INTERACTION BETWEEN HERPESVIRUSES AND GENETIC VARIATION IN SCHIZOPHRENIA PATHOGENESIS: A CANDIDATE GENE APPROACH

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Schizophrenia is a debilitating disorder characterized by disturbances in thought with lifetime prevalence of one percent. The public health burden of schizophrenia due to medical care, social disability, and co-morbid conditions is substantial. Genetic variation, viral infection, or interaction of the two could influence schizophrenia risk. An understanding of these disease pathways could lead to strategies for prevention and treatment of schizophrenia.

We used a positional approach to identify schizophrenia candidate genes that could interact with cytomegalovirus and herpes simplex viruses (HSV). We focused on three groups of genes: *TNF* and *MICB* near D6S2672, which was associated with schizophrenia and CMV in our previous studies; *IL1β, IL1RN,* and *IL10,* immune related genes associated with schizophrenia in published articles; and *IL-18, IL18BP, IL18RAP, IL12A,* and *IL12B*, positional candidate genes in the IL-18 pathway. We used multiple case-control and family-based samples to test these hypotheses.

We comprehensively sequenced *TNF*, and genotyped eight SNPs in a case-control sample. We detected no significant associations. We used a dual-luciferase expression assay to quantify TNF expression driven by common promoter haplotypes. Differences in TNF expression did not correlate with schizophrenia.

To localize the D6S2672 association, we genotyped 26 SNPs spanning 100kb in a case-control sample. Based on suggestive associations, we selected five SNPs to assay among additional samples. A SNP in *MICB* was associated with schizophrenia in these samples. The opposite allele was associated with HSV1 in two non-schizophrenia groups.

We used comprehensive sequencing data to select tag SNPs at *IL1β, IL1RN, IL10*, and IL-18 pathway genes. Tag SNPs were evaluated in a case-control sample. In *IL1β, IL1RN*, and *IL10* significant associations were not detected. However, meta-analysis of rs16944 (IL1β –511) studies suggests modest, but significant, risk for schizophrenia in Caucasian samples. In IL-18 pathway genes, a *IL18RAP* SNP was associated with schizophrenia, the opposite allele was associated with HSV1.

Identified associations with schizophrenia may be due to host gene-virus interaction. These genetic variants could clarify which patients are vulnerable to viral infection. Treatment or prevention may be feasible, if our results are confirmed. Further replicate studies are warranted, as are functional studies of associated polymorphisms.

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1. INTRODUCTION

1.1.SCHIZOPHRENIA

Schizophrenia is a brain disorder characterized by distortions in thought. Persistent hallucinations, delusions, or disordered thought are core components of the diagnosis and other more subtle characteristics such as incongruent mood, flattened affect, or psychomotor slowing may also be clinically apparent (A.P.A., 1994; WorldHealthOrganization, 1989). In addition, cognitive dysfunction, including deficits in executive functioning, attention, and memory, has been shown to be a persistent, although non-specific, component of schizophrenia (Peuskens et al., 2005; Sharma et al., 2003). These symptoms lead to chronic impairment in emotional and social functioning (Carpenter, 1994). In the last half of the 20th century explicit diagnostic criteria for schizophrenia were established (A.P.A., 1994; Jablensky, 1997). These have facilitated larger studies of schizophrenia epidemiology; but important symptoms are not always obvious, so specialized clinical skills are necessary to make accurate diagnoses (Jablensky, 2000). Though schizophrenia is relatively rare compared with other complex diseases, such as diabetes and heart disease, the long-term impairment associated with schizophrenia make it the ninth leading cause of disability worldwide (Lopez et al., 1998). In addition, high suicide rates and other medical complications are associated with schizophrenia and often lead to premature mortality (Heila et al., 2003; Mortensen, 2003)

Numerous studies have suggested alterations in brain structure and function, but the specific pathogenic abnormalities are not known (Lewis et al., 2000). The efficacy of dopamine

receptor antagonists in controlling the most visible symptoms of schizophrenia has led substantial research into the dopamine hyperactivity hypothesis of schizophrenia (Carlsson et al., 1963; Davis et al., 1991). More recent evidence suggests that glutamate and GABA neurotransmitter systems may also be involved, but a consensus has not been well established (Collier et al., 2003; Lewis et al., 1999; Moghaddam, 2003). Brain imaging studies consistently show increased ventricular volume with decreased whole-brain and hippocampal volume (Steen et al., 2006). Recent microarray studies have revealed several genes that appear to be differentially expressed in postmortem schizophrenia brains, but inter-study consistency and implied causality of differential expression are important issues that have not been fully addressed (Mirnics et al., 2006).

After over a century of schizophrenia epidemiology research, the etiology of schizophrenia is still a mystery (Jablensky, 1997). Schizophrenia prevalence estimates range from 1.4-4.6 per 1000, and the disorder has been documented in all ethnic groups investigated to date (Jablensky, 1993; Jablensky et al., 1992). Several general environmental factors such as urban birth, winter birth, and immigration have been consistently associated with increased risk (Boydell et al., 2003; Davies et al., 2003), but the mechanisms involved in this increased risk are uncertain. Perinatal brain damage and birth complications have also been consistently associated with schizophrenia (Jones et al., 1998; Verdoux, 2004), but the population attributable fraction of each of these is less than 10% and the exact pathogenic mechanisms are not clear (Jablensky, 2000).

Liability to schizophrenia is likely to be due to genetic and environmental factors (see section 1.2). The latter include a plausible role for infectious agents (see section 1.3). Despite several promising leads, there is scant consensus on pathogenic etiology at present.

Inconsistencies in etiologic studies involving infectious agents could be explained if interactions between pathogens and host genetic variation were to play a role in susceptibility (see section 1.6), but few studies that have directly investigated interaction have been performed (Clark et al., 2005).

1. 2. GENETIC EPIDEMIOLOGY OF SCHIZOPHRENIA

Schizophrenia can cluster in families. Adoption studies suggest that the familial aggregation is unlikely to be due to environmental factors alone (Heston, 1966; Kety et al., 1992). Also, concordance for monozygotic twins (39%) is significantly higher than that for dizygotic twins (10%) (Gottesman, 1991). Estimates of heritability have ranged from 60-85% after accounting for shared environment (McGue et al., 1983; Rao et al., 1981). The mode of inheritance is controversial. Autosomal dominant and recessive modes of inheritance have been suggested (Hurst, 1972; Slater, 1958) and the possibility of genetic heterogeneity cannot be excluded (Baron, 1985). Nevertheless, complex segregation analyses of published family and twin data suggest that multiple genes of small effect are likely (Carter et al., 1980; McGue et al., 1983; Rao et al., 1981). Modeling of risk to relatives of schizophrenia patients suggests that at least two or three loci, possibly many more, contribute a moderate to small amount of risk (Risch, 1990).

