer of disease to animal.

The putative association between immune dysfunctions and OCD needs further study to determine its specificity, its frequency, and its relation to other psychobiological factors such as genetic variables. There have been no genetic association studies of immune-related genes with OCD. Thus, it would be innovative to explore this area of research.

Figure 2. Autoimmune mechanism in neuropsychiatric disorders: OCD

Autoantibodies cross-reacted with proteins that make up the neurons, causing damages to the CNS, which eventually leads to the development of OCD (Swedo 1994).

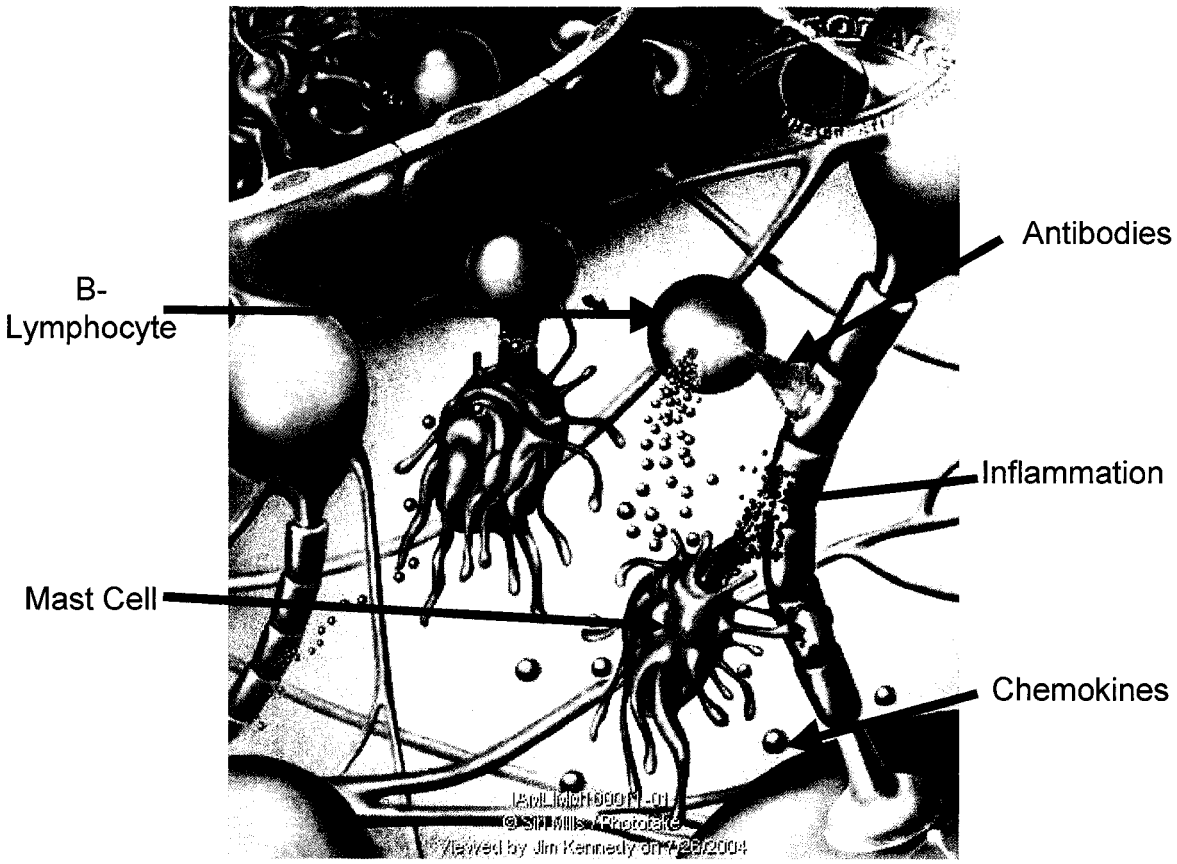


Table 1. Association of infection with autoimmune diseases

Several autoimmune diseases occur after specific infections and are presumably triggered by the infection. The case of post-streptococcal disease is best known. Most of these post-infection autoimmune diseases also show susceptibility to the MHC. Adapted from Janeway et al. (1999).

Associations of infection with immune-mediated tissue damage		
Infection	**HLA association**	**Consequence**
Group A *Streptococcus*	?	Rheumatic fever (carditis, polyarthritis)
Chlamydia trachomatis	HLA-B27	Reiter's syndrome (arthritis)
Shigella flexneri	HLA-B27	Reactive arthritis
Salmonella typhimurium		
Salmonella enteritidis		
Yersinia enterocolitica		
Campylobacteer jejuni		
Borrelia burgdorferi	HLA-DR2, DR4	Chronic arthritis in Lyme disease

1.3.2 Support for Immune Mechanisms in SCZ

Evidence has suggested that immunological abnormalities, which include lymphocyte and cytokine production, have been observed in a subgroup of SCZ. Hyper-secretion of IL-2 and IL-6 (Kaminska et al. 2001), increased level of IL-18 (Tanaka et al. 2000), decreased ratio of CD4+/CD8+, detection of antinuclear, anticytoplasmatic, antiphospholipid antibodies in SCZ patients (Schwartz and Silver 2000) are examples of the immunological findings. Mittleman et al. (Mittleman et al. 1997) reported that there is a relative preponderance of cerebrospinal fluid level profiles toward type 2 cytokines in patients with SCZ. Exposure to infective agents, human leukocyte antigen (HLA) associations, autoimmune associations, and disturbances in lymphocyte populations are supportive of this view (Gaughran 2002). Physiological and pharmacological studies demonstrated that cytokines could markedly influence synaptic transmission and plasticity upon acute or chronic application. Moreover, many of the molecular alterations observed in SCZ brain samples are consistent with abnormalities in cytokine regulation (Nawa et al. 2000). The nonspecific innate immune system shows signs of an over-activation in non-medicated SCZ patients, as indicated by increased monocytes and gamma delta-cell levels. Increased levels of interleukin-6 (IL-6) and the activation of the IL-6 system in SCZ might be the result of the activation of monocytes/macrophages. Additionally, based on the autoimmune rationale, more than 60 genetic association studies of the diagnosis of SCZ and HLA alleles have been reported (Tables 2 to 4). Results are mixed, but there are many positive studies and thus further investigation is warranted. Specifically, this thesis will examine genes in the area of linkage disequilibrium (LD) surrounding the HLA region.

Table 2. HLA association studies of schizophrenia – Class I (A, B, and C) antigens [a,b]

Investigator, year [c]	Ethnicity	Patient subjects Diagnosis (number)	Comparison subjects Origin (number)	Marker Polymorphism	Result (comment)
(Cazzullo et al. 1974)	Caucasian	Feighner (52)	Population (386)		No association
(Eberhard et al. 1975)	Caucasian	Bleuler (47)	Various (1263)	A9	A9 (RR=2.9)
(Ivanyi et al. 1976)	Caucasian	- (148)	Population (1200)	A28	A28 (RR=3.4)
(Smeraldi et al. 1976a)	Caucasian	Feighner (70)	Population (386)		No association
(Smeraldi et al. 1976b)	Caucasian	Feighner (144)	Population (386)	A10	A10 (RR=0.4)
(Julien et al. 1977)	Caucasian	- (65)	Population (250)	A9	A9 (RR=2.5)
(Ivanyi et al. 1977)	Caucasian	- (40)	Population (438)	Cw4	Cw4 with paranoid SCZ (RR=3.7)
			Population (1200)	B18	B18 with paranoid SCZ (RR=3.4)
(Bennahum et al. 1977)	Caucasian	Feighner (38)	- (102)		No association
(Kyner et al. 1978)	Caucasian	Feighner (20)	- (67)		No association
(Ivanyi et al. 1978)	Caucasian	- (200)	Population (1200)	A28	A28 (RR=3.0)
(McGuffin et al. 1978)	Caucasian	ICD-9 (80)	Blood donors (458)		No association
(Perris et al. 1979)	Caucasian	- (50)	Blood donors (449)		No association
(Crowe et al. 1979)	Caucasian	Feighner (45)	Population (1263)	HLA-Aw10	Aw10 (A26 subtype) with hebephrenia (RR=6.6)
(Luchins et al. 1980)	Caucasian	RDC (38)	Published data (743)		No association
	African-USA	RDC (92)	Published data (563)	A2	A2 (RR=2.3)
(Gattaz and Beckmann 1980)	Caucasian	Feighner (100)	- (472)	B27	B27 with poor prognosis patient
(Mendlewicz et al. 1981)	Caucasian	Feigher (64)	Blood donors (113)		No association
(Asaka et al. 1981)	Japanese	- (136)	Blood donors (187)	A9	A9 (Aw24 subtype) (RR=2.0)
				A10	A10 (A26 subtype) (RR=1.9)
(Goudemand et al. 1981)	Caucasian	- (51)	Blood donors (94)		No association
(Singer et al. 1982)	Caucasian	- (75)	Blood donors (184)		No association
(Ivanyi et al. 1983)	Caucasian	Feighner (62)	- (1018)		No association
(Rosler et al. 1983)	Caucasian	Feighner (107)	Blood donors (600)	A28	A28 (RR=3.1)
(Miyanaga et al. 1984)	Japanese	DSM-III (77)	Blood donors (1252)		No association
(Rudduck et al. 1984a)	Caucasian	DSM-III (100)	Blood donors (919)		No association

(Rudduck et al. 1984b)	Caucasian	DSM-III (116)	Blood donors (919)		No association
(Adler et al. 1985)	Caucasian	RDC (37)	Various (246)	A2	A2 (schizoaffective<<SCZ/controls)
(Amar et al. 1988)	Jewish	- (32)	Various (151)	HLA-A, B, C	A2 (P=0.01) and A23 (P=0.02)
(Metzer et al. 1988a)	Caucasian	DSM-III (53)	Blood donors as control (114)	HLA-A1	No association
(Alexander et al. 1990)	Caucasian	DSM-III (55)	Published data (1029)	HLA-A (17 antigens)	No association
				HLA-B (20 antigens)	No association
(Brown and White 1991)	Caucasian	DSM-III (20)	Controls (20)	HLA-A (12 antigens)	No association
				HLA-B (11 antigens)	No association
				HLA-C (7 antigens)	No association
(Campion et al. 1992)	Caucasian	DSM-III (33 families)	Families (33)	HLA-A9	No association (haplotype relative risk)
(Muller et al. 1993)	Caucasian	ICD-9 (86)	Population controls (133)	HLA-A (10 antigens)	No association
				HLA-B (18 antigens)	No association
				HLA-C (7 antigens)	No association
(Wright et al. 1995)	Caucasian	DSM-III-R (93)	Screened controls (141)	HLA-A9, A23, A24	A9 (P=0.02, RR=1.94)
					A24 (P=0.001, RR=2.78)
(Blackwood et al. 1996)	Caucasian	RDC & DSM-III-R (107)	Population controls (133)	HLA-A, B, C	B35 (corrected P=0.004, RR=0.06)
					Cw5 (corrected P=0.05, RR=0.38)
			Blood donors as control (264)		B35
(Ozcan et al. 1996)	Caucasian	DSM-III-R (110)	Population controls (3731)	HLA-A (10 antigens)	A2,A11,A29 (P<0.05, RR=N/A,2,2.48 for SCZ+paranoid)
				HLA-B (16 antigens)	A10,B14,B17,Cw1 (P<0.01, RR=2.38,3.18,3.6,3.07 for SCZ+paranoid)
				HLA-C (4 antigens)	
(Jacobsen et al. 1998)	Caucasian	DSM-IV (28 children)	Population controls (51)	HLA-A (7	No association

				antigens)	
				HLA-B (10 antigens)	No association
(Gibson et al. 1999)*	Caucasian	DSM-III-R (256)	Blood donors (261)	HLA-A, B, C	No association
		DSM-III-R (358)	Population controls (434)	HLA-A (5 antigens)	No association
(Hawi et al. 1999)*	Irish	DSM-III-R (256)	Blood donors (261)	HLA-A	No association
(Matsumoto et al. 2002)*	Japanese	DSM-III-R & ICD-10 (98)	Population controls (392)	HLA-A (9 antigens)	No association
				HLA-B (26 antigens)	No association
				HLA-C (10 antigens)	No association
(Tochigi et al. 2002)*	Japanese	DSM-IV (110)	Bone marrow donors as control (493)	HLA-A (10 antigens)	No association
(Laumbacher et al. 2003)*	Caucasian	DSM-III-R (31+71)	Population controls (367+390)	HLA-A (20 antigens)	A10 (P=0.006, RR 3.071) (with 71 subjects)
				HLA-C (8 antigens)	No association

Total studies	*Total patients per ethnic group*	*Total controls per group* [d]	*Associations reported more than once*
36 serotyping studies	Caucasian 3413 (including 28 children)	12340 population	5 studies: A10
		6075 blood donors	4 studies: A2 or A9
5 genotyping study	Japanese 421	2335 published data	3 studies: A28
	African-USA 92	1679 unknown	2 studies: B35
	Jewish 32	1660 various	1 study: A11, A24, A29, B14, B17, B18, B27, Cw1, Cw4, Cw5
		493 bone marrow donors	
		174 relatives	
		141 screened controls	
		33 families	

[a] Table based on data from (Nimgaonkar et al. 1992), (Hawi et al. 1999), Index Medicus, MEDLINE and EMBASE searches from 1974 to 2004.
[b] RR=relative risk when significant association remains after correction for multiple comparisons; no association=no association remains after correction for multiple comparisons; *n*=number of SCZ patients or number of controls; diagnostic criteria utilized in the above studies are those of (Feighner et al. 1972), the International Classification of Diseases 9 (WHO, 1978), the Diagnostic and Statistical Manual III, III-R and IV (APA 1994) and the Research Diagnostic Criteria of (Spitzer et al. 1978).
[c] Earlier studies utilized HLA serotyping, while more recent studies indicated by * utilized HLA genotyping.
[d] Total controls per group is not equal to total number of controls, because the same control groups were used by some investigators.

Table 3. HLA association studies of schizophrenia – Class II (DP, DQ, and DR) antigens and alleles [a,b]

Investigator, year [c]	Ethnicity	Patient subjects Diagnosis (number)	Comparison subjects Origin (number)	Marker Polymorphism	Result (comment)
(Perris et al. 1979)	Caucasian	- (50)	Blood donors (449)		No association
(Miyanaga et al. 1984)	Japanese	DSM-III (77)	Blood donors (1252)	DRw8	DRw8 (RR=5.8)
(Rudduck et al. 1984a)	Caucasian	DSM-III (100)	Blood donors (919)		No association
(Rudduck et al. 1984b)	Caucasian	DSM-III (116)	Blood donors (919)		No association
(Rabin et al. 1987)	Caucasian	RDC (31)	Published data (263)		No association
	African-USA	RDC (35)	Published data (1926)	DRw6	DRw6 (RR=3.6)
(Amar et al. 1988)	Jewish	- (32)	Various (151)	DR (10 antigens)	No association
(Metzer et al. 1988b)	Caucasian	DSM-III (53)	Blood donors as control (114)		No association
(Muller et al. 1992)	Caucasian	ICD-9 (31)	Published data (1926)		DRw6 (RR=0.0)
(Nimgaonkar et al. 1993)*	African-USA	DSM-III (26)	Screened controls (28)	DQB1 (11 antigens)	DQB1*0602 (P<0.04, RR=0.12)
	Caucasian	DSM-III (32)	Screened controls (44)		No association
(Sasaki et al. 1994)*	Japanese	DSM-III-R (44)	Screened controls (51)	DR (12 antigens)	No association
(Zamani et al. 1994)*	Caucasian	DSM-III-R (100)	Blood donors as control (204)	DRB and DPB1	DPB1*0101 (P=0.018, RR=0.27)
(Nimgaonkar et al. 1995)*	Chinese	ICD-10 (102)	Screened controls (111)	DQB1 (13 antigens)	DQB1*0602 (males only, P<0.02)
(Ozcan et al. 1996)	Caucasian	DSM-III-R (110)	Population controls (3731)	DR (9 antigens) and DQ	DQB1*0303 (P<0.005) DRw52 (P<0.01 for SCZ+paranoid, mania+depression) DQw1 (P<0.01 for SCZ+paranoid, mania+depression
(Wright et al. 1996)	Caucasian	DSM-III-R (94)	Healthy controls (177)	DRB1*04, DRB1*0602	DRB1*0401 to 0411 (P=0.004, OR=0.46)
		Unrelated mothers (92)	Healthy controls (177)	DRB1*04, DRB1*0602	DRB1*0401 to 0411 (P=0.002, OR=0.42)
(Nimgaonkar et al. 1997)*	African-USA	DSM-III-R/DSM-IV (75)	Screened controls (66)	DQB1*0602	DQB1*0602 (female only, P<0.02)
(Jonsson et al. 1998)*	Swedish	DSM-III-R (124)	Population controls (85)	DQB1 (14 antigens)	No association
(Grosskopf et al. 1998)	German-Causian	DSM-III-R (32)	Population controls (202)	DQB1 (3 antigens)	No association
	Caucasian	DSM-III-R (64)	Population controls (646)	DQB1*0602	DQB1*0602 (corrected P=0.028)

(Jacobsen et al. 1998)*	Caucasian	DSM-IV (28 children)	Population controls (51)	DR (11 antigens)	No association
				DQ1 and DQ6	No association
(Wright et al. 1998)*	Caucasian	RDC (23 pedigrees)	Heterozygous parents (23 pedigrees)	DRB1 (12 antigens)	No association
				DQA1 (6 antigens)	No association
				DQB1 (5 antigens)	No association
(Arinami et al. 1998)*	Japanese	DSM-III-R (266)	Various (281)	DR1 and DR4	DRB1*01 (P=0.04, OR=1.87)
					DRB1*04 (P=0.02, OR=0.63)
(Sasaki et al. 1999)*	Japanese	DSM-IV (233)	Population controls (493)	DR1 and DR4	DRB1*0101 (P=0.04)
(Gibson et al. 1999)*	Irish	DSM-III-R (256)	Blood donors (261)	DRB, DQB, DPB	No association
		DSM-III-R (358)	Population controls (434)	DR4	No association
				DQB	No association
(Hawi et al. 1999)*	Irish	DSM-III-R (256)	Blood donors (261)	DR, DO, and DP	No association
(Narita et al. 2000)*	Japanese	DSM-IV (367)	Season of birth	DR1	Feb. and Mar. without DR1 (P=0.003)
(Haider et al. 2000)*	Kuwaiti Arabs	ICD-10 (80)	Population controls (114)	DRB1 (12 antigens)	DRB1*04 (P=0.028)
					DRB1*13 (P=0.015)
(Akaho et al. 2000)*	Japanese	DSM-IV (45)	Population controls (117)	DR1 and DR4	DR1 (P=0.03)
(Li et al. 2001)*	Han Chinese	DSM-III-R or DSM-IV (165)	Parents (330)	DRB1 and DQA1	DRB1*03 (P=0.009)
				DQB1 and DPB1	DRB1*13 (P=0.041)
					DRB1*13-DQA1*01 (P=0.012)
					DRB1*03-DQA1*05 (P=0.002)
(Chowdari et al. 2001)*	Chinese	ICD-10 & DSM-IV (271)	Population controls (229)	DQB1 and DQCAR2	No association
				DQCAR and G5-1152	No association
				G4-12348 and G6-7571	No association
(Schwab et al. 2002)*	Caucasian	DSM-III-R (69)	Siblings (69)	DQA1 and DQB1	DQB CAR (LOD P=0.0004)
					DRB1*11 (P=0.03)
					DQB1*301-DQA1*501-DRB1*11 (P=0.043)
		DSM-III-R (89)	Parents (178)		No association

(Yu et al. 2003)*	Chinese Han - (116)	Parents (232)	DRB1 and DRB3	DRB1 (rs707954) with idea of reference (P=0.019) DRB1 (rs707954) with apathy (P=0.017)

Total studies	*Total patients per ethnic group* [d]	*Total controls per group* [d]	*Associations reported more than once*
19 genotyping studies	Caucasian 2239 (including 28 children)	6235 population	4 studies: DQB1*0602
13 serotyping studies	Japanese 1032	4643 blood donors	3 studies: DRB1*04
	Chinese 654	4115 published data	2 studies: DR1, DRw6, DRB1*01. DRB1*13
	African-USA 136	854 unknown	1 study: DRw8, DRw52, DRB1*03-5, DRB1*11, DPB1*0101, DQw1, DQB1*303, DQB-CAR, DQB1*301-DQA1*501-DRB1*11, DRB1 (rs707954)
	Jewish 32	740 parents	
		432 various	
		300 screened controls	
		69 siblings	
		23 pedigrees	

[a] Table based on data from (Nimgaonkar et al. 1992), (Hawi et al. 1999), Index Medicus, MEDLINE and EMBASE searches from 1974 to 2004.
[b] RR=relative risk when significant association remains after correction for multiple comparisons; no association=no association remains after correction for multiple comparisons; n=number of SCZ patients or number of controls; diagnostic criteria utilized in the above studies are those of (Feighner et al. 1972), the International Classification of Diseases 9 (WHO, 1978), the Diagnostic and Statistical Manual III, III-R and IV (APA 1994) and the Research Diagnostic Criteria of (Spitzer et al. 1978).
[c] Earlier studies utilized HLA serotyping, while more recent studies indicated by * utilized HLA genotyping.
[d] Total controls per group is not equal to total number of controls, because the same control groups were used by some investigators.

Table 4. HLA association studies of schizophrenia – Class III

Investigator, year	Ethnicity	Patient subjects Diagnosis (number)	Comparison subjects Origin (number)	Marker Polymorphism	Result (comment)
(Wang et al. 1992)	Asian	- (60)	Controls (60)	HLA-III (C2, BF, and C4)	BF*F, BF*SO7, C4A*4 (RR=1.81,7.79,4.18 respectively)

1.4 Genetics of OCD and SCZ

Though there is currently no single, proven cause of OCD, many hypotheses in the last several years have been advanced to explain the pathophysiology of this condition. Early researchers have long known that OCD had a strong hereditary component (Janet 1903). Parents with OCD have an elevated risk of transmitting the disorder to their offspring. An early study for example showed that patients with OCD had increased rates of first-degree relatives with OCD (Riddle et al. 1990). The general nature of OCD but not the specific symptoms is usually passed on. This means that a mother with cleaning compulsions is just as likely to have children with different symptoms like checking or counting compulsions. Studies have also shown that the concordance rate of OCD is higher for monozygotic (identical) twins (80-87%) than it is for dizygotic (non-identical) twins (47-50%) (Carey and Gottesman 2000). The prevalence of OCD is increased among the first-degree relatives of patients with OCD when compared with the relatives of control subjects; additionally, the age at onset of the proband is inversely related to the risk of OCD among the relatives (Alsobrook and Pauls 1998; Nestadt et al. 2000). A recent meta-analysis combining five family studies by Hettema et al. (Hettema et al. 2001) calculated the Mantel-Haenszel summary odds ratio to be 4.0 (95% CI = 2.2-7.1). Jonnal et al. (Jonnal et al. 2000) performed a twin study and estimated heritabilities for the variance of compulsions and obsessions to be 33.4% (95% CI: 24.1-42.1%) and 26.2% (95% CI: 16.6-35.3%).

Family, twins, and adoption studies have also supported a strong genetic role for the etiology of SCZ (Bray and Owen 2001). The highest lifetime morbid risks for developing SCZ are found in the offspring of two schizophrenic parents and the co-twins of affected monozygotic twins. These risks are reported to be 46% and 48% respectively, and decline to

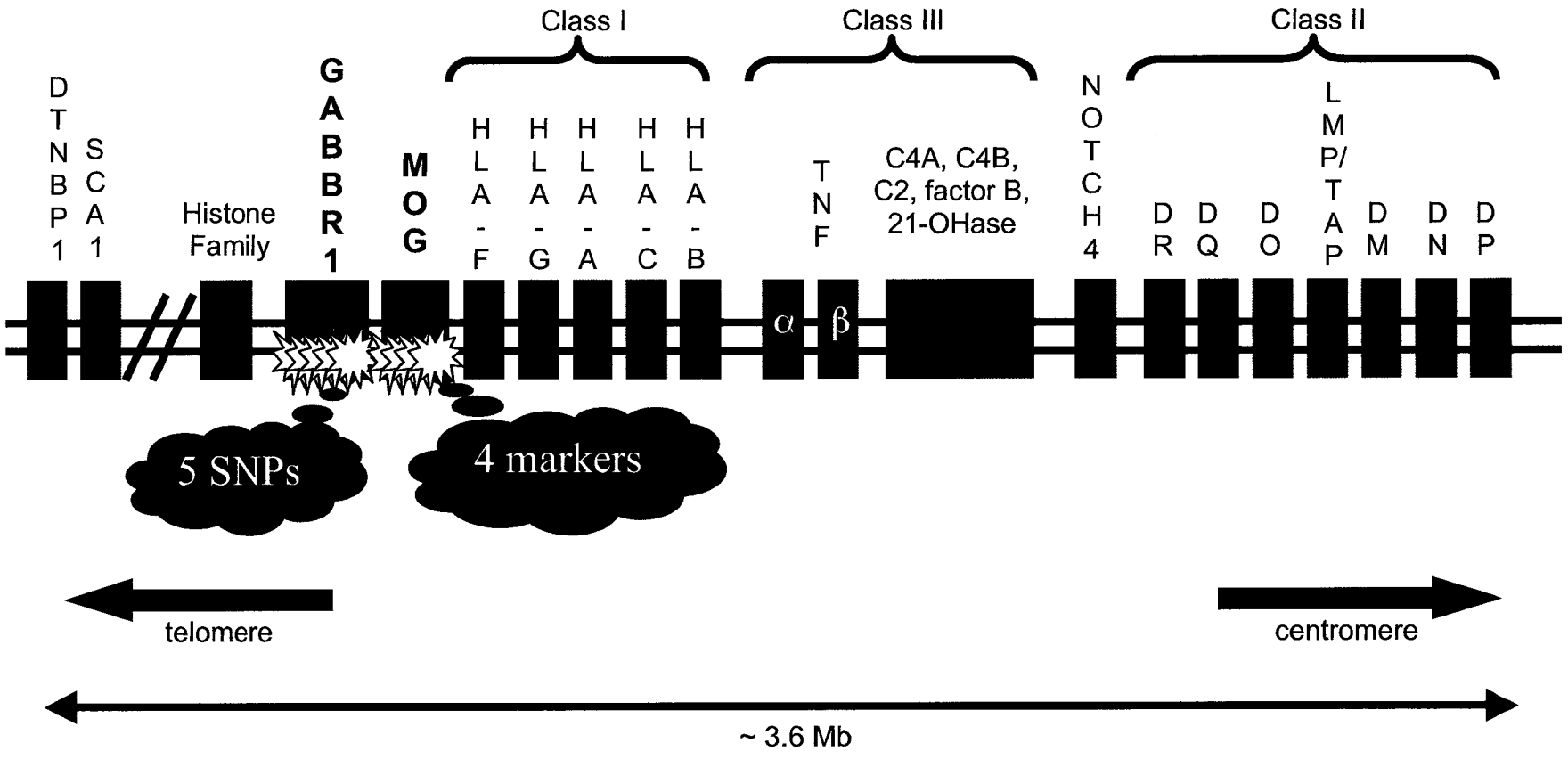
17% in dizygotic twins and to a rate of 2% for third-degree relatives (e.g., cousins) (McGuffin et al. 1994). A combined analysis of adoption data showed that the prevalence of chronic SCZ was significantly greater in biological relatives of adoptees with chronic SCZ than in the biological relatives of control adoptees (Kendler et al. 1994; Kety et al. 1994). Model fitting using twin data from recent studies yielded a heritability of 73% to 90% (Sullivan et al. 2003). Therefore, based on the evidence for strong genetic involvement in SCZ, researchers have examined the causes of SCZ via genetics methodologies and many studies have supported the involvement of neurotransmitter and neuro-immune systems in the development of SCZ.

Since genetics partly determines the probability of an individual in developing either OCD or SCZ or both, we have carried out molecular genetic approaches to identify the genes of interest related to immune response, which are located closely to the HLA region on chromosome 6p. This is also partly because, in genome scan studies, suggestive linkage has been found on chromosome 6p for both OCD (Hanna et al. 2002) and SCZ (Antonarakis et al. 1995; Straub et al. 1995; Wang et al. 1995; Lewis et al. 2003).

1.5 Hypothesis of the Present Study

With the observations of white matter abnormality, the detection of anti-neuronal antibodies (Miller et al. 1988), and the evidence of cross-reactive epitope of streptococcal protein with human brain, genetic susceptibility in PANDAS/OCD and SCZ cases may be a function of an exaggerated immune response from an immunological defect leading to aberrant discrimination between host and foreign antigens, and/or the presence of neuronal cell components with epitopes closely resembling those of the GABHS bacteria. We therefore hypothesized that the pathoetiology in at least some cases of OCD and SCZ results from an exaggerated immune response, and this abnormal response will be associated with genetic markers in the HLA region. Thus, we undertook to examine markers either linked to the genetic determinants of immune response or in LD with those associated with immune response in the MHC region on the short arm of chromosome 6. In particular, we have examined the myelin oligodendrocyte glycoprotein (MOG) gene and the gamma-aminobutyric acid type B receptor 1 (GABBR1) gene, which are 60 Kb (Pham-Dinh et al. 1993) and 130 Kb (Goei et al. 1998; Peters et al. 1998) telomeric from the HLA-F locus respectively (Figure 3).

Figure 3. Simplified human genetic map of chromosome 6p21.3 region (MHC region) and genes within the region



1.6 Myelin Oligodendrocyte Glycoprotein (MOG) Gene

The human MOG gene (NM_002433) has been mapped to chromosomal region of 6p21.3-p22 by in situ hybridization (Pham-Dinh et al. 1993), more specifically at position 29,732,749 to 29,748,087 on chromsome 6 (UCSC July 2003), just distal to the HLA region as mentioned above (Figure 3). The primary nuclear transcript of the human MOG gene from the putative transcription start site to poly(A) site is 15,561 nucleotides in length (see Figure 4) (Roth et al. 1995). The 17 Kb gene is composed of nine exons and eight introns (Pham-Dinh et al. 1995) that display the canonical splice junction sequences and produce six different transcripts through alternative splicing.

MOG is a quantitatively minor component of myelin, expressed preferentially in oligodendrocytes at the extracellular surface of the myelin sheath in the CNS (Figure 5). MOG, which is an intrinsic membrane molecule with two transmembrane domains, contains an immunoglobulin-like variable domain characteristic of members of the immunoglobulin super-family and may be a target for immune-mediated demyelination (Sun et al. 1991; Kerlero de Rosbo et al. 1997); thus, MOG has been implicated in autoimmune demyelinating diseases such as multiple sclerosis (MS). It has been observed that MOG activates C1q, an enzyme that is essential in the activation of the complement cascade *in vitro* (Johns and Bernard 1997; Johns and Bernard 1999) (Figure 6). The external location of MOG in the myelin sheath, its oligodendrocyte specificity, and its possible immune involvement, make this molecule an attractive candidate as a modifier factor in autoimmune diseases in the CNS.

There have been inconsistent findings in linkage studies of MOG polymorphisms with MS (Brown et al. 1998; He et al. 1998). Significant LD has been found for ADHD with the MOG-(CA)n polymorphism together with the Val-145-Ile polymorphism of the MOG

gene (Barr et al. 2001). TDT was also performed in the latter study in family trios with ADHD, but no biased transmission of the alleles of either of these polymorphisms was observed in ADHD probands or siblings (Barr et al. 2001). In MS, specific HLA alleles have been associated with presence of MOG autoantigens (Lutterotti et al. 2002). Further, because of the proximity of the MOG gene to the HLA class I region and evidence for LD extending from markers in the MOG gene to markers in the TNF-α gene (Barr CL, unpublished) located proximal to the class I region, we were able to perform a preliminary test of association of the HLA class I region. Since the MOG and HLA loci are close to each other on chromosome 6p, positive linkage or association with MOG in human diseases could, in fact, reflect genetic risk at the HLA locus rather than MOG itself, and *vice versa.*

Jenike et al. (Jenike et al. 1996) have shown that patients with OCD have significantly less total white matter, greater total neocortex, and opercular volumes when compared with normal controls. Moreover, OCD subjects have been demonstrated in some magnetic resonance imaging (MRI) studies to have a greater relative proportion of water in the white matter within frontal regions (Garber et al. 1989; Jenike et al. 1996).

Dysfunction of neuronal connectivity in SCZ patients, such as factors that affect myelination has also been observed (Davis et al. 2003; Hof et al. 2003). MRI has repeatedly found volume reductions in the white matter of the prefrontal cortex in schizophrenic patients (Breier et al. 1992; Buchanan et al. 1998; Sanfilipo et al. 2000; Sigmundsson et al. 2001) and these reductions have been associated with negative symptoms (Sanfilipo et al. 2000; Sigmundsson et al. 2001). Additionally, a recent study has found a 28% decrease in total numbers (or densities) of cortical layer III oligodendrocytes and a 27% decrease in the white matter in SCZ patients when compared with controls (Hof et al. 2003). Thus, an autoimmune

reaction might have been activated against the myelin sheath of neurons, causing alterations in neurotransmission.

The proposed function of MOG as a complement cascade activator and the increasing evidence of autoimmune pathological mechanism involved in OCD and SCZ suggest that the MOG gene may be a candidate gene for both OCD and SCZ. Thus, the primary aim of our study was to test for the presence of an association between markers within the MOG gene and OCD/SCZ. We hypothesized that anti-MOG antibodies may contribute to the etiology of OCD, via demyelination in the frontal lobe, or basal ganglia, or other neuroanatomic regions in the CNS, or by ectopic activation of the complement cascade leading to loss of self-tolerance.

In the research, we have examined various markers linked to the allelic determinants of immune response. To assess the contribution of the HLA region variants to OCD and SCZ, four markers located within the MOG gene were used in this study, a polymorphic dinucleotide repeat (CA)n located downstream from the MOG 3' untranslated region (3'-UTR), MOG-(CA)n (Genebank Accession #: AL050328 Region: 40030..40069), a putatively functional tetranucleotide repeat (TAAA)n located in the 3'-UTR, MOG-(TAAA)n (Malfroy et al. 1995), a single nucleotide polymorphism (SNP) in intron 1 at position 1334 from the transcription start site, C1334T (rs2252711), and a SNP in intron 5 at position 10991, C10991T (rs2071653) (see Figure 4). Since the MOG-(TAAA)n microsatellite is located within the 3'-UTR, we proposed that the length of the repeats might affect mRNA stability. None of the other polymorphisms have definite functional significance in the MOG protein.

Figure 4. Structural organization of the human MOG gene

The human MOG gene contains 8 exons, separated by 7 introns (later shown to be 9 exons and 8 introns (Pham-Dinh et al., 1995).

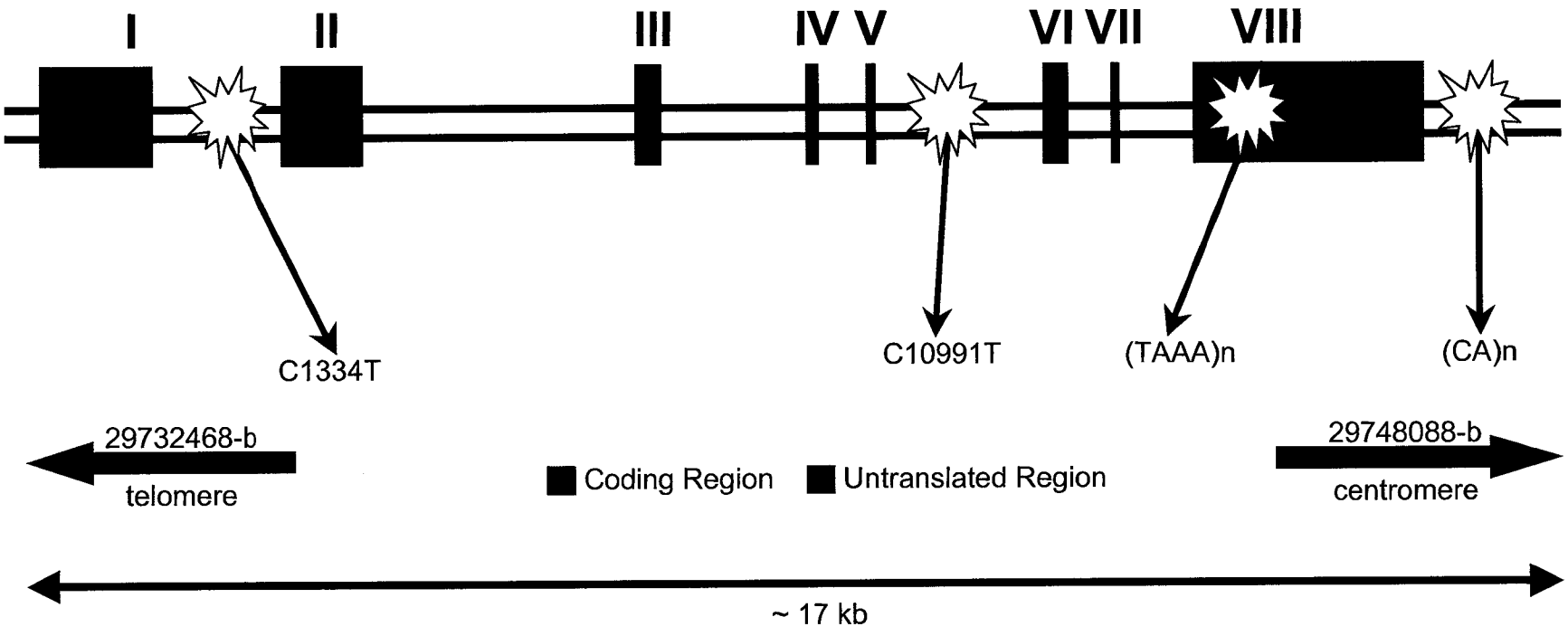


Figure 5. General morphology of a myelinated neuron

Myelin sheath, which acts as an insulator to increase neural transmission, includes MOG.

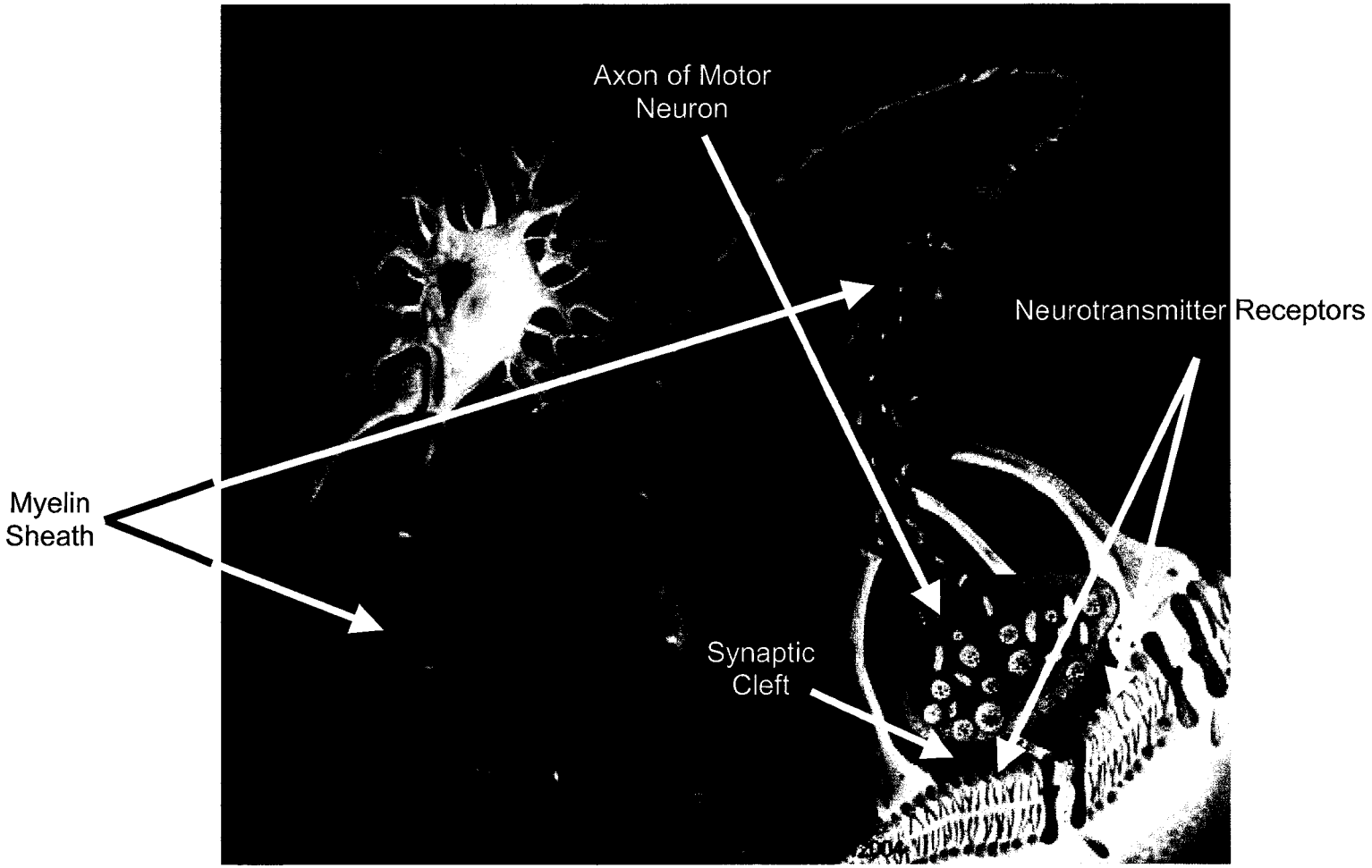
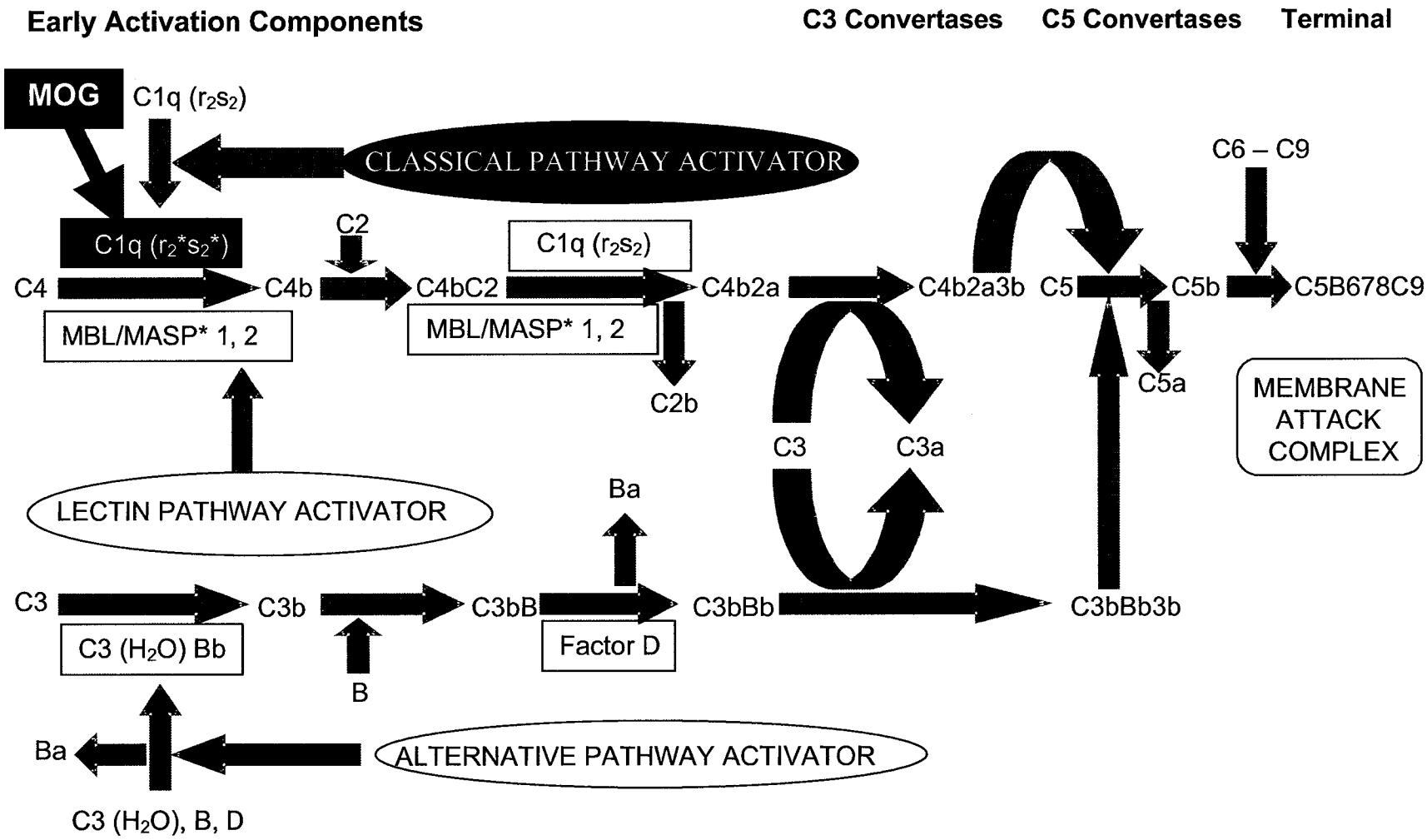


Figure 6. Schematic diagram of the three pathways of complement activation



1.7 Gamma-aminobutyric Acid type B Receptor 1 (GABBR1) Gene

Neurotransmitters in the brain not only mediate fast neuronal signalling, they can act as neurotoxic or neuroprotective substances. The main excitatory neurotransmitter glutamate is well studied for its action as a neurotoxic substance (Choi 1990; Choi and Rothman 1990) while the inhibitory neurotransmitter GABA is reported to be neuroprotective: In a model of white matter anoxia, GABA has been proposed to attenuate neuronal dysfunction acting via $GABA_B$ receptors and Na^+/Ca^{2+} exchange (Fern et al. 1995). Extracellular GABA levels increase during injury. These elevated levels can even be detected in patients with cerebral ischemia, head injury, or a subarachnoid hemorrhage (Hutchinson et al. 2002).