Linkage studies can be a model-free, hypothesis-free method of localizing genes for a specific trait, and as such are often the first step in identifying genes that confer disease susceptibility (Ott et al., 1999). Linkage studies investigate the co-segregation of genetic polymorphisms with the disease state in families with multiple affected members. Statistically significant evidence for co-segregation is termed *linkage*. If the chromosomal location of the

polymorphism is known, it is then possible to localize the region that harbors the disease genes. Linkage analysis is attractive because a judiciously chosen set of polymorphisms can enable coverage of the entire genome. With adequately sized samples, it is possible to exclude genes localized to unlinked regions. On the other hand, the linked regions identified from currently available samples are typically quite extensive (millions of base pairs). Initial linkage scans of schizophrenia focused on large families with multiple affected individuals were not successful at finding genes of major effect, so subsequent linkage scans have incorporated larger samples of smaller families, such as affected sibling pairs (Williams et al., 2002). In the past two decades, significant linkage for schizophrenia has been reported at several chromosomal regions, but there have been relatively few consistent results (Badner et al., 2002; Lewis et al., 2003). This is likely due to inadequate sample size or a multigenic-multifactorial etiology, among other reasons (Owen et al., 2000). Because multiply affected families are infrequent it may only be possible to recruit sufficiently large samples through collaborative initiatives. Meta-analyses have shown that current whole-genome linkage scans are more concordant than expected by chance, but do not conclusively identify a loci of major importance (Lewis et al., 2003; Zintzaras et al., 2005). Several genes identified in putative linkage regions have been found to be associated with schizophrenia. There has been recent excitement about several of these positional candidate genes including DISC1, RGS4, neuregulin, and dysbindin. Although multiple groups have found associations at each of these genes, no allele has been consistently associated or shown to have a functional effect on gene expression or activity (Shirts et al., 2004). Methodological issues that could cause discrepancies in these candidate gene studies will be discussed in section 3.1 and 3.2. While genetic factors underlie susceptibility to schizophrenia, environmental factors also play a role (Murray et al., 2003).

1.3. IMMUNE SYSTEM DYSFUNCTION IN SCHIZOPHRENIA

Findings of immune system imbalance in schizophrenia patients have been reported since the early part of the 20th century (Ganguli et al., 1994). Immune alterations in schizophrenia can be seen in several of the distinct but interrelated branches of the immune system (Muller et al., 1999). The immune system can be divided into innate and adaptive components (Janeway et al., 2005). The innate immune system is phylogenetically older, consists of natural killer (NK) cells, monocytes, and granulocytes as well as compliment and acute phase proteins. The innate immune system has evolved to be an early response to a set of general biological triggers, such as extracellular and double-stranded RNA or bacterial lipopoplysaccarides. On the other hand, the adaptive immune system is pyhlogenetically younger and more specific; it consists of T and B lymphocytes and their molecular products. It is involved in antigen specific cell-cell interactions and specific antibody production. These antigen specific responses can be conditioned and stored in a cellular "memory," to provide a rapid response to reinfection. The adaptive immune system is sometimes grouped into T-helper 1 (Th1) and T-helper 2 (Th2) components. The Th1 response refers mainly to the activation CD8+ T cells and the cellular component of the adaptive immune response, which responds mainly to intracellular pathogens. IL-2, IFN-γ, and TNF are often involved in the Th1 response. The Th2 response mainly refers to the eventual activation of B-cells, which are responsible for antibody production, targeted mainly at extracellular pathogens. IL-4, IL-10, IL-6, and TGF-β are often associated with the Th2 response. This grouping of cytokines can be useful for distinguishing between different immune system pathways, but in actuality the pathways are interrelated and infection may simultaneously

elicit cytokines that have overlapping, antagonistic, or synergistic effects in multiple pathways, so the balance of conflicting cytokine signals may be important in the final immune response.

Early studies that showed apparent immune dysfunction in schizophrenia cases have led to investigation of autoimmune and infectious disease hypotheses of schizophrenia (Crow, 1983; Ganguli et al., 1994; Jones et al., 2005; Kirch, 1993; Wright et al., 2001), (see section 3.2.2 for more discussion on genetic studies of these hypotheses). Recently, there has been renewed interest in cytokine abnormalities as markers of immune imbalance in schizophrenia patients (Kim et al., 2004c; Riedel et al., 2006). Studies of schizophrenia immunology have been prone to the difficulties of small sample size, varying lab techniques, and confounders such as age and medication status (Arolt et al., 2002). Nevertheless, relatively consistent evidence of decreased IL-2 and IFN-γ as well as increased IL10,IL6, and TGF-β suggests Th2 system predominance over Th1 system cytokines among patients (Akiyama, 1999; Kim et al., 2004c; Muller et al., 1999; Rothermundt et al., 2001; Schwarz et al., 2001). This imbalance may be linked to infectious disease exposure, and has recently been invoked in variations of the neurodevelopmental, autoimmune, and infectious disease hypotheses of schizophrenia (Brown, 2006; Eaton et al., 2006; Ganguli et al., 1994; Rapaport et al., 2001). Genetic variation in the immune system may cause cytokine imbalance in schizophrenia. Findings of altered serum cytokine levels have been an important rationale for many association studies of cytokine candidate genes in schizophrenia (Muller et al., 1999); several of these will be discussed in more detail in section 3.3.2.