Studies of GABA physiology and pharmacology in the past forty years provide a context for understanding its potential roles. Both $GABA_A$ and $GABA_B$ receptors are clinical therapeutic targets. Evidence supporting GABAergic involvement in OCD comes from observations of the clinical utility of gabapentin, a synthetic GABA analog. Baclofen, a $GABA_B$ receptor agonist, is used to treat spasticity. Moreover, possible clinical application of $GABA_B$ agonists/antagonists includes treatment for spasticity (Bowery 1993), epilepsy (Caddick and Hosford 1996), drug withdrawal (Shoaib et al. 1998; Capasso 1999), anxiety, depression, and cognitive dysfunction (Kerr and Ong 1995; Getova et al. 1997). $GABA_B$ receptor antagonists improve cognitive processes and memory in animal models as measured by T-maze or forced swim test (Mondadori et al. 1993). The $GABA_B$ receptor-mediated neurotransmission has been implicated in the pathophysiology of several neuropsychiatric disorders including anxiety, depression, alcohol dependence, and cognitive deficits (Bittiger et al. 1993; Kerr and Ong 1995).

In addition to OCD, an abnormality in GABAergic regulation of dopamine cell burst

firing has been postulated to underlie the symptoms of SCZ (Grace and Stiers 1991; Moore et al. 1999). Effects of the GABAergic system in neuro- and cortico-developmental processes have been integrated into developmental hypotheses for psychosis and SCZ. Disarray of cortical neurons suggests neurodevelopmental problems that eventually lead to the development of SCZ.

The $GABA_B$ receptor consists of a metabotrophic receptor, which functions as heterodimer of two related 7-transmembrane receptors, $GABA_B$ receptor subunit 1 (GABBR1) (NM_001470) and $GABA_B$ Receptor subunit 2 (GABBR2) or G protein-coupled receptor 51 (GPR51) (Figure 7). Schuler et al. (Schuler et al. 2001) showed that GABBR1 subunit knockout mice exhibited spontaneous seizures, hyperalgesia, hyperlocomotor activity, and memory impairment and concluded that GABBR1 is an essential component of pre- and postsynaptic $GABA_B$ receptors.

From the above mentioned studies, it is clear that the $GABA_B$ receptor has a known role in synaptic inhibition. Moreover, the $GABA_B$ receptor is expressed in almost all areas in the brain where abnormalities have been observed in OCD and SCZ patients (GNF 2002). The expression of the $GABA_B$ receptor is reduced in the entorhinal cortex and the inferior temporal cortex of the schizophrenic brain, raising the possibility that $GABA_B$ receptor dysfunction is involved in the pathophysiology of SCZ (Mizukami et al. 2002). Furthermore, some OCD and SCZ patients are treated with GABA-regulating drugs (e.g. gabapentin, baclofen) to relieve their symptoms. Most importantly, the gene [position 29,677,897-29,708,753 (UCSC July 2003)] is localized in the proposed candidate susceptibility region on 6p21.3. Therefore, the GABBR1 gene is considered as an attractive candidate gene for neurobehavioural disorders in pedigrees linked to the HLA region. The aim of our study is

thus to investigate for the presence of an association between markers in the GABBR1 gene and OCD/SCZ.

Five polymorphisms located within the GABBR1 gene were used in this study, all of them being SNPs: A-7265G – an A → G base exchange at position -7265 in the promoter region from the transcription start site (rs29220), C10497G – a C → G base exchange at position 10497 in intron 9 (rs29220), Ser-491-Ser – an A → G substitution at exon 12 (rs29225), Phe-659-Phe – an A → G substitution at exon 16 (rs29230), and A33795G – an A → G base exchange at position 33795 in the 3'-UTR (rs3095273) (Figure 8). Synonymous SNPs might not have a significant impact on the GABBR1 protein but the SNP located at the promoter region could affect the transcription of the GABBR1 protein, whereas the SNP located in the 3'-UTR might influence mRNA stability.

Support for examining these two genes, MOG and GABBR1 in psychiatric phenotypes also come from other association studies in related disorders (Table 5). Table 5 shows that these genes can indeed be associated with neuropsychiatric diseases, thus providing additional support for their examination in OCD and SCZ.

Figure 7. $GABA_B$ receptor heterodimer activation and at rest

Agonist binding causes a conformational change in the GABBR heterodimer resulting in receptor coupling to effector systems.

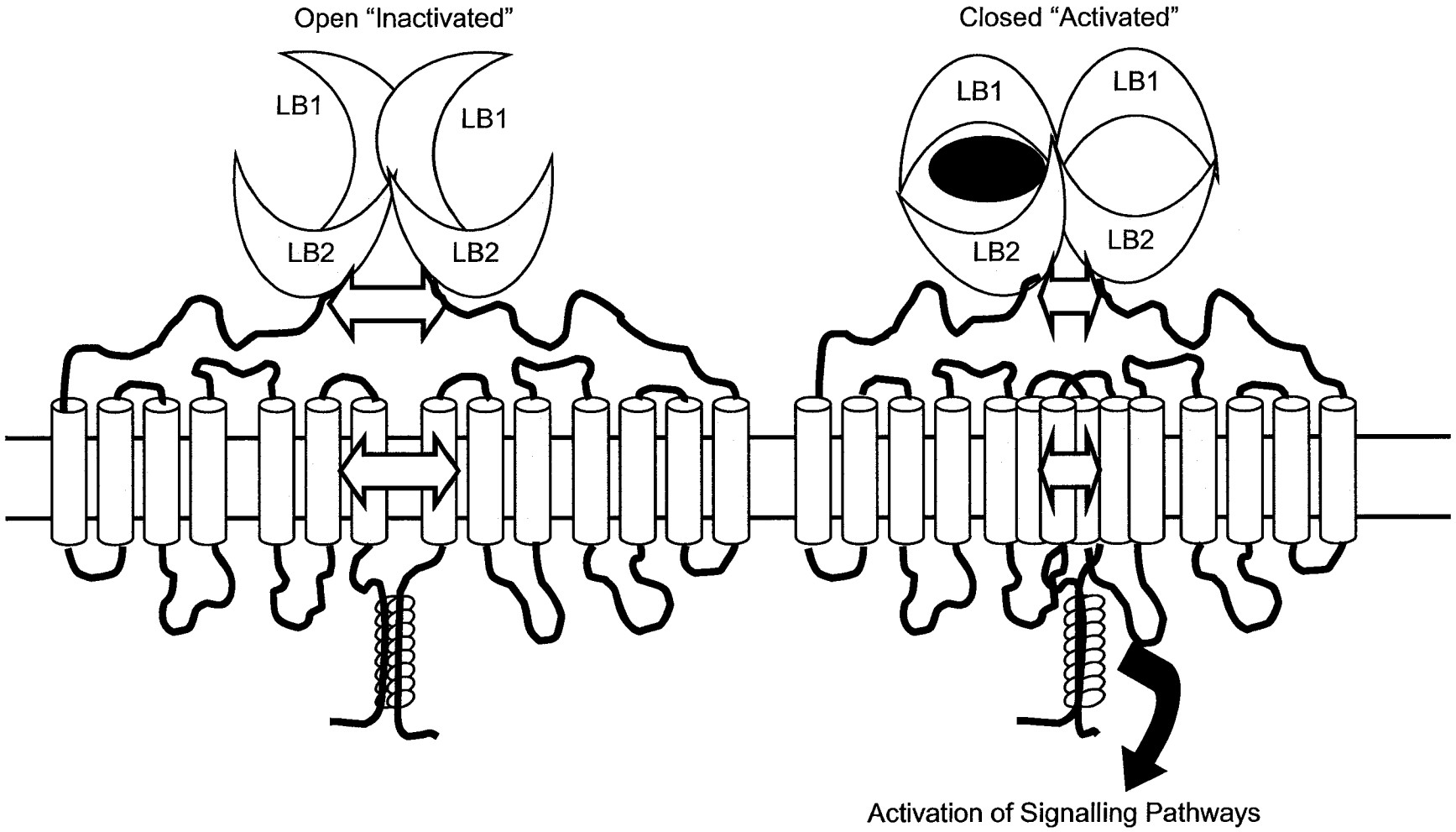


Figure 8. Structural organization of the human GABBR1 gene

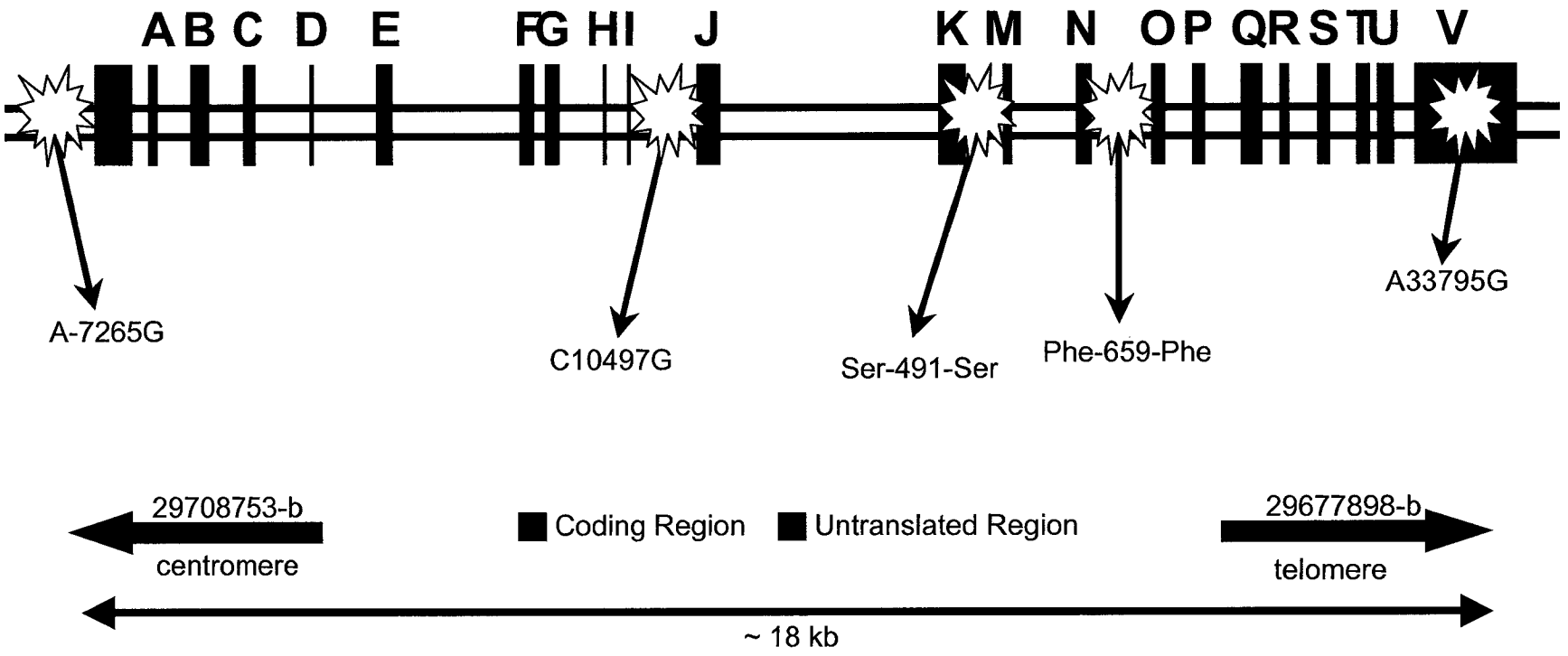


Table 5. Association studies of the myelin oligodendrocyte glycoprotein gene and $GABA_B$ receptor 1 gene in neuropsychiatric, neurological, autoimmune disorders

Disease/Gene	Results	Reference
MOG		
Autism	anti-MOG antibody	(Vojdani et al. 2002)
Attention-deficit hyperactivity disorder (ADHD)	no association	(Barr et al. 2001)
Gilles de la Tourette's syndrome (TS)	$\chi^2 = 4.000$, $P = 0.046$	(Huang et al. 2004)
Reading disabilities (RD)	LD between D6S109 and D6S1260 ($P<0.04$)	(Turic et al. 2003)
GABBR1		
ADHD	no association	(Barr et al. 2000b)
Childhood absence epilepsy (CAE)	no association	(Lu et al. 2003)
Juvenile myoclonic epilepsy (JME)	z-score = 3.17 at HLA-DQ	(Peters et al. 1998)

1.8 Overview Summary of Statistical Analyses

We genotyped a sample of 159 small nuclear families with an OCD proband and 101 small nuclear families with a SCZ proband, and examined alleles of these five polymorphisms for evidence of biased transmission of alleles and haplotypes in order to determine whether variants of the MOG and GABBR1 genes are associated with OCD and/or SCZ using the transmission disequilibrium test (TDT-STDT program version 1.1) (Spielman and Ewens 1998) and the TRANSMIT program (version 2.5) (Clayton and Jones 1999) or the TDTPHASE program (version 2.40) (Dudbridge 2003) to perform migrating window haplotypes, quantitative analysis with the family-based association test (FBAT, version 1.0) (Laird et al. 2000) or Kruskal-Wallis test, and finally LD across the nine markers with the Haploview program (version 2.04, http://www.broad.mit.edu/personal/jcbarret/haploview/) to determine haplotype blocks. We also genotyped a sample of 182 SCZ case-control pairs matched by age, gender, and ethnicity, and examined the distribution of alleles and genotypes with Pearson χ^2 test using SPSS version 10.0 (SPSS Inc., Chicago, IL) and Monte Carlo test using the CLUMP program (Sham and Curtis 1995). Power for the case-control samples was determined with the Genetic Power Calculator (Purcell et al. 2003). The TDT Power Calculator (version 1.2.1) (Chen and Deng 2001) and the PBAT program (Lange et al. 2002) were used to determine the power for the TDT and FBAT samples respectively.

CHAPTER 2: Myelin Oligodendrocyte Glycoprotein (MOG) Gene Associated with Obsessive-Compulsive Disorder

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2.1 Introduction

Obsessive-compulsive disorder (OCD) is a severe psychiatric condition characterized by intrusive, unwanted thoughts (obsessions), and the need to perform repetitive acts or rituals (compulsions) in order to alleviate anxiety (American Psychiatric Association 1994). The lifetime prevalence in the general population has been estimated at 2 to 3% (Sasson et al. 1997). There is broad support for involvement of genetic factors in OCD, primarily based on family and twin studies (Hettema et al. 2001).

Evidence for pathophysiological abnormalities in OCD patients comes from neuroimaging studies implicating the orbitofrontal cortex, basal ganglia, and thalamus (Giedd et al. 2000; Rosenberg et al. 2001; Bottas and Richter 2002). These brain regions are functionally linked in cortico-striatal-thalamic circuits (Rauch et al. 1998). Jenike et al. (Jenike et al. 1996) have shown that OCD patients have significantly less total white matter, greater total neocortex, and greater opercular volumes when compared with normal controls. Moreover, OCD subjects have been demonstrated in some MRI studies to have white matter abnormalities in frontal regions (Garber et al. 1989; Jenike et al. 1996).

In a heterogeneous population, a specific candidate gene may not reveal any statistically significant effect if it has a small effect on the phenotype. Alternatively the specific gene may have a large effect in a small percentage of patients.

A series of studies (Swedo et al. 1994; Swedo et al. 1997; Swedo 2002; Snider and Swedo 2003) identified a subgroup of children with episodic OCD, in whom abrupt and dramatic symptom exacerbations were clearly preceded by group A β-hemolytic streptococcal (GABHS) infections. These cases appeared to be mediated through antineuronal antibodies (Snider and Swedo 2003). This subgroup has been termed pediatric

autoimmune neuropsychiatric disorders associated with (group A β-hemolytic) streptococcal infection" (PANDAS) (Snider and Swedo 2003). Evidence that PANDAS is familial, with increased rates of clinical and subclinical OCD observed in parents of children with this disorder (Snider and Swedo 2003), further suggests that this disorder may represent a distinct genetic subtype of OCD.

An autoimmune mechanism has thus been postulated for the PANDAS-related subtype of OCD, using rheumatic fever as a model (Arnold and Richter 2001; Snider and Swedo 2003). According to this model, GABHS infection presents antigens that stimulate circulating B-lymphocytes, which then cross into the brain (Alter et al. 2003) where they encounter a similar antigen stimulus in the form of brain tissue with cross-reactive epitopes. B-lymphocytes then set up an active cascade, which results in specific antigen-antibody complexes being formed against host brain tissue, and a corresponding inflammatory response. With respect to clinical presentation, it has been suggested that neuroimmunological dysfunction secondary to antineuronal antibodies, may result in behavioural disturbances such as anxiety, emotional lability, OC symptoms, hyperactivity, and sleep disturbances, and such neurological abnormalities as motor and phonic tics, ballismus, chorea, and choreiform movements (Snider and Swedo 2003).

These observations suggest that genetic susceptibility in PANDAS cases may be a function of an inappropriate immune response, an immunological deficit leading to aberrant discrimination between host and foreign factors, and/or the presence of neuronal cell components with epitopes more closely resembling those of the GABHS bacteria. Therefore, we have begun to examine markers linked to the genetic determinants of immune response.

Myelin oligodendrocyte glycoprotein (MOG) (NM_002433) is a component of the

myelin sheath, and has been implicated in autoimmune demyelinating diseases such as multiple sclerosis. It has been proposed that MOG functions as a cellular adhesion molecule, a regulator of oligodendrocyte microtubule stability, and/or as a mediator of interactions between myelin and the immune system, particularly as an activator of the complement cascade (Johns and Bernard 1997; Johns and Bernard 1999). Variants of this gene may therefore contribute to the development or progression of autoimmune disorders by various mechanisms, including altered expression, amino acid substitutions, or loss of function rendering myelin more susceptible to autoimmune attack. We hypothesized that anti-MOG antibodies may contribute to the etiology of OCD, via demyelination in the frontal lobe or other neuroanatomic regions in the central nervous system (CNS), or by ectopic activation of the complement cascade leading to loss of self-specificity. MOG is located on chromosome 6p21.3, just distal to the human leukocyte antigen (HLA) region, which contains genes that contribute to the immune response.

The primary aim of our study was thus to investigate for the presence of an association between markers in MOG and OCD. Four markers located within the MOG gene were used in this study, a polymorphic dinucleotide repeat (CA)n located downstream from the MOG 3' untranslated region (UTR), MOG-(CA)n (Genebank Accession #: AL050328 Region: 40030..40069), a tetranucleotide repeat (TAAA)n located in the 3' UTR, MOG-(TAAA)n (Malfroy et al. 1995), a single nucleotide polymorphism (SNP) in intron 1 at position 1334, C1334T (rs2252711), and a SNP in intron 5 at position 10991, C10991T (rs2071653). We genotyped a sample of 160 small nuclear families, and examined alleles of these four polymorphisms for evidence of biased transmission in order to determine whether variants of MOG are associated with OCD.