1.4. EPIDEMIOLOGIC EVIDENCE OF INFECTIOUS DISEASE RISK

Early studies investigated the possibility of an infectious etiology for schizophrenia (Kirch, 1993). Several infectious agents have received prominent attention (Brown, 2006; Torrey et al., 2003; Yolken, 2004). As well as being consistent with immune imbalance, the infectious disease hypothesis of schizophrenia is consistent with robust epidemiological findings including increased risk caused by urban birth, winter birth, and migration (Boydell et al., 2003). Neonatal CNS infection with viruses, such as rubella and Coxsackie virus, is rare but can cause a substantial increase in schizophrenia risk (Brown et al., 2001; Koponen et al., 2004; Rantakallio et al., 1997). However, rare viral CNS infection contributes less than 5% to the population-attributable risk (Koponen et al., 2004). Although evidence implicating any specific common infection has been inconsistent, recent studies of CMV, herpes viruses, *Toxoplasma gondii,* and influenza have been more promising (Brown et al., 2005a; Brown et al., 2005b; Leweke et al., 2004; Pearce, 2003; Wang et al., 2006). The following section will discuss the possible influence of a family of related common viral infections on schizophrenia risk.

1.5. HERPES VIRUSES AND SCHIZOPHRENIA

Herpes viruses are enveloped double-stranded DNA viruses. Eight members of the *Herpesviridae* family are known to infect humans including cytomegalovirus, varicella zoster virus, Epstein-Barr virus, and herpes simplex virus 1 and 2 (Cleator et al., 2004). Each of these viruses causes changes in the expression of many immune system genes enabling them to establish latent infection in humans.

A role for some herpes viruses in schizophrenia has been hypothesized because they are common and neurotropic (Mettenleiter, 2003; Whitley, 2004). Herpes encephalitis often

presents with psychiatric symptoms, which can persist for years after the encephalitis resolves (McGrath et al., 1997; Wilson, 1976). CMV infection of the central nervous system is common in immunocompromised patients and can lead to apoptosis and glial cell activation, which have been noted in postmortem studies of schizophrenia (Cotter et al., 2001; DeBiasi et al., 2002; Lokensgard et al., 2002).

Interest in CMV and schizophrenia began with positive studies of CMV antibodies in the CSF and sera of schizophrenia patients (Albrecht et al., 1980; Torrey et al., 1982). Researchers attempting to replicate these results have had mixed results (DeLisi et al., 1986; Fux et al., 1992; Rimon et al., 1986; Stevens et al., 1984), as both the quality of design and the results of these studies were inconsistent (Pearce, 2003). PCR-based investigations of CMV and HSV in postmortem brains of schizophrenia cases have been largely negative (Sierra-Honigmann et al., 1995; Taller et al., 1996). However, recent investigations have shown higher antibody titers when examining recent onset schizophrenia cases (Leweke et al., 2004) and specific subtypes of schizophrenia (Dickerson et al., 2006). In addition, schizophrenia patients seropositive for HSV1 do worse on cognitive tests and have decreased dorsolateral prefrontal cortex volumes when compared with HSV1 seronegative patients and controls (Dickerson et al., 2003a; Prasad et al., in press).

Studies have hinted that other herpes viruses may have an additive effect on schizophrenia risk (Yolken, 2004). It is possible that herpes viruses contribute to the cytokine imbalance that has been observed in schizophrenia patients (Almanzar et al., 2005; Kaminska et al., 2001; Kim et al., 2004b; Kipnis et al., 2004; Schwarz et al., 2001). In addition, interactions between genes and viruses may account for the variability that has been noted in studies of viral risk for schizophrenia.

1.6. GENE-VIRUS INTERACTION AND SCHIZOPHRENIA

Genetic variation in host response to infectious disease has been well documented (Cooke et al., 2001; Lipoldova et al., 2006; Somech et al., 2003). For example, the severity of HIV infection is dependent on the CCR5 genotype of the host, and hemoglobin variation can be a major factor in host response to malaria infection (Hill, 2001; Segal et al., 2003). Genetic modifiers of susceptibility to HSV and CMV have been demonstrated in experimental strains of mice (Beutler et al., 2005; Lee et al., 2001; Norose et al., 2002). Though genetic variation in human susceptibility to herpes virus risk has not been extensively studied, candidate genes have been associated with human susceptibility to several members of the *herpesviridae* family (Hurme et al., 2003; Hurme et al., 1998). Genetic differences also modify herpes virus resistance in human oligodendrocytes (Kastrukoff et al., 2002). Researchers who have investigated viral risk factors for schizophrenia have noted that specific genetic polymorphisms are likely to modulate the action of viruses in schizophrenia risk (Pearce, 2003). Consistent with this hypothesis, Krabbendam et al. (2005) found that increased schizophrenia risk associated with urban birth was conditional on genetic liability. Furthermore, several cytokine genes have been associated with schizophrenia, some of which are involved in controlling HSV1 and CMV infection (Chiavetto et al., 2002; Duan et al., 2004; Hurme et al., 2003; Katila et al., 1999b) (see also section 1.3, 1.5, and 3.3.2).

Nevertheless, difficulties in investigating the possibility of gene-environment interaction using epidemiological tools have limited formal tests of interaction (Kraft et al., 2005; Wong et

al., 2003). These difficulties are due to challenges in measuring risk caused by infectious agents in schizophrenia, lack of genes of large effect contributing to schizophrenia risk, and difficulties inherent in measuring interactions. The peak incidence of initial schizophrenia onset is in late adolescence and early adulthood, and symptoms often persist for months before a definitive diagnosis is made (Hafner, 2003). Environmental insults in utero or in early childhood, while the brain is developing, may be more important than proximal events in schizophrenia pathogenesis (Cannon et al., 2003). Thus, measuring the extent and timing of exposures that may precede diagnosis by months to decades are a major challenge in schizophrenia epidemiology. In addition, lack of consistency in genetic linkage and association studies of schizophrenia are common despite significant heritability (Owen et al., 2000), so there are no clear genetic candidates for investigation of interactions. Difficulties in schizophrenia epidemiology compound the limitations inherent in investigations of interaction, specifically a larger space of possible tests, smaller cell size due to sample subdivision, and possible additional reductions in power due to the necessity of more complex statistical models (Botto et al., 2001; Ottman, 1996).