2.2 Methods

2.2.1 Samples

One hundred and sixty OCD probands and their parents were recruited from consecutive referrals to the Anxiety Disorders Clinic of the Centre for Addiction and Mental Health (CAMH), Toronto. All participants were assessed using the Structured Clinical Interview for the DSM-IV (SCID) (First et al. 1996) and using the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) score to determine the severity of their symptoms (Goodman et al. 1989). Assessments and interviews were done by an experienced research assistant and diagnoses were assigned according to DSM-IV criteria following review by a research psychiatrist. This study was approved by the Research Ethics Board of CAMH and all subjects gave their written informed consent to participate. A total of 160 families, which includes 62 triads, 25 diads, 20 families with both parents, a proband, and a sibling, 22 families with a single parent and a sibling, 31 families with a proband and a sibling, were analyzed at four MOG markers.

2.2.2 Diagnostic Criteria

The inclusion criteria for the affected probands were DSM-IV diagnosis of primary OCD. Family members were deemed to be affected if they had OCD at either a clinical or subclinical OC behaviour level, or for an OCD spectrum disorder, which in our sample included body dysmorphic disorder, trichotillomania, skin picking, Gilles de la Tourette's syndrome (TS), or chronic motor/vocal tics. The exclusion criteria included any metabolic or chronic neurological disease, schizophrenia, schizoaffective disorder, or bipolar disorder.

2.2.3 Isolation of DNA and Marker Genotyping

Venous blood was drawn from the probands and their family members in two 10 cc EDTA tubes, and genomic DNA extracted from lymphocytes using the high salt method as described in Lahiri and Nurnberger (Lahiri and Nurnberger 1991). The MOG-(CA)n polymorphism was genotyped according to procedures described in Barr et al. (Barr et al. 2001). Amplification of the region containing the MOG-(TAAA)n was achieved using a reaction mixture of 60 ng of genomic DNA, 5 μM of each of the 6-FAM labelled primers MOG4(F): 5'-AGA TAC GAG TTT TGG CCG G-3' and MOG4(R): 5'-GCC TCT GGG GTA ATG AGG CT-3', 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP (dATP, dCTP, dGTP, and dTTP), 1× standard PCR buffer, and 1 Unit of *Taq* polymerase (Applied Biosystems, Foster City, CA). Amplification of the fragments by PCR was done as follows: an initial denaturation stage at 95°C for 5 min followed by 30 cycles of denaturing at 94°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 30 sec. A final extension step of 72°C for 10 min was added after the last cycle. One microliter (μL) of the PCR product was then mixed with 10.5 μL of formamide and 0.5 μL of ROX size standard (500 bp) (Applied Biosystems, Foster City, CA). The mixture of products was genotyped using the ABI Prism® 3100 Genetic Analyzer (Applied Biosystems), and scored with the ABI Prism® 3100 GeneScan (version 3.7) and Genotyper (version 3.7).

The C1334T and C10991T polymorphism sites were amplified by PCR using the following primers: forward primer 5'-AGG ACT GAA TAG TGA GAA GTG ATG GA-3' and reverse primer 5'-ACC CTT AGA GGT TTT GCT TTT GTC A-3', forward primer 5'-TGC CAG GCA CTA TTC TAA CTA CTT TAC-3' and reverse primer 5'-GCA TTC CTG AAA AGA GAG ATC ATG TG-3' respectively. The probes used were labelled with 5'-

reporter fluors VIC or 6-FAM and 3'-quencher. The probe sequences were: VIC-CCA TCT ACT TTA GCT GTC TC and 6-FAM-CCA TCT ACT TTA GCT GTC TC for C1334T and VIC-AGA GAA TAA GAT AAC GTT TTT and 6-FAM-CAG AAT AAG ATA ACA TTT TT for C10991T. PCR amplifications for both markers were assessed by the TaqMan allele specific assay method using the ABI Prism® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) with the reaction mixture in a total volume of 25 μl, consisting of 50 ng of genomic DNA, 1× TaqMan Universal Master Mix, and 1× Assay-By-Design SNP Genotyping Assay Mix (Applied Biosystems), which includes the primers and labelled probes above. After denaturing at 95°C for 10 min, 45 cycles of PCR were performed under the following conditions: denaturing at 92°C for 15 sec and annealing at 60°C for 1 min. All genotypes were determined with the allelic discrimination program using the ABI software and confirmed by two experienced researchers.

2.2.4 Statistical Analyses

We tested for an association between alleles of the MOG-(CA)n, MOG-(TAAA)n, C1334T, and C10991T polymorphisms and OCD diagnosis using the transmission disequilibrium test (TDT) with the TDT-STDT program (version 1.1) (Spielman and Ewens 1998). Haplotype analysis was performed using the TRANSMIT program (version 2.5) (Clayton and Jones 1999). We also applied the family based association test (FBAT, version 1.0) (Laird et al. 2000) to allow for the analysis of the diagnosis of OCD with clinically relevant quantitative phenotypes including the Y-BOCS score and age at onset. All of the FBAT analyses were performed under the assumption of an additive model. Power for FBAT analyses was determined with the PBAT program (Lange et al. 2002). Linkage

disequilibrium (LD) was tested with the LDMAX program (Excoffier and Slatkin 1995). Results were assumed to be significant if $P<0.05$ in all cases; the results were not corrected for multiple comparisons.

2.3 Results

The sample of OCD patients had a mean age of 34.9±10.7 years, a mean age at onset of 14.7±9.0 years (N=121), and a mean total current Y-BOCS score of 22.2±8.1 (N=103). The sample consisted of 96.8% Caucasian, 1.6% Asian, and 1.6% African Americans.

Significant association with OCD was observed for allele 2 at MOG-(TAAA)n ($\chi^2=5.255$, $P=0.022$, Table 6). However, no significant results were observed for any of the alleles at MOG-(CA)n, C1334T (allele 1 transmitted 33 times and not transmitted 29 times, $\chi^2=0.258$, $P=0.611$), or C10991T (allele 1 transmitted 28 times and not transmitted 31 times, $\chi^2=0.153$, $P=0.696$) although a trend was detected at allele 13 of MOG-(CA)n ($P=0.061$, Table 6). For the transmission of haplotypes (global $\chi^2=12.418$, 9 df, $P=0.191$), we observed that the haplotype 1.13.2.2 [C1334T.MOG-(CA)n.C10991T.MOG-(TAAA)n] was significantly over-transmitted from parents to their affected offspring ($\chi^2=6.426$, $P=0.011$). FBAT analyses revealed significant results (Table 7) for allele 2 in MOG-(TAAA)n for total Y-BOCS score ($P=0.020$), obsession subscale ($P=0.022$), and compulsion subscale ($P=0.020$). Quantitative analyses for age at onset did not demonstrate an association at any markers. Significant LD was observed between polymorphisms as follows: C1334T and MOG-(CA)n (D'=0.961, $P<0.0001$), C1334T and C10991T (D'=1.000, $P=0.078$), C1334T and MOG-(TAAA)n (D'=0.570, $P<0.0001$), MOG-(CA)n and C10991T (D'=0.934, $P<0.0001$).

Table 6. Transmission disequilibrium analyses at the MOG-(CA)n and MOG-(TAAA)n markers

[a] Rare alleles less than 5% have been excluded.

[b] Global Chi-Square Value = 6.94; 3 df; P = 0.074 (calculated with alleles 11, 12, 13 and other alleles combined).

[c] Global Chi-Square Value = 7.56; 4 df; P = 0.109 (calculated with alleles 2, 3, 4, 5, and other alleles combined).

Marker	Allele [a]	Allele Frequency	Transmitted	Not Transmitted	χ^2 (1 df)	*P*
MOG-(CA)n [b]	3	0.098	12	19	1.581	0.209
MOG-(CA)n	5	0.053	8	9	0.059	0.808
MOG-(CA)n	8	0.051	6	9	0.600	0.439
MOG-(CA)n	9	0.084	6	9	0.600	0.439
MOG-(CA)n	11	0.236	29	28	0.018	0.893
MOG-(CA)n	12	0.221	32	29	0.148	0.700
MOG-(CA)n	13	0.215	35	21	3.500	0.061
MOG-(TAAA)n [c]	1	0.045	4	11	3.267	0.071
MOG-(TAAA)n	2	0.175	36	19	5.255	0.022
MOG-(TAAA)n	3	0.176	26	24	0.080	0.777
MOG-(TAAA)n	4	0.239	33	36	0.130	0.718
MOG-(TAAA)n	5	0.220	27	32	0.424	0.515
MOG-(TAAA)n	6	0.078	9	16	1.960	0.162
MOG-(TAAA)n	7	0.051	10	10	0.000	1.000

Table 7. Selected results of FBAT analyses of MOG and quantitative measures

[a] S represents the test statistic for observed number of alleles.

[b] E represents the expected value of S under null hypothesis.

[c] Variance between the observed and expected transmission.

[d] These are bi-allelic markers and only the *z* score for the over-transmitted allele is reported here.

[e] Global χ^2 of all alleles instead of a *z* score statistic and therefore has no S and E.

Marker	Quantitative Trait	Allele	S [a]	E (S) [b]	Var (O-E) [c]	*z* score	*P*
MOG-(CA)n	Total Y-BOCS	All	N/A	N/A	N/A	χ^2 (6 df) = 2.800 [e]	0.833
MOG-(CA)n	Obsessions	11	361	302.500	1207.750	1.683	0.092
MOG-(CA)n	Age at Onset	All	N/A	N/A	N/A	χ^2 (6 df) = 3.374 [e]	0.761
MOG-(TAAA)n	Total Y-BOCS	2	651	494.750	4483.438	2.334	0.020
MOG-(TAAA)n	Obsessions	2	322	244	1170	2.280	0.022
MOG-(TAAA)n	Compulsions	2	328	250.250	1119.688	2.324	0.020
MOG-(TAAA)n	Age at Onset	All	N/A	N/A	N/A	χ^2 (7 df) = 7.844 [e]	0.347
C1334T	Total Y-BOCS	2 [d]	806	745.165	5518.361	0.819	0.413
C1334T	Age at Onset	1 [d]	675	593.667	2533.722	1.616	0.106
C10991T	Total Y-BOCS	2 [d]	1119	1103.667	4101.778	0.239	0.811
C10991T	Age at Onset	2 [d]	827	804.167	3656.528	0.378	0.706

2.4 Discussion

In this paper, we investigated the possibility that OCD is associated with the MOG gene, using a family-based association approach. Our tests for association with the diagnosis of OCD revealed a trend towards biased transmission for allele 13 of MOG-(CA)n (P=0.061), and a significantly biased transmission of allele 2 of MOG-(TAAA)n (P=0.022) with an accompanying trend towards biased non-transmission for allele 1 (P=0.071) (Table 6). A significant association (P=0.011) was also observed between a MOG haplotype involving allele 13 of MOG-(CA)n and allele 2 of MOG-(TAAA)n with OCD. It remains unclear whether the MOG locus itself is the putative genetic risk site, or if the risk is conferred by HLA-related site in LD with this MOG marker.

In the quantitative trait analysis of Y-BOCS severity score, we found significant association with the MOG-(TAAA)n polymorphism (Table 7). Furthermore, allele 2 from MOG-(TAAA)n was found to be associated with high Y-BOCS severity scores. We also observed a significant association between allele 2 of MOG-(TAAA)n and both the obsession and compulsion subscales, indicating that the significant results were associated with both of the major symptom types. Our significant findings were strong despite the fact that the cases were not selected for PANDAS subtype. This implies that MOG may have important implications for OCD in general. A study enriched with childhood-onset probands would presumably be more likely to identify autoimmune-related genes since these are likely more relevant to the PANDAS subtype.

Our research focused on the contribution of MOG to risk for OCD. Furthermore, a recent TDT analysis suggests that there is a weak association between MOG and TS [148 bp allele in MOG-(CA)n: χ^2=4.000, P=0.046] (Huang et al. 2004). In several MRI studies,

white matter abnormalities were observed in frontal regions of OCD patients, which may be consistent with altered myelination that might arise from changes in MOG function (Jenike et al. 1996).

To our knowledge, this is the first attempt to study genes involved in immune processes in OCD. The need for further study of other genes that may be associated with the PANDAS group of disorders is indicated, particularly genes lying in or near the HLA region. Various reports indicate that the monoclonal antibody, D8/17, a known marker for susceptibility to rheumatic fever, is present at dramatically high rates in OCD and PANDAS disorders. However, such positivity has not been associated with the HLA class I or class II MHC complex antigens (Swedo et al. 1997; Murphy and Goodman 2002). Further studies may help clarify the HLA background of individuals with PANDAS, and elucidate the contribution of HLA haplotypes to the etiology of this group of disorders.

Evidence for the possible role of MOG in PANDAS comes from studies implicating MOG as an activator of complement. Johns and Bernard (Johns and Bernard 1997; Johns and Bernard 1999) demonstrated the ability of MOG to bind C1q in a dose dependent and saturating manner, and to inhibit antibody dependent lysis of red-blood cells by complement. Clinical research has indicated a rapid increase in C1q levels with CNS inflammation (Dietzschold et al. 1995). These findings suggest the intriguing possibility that MOG may potentiate an autoimmune mechanism in the PANDAS disorders by complement activation. Consistent with this hypothesis is the proposition by Johns and Bernard (Johns and Bernard 1997) that the inhibition of C1q-MOG interaction could be a mechanism for reducing excessive inflammation in diverse neurodegenerative disorders, including chronic viral infection.

Failure to identify cases of PANDAS, which may account for a subtype of OCD, may conceivably confound genetic studies due to cryptic etiologic heterogeneity. Likewise, the factors involved in predisposing an individual to develop OCD under a particular environmental condition (exposure to GABHS infection) are likely complex, and may involve one or more loci of small effect - again rendering traditional genetic techniques difficult. Therefore, it is particularly important to examine potential homogeneous phenotypes of OCD such as PANDAS. Our own study presents an intriguing finding of positive association of the MOG locus with OCD and may suggest a pathophysiological explanation accounting for a subtype of OCD patients. Our study has power of 80% to detect a relative risk of 3.1, thus small gene effects would not be found by our current sample. Therefore, larger and well-characterized samples are needed, in order to extend these results, to better delineate subgroups of OCD, and to enable more directed studies regarding the localization and characterization of genetic factors. Finally, we suggest further study on the function of MOG in the context of autoimmune disorders, to clarify its role in the immune system and the complement cascade.

CHAPTER 3: Genetic Study of the Myelin Oligodendrocyte Glycoprotein (MOG) Gene in Schizophrenia

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3.1 Introduction

Schizophrenia (SCZ) is a neuropsychiatric disorder that affects approximately 1% of the world population and usually first emerges in early adulthood (American Psychiatry Association 1994). This disease is characterized by diverse and variably expressed symptoms, which include disorganized thought patterns and behaviours, delusional beliefs, auditory hallucinations, cognitive difficulties, apathy and social withdrawal. Evidence for a strong genetic component to the etiology of SCZ has been drawn from family, twin and adoption studies (Bray and Owen 2001). The cause of SCZ remains unknown. Of many contemporary theories of SCZ, the most enduring is the dopaminergic hypothesis due to dopamine receptors' role as sites for anti-psychotic drug action (van Rossum 1966; Carlsson 1978; Seeman 1993). Since the dopamine hypothesis has not yielded any definitive answers regarding the etiology of SCZ, we have investigated the MOG gene located near the human leukocyte antigens (HLA) region, based on the rationale below.

Several studies have examined the possible existence of a susceptibility locus for SCZ on the short arm of chromosome 6, close to the HLA region at 6p21.3 (Antonarakis et al. 1995; Gurling et al. 1995; Moises et al. 1995; Mowry et al. 1995; Schwab et al. 1995; Straub et al. 1995; Wang et al. 1995). Although three investigations reported non-significant results (Gurling et al. 1995; Moises et al. 1995; Mowry et al. 1995), linkage to SCZ was found for markers D6S274 (Schwab et al. 1995) and D6S296 (Straub et al. 1995), which map within 20 cM telomeric to the HLA region, and for D6S291 (Schwab et al. 1995), which maps centromeric to HLA (Straub et al. 1996). Furthermore, Straub et al. (Straub et al. 2002b) and Schwab et al. (Schwab et al. 2000) recently reported a significant linkage in the HLA region (6p24-22) for SCZ and they also presented evidence supporting a small but significant role

for dysbindin (DTNBP1; 6p22.3) as a SCZ candidate gene in this region (Straub et al. 2002a; Schwab et al. 2003). These results provide some evidence for a susceptibility locus for SCZ on the short arm of chromosome 6. The exact location of this locus is uncertain, but it may be within or near the HLA region at 6p21.3.

The major histocompatibility complex (MHC) within the HLA region plays a fundamental role in the control of immune responses by lymphocytes (Sette et al. 1987). The HLA region contains a number of closely linked genes that fulfill their biological role by modulating immune responses to foreign antigens and discriminating foreign antigens from host tissue; thus, they are highly polymorphic. The affinity of an HLA antigen directly impacts on the capability of an individual to mount an immune response - even a single base-pair difference can greatly increase or reduce this capacity. Many studies have therefore examined HLA polymorphisms in the context of various autoimmune disorders, with the assumption that certain alleles may be associated with a deficiency in discriminating self from non-self (Thomson 1988). Furthermore, sites near the HLA locus have been implicated in neuropsychiatric disorders (Mytilineos et al. 1990; Sasaki et al. 1994; Wright et al. 1996; Wright et al. 2001) and may contribute to behavioural changes (Weitkamp et al. 1981). Moreover, more than 60 genetic associations of HLA alleles with SCZ have been published with the first association study reported by Cazzullo et al. in 1974 (Cazzullo et al. 1974).

The hypothesis of the dysregulation of the immune system is also strengthened with the indication of immune activation in SCZ patients due to their abnormal levels of proinflammatory cytokines and their receptors in peripheral blood and cerebrospinal fluid (Boin et al. 2001). Cytokines are involved in normal central nervous system (CNS) development as well as in the pathogenesis of many neuropsychiatric disorders, altering

neurotransmitter and neuropeptide systems or acting directly on neural cells.

Thus, we examined markers linked to the allelic determinants of immune response. To assess the contribution of the HLA region variants to SCZ, we studied four polymorphisms within the myelin oligodendrocyte glycoprotein (MOG) gene, which is located 60 Kb telomeric to HLA-F (Amadou et al. 1995). MOG, which has been implicated in autoimmune demyelinating diseases such as multiple sclerosis (Hartung and Rieckmann 1997), is a member of the immunoglobulin super family and a component of the myelin sheath. It has been proposed that MOG might function as a cellular adhesion molecule, a regulator of oligodendrocyte microtubule stability, and/or as a mediator of interactions between myelin and the immune system, in particular as an activator of the complement cascade (Johns and Bernard 1999). Variations or mutations in this gene may therefore contribute to the development or progression of autoimmune disorders by various mechanisms, including altered gene expression, amino acid substitutions increasing the possibility of interactions between MOG and environmental factors, or loss of function rendering myelin more susceptible to attack. Moreover, dysfunction of neuronal connectivity in SCZ patients, such as factors that affect myelination has been observed (Davis et al. 2003; Hof et al. 2003). Volume reductions in the white matter of the prefrontal cortex (PFC) have been repeatedly found in schizophrenic patients with magnetic resonance imaging (MRI) (Breier et al. 1992; Buchanan et al. 1998; Sanfilipo et al. 2000; Sigmundsson et al. 2001) and these reductions have been associated with negative symptoms (Sanfilipo et al. 2000; Sigmundsson et al. 2001). Additionally, a recent study has found a 28% decrease in total numbers (or densities) of cortical layer III oligodendrocytes and a 27% decrease in the white matter in SCZ patients when compared with controls (Hof et al. 2003). Furthermore,

Tkachev et al. (Tkachev et al. 2003) reported decreased MOG mRNA expression in the prefrontal cortex of SCZ patients when compared with healthy controls. The four polymorphisms examined consist of a dinucleotide repeat located downstream from the MOG 3'-untranslated region (3'-UTR) (Genebank Accession #: AL050328 Region: 40030..40069), a tetranucleotide repeat located in the 3'-UTR (Malfroy et al. 1995; Roth et al. 1995), entitled (CA)n and (TAAA)n, respectively, and two intronic C to T base exchange polymorphisms located at position 1334 and 10991, entitled C1334T and C10991T.

We genotyped a sample of 111 small nuclear families and 182 SCZ case and healthy control pairs matched with age, sex, and ethnicity. We examined the transmission of alleles of these four polymorphisms within the MOG gene for evidence of biased transmission using several statistical analyses, including the transmission disequilibrium test (TDT) (Spielman et al. 1993; Spielman and Ewens 1998) in order to determine whether the MOG gene is associated with SCZ. The Kruskal-Wallis test was used to compare mean age at onset (AAO) with genotypes. Finally, we also performed the Pearson χ^2 and Monte Carlo tests in our case-control sample.

3.2 Methods

3.2.1 Clinical Diagnostic Criteria

Structured diagnostic interviews were performed on probands by the clinical team with extensive experience with the Structured Clinical Interview for DSM-IV (SCID-I) (First et al. 1996), which was used as the primary diagnostic tool for this study. A clinical narrative summary was prepared for each patient to provide a more detailed phenotype description and information about the context, severity, and sequence of symptoms during the illness

(Mellman et al. 2001). Research psychiatrists then reviewed the clinical summary, the SCID interview response, and the medical records. The evidence for the patient meeting DSM-IV criteria was reviewed, diagnostic uncertainties were discussed, and a best-estimate consensus diagnosis was reached (Endicott 2001). For the diagnostic packages wherein disagreement occurred, review was performed by a third psychiatrist to make the final decision. In the present study, 97% of our sample had medical records included as part of their clinical data. The inclusion criteria for adult probands were DSM-IV diagnosis of SCZ, or schizoaffective disorder (depressed type). The exclusion diagnostic criteria included history of drug-induced psychosis or history of drug dependence, major neurological disorder including epilepsy, or head injury with significant loss of consciousness. For the purposes of this study, we characterized AAO according to the distinct definition below. AAO has been defined as the age at which the subject was first hospitalized for a psychotic episode. All the information provided by the probands regarding their AAO is validated when possible by participating parents and/or siblings.

3.2.2 Families and Case-Control Samples

The analysis included 33 parents/proband trios, 9 parents/proband/sibling families, 8 families consisting of a single parent and a sibling, 49 single parent/proband diads, and 12 families with a proband and a sibling (5 affected siblings) for a total of 111 families. The ethnic background of the probands consists of 3% Asians, 2% African North Americans, and the remaining 95% Caucasians. We collected a total of 182 affected unrelated individuals and 100 healthy controls matched with their age, gender, and ethnicity.

3.2.3 DNA Isolation, Polymorphism Detection, and Genotyping

Venous blood was drawn from the probands and their family members in two 10cc EDTA tubes, and DNA was extracted from blood lymphocytes using a high salt method (Lahiri and Nurnberger 1991). The (CA)n polymorphism within the MOG gene was genotyped as described by Barr et al. (Barr et al. 2001). For the PCR protocol of the (TAAA)n polymorphism (Malfroy et al. 1995), reaction components included 60 ng nuclear DNA, 5 μM of each primer tagged with 6-FAM fluorescence [MOG4(F): 5'- AGA TAC GAG TTT TGG CCG G and MOG4(R): 3'- GCC TCT GGG GTA ATG AGG CT], 1× GeneAmp PCR Buffer II (Applied Biosystems, Foster City), 1.5 mM $MgCl_2$, 200 μM each of dATP, dTTP, dGTP, dCTP, 1 Unit of *Taq* polymerase (Applied Biosystems, Foster City), and with distilled and deionized water to make a total reaction volume of 25 μl. The conditions for the PCR reaction were as follows: an initial incubation/denaturation at 95°C for 5 min, followed by 25 cycles of denaturing at 94°C for 30 sec, annealing at 58°C for 30 sec, and 72°C for 30 sec. A final extension at 72°C was carried out for 10 min. Genotyping was performed on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City), according to manufacturer's protocol.

For the C1334T (rs2252711) and C10991T (rs2071653) markers, genotypes were assessed by the TaqMan allele specific assay method using the ABI Prism® 7000 Sequence Detection System according to the manufacturer's protocols (Applied Biosystems, Foster City, CA). The C1334T polymorphism site was amplified by PCR using the following primers: forward primer 5'-AGG ACT GAA TAG TGA GAA GTG ATG GA-3' and reverse primer 5'-ACC CTT AGA GGT TTT GCT TTT GTC A-3'. The probes used were labelled with 5'-reporter fluors VIC or 6-FAM and 3'-quencher. The probe sequences were: VIC-

CCA TCT ACT TTA GCT GTC TC and 6-FAM-CCA TCT ACT TTA GCT GTC TC. The C10991T polymorphism site was amplified by PCR using the following primers: forward primer 5'-TGC CAG GCA CTA TTC TAA CTA CTT TAC-3' and reverse primer 5'-GCA TTC CTG AAA AGA GAG ATC ATG TG-3'. The probe sequences were: VIC-AGA GAA TAA GAT AAC GTT TTT and 6-FAM-CAG AAT AAG ATA ACA TTT TT. PCR amplification were performed with the same conditions for C1334T and C10991T on an ABI Prism® 7000 Sequence Detection System with the reaction mixture in a total volume of 25 μl, consisting of 50 ng of genomic DNA, 1× TaqMan Universal Master Mix, and 1× Assay-By-Design SNP Genotyping Assay Mix (Applied Biosystems, Foster City, CA), which includes the primers and labelled probes above. After denaturing at 95°C for 10 min, 45 cycles of PCR were performed under the following conditions: denaturing at 92°C for 15 sec and annealing at 60°C for 1 min. All genotypes were determined with the allelic discrimination program using the ABI software and confirmed by two experienced researchers.

3.2.4 Statistical Analyses

The TDT/S-TDT (Transmission Disequilibrium Test/sib TDT version 1.1) statistical program, which is a program to test for association in the presence of linkage disequilibrium, was used to calculate transmission disequilibrium (Spielman et al. 1993; Spielman and Ewens 1998). Global χ^2 values from the microsatellites were calculated using SPSS version 10.0 (SPSS Inc., Chicago, IL) by pooling the rare alleles with allele frequency less than 10%. Haplotype analysis was also performed in the MOG gene utilizing all four markers using the TRANSMIT program (version 2.5) (Clayton and Jones 1999). TRANSMIT tests for association between markers and disease by examining the transmission of multi-allele

haplotypes from parents to affected offspring, allowing for phase unknown and missing parental genotypes. The tests are based on a score vector, which is averaged over all possible configurations of parental haplotypes and transmissions consistent with the observed data, and produce asymptotic χ^2 tests and *P* values empirically using ≥100,000 replicates. For the haplotype analysis, in order to protect against misleading results due to rare alleles and haplotypes, the command-line switch (i.e., flags) "-c5" was used to aggregate all alleles or haplotypes with frequencies less than 0.05 before haplotype construction. Power for TDT analyses was determined with the TDT Power Calculator, version 1.2.1 (Chen and Deng 2001) and power for case-control analyses was determined with the Genetic Power Calculator (Purcell et al. 2003). Linkage disequilibrium (LD) between markers was tested with the LDMAX program (Excoffier and Slatkin 1995). We also analyzed the AAO, and since the distributions of the AAO and the genotype frequency deviate from the normal distribution, instead of using one-way ANOVA, we have chosen to apply the Kruskal Wallis Test using SPSS version 10.0 (SPSS Inc., Chicago, IL). For the case-control analyses, Pearson χ^2 test (Fisher's Exact Test) using SPSS version 10.0 (SPSS Inc.), Monte Carlo test using CLUMP program (Sham and Curtis 1995), odds ratio (OR) and confidence interval (CI) using 2BY2 program version 1.50 (Ott 1999), and Hardy Weinberg Equilibrium (HWE) using HWE program (Ott 1999) were performed. Conversion of the χ^2 values from the TDT and case-control analyses into *z*-scores is obtained from the following equations: for degrees of freedom>1: $(\chi^2\text{-}n)/(2n)^{1/2} = z\text{-score}$ and for 1 degree of freedom test: $\sqrt{\chi^2} = z\text{-score}$. A combined *z*-score was then calculated using the *z*-scores from the TDT and case-control analyses $[(z_{\text{case-control}} + z_{\text{TDT}})/\sqrt{2}]$. All of the statistical analyses in this study were based on $P<0.05$ as significant and results were not corrected for multiple comparisons.

3.3 Results

The mean age for the probands used in the TDT analysis is 37.2±9.6 and the mean AAO is 19.4±5.2. Allelic counting indicated a heterozygosity of 79.1% and 76.1% for the (CA)n and (TAAA)n respectively. The TDT-STDT results are presented in Table 8. We did not observe any biased transmission of alleles at all four MOG polymorphisms.

The results for the TDT haplotype analysis are presented in Table 9 and there was no significant result. The Kruskal-Wallis Test results for the phenotypic analysis between mean AAO for each genotype were not significant at all four polymorphisms [(CA)n: χ^2=28.655, 27 df, *P*=0.378; (TAAA)n: χ^2=25.863, 18 df, *P*=0.103; C1334T: χ^2=0.951, 2df, *P*=0.621; C10991T: χ^2=0.051, 1df, *P*=0.821].

For the case-control analysis, the mean age for the affected males was 39.8±10.4 and for females was 42.4±10.0. Healthy males had a mean age of 38.5±9.9 and healthy females had a mean age of 41.1±9.9. For the results of the case-control analysis, we observed slight trends in the allelic frequencies between cases and controls for allele 11 in (CA)n with 41 counts in cases and 55 in controls (χ^2=2.042, *P*=0.153) and for allele 7 in (TAAA)n with 17 in cases and 9 in controls (χ^2=2.462, *P*=0.117). The global χ^2 values were not significant for either microsatellite [(CA)n: χ^2=4.570, 6 df, *P*=0.600 and (TAAA)n: χ^2=4.900, 5 df, *P*=0.428]. The case-control analyses of the C1334T (χ^2=0.630, *P*=0.427; OR=1.17, 95% CI=0.83-1.65) and C10991T (χ^2=0.150, *P*=0.699; OR=1.10, 95% CI=0.75-1.60) polymorphisms were also not significant (Table 10). We obtained a combined *z*-score of –1.035 (*P*=0.150), –0.591 (*P*=0.277), 0.703 (*P*=0.241), and 0.551 (*P*=0.291) respectively for (CA)n, (TAAA)n, C1334T, and C10991T.

Significant LD was observed between markers as follows: C1334T and C10991T (D'=1.000, P=0.003), C1334T and (CA)n (D'=0.933, P<0.0001), C10991T and (CA)n (D'=0.902, P<0.0001).

Table 8. Transmission disequilibrium test for the four polymorphisms in the MOG gene with the diagnosis of SCZ as the affection trait

[a] T is the number of transmission from heterozygous parents to affected proband.

[b] NT is the number of non-transmission from heterozygous parents to affected proband.

[c] (TAAA)n: Global χ^2=3.720; 5 df; P=0.445 (calculated with alleles that have a frequency >10%, which include alleles 2, 3, 4, 5, and other alleles combined).

[d] (CA)n: Global χ^2=0.740, 4 df, P=0.864 (calculated with alleles that have a frequency >10%, which include 11, 12, 13, and other alleles combined).

Marker	Allele	Frequency	T [a]	N [b]	χ^2 (1 df)	*P*
C1334T	1	0.801	12	13	0.040	0.841
C1334T	2	0.199	13	12	0.040	0.841
C10991T	1	0.217	12	14	0.154	0.695
C10991T	2	0.783	14	12	0.154	0.695
(TAAA)n [c]	2	0.142	9	13	0.727	0.394
(TAAA)n	3	0.184	22	16	0.947	0.330
(TAAA)n	4	0.271	26	23	0.184	0.668
(TAAA)n	5	0.216	16	24	1.600	0.206
(TAAA)n	6	0.092	9	9	0.000	1.000
(TAAA)n	7	0.085	9	6	0.600	0.439
(TAAA)n	9	0.002	0	1	1.000	0.317
(TAAA)n	10	0.001	1	0	1.000	0.317
(CA)n [d]	2	0.022	4	2	0.667	0.414
(CA)n	3	0.098	8	13	1.190	0.275
(CA)n	4	0.002	0	1	1.000	0.317
(CA)n	5	0.055	7	8	0.067	0.796
(CA)n	7	0.001	1	0	1.000	0.317
(CA)n	8	0.045	8	6	0.286	0.593
(CA)n	9	0.090	13	10	0.391	0.532
(CA)n	10	0.030	3	4	0.143	0.705
(CA)n	11	0.246	23	20	0.209	0.648
(CA)n	12	0.149	18	17	0.029	0.865
(CA)n	13	0.231	14	18	0.500	0.480

Table 9. TDT haplotype analysis across the four polymorphisms in the MOG gene with the diagnosis of SCZ as the affection trait

[a] Rare haplotypes with frequency less than 5% have been excluded.

[b,c] Observed and expected transmission of haplotype from parents to the probands.

[d] Variance (difference between observed and expected transmissions).

[e] Global χ^2=7.954, 9 df, P=0.539.

[f] Rare alleles with allele frequency <10% in the (TAAA)n marker have been pooled and renumbered as allele 11.

[g] Rare alleles with allele frequency <10% in the (CA)n marker have been pooled and renumbered as allele 14.

Haplotype (C1334T.C10991T.TAAA.CA) [a,e]	Frequency	Observed [b]	Expected [c]	Var (O-E) [d]	χ^2 (1 df)	*P*
2.2.4.11	0.061	11.514	11.942	4.618	0.040	0.842
2.2.5.11	0.096	18.423	20.028	7.969	0.323	0.570
1.1.4.12	0.103	21.222	20.518	7.534	0.066	0.798
1.1.5.12	0.06	8.739	11.664	4.730	1.809	0.179
1.2.3.13	0.058	15.121	13.253	4.964	0.702	0.402
1.2.4.13	0.089	15.200	18.043	5.313	1.520	0.218
1.2.3.3	0.051	12.982	10.407	3.246	2.041	0.153
1.2.11 [f].9	0.067	14.000	13.666	5.638	0.020	0.888
1.2.11.14 [g]	0.058	14.324	11.781	4.722	1.369	0.242

Table 10. Case-control analysis at the C1334T and C10991T polymorphisms in the MOG gene

[a] Hardy Weinberg Equilibrium (HWE) test for SCZ cases: χ^2=0.021, *P*=0.885 and HWE for healthy controls: χ^2=2.992, *P*=0.084.

[b] HWE test for SCZ cases: χ^2=0.496, *P*=0.481 and HWE for healthy controls: χ^2=0.494, *P*=0.482.

Marker	Allele or Genotype	Cases (N = 182)	Controls (N = 182)	χ^2 (df)	*P*
C1334T	1	309	299	0.630 (1)	0.427
C1334T	2	77	87		
C1334T	1/1 [a]	124	120		
C1334T	1/2	61	59	1.735 (2)	0.420
C1334T	2/2	8	14		
C10991T	1	69	64	0.150 (1)	0.699
C10991T	2	295	300		
C10991T	1/1 [b]	8	7		
C10991T	1/2	53	50	0.219 (2)	0.896
C10991T	2/2	121	125		

3.4 Discussion

In this study, we investigated the possibility of transmission disequilibrium between the alleles in the (CA)n, (TAAA)n, C1334T, and C10991T polymorphisms and SCZ. Moreover, we also examined variations in the MOG gene using a case-control approach. However, no biased transmissions of alleles and haplotypes were observed and no significant difference was found between the allelic frequencies of SCZ cases and controls. Although our results did not support a role of MOG in the pathogenesis of SCZ, other variants in the MOG gene may still be worthwhile for investigation because of the overall rationale including support from MRI data that indicate reduction of white matter in SCZ patients when compared with healthy controls (Breier et al. 1992; Buchanan et al. 1998; Sanfilipo et al. 2000; Sigmundsson et al. 2001).

Autoimmune disease begins when susceptible individuals lose tolerance for nuclear self-antigens, which then leads to the production of anti-nuclear autoantibodies that contribute to the dysregulation of the immune system and targets the destruction of tissues in the body to cause vasculitis, neurological disorders, arthritis, and nephritis for example. Evidence has been found suggesting that the streptococcal M proteins may evoke anti-neuronal antibodies that cross-react with the human brain, possibly causing autoimmune diseases and neuropsychiatric disorders (Gibofsky et al. 1991; Bronze and Dale 1993; Swedo et al. 1994; Swedo et al. 1998; Garvey et al. 1999). These reports, along with the genetic association results between HLA markers and SCZ encouraged us to hypothesize a link between autoimmune disease and SCZ.

Evidence for the possible role of MOG in SCZ comes from recent studies implicating MOG as an activator of the classical pathway of complement. Johns and Bernard (Johns and

Bernard 1997) demonstrated the ability of MOG to bind C1q, a component of complement, in a dose dependent and saturating manner, and to inhibit antibody dependent lysis of red blood cells (RBC) by complement. Clinically, research indicates a rapid increase in C1q levels with CNS inflammation (Dietzschold et al. 1995). The activation of complement via the binding of C1q to myelin oligodendrocytes leads to the intriguing possibility that MOG may potentiate an autoimmune mechanism in SCZ. The usual progression of an autoimmune disease begins with polygenic susceptibility coupled with environmental and epigenetic factors, which then leads to cell infiltration, inflammation, tissue destruction, and finally progresses to the clinical disease. Of interest, Johns and Bernard (Johns and Bernard 1997) present the proposition that the inhibition of C1q-MOG interaction could be the basis for a treatment that would reduce excessive inflammation in diverse neuro-degenerative disorders, including chronic viral infection. Moreover, recent imaging and gene expression studies have reported a decrease in MOG expression in SCZ and bipolar disorder patients, and abnormal myelination in SCZ patients (Flynn et al. 2003; Tkachev et al. 2003). Thus, although our results did not suggest a role of MOG in the development of SCZ, MOG should still be considered as an interesting candidate gene in the pathogenesis of SCZ.

Furthermore, the importance of myelination to the normal brain functioning has been implicated in the diagnosis, treatment, and prognosis of SCZ (Bartzokis et al. 2003). Thus, other myelin-related proteins such as myelin-association glycoprotein (MAG) and myelin basic protein (MBP), and the enzyme cyclic nucleotide phosphodiesterase (CNPase), are important for investigation with the diagnosis of SCZ because recent studies have reported white matter abnormalities in SCZ patients due to decreased CNPase (Flynn et al. 2003) and MBP (Honer et al. 1999) protein expressions in the anterior frontal cortex, and decreased

MAG (Hakak et al. 2001; Tkachev et al. 2003) and CNPase (Hakak et al. 2001) mRNA expressions in the frontal cortex of SCZ. Furthermore, a specific isoform of the MBP protein produces lower mRNA in the frontal cortex of SCZ (Tkachev et al. 2003).

We chose to combine the case-control and TDT samples to calculate the power of our data set, which is of moderate size. Our sample of 182 controls and 182 cases has 86% power to detect an odds ratio of 1.9 with 95% certainty. Our TDT sample of 111 families has 67% power to detect a significant effect with a genotypic relative risk of 1.9. We converted the TDT families to their equivalency in case-controls in the following conservative manner: 33 trios + 57 diads + 12 proband-sibling ≈ 90 to 100 case-control samples; 9 trios and sibling pairs ≈ at least 9 case-control samples. Thus the total is 281 (182 [original case-control samples] + 90 [being conservative] + 9 [being conservative]). This combined case-control sample has a power of 96% to detect a relative risk as low as 1.5. Furthermore, the large number of alleles in each of the highly polymorphic microsatellites increased the degrees of freedom.

Since we observed no trend in our analyses of all four markers, we could recommend for future studies that larger and well-characterized samples be used and further exploration of the phenotype be pursued. Finally, we suggest further study regarding the function of MOG in the context of autoimmune disorders, to clarify its role in the immune system and the complement cascade since the function of MOG remains largely unknown. Additional clinical studies on the expression of the different gene variants together with more sophisticated definition of subgroups in SCZ may help in providing more conclusive results.

CHAPTER 4: Evidence for the Gamma-Amino-Butyric Acid Type B Receptor 1 (GABBR1) Gene as a Susceptibility Factor in Obsessive-Compulsive Disorder

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Ms. Zai has performed all of the experiments including genotyping and statistical analyses and she has written all the materials in this manuscript.

4.1 Introduction

Obsessive-compulsive disorder (OCD), which has a lifetime prevalence of 2 to 3% in the general population (Sasson et al. 1997), is a severe neuropsychiatric disorder characterized by unwanted intrusive thoughts (obsessions), and the need to perform rituals or repetitive acts (compulsions) in order to alleviate anxiety (American Psychiatric Association 1994). Twin and family studies have supported the genetic basis in OCD (Hettema et al. 2001).

While it is widely accepted that serotonergic mechanisms are important in the neurobiology of OCD, other neurotransmitter systems may also be involved. The neurotransmitter gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the central nervous system (CNS), and might be functionally important in cortical disinhibition (Krogsgaardlarsen et al. 1997). It is therefore relevant to explore the potential role of the GABAergic mechanisms in OCD.

Evidence supporting GABAergic involvement in OCD comes from observations of the clinical utility of gabapentin, a synthetic GABA analog. Gabapentin has been investigated in three small open OCD studies; all reported some benefit from gabapentin augmentation of a selective serotonin reuptake inhibitor (SSRI) (Chouinard et al. 1998; Cora-Locatelli et al. 1998) (Kahn, Katzman, and Richter, unpublished). There have also been a number of studies suggesting clinical utility of this agent in other anxiety disorders including panic, posttraumatic stress disorder (PTSD), generalized anxiety disorder (GAD), and social phobia (Schaffer and Schaffer 1997; Pollack et al. 1998; Pande et al. 1999; Brannon et al. 2000; Pande et al. 2000).

GABA exerts its effects by the alteration of intracellular signal transduction through

two distinct receptor types: the ionotrophic $GABA_A$ and $GABA_C$ receptors that are associated with Cl^- channels and are involved in fast inhibitory synaptic transmission, and the metabotrophic GABA type B ($GABA_B$) receptors, which are associated with K^+/Ca^{2+} channels and act via guanosine 5'-triphosphate binding (G-) proteins to produce slow, prolonged inhibitory signals (Bowery 2000; Olsen and Homanics 2000). The modulation of GABA release via an autoreceptor and neurotransmitter [e.g. noradrenaline (Bowery et al. 1980), 5-hydroxytryptamine (Gray and Green 1987), cholecystokinin (Conzelmann et al. 1986), and somatostatin (Bonanno et al. 1991)] release are thought to be an essential physiological role of $GABA_B$ receptors. Additionally, the stimulation of $GABA_B$ receptors reportedly induced the inhibition of glutamate release (Travagli et al. 1991; Bonanno and Raiteri 1992).

$GABA_B$ receptor-mediated neurotransmission has been implicated in the pathophysiology of several neuropsychiatric disorders including anxiety, depression, alcohol dependence, and cognitive deficits (Bittiger et al. 1993; Kerr and Ong 1995). Moreover, possible clinical application of $GABA_B$ agonists/antagonists include spasticity (Bowery 1993), epilepsy (Caddick and Hosford 1996), drug withdrawal (Shoaib et al. 1998; Capasso 1999), anxiety, depression, and cognitive dysfunction (Kerr and Ong 1995; Getova et al. 1997).