Despite these difficulties the nature of herpes viruses, advances in understanding of schizophrenia genetics, and more sophisticated statistical techniques have made the investigation of possible interactions more tenable. Challenges in the temporal measurement of infection are mitigated somewhat in investigations of infections that cause recurrent infection, such as HSV and CMV, because serological evidence of infection is attenuated with recrudescence (Yolken, 2004). Growing consensus on schizophrenia linkage regions allows selection of candidate genes for which there is reasonable combined positional evidence (Lewis et al., 2003). Larger samples with better clinical, genetic, and serological data are becoming available. In addition, recent advances in statistical techniques have demonstrated the feasibility of evaluating of gene-

environment interaction in family based samples; these techniques control for the possibility of genetic population stratification, but are dependant on assumptions of gene-environment independence (Chatterjee et al., 2005)(see section 4.6.2). Case only analysis of interaction is another recently developed method for investigating gene environment interaction (Khoury et al., 1996a)(see section 4.6.3). Because HSV1 and CMV are common viruses, stratification by exposure divides samples into relatively even segments, so for a common allele only moderately sized samples may be necessary to achieve meaningful cell sizes and estimates of risk. Studying common viruses not only mitigates difficulties in study design, it also enhances the potential significance of findings. A small increase in risk conferred by common herpes viruses or common-gene-common-virus interactions could contribute a relatively large amount to the population-attributable risk for schizophrenia, so the potential public health benefits are great.

1.7. SIGNIFICANCE

Although there is convincing evidence for the presence of environmental and genetic factors, pinpointing risk factors for schizophrenia has been difficult. Exposure to infectious agents is a plausible environmental risk factor, but prior studies have not been consistent. Despite excitement about the topic, there is a paucity of studies directly investigating gene-environment interaction (Clark et al., 2005). One reason may be that most research groups investigate infectious factors in isolation from genetic variation in the patients.

The investigation of genes that may interact with HSV1 and CMV infection in schizophrenia pathogenesis has several possible outcomes and benefits. Several candidate genes we examine have previously been associated with schizophrenia, but previous studies did not take infection into consideration. Our investigation may clarify whether these genetic

associations are dependent on viral infection as well as acting as and independent replication of genetic associations. Several candidate genes we examine have not been previously investigated. Our studies will be the first to investigate candidate genes chosen specifically to test specific interaction hypotheses. Negative findings in well-designed, comprehensive evaluation of common polymorphisms in genes of interest will at least demonstrate lack of association strong enough to be detect given the power of the study. Positive findings will have clear utility in the understanding of schizophrenia. Regardless of association with schizophrenia associations with CMV or HSV infection will potentially lead to greater understanding of human variation in response to viral infection.

Greater understanding of putative gene-virus interactions in schizophrenia may clarify which patients are vulnerable to the deleterious effects of viral infections. Understanding of gene-environment interaction may shed light on studies of decreased cognitive functioning and brain volume in HSV1 infected schizophrenia patients (Dickerson et al., 2003a)(Prasad 2006 in press). In light of recent open trials suggesting the benefit of anti-viral therapy among patients with prior exposure to CMV (Dickerson et al., 2003b), our genetic analyses may also help identify candidates for such treatment. Indeed, preventive strategies for at risk individuals may also be feasible if associations are confirmed in larger studies. Thus, this project may hold one of the keys to the molecular etiology of schizophrenia, and lead to new methods for diagnosis, therapy, and prevention.

2. SPECIFIC AIMS

Hypothesis: Variations in immune-related positional candidate genes in humans interact with common infectious agents to influence schizophrenia risk.

Aim 1 – Select candidate genes and SNPs

1a. Select positional candidate genes for gene-virus interaction studies based on published linkage and association studies and our previous data on chromosome 6p.

1b. Using public databases and pooled DNA resequencing , select polymorphic "tag" SNPs that represent common polymorphisms at candidate genes identified in Aim 1a.

Aim 2 – Genotype selected SNPs in case and control samples, and compare allele frequencies in subgroups of these samples defined by infectious exposure.

2a. Genotype selected SNPs using multiplex assay systems.

2b. Compare allele and genotype frequencies between subgroups defined by antibody titer status for viral antigens while adjusting for differences between groups using various statistical methods including genomic control.

Aim 3 – Refine associations and investigate additional samples.

3a. Use linkage disequilibrium data, association data and pooled sequencing of polymorphisms near associated SNPs to identify additional putative risk polymorphisms.

3b. Investigate associations identified in Aim 2 using additional adequately powered samples. These samples include case-parent trio samples and samples from different ethnic populations.

3c. Explore statistical methods that may be used to investigate additional hypotheses, such as uniparental transmission of risk alleles, likelihood of neonatal transmission of viral disease, or gene-gene interaction.

3d. Check specificity for schizophrenia by using a sample diagnosed with bipolar disorder.

Aim 4 – Synthesis of results from previous aims.

2.1. COMPLETION OF SPECIFIC AIMS FOR INDIVIDUAL CANDIDATE GENES

All the individual specific aims were addressed. The selection of candidate genes (Aim 1a) is described in chapter 3. Following a description of general methods used (chapter 4), four separate chapters present results for specific candidate genes (chapters 5-8). Chapter 5 discusses studies of *TNF*. Chapter 6 discusses evaluation of a region on chromosome 6p and the gene *MICB*. Chapter 7 discusses studies of *IL-10, IL1β,* and *IL1RN*. And, chapter 8 discusses studies of six IL-18 pathway genes. Each of these chapters describes the selection of candidate genes (Aim 1b), evaluation of associations with schizophrenia and interactions in at least one case-control sample (Aim 2a and 2b), and a discussion and synthesis of findings (Aim 4). Finally, a general discussion and synthesis of all results is presented (chapter 9).

Aim 3 was completed to varying degrees for each gene, depending on the availability of public resources, the results of Aim 2, and the availability of additional samples. Aim 3a was

redundant for genes covered in chapters 7 and 8 (*IL-10, IL1β, IL1RN,* and IL-18 pathway genes) because comprehensive sequencing data was already available for these genes. The findings of no association in Aim 2 for genes covered in chapters 5 and 7 (*TNF, IL-10, IL1β,* and *IL1RN*) suggested further investigation of these genes as described in Aim 3 would not be fruitful. All parts of Aim 3 are described in Chapter 6 (*MICB*) including investigations in multiple additional case-control samples, a case-parent trio sample, a bipolar sample, and a mixed outpatient sample. Exploratory analysis proposed in Aim 3c is also described in chapter 8 (IL18 pathway genes). Genotyping of more samples for associated SNPs reported in chapters 6 and 8 (*MICB* and *IL18RAP*) is in progress, but technical and bureaucratic difficulties have prevented a more timely evaluation of these SNPs.