The $GABA_B$ receptor consists of a heterodimer of two related 7-transmembrane subunits, $GABA_B$ Receptor subunit 1 (GABBR1) and $GABA_B$ Receptor subunit 2 (GABBR2) or G Protein-Coupled Receptor 51 (GPR51). The GABBR1 gene (Genebank NM_001470, NP_001461; MIM# 603540) has been mapped to the chromosomal region 6p21.3 (Goei et al. 1998), 130 Kb telomeric to the HLA-F locus. A susceptibility region for

OCD (Lod score of 1.4 on chromosome 6p) (Hanna et al. 2002) has been mapped in this chromosomal area, thus providing additional support for an investigation of the role of this gene in OCD.

Given the physiologic significance of the $GABA_B$ receptor to neurobiology including its known role in synaptic inhibition, the pharmacological support, and the genetic localization in a proposed susceptibility region, GABBR1 is an attractive candidate gene for OCD. The aim of our study is thus to investigation for the presence of an association between markers in the GABBR1 gene and OCD. Five single nucleotide polymorphisms (SNPs) located within the GABBR1 gene were examined in this study: an A to G base exchange at position 7265 in the promoter region (A-7265G; rs29218), a C to G base exchange at position 10497 in intron 9 (C10497G; rs29220), a T to C substitution at exon 12 (Ser-491-Ser; rs29225), an A to G substitution at exon 16 (Phe-659-Phe; rs29230), and an A to G base exchange at position 33795 in the 3'-UTR (untranslated region) (A33795G; rs3095273). We genotyped a sample of 159 small nuclear families, and examined alleles of these five polymorphisms for evidence of biased transmission in order to determine whether variants of GABBR1 are associated with OCD.

4.2 Methods

4.2.1 Samples

The Anxiety Disorders Clinic of the Centre for Addiction and Mental Health (CAMH), Toronto recruited 159 OCD probands and their parents from consecutive referrals. The Structured Clinical Interview for the DSM-IV (SCID) (First et al. 1996) and the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) score, which determines the severity of the

symptoms (Goodman et al. 1989), were used to assess participants. An experienced research assistant did assessments and interviews and diagnoses were assigned according to DSM-IV criteria following review by a research psychiatrist. The Research Ethics Board of CAMH approved this study and all subjects gave their written informed consent to participate. The sample included 58 triads, 20 triads and a sibling, 28 families with a single parent and an affected proband, 21 families with a single parent and unaffected sibling, and 32 families with an affected proband and a sibling, a total of 159 families. Twenty unaffected siblings of the probands were included in the analysis.

4.2.2 Diagnostic Criteria

DSM-IV diagnosis of primary OCD was the inclusion criteria for the affected probands. Family members were reported as affected if they had OCD at either a clinical or subclinical OC behaviour level, or they had an OCD spectrum disorder, which in our sample included body dysmorphic disorder, chronic motor/vocal tics, Gilles de la Tourette's syndrome (TS), trichotillomania, or skin picking. The exclusion criteria included any metabolic or chronic neurological disease, bipolar disorder, schizoaffective disorder, or schizophrenia.

4.2.3 Isolation of DNA and Marker Genotyping

Blood samples were drawn from the veins of the probands and their family members in two 10 cc EDTA tubes, and genomic DNA was extracted from lymphocytes using the high salt method as described in Lahiri and Nurnberger (Lahiri and Nurnberger 1991). The Ser-491-Ser polymorphism was genotyped according to Barr et al. (Barr et al. 2000a). For the A-

7265G (Assays-on-Demand Assay ID: C_2986495_10), C10497G (C_2986501_1_), Phe-659-Phe (C_596215_1_), and A33795G (C_2500843_10) markers, genotypes were assessed by the TaqMan allele specific assay method using the ABI Prism® 7000 Sequence Detection System according to the manufacturer's protocols (Applied Biosystems, Foster City, CA). All genotypes were scored with the allelic discrimination program using the ABI software and confirmed by two experienced researchers.

4.2.4 Statistical Analyses

We tested for an association between alleles of the A-7265G, C10497G, Ser-453-Ser, Phe-659-Phe, and A33795G polymorphisms and OCD using the transmission disequilibrium test (TDT) with the TDT-STDT program (version 1.1) (Spielman and Ewens 1998). Haplotype analysis of the combined polymorphisms was applied using the TRANSMIT program (version 2.5) (Clayton and Jones 1999). We also performed the family-based association test (FBAT, version 1.0) (Laird et al. 2000) to allow for the analysis of both qualitative and quantitative traits. We applied FBAT under the assumption of an additive model when considering the diagnosis of OCD and quantitative phenotypes including the Y-BOCS score and age at onset. The PBAT program (Lange et al. 2002) was used to determine the power for FBAT analyses. Linkage disequilibrium (LD) was tested using the LDMAX program (Excoffier and Slatkin 1995). All of the results were assumed to be significant if $P<0.05$. Uncorrected *p*-values were given because a Bonferroni correction for multiple comparisons is too conservative given the non-independence of the markers due to high degree of intermarker LD.

4.3 Results

Our sample of OCD patients has a mean age of 34.9±10.7 years, a mean age at onset of 14.7±9.0 years (N=121), and a mean total current Y-BOCS score of 22.2±8.1 (N=103). The sample is comprised of 96.8% Caucasian, 1.6% Asian, and 1.6% African Americans.

A trend of over-transmission of allele 2 (-7265A) to the affected probands was observed for the promoter SNP, A-7265G (χ^2=3.270, P=0.071), in the TDT analysis alone (Table 11). The combined TDT and S-TDT results indicate a significant over-transmission of allele 2 (-7265A) with a z-score of 2.494, which corresponds to a p-value of 0.006. Furthermore, a significant combined score was detected in allele 2 (A) of the Phe-659-Phe polymorphism (z=1.859, P=0.032). However, no significant results were observed for the other three polymorphisms (Table 11), although a trend in the combined score was detected at allele 1 (T) of the Ser-491-Ser polymorphism (z=1.458, P=0.072). For the transmission of haplotypes in the GABBR1 gene (Table 12), we observed that haplotype 2.1.1.2.1 (A-7265G.C10497G.Ser-491-Ser.Phe-659-Phe.A33795G) was transmitted more often from parents to their affected offspring with a trend to significance (χ^2=3.418, P=0.065). FBAT analysis of quantitative traits revealed a trend (Table 13) for allele 2 (A) in A-7265G for total Y-BOCS score (z=1.934, P=0.053) and compulsion sub-scale score (z=1.806, P=0.071).

The following GABBR1 polymorphisms have shown significant LD: A33795G and Phe-659-Phe (D'=1.000, P<0.0001), A33795G and Ser-491-Ser (D'=1.000, P=0.001), A33795G and C10497G (D'=1.000, P<0.0001), A33795G and A-7265G (D'=0.926, P<0.0001), Phe-659-Phe and Ser-491-Ser (D'=1.000, P<0.0001), Phe-659-Phe and C10497G (D'=0.926, P<0.0001), Ser-491-Ser and C10497G (D'=1.000, P=0.002), Ser-491-Ser and A-7265G (D'=1.000, P=0.003).

Table 11. Transmission disequilibrium test (including STDT) for the five polymorphisms in the GABBR1 gene with the diagnosis of OCD as the affection trait

[a] T is the number of transmission from heterozygous parents to affected proband.

[b] NT is the number of non-transmission from heterozygous parents to affected proband.

[c] Number of transmission from heterozygous parents to affected offspring.

[d] Transmitted + Y.

			TDT				STDT				Combined Scores				
Marker	Allele	Frequency	T [a]	NT [b]	χ^2	*P*	Y [c]	Mean (A)	Var (V)	Z'	W [d]	Mean (A)	Var (V)	Z'	*P*
A-7265G	1 (G)	0.181	13	24	3.270	0.071	9	9.167	2.994	-0.193	22	27.667	12.244	1.477	0.070
A-7265G	2 (A)	0.819	24	13	3.270	0.071	35	29.667	7.911	1.718	59	48.167	17.161	2.494	0.006
C10497G	1 (G)	0.281	28	28	0.000	1.000	11	12.833	3.472	0.716	39	40.833	17.472	0.319	0.375
C10497G	2 (C)	0.719	28	28	0.000	1.000	33	28.000	6.944	1.708	61	56.000	20.944	0.983	0.163
Ser-491-Ser	1 (T)	0.903	13	10	0.391	0.532	27	22.250	9.799	1.358	40	33.750	15.549	1.458	0.072
Ser-491-Ser	2 (C)	0.097	10	13	0.391	0.532	5	4.417	2.354	0.054	15	15.917	8.104	0.146	0.442
Phe-659-Phe	1 (G)	0.188	15	25	2.500	0.114	6	6.750	3.021	0.144	21	26.75	13.021	1.455	0.073
Phe-659-Phe	2 (A)	0.812	25	15	2.500	0.114	32	28.583	8.132	1.023	57	48.583	18.132	1.859	0.032
A33795G	1 (A)	0.692	31	32	0.016	0.899	40	38.583	11.104	0.275	71	70.083	26.854	0.080	0.468
A33795G	2 (G)	0.308	32	31	0.016	0.899	18	15.75	7.160	0.654	50	47.25	22.910	0.470	0.319

Table 12. TDT haplotype analysis across the five polymorphisms in the GABBR1 gene with the diagnosis of OCD as the affection trait

[a] Rare haplotypes with frequency less than 5% have been excluded.

[b,c] Observed and expected transmission of haplotype from parents to the probands.

[d] Variance (difference between observed and expected transmissions).

[e] Global χ^2=6.353; 5 df; P=0.273.

Haplotype (A-7265G.C10497G.Ser-491-Ser.Phe-659-Phe.A33795G) [a]	Haplotype Frequency	Observed [b]	Expected [c]	Var (O-E) [d]	χ^2 (1 df)	P [e]
2.1.1.2.1	0.217	91.622	82.052	26.803	3.418	0.065
2.2.2.1.1	0.092	33.910	32.316	11.608	0.219	0.640
1.2.1.2.1	0.086	28.019	30.032	10.123	0.400	0.527
2.2.1.2.1	0.169	63.665	60.991	19.243	0.372	0.542
2.2.1.2.2	0.288	100.02	105.77	29.107	1.137	0.286

Table 13. Selected results of FBAT analysis of the GABBR1 gene with quantitative measures

[a] S represents the test statistic for observed number of alleles.

[b] E represents the expected value of S under null hypothesis.

[c] Variance between the observed and expected transmission.

[d] These are bi-allelic markers and therefore both alleles have the same *z* score and only the positive *z*-score has been recorded.

Quantitative Trait	Marker Polymorphism	Allele	S [a]	E (S) [b]	Var (O-E) [c]	*z*-score	*P*
Obsession Score	A-7265G	2 [d]	553.000	507.000	978.444	1.471	0.141
Compulsion Score	A-7265G	2 [d]	576.000	517.500	1049.194	1.806	0.071
Total Y-BOCS Score	A-7265G	2 [d]	1007.000	888.000	3784.278	1.934	0.053
Total Y-BOCS Score	C10497G	2 [d]	1155.000	1094.000	5180.000	0.848	0.397
Total Y-BOCS Score	Ser-491-Ser	2 [d]	225.000	183.167	1995.472	0.936	0.349
Total Y-BOCS Score	Phe-659-Phe	1 [d]	519.000	506.667	3909.500	0.197	0.844
Total Y-BOCS Score	A33795G	1 [d]	1028.000	1010.000	4369.500	0.272	0.785
Age at Onset	A-7265G	1 [d]	489.000	435.500	3010.917	0.975	0.330
Age at Onset	C10497G	1 [d]	629.000	611.833	3709.194	0.282	0.778
Age at Onset	Ser-491-Ser	1 [d]	282.000	257.667	758.056	0.884	0.377
Age at Onset	Phe-659-Phe	2 [d]	648.000	613.000	2148.000	0.755	0.450
Age at Onset	A33795G	1 [d]	783.000	757.667	2795.889	0.479	0.632

4.4 Discussion

This is the first attempt to perform an association study of the phenotype/allele relationship between the diagnosis of OCD and the variants of the GABBR1 gene using the TDT and FBAT methodologies. Our tests for association between GABBR1 and the diagnosis of OCD showed significant biased transmission of the –7265A allele when the transmission of alleles in the affected siblings was taken into account (combined TDT-STDT). Also, the Phe-659-Phe marker showed a significant biased transmission of the A allele for increased risk in the combined TDT-STDT analysis (Table 11). Moreover, a borderline association was also observed between a GABBR1 haplotype (2.1.1.2.1) and OCD (Table 12). It remains unclear whether the GABBR1 gene itself is the putative genetic risk site, or if the risk is conferred by another HLA-related site in linkage disequilibrium with this A-7265G marker.

In the quantitative trait analysis with the total Y-BOCS severity score and the compulsion sub-scale score, we found an association with the –7265A polymorphism (Table 13) and elevated scores on these measures. Our results suggest that GABBR1 may have important implications for OCD although our findings were not strong enough to make any firm conclusions. To our knowledge, this is the first attempt to study variants in the GABBR1 gene in OCD. Further study of other genes involved in inhibitory neurotransmission, particularly in cortical-striatal-thalamic circuitry is warranted.

Our genetic GABA finding supports the parallel neurobiological work by Krogsgaardlarsen et al. (Krogsgaardlarsen et al. 1997) that indicates the role of GABA in OCD.

Since the $GABA_B$ receptor plays an important role in signal transductions such as

transcriptional activity, extending genetic analyses of the pathway to include examining variants within the GPR51 gene might provide further insights in the involvement of the $GABA_B$ system in the development of OCD. This is because the GPR51 gene, which has been localized to chromosome 9q, encodes the GPR51 protein that must interact with GABBR1 as a heterodimer to form a functional $GABA_B$ receptor.

Our study presents an intriguing finding of positive association of GABBR1 markers with OCD and may point to a pathophysiological mechanism for the disorder. Although our current sample size did achieve a power of 80% to detect a relative risk as low as 3.5, larger and well-characterized samples are needed, in order to extend these results. More work is required to better delineate subgroups of OCD, and to define in more detail the role of GABA system genes in the etiology of OCD and its related disorders.

CHAPTER 5: Possible Association between the Gamma-Amino-Butyric Acid Type B Receptor 1 (GABBR1) Gene and Schizophrenia

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Ms. Zai has performed all of the experiments including genotyping and statistical analyses and she has written all the materials in this manuscript.

5.1 Introduction

Schizophrenia (SCZ), which affects approximately 1% of the general population, is a serious neuropsychiatric disorder that is characterized by diverse and variably expressed symptoms, which include disorganized thought pattern and behaviours, delusional beliefs, auditory hallucinations, cognitive difficulties, apathy and social withdrawal. Family, twin, and adoption studies support a strong genetic component to the etiology of SCZ (Bray and Owen 2001). The cause of SCZ remains unknown. While the most enduring theory for the etiology of SCZ is the dopaminergic hypothesis due to the role of the dopaminergic receptors as sites for anti-psychotic drug action, (van Rossum 1966; Carlsson 1978; Baron 2001), other neurotransmitter systems might also be involved in the etiology of SCZ.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). GABA was first implicated in the pathophysiology of SCZ via a defect in the inhibitory GABAergic neurons' control of neural circuits governing behavioural responses, proposed by Eugene Roberts (Roberts 1972). An abnormality in GABAergic regulation of dopamine cell burst firing has been postulated to underlie the symptoms of SCZ (Moore et al. 1999). Olney and Farber (Olney and Farber 1995) developed a model of SCZ in which a state of "NMDA receptor hypofunction" is caused by either intrinsically hypofunctioning NMDA receptors or through excitotoxic loss of NMDA receptor-bearing GABAergic neurons. Disruption of interactions between the cholinergic system and the serotonin (5-HT) 2A receptor on GABAergic interneurons in the frontal cortex (Dean 2001) also has been proposed in the pathophysiology in SCZ. Effects of the GABAergic system in neuro- and in particular cortico-developmental processes have been integrated into etiologic hypotheses of psychosis and SCZ (Keverne 1999; Reynolds and

Beasley 2001).

The two distinct receptor types that GABA interacts with to achieve its effects via the alteration of intracellular signal transduction are the ionotrophic $GABA_A$ and $GABA_C$ receptors. These are associated with Cl^- channels and are involved in fast inhibitory synaptic transmission. The metabotrophic GABA type B ($GABA_B$) receptors are associated with K^+/Ca^{2+} channels and act via guanosine 5'-triphosphate binding (G-) proteins to produce slow, prolonged inhibitory signals (Bowery 2000; Olsen and Homanics 2000). The $GABA_B$ receptors are highly and almost exclusively expressed in the human brain in regions such as the thalamic nuclei, the molecular layer of the cerebellum, the cerebral cortex, the interpeduncular nucleus, and the dorsal horn of the spinal cord (Bowery et al. 1987; Chu et al. 1990). The $GABA_B$ receptor is thought to play a physiological role in the modulation of GABA release via an autoreceptor and by release of other neurotransmitters [e.g. noradrenaline (Bowery et al. 1980), 5-hydroxytryptamine (Gray and Green 1987), cholecystokinin (Conzelmann et al. 1986), and somatostatin (Bonanno et al. 1991)].

The functional $GABA_B$ receptor comprises of a heterodimer of two related 7-transmembrane subunits, $GABA_B$ 1 (GABBR1) (NM_001470) and $GABA_B$ 2 (GABBR2) or G-protein-coupled receptor 51 (GPR51). The GABBR1 gene (Genebank NP_001461; MIM# 603540) has been mapped to the chromosomal region 6p21.3 (Goei et al. 1998), 130 Kb telomeric to the HLA-F (human leukocyte antigen-F) locus, where linkage to SCZ has been detected in several independent studies (Antonarakis et al. 1995; Schwab et al. 1995; Wang et al. 1995; Lindholm et al. 1999).

With the physiologic significance of the $GABA_B$ receptor to neurobiology including its known role in synaptic inhibition, its tissue pattern of expression, and the genetic

localization in a proposed candidate susceptibility region, GABBR1 is considered an attractive candidate gene for neuro-behavioural disorders. Thus, the purpose of our study is to examine the presence of an association between markers in the GABBR1 gene and SCZ. We tested five biallelic single nucleotide polymorphisms (SNPs) located within the GABBR1 gene in this study, including A-7265G – an A → G base exchange at position 7265 in the promoter region (rs29218), C10497G – a C → G base exchange at position 10497 in intron 9 (rs29220), Ser-491-Ser – a T → C substitution at exon 12 (rs29225), Phe-659-Phe – an A → G substitution at exon 16 (rs29230), and A33795G – an A → G exchange in the 3'-UTR (untranslated region) (rs3095273). We genotyped a sample of 101 small nuclear families and 150 case-control pairs matched for gender, age, and ethnicity, and examined alleles of these five polymorphisms for evidence of biased transmission in the families and differences in allelic and genotype distributions between SCZ cases and healthy controls in order to determine whether variants of GABBR1 are associated with SCZ.

5.2 Methods

5.2.1 Clinical Diagnostic Criteria

One hundred and fifty SCZ unrelated patients and 101 SCZ probands plus available first-degree relatives were recruited. The experienced clinical team performed structured diagnostic interviews on probands with the Structured Clinical Interview for DSM-IV (SCID-I) (First et al. 1996), which was used as the primary diagnostic tool for this study. A clinical narrative summary that provides a more detailed phenotype description and information about the context, severity, and sequence of symptoms during the illness was prepared for each patient (Maxwell 1992). Research psychiatrists then reviewed the SCID

interview response, the clinical summary, and the medical records. Evidence for the patient meeting DSM-IV criteria was reviewed, diagnostic uncertainties were discussed, and a best-estimate consensus diagnosis was reached (Endicott 2001). A third psychiatrist performed review to make the final decision for the diagnostic packages wherein disagreement occurred. The inclusion criteria for adult probands were DSM-IV diagnosis of SCZ, or depressed-type schizoaffective disorder. The exclusion diagnostic criteria included history of drug dependence or history of drug-induced psychosis, major neurological disorder including epilepsy, or head injury with significant loss of consciousness. We characterized age at onset (AAO) for the purposes of this study as defined as the age at which the subject was first hospitalized for a psychotic episode. Participating parents and/or siblings validated all the information provided by the probands regarding their AAO whenever possible.

5.2.2 Samples

The analysis at the five markers included 32 triads, 7 triads and a sibling, 43 diads, 8 families with a single parent and a sibling, and 11 families with an affected proband and a sibling, thus a total of 101 families was analyzed. For the case-control analyses, 150 case/control (gender, age ± 5 years, and ethnicity-matched) pairs were analyzed.

5.2.3 Isolation of DNA and Marker Genotyping

Blood samples was obtained from the veins of the probands and their family members in two 10 cc EDTA tubes, and the genomic DNA was extracted from lymphocytes using the high salt method as described in Lahiri and Nurnberger (Lahiri and Nurnberger 1991). We genotyped the Ser-491-Ser polymorphism according to the protocol of Barr et al. (Barr et al.

2000a). Genotypes for the A-7265G (Assays-on-Demand ID: C_2986495_10), C10497G (C_2986501_1_), Phe-659-Phe (C_596215_1_), and A33795G (C_2500843_10) markers were assessed by the TaqMan allele specific assay method using the ABI Prism® 7000 Sequence Detection System according to the manufacturer's protocols (Applied Biosystems, Foster City, CA). All genotypes were scored with the allelic discrimination program of the ABI software.

5.2.4 Statistical Analyses

Using the transmission disequilibrium test (TDT) with the TDT-STDT program (version 1.1) (Spielman and Ewens 1998), we tested for an association between alleles of the A-7265G, C10497G, Ser-453-Ser, Phe-659-Phe, and A33795G polymorphisms and the diagnosis of SCZ. Power for TDT analyses was determined with the TDT Power Calculator (version 1.2.1) (Chen and Deng 2001) and power for case-control analyses was determined with the Genetic Power Calculator (Purcell et al. 2003). Using the TRANSMIT program (version 2.5) (Clayton and Jones 1999), haplotype analysis of the combined polymorphisms was performed. To assess the role of the GABBR1 gene in the onset of SCZ, we have performed the Kruskal-Wallis Test to test for association between genotype and the mean AAO of the affected probands using SPSS version 10.0 (SPSS Inc., Chicago, IL). Linkage disequilibrium (LD) was calculated with the LDMAX program (Excoffier and Slatkin 1995). For the case-control analyses, Pearson χ^2 test (Fisher's Exact Test) using SPSS version 10.0 (SPSS Inc.), Monte Carlo test using CLUMP program (Sham and Curtis 1995), odds ratio (OR) and confidence interval (CI) using 2BY2 program version 1.50 (Ott 1999), and Hardy Weinberg Equilibrium (HWE) using the HWE program (Ott 1999) were performed.

Conversion of the χ^2 values from the TDT and case-control analyses into z-scores is obtained from the following equation: for 1 degree of freedom test: $\sqrt{\chi^2}$ = z-score. A corrected combined z-score was then calculated using the z-scores from the TDT and case-control analyses [$(z_{case\text{-}control} + z_{TDT})/\sqrt{2}$]. Statistical analyses in this study were based on $P<0.05$ as significant and results were uncorrected for multiple comparisons due to the high degree of intermarker LD and the inappropriately high conservativeness of the Bonferroni correction in this non-independence of tests situation.

5.3 Results

The SCZ sample is comprised of 95.0% Caucasian, 3.0% Asian, and 2.0% African Americans. The TDT results are presented in Table 14 for the polymorphisms within the GABBR1 gene. We did not observe any biased transmission of alleles at all five GABBR1 markers. The TDT haplotype analysis was not significant (Table 15). Phenotypic analyses comparing mean AAO in each genotype were also not significant at A-7265G (χ^2=2.241, 2 df, P=0.326), C10497G (χ^2=1.152, 2 df, P=0.562), Ser-491-Ser (χ^2=1.777, 2 df, P=0.411), Phe-659-Phe (χ^2=0.064, 2 df, P=0.969), A33795G (χ^2=0.510, 2 df, P=0.775) within the GABBR1 gene.

The following GABBR1 polymorphisms showed strong LD: A33795G and Phe-659-Phe (D'=1.000, P=0.005), A33795G and Ser-491-Ser (D'=0.997, P=0.118), A33795G and C10497G (D'=1.000, $P<0.0001$), A33795G and A-7265G (D'=0.817, P=0.004), Phe-659-Phe and Ser-491-Ser (D'=1.000, $P<0.0001$), Phe-659-Phe and C10497G (D'=1.000, $P<0.0001$), Ser-491-Ser and C10497G (D'=1.000, P=0.001), Ser-491-Ser and A-7265G (D'=1.000, P=0.032).

For the case-control analysis in the GABBR1 gene, the allelic χ^2 test was significant (χ^2=4.310, *P*=0.038; OR=0.63, 95% CI=0.42-0.96, *P*=0.038; Table 16) and the genotype χ^2 almost reached a significant level (χ^2=4.970, 2 df, *P*=0.083) for the A-7265G polymorphism. However, the other four GABBR1 polymorphisms did not show significant difference between allelic and genotype frequencies (Table 16) although a trend was detected for the allelic frequencies at the Ser-491-Ser polymorphism (χ^2=2.940, *P*=0.086). The combined *z*-scores for A-7265G (*z*=1.468, *P*=0.071) and C10497G (*z*=1.346, *P*=0.089) polymorphisms showed a trend and a significant association for the Ser-491-Ser polymorphism (*z*=1.712, *P*=0.043). However, the combined *z*-scores for the Phe-659-Phe (*z*=0.582, *P*=0.280) and A33795G (*z*=1.051, *P*=0.147) polymorphisms were not significant.

Table 14. Transmission disequilibrium test (including STDT) for the five polymorphisms in the GABBR1 gene with the diagnosis of SCZ as the affection trait

[a] T is the number of transmission from heterozygous parents to affected proband.

[b] NT is the number of non-transmission from heterozygous parents to affected proband.

[c] Number of transmission from heterozygous parents to affected offspring.

[d] Transmitted + Y.

		TDT					STDT				Combined Scores				
Marker	Allele	Frequency	T [a]	NT [b]	χ^2	*P*	Y [c]	Mean (A)	Var (V)	Z'	W [d]	Mean (A)	Var (V)	Z'	*P*
A-7265G	1 (G)	0.204	10	10	0.000	1.000	0	0.000	0.000	N/A	10	10.000	5.000	-0.224	0.411
A-7265G	2 (A)	0.796	10	10	0.000	1.000	0	0.000	0.000	N/A	10	10.000	5.000	-0.224	0.411
C10497G	1 (G)	0.326	9	15	1.500	0.221	0	0.000	0.000	N/A	9	12.000	6.000	1.021	0.154
C10497G	2 (C)	0.674	15	9	1.500	0.221	0	0.000	0.000	N/A	15	12.000	6.000	1.021	0.154
Ser-491-Ser	1 (T)	0.853	5	3	0.500	0.480	12	17.267	10.029	1.505	17	21.267	12.029	1.086	0.139
Ser-491-Ser	2 (C)	0.147	3	5	0.500	0.480	2	2.667	1.722	0.127	5	6.667	3.722	0.605	0.273
Phe-659-Phe	1 (G)	0.220	14	16	0.133	0.715	0	0.000	0.000	N/A	14	15.000	7.500	0.183	0.427
Phe-659-Phe	2 (A)	0.780	16	14	0.133	0.715	0	0.000	0.000	N/A	16	15.000	7.500	0.183	0.427
A33795G	1 (A)	0.780	10	9	0.053	0.818	0	0.000	0.000	N/A	10	9.500	4.750	0.000	0.500
A33795G	2 (G)	0.220	9	10	0.053	0.818	0	0.000	0.000	N/A	9	9.500	4.750	0.000	0.500

Table 15. TDT haplotype analysis across the five polymorphisms in the GABBR1 gene with the diagnosis of SCZ as the affection trait

[a] Rare haplotypes with frequency less than 5% have been excluded.

[b,c] Observed and expected transmission of haplotype from parents to the probands.

[d] Variance (difference between observed and expected transmissions).

[e] Global χ^2=5.782, 6 df, P=0.448.

Haplotype (A-7265G.C10497G.Ser-491-Ser.Phe-659-Phe.A33795G) [a]	Frequency	Number of Families	Observed [b]	Expected [c]	Var (O-E) [d]	χ^2 (1 df)	P [e]
1.1.1.2.1	0.052	6	12.799	12.338	3.941	0.054	0.816
1.1.2.2.2	0.133	14	26.903	28.669	10.460	0.298	0.585
1.2.1.1.1	0.095	10	23.115	21.141	6.022	0.647	0.421
1.2.1.1.2	0.223	24	43.346	47.318	12.025	1.312	0.252
1.2.1.2.2	0.183	20	35.910	39.855	11.436	1.361	0.243
2.2.1.2.2	0.227	25	57.327	49.915	14.675	3.744	0.053

Table 16. Case-control analyses of the five polymorphisms in the GABBR1 gene

[a] HWE test for SCZ cases: χ^2=1.349, *P*=0.245 and HWE for healthy controls: χ^2=0.079, *P*=0.778; OR=0.63, 95% CI=0.42-0.96, *P*=0.038.

[b] HWE test for SCZ cases: χ^2=0.074, *P*=0.785 and HWE for healthy controls: χ^2=2.806, *P*=0.094; OR=1.16, 95% CI=0.79-1.70, *P*=0.498.

[c] HWE test for SCZ cases: χ^2=0.175, *P*=0.676 and HWE for healthy controls: χ^2=0.083, *P*=0.774; OR=0.63, 95% CI=0.39-1.03, *P*=0.086.

[d] HWE test for SCZ cases: χ^2=0.015, *P*=0.903 and HWE for healthy controls: χ^2=1.570, *P*=0.210; OR=0.96, 95% CI=0.64-1.44, *P*=0.917.

[e] HWE test for SCZ cases: χ^2=1.040, *P*=0.308 and HWE for healthy controls: χ^2=0.074, *P*=0.785; OR=1.11, 95% CI=0.74-1.66, *P*=0.681.

Marker	Allele and Genotype	Cases (N = 150)	Controls (N = 150)	χ^2 (df)	*P*
A-7265G [a]	1	48	69	4.310 (1)	0.038
	2	236	215		
	1/1	6	9		
	1/2	36	51	4.970 (2)	0.083
	2/2	100	82		
C10497G [b]	1	74	66	0.460 (1)	0.498
	2	218	226		
	1/1	10	11		
	1/2	54	44	1.540 (2)	0.463
	2/2	82	91		
Ser-491-Ser [c]	1	242	257	2.940 (1)	0.086
	2	46	31		
	1/1	101	115		
	1/2	40	27	3.630 (2)	0.149
	2/2	3	2		
Phe-659-Phe [d]	1	56	58	0.040 (1)	0.841
	2	244	242		
	1/1	5	8		
	1/2	46	42	0.880 (2)	0.644
	2/2	99	100		
A33795G [e]	1	187	182	0.170 (1)	0.680
	2	61	66		
	1/1	76	69		
	1/2	35	44	1.530 (2)	0.465
	2/2	13	11		

5.4 Discussion

We have performed an innovative association study of the phenotype/allele relationship between the diagnosis of SCZ and the variants of the GABBR1 gene. We investigated the possibility that SCZ is associated with the GABBR1 gene using the TDT and case-control methodologies. We did not observe biased transmission of alleles in any of the five polymorphisms examined with the diagnosis of SCZ. We did not find an association in the quantitative trait analysis with AAO with SCZ. However, we did observe a significantly greater count of allele 2 (-7265A) and less count of allele 1 (-7265G) at the A-7265G polymorphism in SCZ patients when compared with healthy controls (P=0.038, Table 16), suggesting that the variant –7265A allele might predispose a person to risk for SCZ or the variant –7265G allele might have a protective role against the development of SCZ. Nonetheless, the GABBR1 locus is still valuable to the genetic study of SCZ due to its functional role in the GABAergic system, although it is uncertain in our current sample whether the GABBR1 gene alone or another HLA-related site in LD with the GABBR1 markers is the putative genetic risk site for SCZ.

Our research focused on the contribution of the GABBR1 gene variants to the risk for SCZ. This gene has been implicated in the etiology of several neurobehavioural disorders such as SCZ, juvenile myoclonic epilepsy, and dyslexia, and has been localized just distal to the HLA class I region. To our knowledge, this is the first attempt to study the variant in the GABBR1 gene in SCZ. Based on the solid rationale described in the Introduction, the need for further study of GABA system genes in SCZ remains an important goal.

Since the $GABA_B$ receptor functions in the GABAergic neurotransmission system and is involved in signal transductions such as transcriptional activity, examining markers in

the GPR51 gene might assist us to understand the involvement of $GABA_B$ receptor variant in the development of SCZ. This GPR51 gene, which is mapped to chromosome 9q, encodes the other protein that forms the functional heterodimer $GABA_B$ receptor, GPR51 or GABBR2.

Our TDT sample of 101 families has 80% power to detect a significant effect with a genotypic relative risk as low as 3.1. Gene variants of small effect or those that are rare major determinants of risk for developing SCZ would not be detected in our TDT sample. Thus, larger studies, which take into account confounding factors, subgroups, and heterogeneity, are needed to clarify these issues. Our sample of 150 controls and 150 cases has 81% power to detect an odds ratio as low as 1.9 with 95% certainty. This case-control study presents an intriguing finding of slight positive association of the GABBR1 marker with SCZ and may suggest a pathophysiological explanation in SCZ. We have chosen to use a combined case-control and TDT approach and we have calculated the power of our data set, which is of moderate size. We have converted the TDT samples into case-control samples (N>99) and combined them with the original case-control samples (N=150), and the combined sample (N>249) has a power of 95% to detect a relative risk as low as 1.9. In conclusion, well-characterized and larger samples are needed, in order to replicate and extend these results including better delineation of phenotype subgroups of SCZ.

CHAPTER 6: Other Results

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6.1 Haplotype Blocks

There are abundant single-nucleotide polymorphisms (SNPs) within the human genome that permit geneticists to screen for diseases mutations. High linkage disequilibrium between close-by SNPs allows for maximum coverage within the chromosomal region with minimum number of SNPs due to allelic dependence that yields redundancy among markers. Knowledge of the haplotype structure would therefore be useful to uniquely tag all the haplotypes to search for disease mutations (Johnson et al. 2001). Haplotype block is typically defined based on the concept of "chromosome coverage" with a haplotype block containing a minimum number of SNPs that account for a majority of common haplotypes (Patil et al. 2001; Zhang et al. 2002), or a reduced level of haplotype diversity (Daly et al. 2001). There is an inverse relationship between the LD-based definitions; low haplotype diversity is associated with increased LD. The eventual goal of identifying haplotype blocks is to minimize genotyping while maximizing information content, and does not contain an explicit reference to the structure of the LD. The answer of whether a haplotype exists requires more extensive studies and replications; nonetheless, haplotype blocks have been hypothesized by a number of independent research groups (Jeffreys et al. 2001; Cullen et al. 2002; Dawson et al. 2002; van den Oord and Neale 2004). Although haplotype blocks do not appear to be discrete entities with clear-cut boundaries, there is evidence that LD in some regions of the human genome can be modelled via a block structure.

In the present study, we have identified haplotype blocks for two-disease populations, OCD and SCZ, using the simple Solid Spine of LD method in the Haploview program (version 2.04, http://www.broad.mit.edu/personal/jcbarret/haploview/). This method allows for the identification of haplotype blocks or search for a "spine" of strong LD from adjacent

SNPs. SNPs that have strong LD form a haplotype block. In our disease populations, they have the same haplotype block structures (Figures 9 and 10). It is worthwhile to note that there is a separation of blocks between the GABBR1 gene, more specifically between the promoter region SNP, A-7265G, and the intronic SNP, C10497G. When compared to the HapMap haplotype block structure in this region, we found that they were similar.

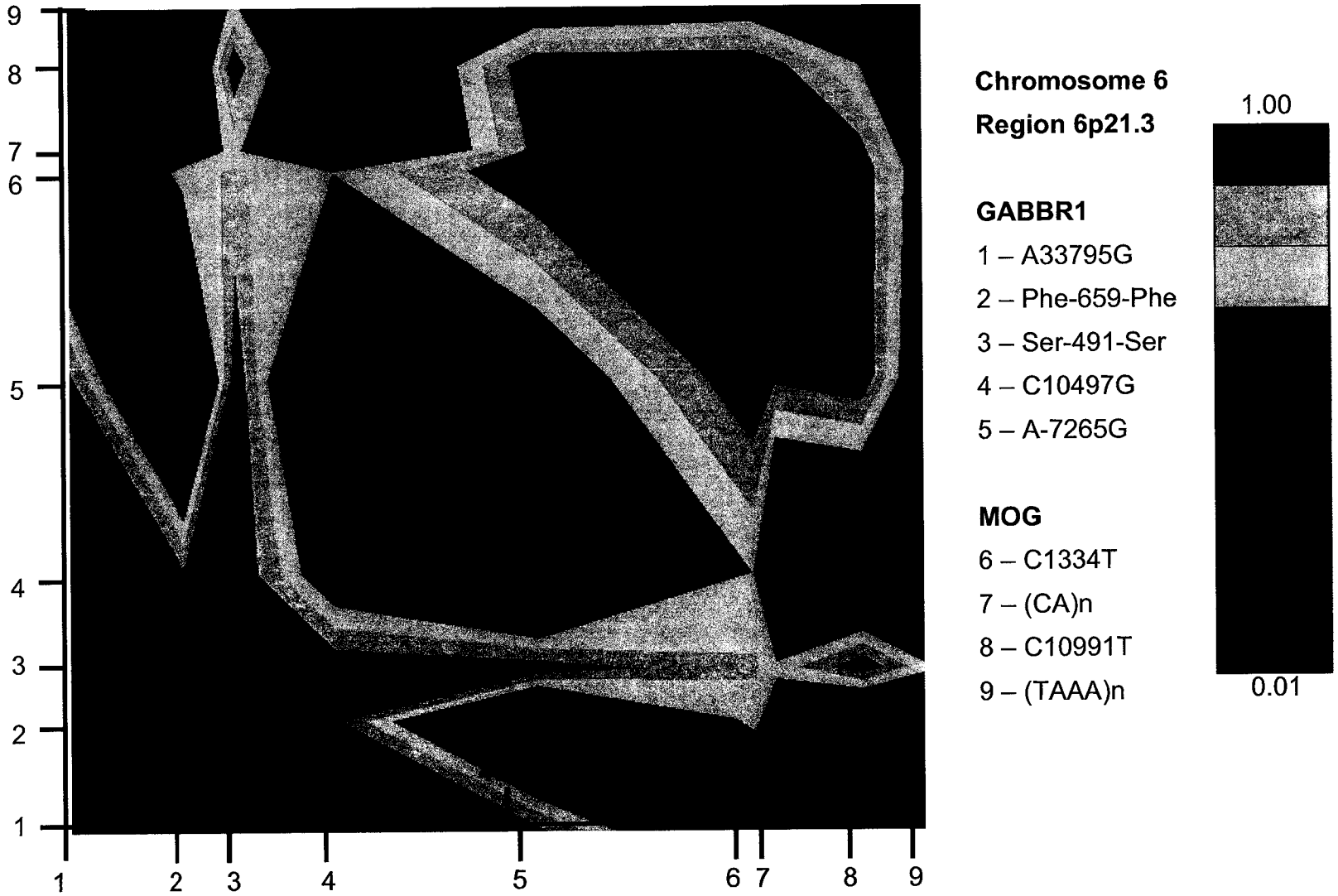
Sample size may potentially influence the validity of inferred haplotype structure, since D' estimates appear to become inflated in small samples and for unbalanced allele frequencies (Teare et al. 2002). However, since both samples in this study have yielded the same haplotype blocks as the HapMap project, our sample size for both populations is sufficient to provide reliable and valid results.

Our results on the LD distribution in the area containing the MOG and GABBR1 genes on chromosomal region 6p21.3 should prove useful in the efficient assembly of SNP panels for association studies of OCD and SCZ, which are genetically linked to this region. Haplotype blocks clearly represent a step forward in optimizing association studies. They have also directed the attention to the potential importance of LD patterns and resulted in the development of techniques for maximizing the genetic information with a minimum number of SNPs. General public haplotype maps have the potential to further facilitate LD studies that are aimed at identifying more disease mutations. This will be very important to advance our genetic knowledge of complex diseases. Nevertheless, currently, the empirical data that are required to evaluate these conditions are lacking, so that time will tell as to what extent this potential of public haplotype maps can be realized.

6.2 Haplotype Analyses with TDTPHASE

Since we have determined the haplotype block for the markers spanning the MOG and GABBR1 genes, we have performed a haplotype analysis utilizing three and four markers at a time with the migrating window method using the TDTPHASE program (version 2.40) (Dudbridge 2003). Our results for this analysis showed a trend for the haplotype with three markers of MOG, C1334T, C10991T, and (TAAA)n (P=0.06947) in our OCD sample but none of the other haplotype analyses with other markers were significant. Interestingly, we detected a trend in the haplotype analyses with three GABBR1 markers, Ser-491-Ser, Phe-659-Phe, and A33795G (P=0.081) in our SCZ sample, which supports our findings with TDT and case-control analyses. Nonetheless, with four-markers migrating window method, we did not observe any significant results with either sample.