2.2. SELECTION OF INFECTIOUS AGENTS TO BE EVALUATED

Throughout the project there was an open discussion about which infectious agents to evaluate for associations with specific genes. Serum antibody titers for CMV, HSV1, HSV2, and *Toxoplasma gondii* were assayed by Dr. Robert Yolken. CMV and HSV1 were investigated for reasons listed in sections 1.4-1.6; in addition, the relatively high prevalence of these diseases gave our samples adequate power to genetic detect associations with these viruses. The prevalence of HSV2 and *Toxoplasma gondii* is relatively low, so our samples had less power to detect genetic associations with these infectious agents. We decided to include analysis of HSV2 in this dissertation because, as a herpes virus, it was likely to have similar immune-system interactions as HSV1 and CMV, so observed trends could possibly compliment association findings with other viruses even in underpowered samples. *Toxoplasma gondii* was also of interest because of epidemiological evidence supporting association of this parasite with

schizophrenia (see section 1.4). We decided not to include *Toxoplasma gondii* in the analysis presented in this dissertation because the immune response to protozoan parasites differs from the immune response to herpes viruses; however, it was often possible to evaluate genetic associations with *Toxoplasma gondii* with minimal additional effort. Though not included in the formal analysis and discussion, tables generated showing tests of genetic associations with *Toxoplasma gondii* are presented in Appendix C.

2.3. MANUSCRIPTS

Four peer-reviewed articles in various stages have resulted from the work presented in this dissertation. These are listed below:

1. **Shirts BH,** Bamne M, Kim JJ, Talkowski M, Wood J, Yolken R, Nimgaonkar VL. "A comprehensive genetic association and functional study of TNF in schizophrenia risk" Schizophrenia Research 2006 Mar;83(1):7-13. Chapter 5 of the dissertation is based on the work presented in this paper.
2. **Shirts BH,** Wood J, Yolken RH, Nimgaonkar VL. "Association study of IL10, IL1β, and IL1RN and schizophrenia using tag SNPs from a comprehensive database: suggestive association with rs16944 at IL1β." Schizophrenia Research (In press). Chapter 7 of the dissertation is based on the work presented in this paper.
3. **Shirts BH,** Kim JJ, Reich S, Yolken RH, Devlin B, Nimgaonkar VL. "Polymorphisms in MICB are associated with human herpes virus seropositivity and schizophrenia risk." Chapter 6 of the dissertation is based on the work presented in this manuscript, which has been submitted to Genes, Brain, and Behavior.

4. **Shirts BH,** Wood J, Yolken RH, Nimgaonkar VL. "Systematic association studies with IL-18 pathway gene localized to schizophrenia linked regions: Significant associations at IL18RAP and possible epistatic and gene-environment interactions." ." Chapter 8 of the dissertation is based on the work presented in this manuscript, which will be submitted to Molecular Psychiatry.

3. POSITIONAL AND FUNCTIONAL CANDIDATES FOR VIRUS-ENVIRONMENT INTERACTION

3.1. DESIGN OF CANDIDATE GENE ASSOCIATION STUDIES

Several interrelated issues are germane to genetic association studies. Some of the issues are shared with other fields of psychiatric research. Differences in ascertainment sources such as community versus hospital-based or community-based versus screened controls can lead to variations in findings (Merikangas et al., 2002). In addition, the schizophrenia phenotype is not easily defined and requires specific clinical skills to diagnose. There have been several diagnostic schedules used in the past three decades; thus, diagnostic criteria are an issue, and disease heterogeneity is possible (Jablensky, 2000). Relatively low incidence has made the cost of large-scale and prospective studies of schizophrenia risk prohibitive. Identification of 'prodromal' symptoms may facilitate future prospective studies (Cannon et al., 2005). These issues have made small sample size a concern for many studies.

Association studies rely on linkage disequilibrium (LD), the nonrandom segregation of alleles at linked loci in physical proximity in the genome. Selected polymorphisms are examined among cases and unrelated controls. If differences in the distribution of particular alleles (variants) are detected, this suggests that the polymorphism itself confers susceptibility or is located very close to the disease locus. LD typically dissipates over short genomic regions (100 bp–10 kb). Therefore, association studies can only be used to interrogate relatively small genomic regions. Often they are employed to investigate genes in regions where linkage has been detected.

Frustrated by the lack of consistent evidence from schizophrenia linkage studies, some investigators have employed association studies to investigate functional candidate genes, that is, genes encoding proteins implicated by biochemical, pharmacologic, or other investigations (Nimgaonkar, 1997; O'Donovan et al., 1999). To date, several-hundred candidate gene association studies of schizophrenia have been reported. The majority failed to detect significant associations. Over 90% of past studies have only investigated one polymorphism per gene, so failure to detect a significant association does not 'rule out' investigated genes. Most reported associations have been modest (odds ratios less than 2.0) (The Chinese National Human Genome Center, 2003). For many association studies there are serious design problems. Of prime concern is the definition of a functional candidate gene for a disorder with unknown pathogenesis. Hence the prior probability of selecting the correct candidate is very low, and corrections for multiple comparisons should be applied or multiple samples examined (Weiss et al., 2000). Multiple corrections are not usually possible, because most studies comprised samples of 200 individuals or less. Replication has been attempted for only a minority of loci, and most of these attempts have been inconsistent and underpowered (Baron, 2001).

Additional study design issues are specific to genetic association studies (Terwilliger et al., 2000). A contested debate relates to the choice of controls: unrelated, population-based versus family-based controls. Classical association studies have employed the former design, but lately family based analyses have come to the fore. Family studies use hypothetical parental genotypes not transmitted to cases as controls (haplotype relative risk) (Falk et al., 1987) or examine biased transmission of alleles from heterozygous parents to cases (transmission disequilibrium test (TDT)) (Spielman et al., 1993b). Family-based designs are less prone to artifacts introduced by differences in ethnic background between the cases and population-based

controls (Lander et al., 1994). On the other hand, methods to estimate and account for ethnic admixture are available for case-control studies (Bacanu et al., 2000; Devlin et al., 2004; Pritchard et al., 1999). The case-control design is more economical, and in many settings has more power than family based designs (Bacanu et al., 2000). Still, the TDT detects association only if linkage is present, so is considered more reliable than case-control studies (Spielman et al., 1996).