Figure 9a. Linkage disequilibrium across the 9 polymorphisms within the MOG and GABBR1 genes with the diagnosis of OCD as the affection trait



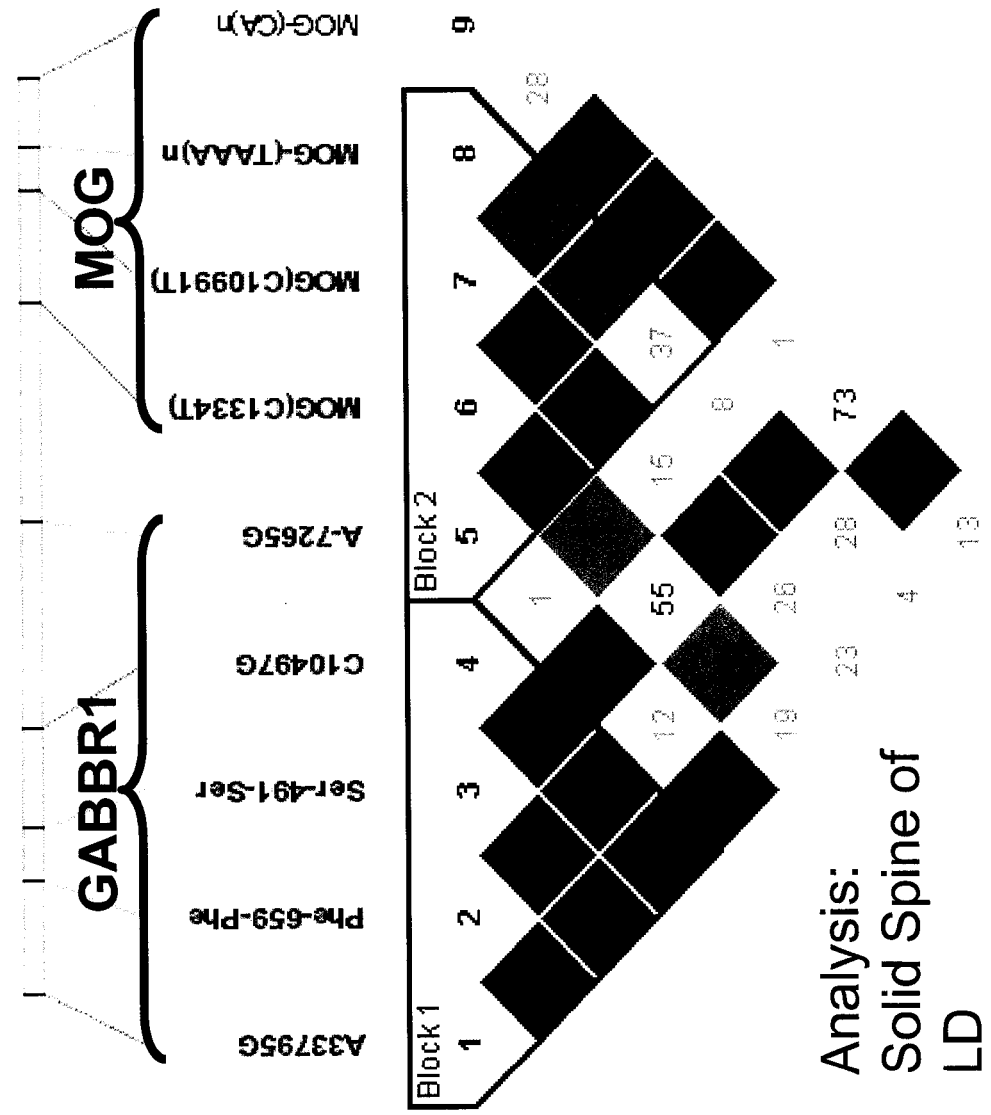
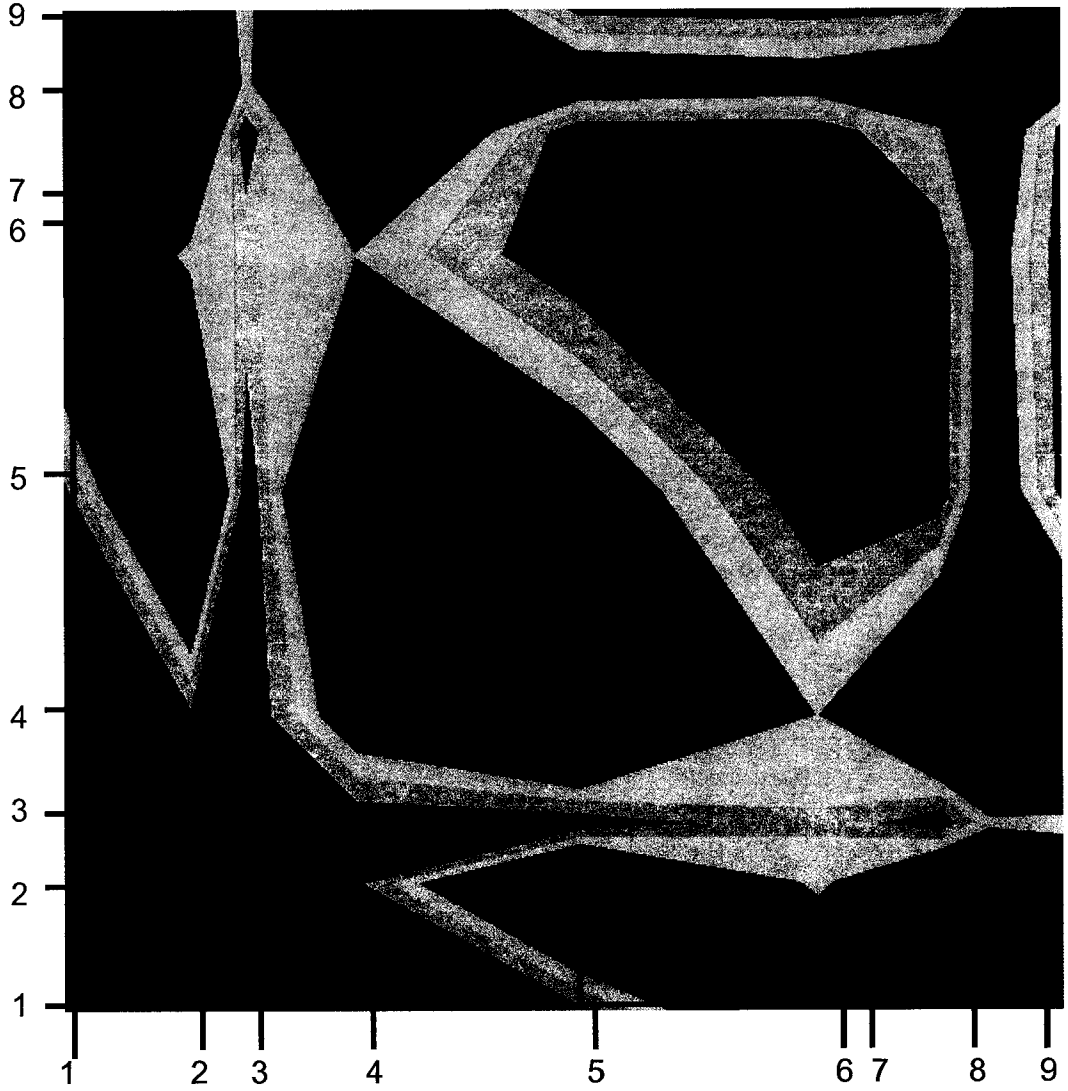


Figure 9b. Linkage disequilibrium across the 9 polymorphisms within the MOG and GABBR1 genes with the diagnosis of OCD as the affection trait

Figure 10a. Linkage disequilibrium across the 9 polymorphisms within the MOG and GABBR1 genes with the diagnosis of SCZ as the affection trait



Chromosome 6

Region 6p21.3

GABBR1

1 – A33795G

2 – Phe-659-Phe

3 – Ser-491-Ser

4 – C10497G

5 – A-7265G

MOG

6 – C1334T

7 – (CA)n

8 – C10991T

9 – (TAAA)n

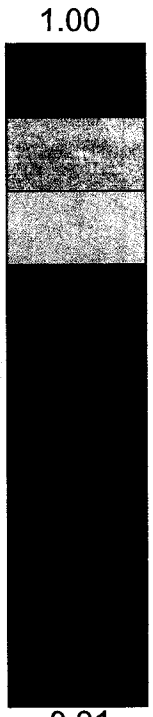
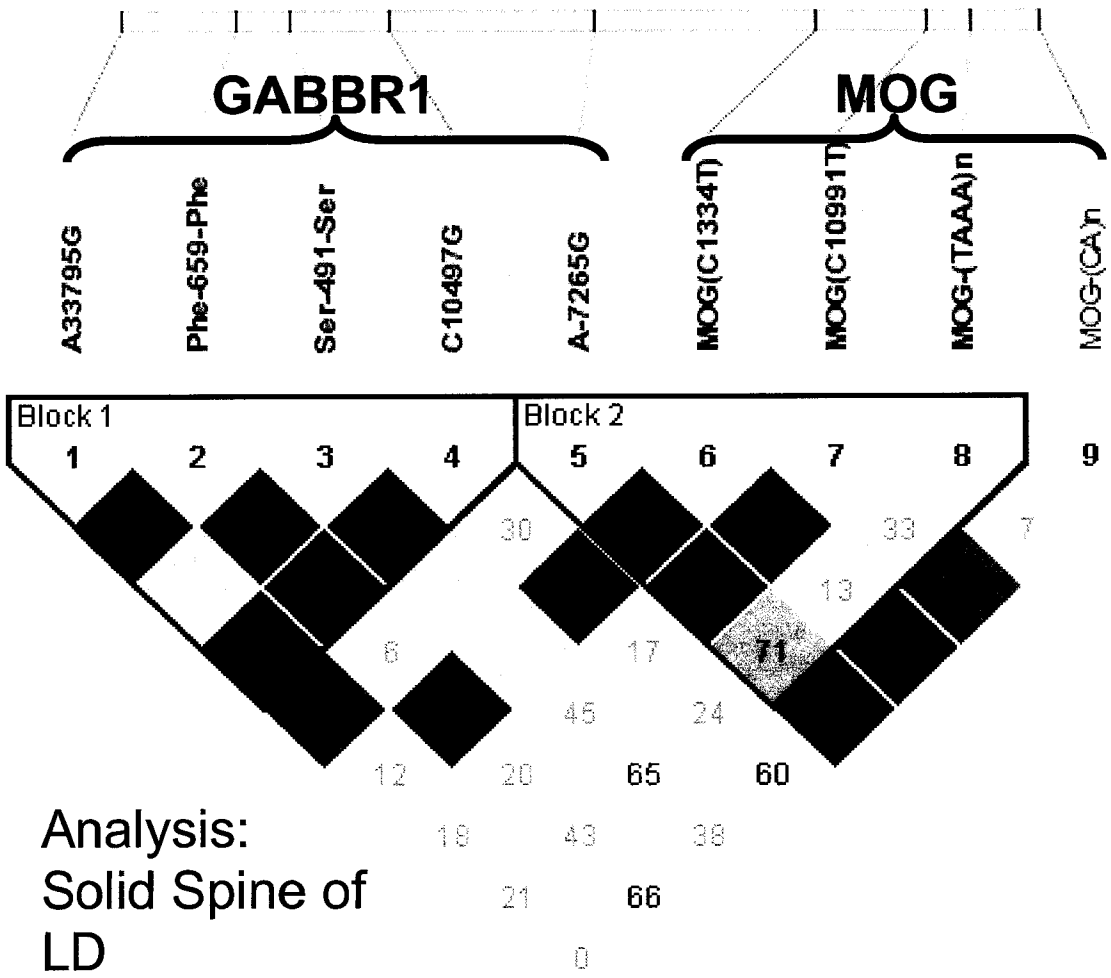


Figure 10b. Linkage disequilibrium across the 9 polymorphisms within the MOG and GABBR1 genes with the diagnosis of SCZ as the affection trait



CHAPTER 7: Discussion

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7.1 Summary of and Implications from Results

This is the first preliminary study of association between the phenotype and allele/haplotype relationships of the variants in the MOG and/or the GABBR1 genes and the diagnosis of OCD and/or SCZ using the TDT, FBAT, and case-control methodologies.

7.1.1 TDT and TDT Haplotype Analyses in OCD

Our tests for association between the MOG or the GABBR1 genes and the diagnosis of OCD showed: a trend towards biased transmission for allele 13 of MOG-(CA)n (P=0.071); a significant biased transmission of allele 2 of MOG-(TAAA)n for increased risk (P=0.022) with an accompanying trend towards biased non-transmission for allele 1 (P=0.071); a trend towards biased transmission of the –7265A (allele 2) allele of GABBR1(A-7265G) (P=0.071) and a significant biased transmission of the –7265A allele when the transmission of alleles in the affected siblings was taken into account (P=0.006); and a significant biased transmission of the A allele of GABBR1(Phe-659-Phe) for increased risk in the combined TDT-STDT analysis (P=0.032). Moreover, a significant association was also observed between a MOG haplotype involving allele 13 of MOG-(CA)n and allele 2 of MOG-(TAAA)n and the diagnosis of OCD (P=0.006); and a borderline association was also observed between a GABBR1 haplotype (A-7265G.C10497G.Ser-491-Ser.Phe-659-Phe.A33795G – 2.1.1.2.1) and the diagnosis of OCD (P=0.065). It remains unclear whether the MOG or the GABBR1 locus itself is the putative genetic risk site, or if the risk is conferred by another HLA-related site in LD with this MOG marker [MOG-(TAAA)n] or this GABBR1 marker (A-7265G).

7.1.2 TDT, TDT Haplotype, and Case-Control Analyses in SCZ

Our tests for association between the MOG or the GABBR1 markers and the diagnosis of SCZ did not show biased transmission of alleles in any of the nine polymorphisms examined. We did not find an association in the quantitative trait analysis with AAO with SCZ. However, we did observe a significantly greater count of the -7265A allele (allele 2) and less count of the -7265G allele (allele 1) at the GABBR1(A-7265G) polymorphism in SCZ patients when compared with healthy controls (P=0.038), suggesting that the variant –7265A allele might predispose a person to risk for SCZ or the variant –7265G allele might have a protective role against the development of SCZ. Nonetheless, it remains unclear whether the GABBR1 locus itself is the putative genetic risk site, or if the risk is conferred by another HLA-related site in LD with this GABBR1 marker. Although our results did not support a role of MOG in the pathogenesis of SCZ, other variants in the MOG gene may still be worthwhile for investigation because of the overall rationale including support from MRI data that indicate reduction of white matter in SCZ patients when compared with healthy controls (Breier et al. 1992; Buchanan et al. 1998; Sanfilipo et al. 2000; Sigmundsson et al. 2001).

7.1.3 Quantitative Analyses

In the quantitative trait analysis with Y-BOCS severity score, we found significant association with the MOG-(TAAA)n and the GABBR1(A-7265G) polymorphism. Allele 2 from MOG-(TAAA)n (P=0.020) and the –7265A allele (P=0.053) were found to be associated with high Y-BOCS severity scores. We also observed a significant association between allele 2 of MOG-(TAAA)n and both the obsession and compulsion subscales,

indicating that the significant results were associated with both of the major symptom types, whereas the –7265A allele was found to have a trend toward association with only the high compulsion sub-scale score (P=0.071). Our significant findings in the MOG gene were strong despite the fact that the cases were not selected for PANDAS subtype. This implies that MOG may have important implications for OCD in general. A study enriched with childhood onset probands would presumably be more likely to identify autoimmune-related genes since these are likely more relevant to the PANDAS subtype, which is predominantly found in children. Our results in the GABBR1 gene imply that GABBR1 may have important implications for OCD in general although our findings were not strong enough to conclude a strong association between the promoter region polymorphism, A-7265G, with OCD. Nonetheless, this A-7265G marker might be in LD with another MOG marker if it was the variant of the MOG gene that confers susceptibility of an individual to OCD.

7.1.4 Implications from Results

There are several possible ways to interpret our findings.

The significant association between MOG and the diagnosis of OCD support the autoimmune hypothesis in its etiology. Thus, OCD might be considered as an immune-mediated basal ganglia disorder with consequent movement abnormalities in some cases and manifestation of psychiatric symptoms similar to PANDAS. This is important because it has broad implications for the understanding of causation, and perhaps for treatment. If an autoimmune etiology has been proven confidently, immunotherapies might be indicated in common psychiatric disorders to induce autoimmune remission and therefore symptom remediation. In order to consider such treatments, there must be clear clinical and laboratory

diagnostic criteria. Positive streptococcal serology alone is inadequate for such a diagnosis, as GABHS is so prevalent in the pediatric community. The ultimate molecular goal would be to characterize the basal ganglia proteins involved in antibody binding, which would provide central clues to the neurotransmitter or second messenger systems involved, and even point towards novel drug targets for neuropsychiatric disorders in children.

However, since our OCD samples were not selected for PANDAS criteria, the hypothesis of demyelination or neurodevelopment might also be a plausible reason for the positive findings. MOG itself could potentially be a neuro-developmental gene of interest due to its known role in the formation of the myelin sheath. Variants of this gene might alter myelination of neurons, which in turn may affect neurotransmission, such as rate and frequency, and cause abnormalities in the brain.

Since there is strong LD between the promoter SNP, A-7265G, of GABBR1 and several markers of MOG (Figures 9b and 10b), the significant findings of GABBR1 and OCD might have been linked to the positive association observed in MOG-(TAAA)n. Further studies into other MOG markers should be explored. In addition, many studies have found high LD between markers within the HLA region and adjacent non-HLA markers; thus, our autoimmune hypothesis might still be of strong interest since our significant association might have come from adjacent HLA markers, which are in strong LD with the MOG and GABBR1 variants that showed association with OCD in this study.

Nonetheless, an entirely different mechanism such as the GABAergic neurotransmitter system rather than the autoimmune mechanism might be involved since the positive findings of MOG in OCD might have been linked to GABBR1. Variants of the GABBR1 gene might disrupt the function of the major inhibitory neurotransmitter system,

which may lead to disturbance in homeostasis and eventually to the development of neuropsychiatric disorders; thus, other genes involved in the GABAergic system should also be examined.

There is always a possibility that our positive results might be due to chance; thus, the significant association could be a false positive. Since these investigations were exploratory in nature, we have not corrected for multiple comparisons. Also the fact that the markers across both genes are, to varying degrees, related to each other means that each marker is not an independent test and thus stringent Bonferroni-type correction is not indicated.

In all, it is difficult to truly prove an association of a gene with OCD and/or SCZ, and though we found significant association, replications with a different sample are required to support and extend our findings and to eliminate false positive results.

7.2 Limitations

This study presents an intriguing finding of significant association of the MOG and GABBR1 markers with OCD and may suggest a pathophysiological explanation in OCD. Failure to identify cases of PANDAS, which may account for a subtype of OCD, or a subgroup of SCZ, may conceivably confound genetic studies due to cryptic etiologic heterogeneity. Likewise, the factors involved in predisposing an individual to develop OCD and/or SCZ under a particular environmental condition (such as exposure to GABHS infection) are likely complex, and may involve one or more loci of small effect. Thus the polygenic nature of these diseases plus possible interaction with environmental factors renders traditional genetic techniques difficult. Therefore, it is particularly important to examine potential homogeneous phenotypes of OCD such as PANDAS, and potentially more

homogeneous phenotypes of SCZ such as those cases with high levels of negative symptoms.

Our results have not been corrected for multiple comparisons because of strong intermarker LD and the relatively high conservativeness of the Bonferroni correction, particularly in this situation of the non-independence of the tests. The Bonferroni correction is the most stringent test, which offers the most conservative approach to control for false positive results (Bonferroni 1936). When high degree of LD exists between markers, which are assumed to be completely independent, then the Bonferroni correction would markedly overcorrect for the inflated false-positive rate, resulting in a reduction in power (Perneger 1998). However, multiple comparisons should always be taken into account when implicating the involvement of a gene variant to the diagnosis of a disorder. The Westfall and Young Permutation is the only correction accounting for genes coregulation (Westfall and Young 1993); nonetheless, computation is very slow and is also very conservative. The least stringent of all corrections is the Benjamini and Hochberg False Discovery Rate (Benjamini and Hochberg 1995). This correction provides a good balance between discovery of statistically significant genes and limitation of false positive occurrences.

Our current OCD sample did achieve a power of 80% to detect a relative risk as low as 3.5. Nonetheless, larger and more well-characterized samples are needed, in order to extend these results, to better delineate subgroups of OCD, and to enable more directed studies regarding the localization and characterization of genetic factors in the etiology of OCD and its related disorders. Our analyses performed for SCZ have included both the TDT and case-control samples and our samples have powers of 67% and 82% to detect a genotypic relative risk of 1.9 respectively. When we combine the TDT and case-control samples for SCZ the combined power analysis has shown a robust power of 96% to detect a

relative risk as low as 1.5. Thus, we should be able to detect small gene variants in the SCZ sample but we do not have comparable power in the OCD sample.

Furthermore, the large number of alleles in each of the highly polymorphic microsatellites increased the degrees of freedom. Therefore, larger samples would be required to detect statistical significance of small effect genes.

7.3 Future Directions

Finally, since we have detected a haplotype block that contains several markers that were associated with the diagnosis of OCD in our sample, our next step should try to identify additional SNPs, which have a relatively high heterozygosity, to cover the regions in the gaps between the markers in our present study. In addition, we suggest further study on the function of MOG in the context of autoimmune disorders, to clarify its role in the immune system and the complement cascade since the function of MOG remains largely unknown. Furthermore, the importance of myelination in normal brain functioning has been implicated in the diagnosis, treatment, and prognosis of SCZ (Bartzokis et al. 2003). Thus, other myelin-related proteins such as myelin-association glycoprotein (MAG) and myelin basic protein (MBP), and the enzyme cyclic nucleotide phosphodiesterase (CNPase), are important for investigation in SCZ. The examination of the myelin molecular system in SCZ is increasingly important because recent studies have reported white matter abnormalities in SCZ patients due to decreased CNPase (Flynn et al. 2003) and MBP protein expression (Honer et al. 1999) in the anterior frontal cortex. Decreased mRNA expression of MAG (Hakak et al. 2001; Tkachev et al. 2003) and CNPase (Hakak et al. 2001) have been found in the frontal cortex of SCZ. In addition, decreased mRNA level in the frontal cortex of SCZ has been associated with a specific isoform of the MBP protein (Tkachev et al. 2003).

Another valuable investigation would be to combine brain-imaging data with genetic data to facilitate the identification of pathophysiological dysfunction associated with genetics. For example, the measurement of brain white matter changes using specific imaging methods such as Diffusion Tensor Imaging may provide a more biologically direct phenotype for correlation to genetic variants in the myelin system.

In addition, the need for further study of other genes involved in the inhibitory neurotransmission system that may be associated with OCD and SCZ should also be an important goal. The $GABA_B$ receptor plays an important role in the GABAergic neurotransmission system and in signal transduction including altering transcriptional activity. Thus, examining variants within the GPR51 gene might provide further insights in the involvement of $GABA_B$ receptor in the development of OCD and SCZ.

Further clinical studies on the expression of the different gene variants together with more sophisticated definition of subgroups in OCD and SCZ may help in providing more conclusive results. Recently, researchers have begun to ask if the OC symptoms that frequently occur in SCZ are not simply expressions of persistent schizophrenic psychosis, but rather they may constitute a distinct subtype of SCZ similar to OCD. Better definitions of OCD and SCZ in general are needed in order to elucidate the symptoms associated with or comorbid with other diseases.

In all, additional work must be performed in order to clarify the autoimmune background in OCD and SCZ. If the positive genetic results reported here replicate in other samples, and additional convergent supporting evidence arrives from immunology and neuroimaging studies, then ultimately the work may lead to identification of at-risk children and of new treatments.

CHAPTER 8: References

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