Another critical issue is the choice of polymorphisms. Short tandem repeat polymorphisms (STRPs) have been used extensively for linkage studies, but are less favored for association studies than single nucleotide polymorphisms (SNPs) because they are relatively sparse in the genome. SNPs are encountered frequently in the genome and can be assayed more accurately than STRPs, however, the allele frequency varies from SNP to SNP. Allele frequency impacts on "informativeness" and hence on the power to detect case-control differences. To improve informativeness, investigators use haplotypes, or combinations of SNPs. This benefit is offset by uncertainty about assigning haplotypes for heterozygous individuals, the so-called phase problem (Sinsheimer et al., 2001). Furthermore, haplotypes may not provide additional information if SNPs in tight LD are used.

The recent completion of the human Hapmap project (HapMap, 2003) has facilitated association studies using SNPs by providing an overview of LD structure and haplotype blocks throughout the human genome. The Hapmap project is a database of genotypes for common polymorphisms spaced at ~5 kb intervals in a defined set of Caucasian, Asian, and African population samples. With this resource an investigator can select "tag" SNPs: the smallest set of SNPs that, based on LD estimates, will represent other common SNPs. Several applications use related algorithms to select tag SNPs (Barrett et al., 2005; Rinaldo et al., 2005). These usually

group SNPs based on pairwise correlation (r^2) and select one SNP per group correlated above a preset minimum r^2 (often > 0.8).

Beyond Hapmap, the next phase of understanding variation in the human genome is complete sequencing of selected genomic regions in population-based samples. This has already begun for candidate genes in inflammatory pathways by a network of Programs for Genetic Applications (PGA) sponsored by the National Heart Lung and Blood Institute (i.e. Seattle SNPs PGA and Innate Immunity PGA)(Martinez et al., 2005; Nickerson et al., 2005). These projects provide comprehensive information about common variation in genes sequenced in a defined set of 23 individuals of European descent and 25 individuals of African descent. Previously, limited knowledge of genomic variation forced candidate gene association studies to investigate one or two polymorphisms within a candidate gene. This made it impossible to 'rule out' candidate genes with negative association results. Now it is possible to perform comprehensive evaluation of all common variations within well-characterized genes. In this project several methods have been used for tag SNP selection as the Hapmap project and PGA resources have evolved during the course of this project. More details on specific methods used for SNP selection in this project can be found in sections 5.2.4, 6.2.3, 7.3.2, and 8.2.2.

Factors such as migration, drift, and selection can have significant impact on genetic associations. Such factors are difficult to estimate or model for particular populations (Jorde, 2000); hence, a detected association may not reflect an etiologic process and independent replications are crucial. Replications can also mitigate the fact that studies that initially reported associations are likely to be uncorrected for multiple comparisons. An ideal replicate examines the same markers in a separate, but diagnostically comparable, sample that is large enough to statistically replicate the initial finding. Even if replicate samples are not large enough to prove

initial findings, concordant data from several samples lends some credence to putative associations. Several methods of meta-analysis provide well-developed techniques to combine and synthesize data from multiple studies with similar or varied study designs (Rosenthal et al., 2001).

3.2. CONSIDERATIONS FOR SELECTION OF POSITIONAL CANDIDATE GENES FOR GENE-ENVIRONMENT INTERACTION

Schizophrenia researchers have begun analyzing genes that are both functional and positional candidates (i.e., those that have a plausible pathogenic role and are localized to putative linked regions). Predictably, these studies have revealed more consistent associations than previous studies (Harrison et al., 2003). Selecting candidate genes for association studies focuses on creating hypotheses from available data. This method benefits from the ability to assimilate all forms of evidence to generate testable hypotheses. The process is limited by the availability of data, and is subject to the possibility that additional data may make current candidate gene selections less plausible.

There is current excitement that whole genome association tests using high density SNPs will bring a new wave of genetic discoveries that linkage studies lacked precision to detect (Carlson et al., 2004; John et al., 2004). Even if whole genome association studies are able to identify novel risk alleles, it is not clear whether they will be as useful in identifying interactions, largely due to the problem of multiple testing (Heidema et al., 2006; Lunetta et al., 2004). Hypothesis-free exploration of interactions of multiple polymorphisms with a number of environmental variables or other polymorphisms leads to a dramatic increase in statistical tests,

so advanced statistical methods such as multifactor dimensionality reduction or the random forests approach will be necessary (Bureau et al., 2005; Hahn et al., 2003). The debate over how to deal with thousands or millions of tests in a whole-genome SNP scan with or without looking at interactions has not been resolved (Evans et al., 2006; Ohashi et al., 2005; Wang et al., 2005). In the coming years geneticists will find out whether the results of whole genome SNP scans will be as fruitful as anticipated. In the meantime well-designed hypothesis-driven candidate gene studies may be the only way to investigate gene-environment interaction.

Difficulties in evaluating the possibility of gene-environment interaction have already been discussed (section 1.6). Positive results of candidate gene association studies depend on the selection of 'true' risk genes. This is not a trivial task. Extensive lists of positional schizophrenia candidates and genes that might influence viral infection can be produced. In this investigation of gene-environment interaction the list of reasonable candidates was shortened by searching schizophrenia linkage regions for infection-related candidate genes. Combined evidence from the diverse disciplines of psychiatry, neurology, virology, immunology, and genetics were then utilized to investigate plausible common pathways and build hypotheses. Clearly genes for which evidence from all of these fields combined supports a biologically plausible pathogenic mechanism represent ideal candidate genes. Selecting ideal candidates becomes more challenging when evidence from some fields is lacking and there is conflicting evidence from different sources.

For this project genetic evidence supporting a specific region or gene was taken from published linkage and association studies of schizophrenia, particularly the Lewis et al (Lewis et al., 2003) meta-analysis. Our own previous data and previous schizophrenia association studies conducted by others were evaluated as well (Williams et al., 2003). Evidence linking genes with

CMV or HSV was taken from published studies of the molecular mechanisms of virus/host interaction. Recent microarray analysis of gene expression changes caused by latent and active viral infection were used (Ray et al., 2004; Song et al., 2002).

At the end of the search three groups of genes were selected: 1. Genes in the HLA region of chromosome 6p, which were associated with schizophrenia and infection in our previous studies. 2. Genes significantly associated with schizophrenia in at least one previous study and known to be involved in HSV1 or CMV infection. 3. The IL-18 pathway, which is involved in the innate and adaptive immune response to several infections and contains multiple positional candidate genes.

3.3. CANDIDATE GENES INVESTIGATED

The strengths of different sources of evidence varied for each candidate gene or group of candidate genes. Location, size, and linkage meta-analysis bin rank of selected candidate genes are listed in Table 3.1. Selected publications that link genes with schizophrenia and CMV or HSV1 are summarized in Table 3.2. Detailed gene specific data is also presented in the following subsections.

Table 3.1. Candidate Genes: Location, Size, and Meta-analyses Rank

Gene	Name	Location	Position		Linkage Meta-analyses	
			Mb	Size Kb	Lewis (2003) Rank	Badner (2002) p-value range
MICB	MHC Class 1 Chain-Related Gene B	6p21.3	31.5	12.9	5	p=0.03-0.04
TNF	Tumor Necrosis Factor	6p21.3	31.6	2.8	5	p=0.03-0.04
IL-10	Interleukin 10	1q31-32	203	4.9	16	-
IL1RN	IL-1 receptor antagonist	2q14.2	113.6	16.1	1	p=0.1-0.008
IL1-beta	Interleukin 1 beta	2q14	113.6	7.0	1	p=0.1-0.008
IL12A	Interleukin 12 alpha	3q25.32	161.2	7.0	117	-
IL12B	Interleukin 12 beta	5q31.1	158.7	15.7	2	-
IL-18	Interleukin 18	11q22.2	111.5	20.9	4	-
IL18BP	Interleukin 18 Binding Protein	11q13	71	6.8	109	-
IL18R1	Interleukin 18 Receptor 1	2q12	102	36.1	1	p=0.02-0.008
IL18RAP	IL-18 Receptor Accessory Protein	2q12	102	33.7	1	p=0.02-0.008

Table 3.2. Candidate Genes: Summary of Published Supporting Evidence

Gene	Previous Association Studies		Serum or CNS levels altered in schizophrenia	Role in neurodevelopment neurodegeneration	Involvement in Infection	
	Positive	Negative			CMV	HSV
MICB	(Kim et al., 2004a)	-	-	(Azimi et al., 2006)	(Cosman et al., 2001; Wu et al., 2003)	-
TNF	(Boin et al., 2001; Meira-Lima et al., 2003; Schwab et al., 2003b; Tan et al., 2003)	(Duan et al., 2004; Hashimoto et al., 2004; Riedel et al., 2002; Tsai et al., 2003)	(Kowalski et al., 2001; Urakubo et al., 2001)	(Beattie et al., 2002; Stellwagen et al., 2006)	(Hurme et al., 1998), (Cheeran et al., 2001)	(Halford et al., 1996; Irie et al., 2005)
IL-10	(Chiavetto et al., 2002; Yu et al., 2004)	(Jun et al., 2003)	(Cazzullo et al., 1998; Rothermundt et al., 1996)	-	(Hurme et al., 2003; Raftery et al., 2004)	(Lokensgard et al., 2002; Marques et al., 2004)
IL1RN	(Katila et al., 1999a; Kim et al., 2004b)]	(Chowdari et al., 2001; Papiol et al., 2004)	(Akiyama, 1999; Maes et al., 1996)	(Allan et al., 2005)	(Hurme et al., 1998; Witkin et al., 2002)	-
IL1-beta	(Papiol et al., 2004; Zanardini et al., 2003)	(Rosa et al., 2004; Yang et al., 2003)	(Gilmore et al., 2004; Marx et al., 2001)	(Ashdown et al., 2006; Hensley et al., 2000; Viviani et al., 2004)	(Randolph-Habecker et al., 2002; Wara-aswapati et al., 1999)	(Lokensgard et al., 2001)
IL12A	-	-	(Kim et al., 2002)	(Sitaraman et al., 2000)	(Pien et al., 2000)	(Kumaraguru et al., 2002)
IL12B	-	-				
IL-18	-	-	(Tanaka et al., 2000)	(Felderhoff-Mueser et al., 2005a; Hedtjarn et al., 2005a; Kanno et al., 2004)	(Andoniou et al., 2005; Andrews et al., 2003; Pien et al., 2000; Vliegen et al., 2004)	(Cui et al., 2005; Lee et al., 2003; Malmgaard et al., 2003; Zhu et al., 2003)
IL18BP	-	-				
IL18R1	-	-				
IL18RAP	-	-				

3.3.1. Regions Associated with Schizophrenia and CMV Infection in Our Previous Studies

In collaboration with Dr. R H Yolken at the Stanley Laboratory of Developmental Neurovirology (Baltimore, MD), we investigated the joint impact of genetic variation and exposure to infectious disease (Kim et al., 2004a). We investigated antibody levels and genotyped 151 US Caucasian patients with schizophrenia or schizoaffective disorder (DSM IV criteria) and their parents. A sample of 183 neonatal community-based controls were also genotyped, but immunoassays could not be conducted. We simultaneously assessed genetic variation in the HLA region and exposure to four infectious agents implicated in schizophrenia: CMV, *Toxoplasma gondii*, HSV-1, and HSV-2 (Yolken et al., 1995). Associations of exposure to infectious agents in simplex and multiplex families were evaluated by Dr. Bernie Devlin. Exposure was increased amongst multiplex families (more than one affected child) versus simplex families (one affected child), significantly so for CMV, HSV1, and HSV2. The difference was most consistent for CMV, and supported CMV exposure as a risk factor for schizophrenia.

Initial genetic analysis was conducted by Dr. Jung Jin Kim, and involved 18 anonymous STRPs selected on the basis of known recombination patterns in the HLA region. Significant association was detected with marker D6S2672 in the HLA Class I region among patients with elevated levels of antibodies to CMV (CMV seropositive cases, $n = 27$; total $n = 151$ cases, $p = 0.0085$). The association was detectable using family-based or unrelated community based controls. A nearby STRP, D6S2676, showed significant differences between HSV seropositive cases and controls, and SNPs D6S2676, D6S2694, D6S2707 showed significant differences between *Toxoplasma gondii* seropositive cases and controls, but overtransmissions were not apparent in the family-based sample. To identify specific genes associated with schizophrenia, we next examined 59 SNPs in twelve genes selected on the basis of their known involvement in

the pathogenesis of CMV infections or association with schizophrenia. We genotyped these selected SNPs in the initial sample. When all cases (regardless of CMV status) were analyzed using the TDT test no significant associations were detected. However, among CMV seropositive patients, TDT showed statistically significant associations with three polymorphisms in the 5' untranslated region (UTR) of *MICB* and one in the 5' UTR of *TNF* (see Table 3.3). Transmission distortion was also observed at haplotypes incorporating these SNPs. The associations were not observed among CMV seronegative cases.

Table 3.3 Associations with Schizophrenia in CMV Seropositive Schizophrenia Cases from Our Previous Studies

Gene	MICB	MICB	MICB	MICB	TNF
SNP	rs2244839	rs2596536	rs2904776	rs2242955	rs1799964
TDT p-value (CMV+ patients only)	**0.00369**	**0.01544**	0.30362	**0.00647**	**0.03841**
Trends CMV+/Control p-value	**0.01958**	0.11013	0.31626	**0.00023**	**0.00041**
Trends CMV+/CMV- p-value	**0.00047**	0.06193	**0.017944**	**0.00027**	**0.00247**

Consistent with the TDT, comparison of CMV seropositive cases with community-controls also revealed significant associations with the SNPs at *MICB* and *TNF*. These associations were also detectable when CMV seropositive cases were compared with CMV- cases. Notably, D6S2672 is localized only 6kb from *MICB* and 71kb from *TNF* raising the possibility that linkage disequilibrium between markers may account for these associations.

The associations at *MICB* and *TNF* may reflect an interaction between particular genotypes and exposure to CMV, but need not impact on schizophrenia pathogenesis. If this were true, associations with the same SNPs should be present among CMV seropositive parents who did not have schizophrenia. Separate comparison of CMV seropositive fathers and CMV seropositive mothers with the unrelated controls did not yield similar genetic associations. The

lack of association among the parents, despite the alleles they share with the probands suggests that the genetic associations are germane to schizophrenia pathogenesis, rather than CMV infection per se. However, analysis of a separate control group is necessary.

These findings suggest an association between the schizophrenia and immune response genes on chromosome 6p in CMV exposed individuals. To localize the association further and identify and evaluate candidate genes in this region we conducted a multi-staged study using individuals of Caucasian ancestry. This work is described in detail in chapters 5 and 6.

3.3.2. Immune-Related Genes Previously Associated with Schizophrenia

Because of the epidemiological evidence suggesting an infectious etiology, geneticists have investigated several immune-related genes for association with schizophrenia. Initially researchers focused on the HLA region on chromosome 6, which harbors many immune system genes. Wright et al reviewed multiple association studies of genes in this region and found inconsistencies possibly because of differences in study design and genes selected (Wright et al., 2001). Recently there has been renewed interest in cytokine genes because of consistent findings of Th1 and Th2 cytokine imbalance in schizophrenia (Akiyama, 1999; Kim et al., 2004c; Maes et al., 2000; Muller et al., 1999; Schwarz et al., 2001). Association studies have focused on candidate genes in the HLA class III region on chromosome 6p21, the IL-1 cluster on chromosome 2q13, and IL-10 on chromosome 1q31. Each of these regions has been implicated in schizophrenia susceptibility by multiple linkage studies (DeLisi et al., 2002; Garver et al., 2001; Levinson et al., 1998; Lewis et al., 2003; Schwab et al., 2003a; Straub et al., 2002).

Among genes localized to chromosome 6p, tumor necrosis factor (TNF) has received the most attention (Duan et al., 2004; Schwab et al., 2003b; Zhang et al., 2004a). Several lines of

evidence implicate *TNF* in the pathogenesis of schizophrenia. *TNF* localizes to chromosome 6p in a region that has been linked with schizophrenia (Schwab et al., 2000; Straub et al., 2002). TNF involvement would fit the infectious hypothesis of schizophrenia genesis: that immune disregulation caused by genetic variants, infectious agents, or a combination of both may be important (Muller et al., 2000; Torrey et al., 2000; Verdoux, 2004). During prenatal exposure to infection, placental TNF levels are elevated (Buka et al., 2001; Urakubo et al., 2001). Elevated TNF has been shown to reduce neuronal survival and dendrite growth in vitro and in animal models (Gilmore et al., 2004) (Marx et al., 2001) providing a mechanism for TNF involvement in brain development. Furthermore, atypical antipsychotic drugs have immunomodulatory effects including reduced expression of TNF, which may be responsible for some of their therapeutic effects (Kowalski et al., 2001; Kowalski et al., 2003; Monteleone et al., 1997).

Evidence suggesting elevated levels of TNF in the serum of schizophrenia patients (Monteleone et al., 1997; Naudin et al., 1997) motivated Boin and colleagues to examine polymorphisms in *TNF* (Boin et al., 2001). The -308A allele had been shown to increase TNF expression in human B-cell lines (Wilson et al., 1997). Boin and colleagues found the A allele of the -308G→A polymorphism (rs1800629) to be over-represented among Italian schizophrenia patients. Multiple groups have attempted to replicate this finding with mixed results (Duan et al., 2004; Handoko et al., 2003; Hanninen et al., 2005; Hashimoto et al., 2004; Kampman et al., 2004; Meira-Lima et al., 2003; Pae et al., 2003; Riedel et al., 2002; Schwab et al., 2003b; Tan et al., 2003; Tsai et al., 2003). Duan et al (2004) included a meta-analysis of previously published -308G→A association studies and concluded that there was evidence for association at this polymorphism only among Caucasian populations.

Multiple schizophrenia associations studies have been conducted for three other positional candidates genes: interleukin 1-beta (*IL1β*), interleukin 1 receptor antagonist (*IL1RN*), and interleukin 10 (*IL10*). *IL1β* and *IL1RN* localize to the 400kb IL1 gene cluster on chromosome 2q14; a region that achieved genome wide significance in a recent meta-analysis of schizophrenia linkage (Lewis et al., 2003). *IL10* localizes to chromosome 1q32; a region that has also been linked with schizophrenia (Garver et al., 2001; Gurling et al., 2001). Published investigations of these candidate genes have focused on one to three polymorphisms, and have not explored the extent of common variation or LD at these loci.

There have been ten published association studies of *IL1β* and *IL1RN* and schizophrenia. The IL1β promoter and binding sites have been highly conserved through vertebrate evolution (Bird et al., 2002). SNP rs16944, which may be associated with increased IL1β production, has been found to be associated with a myriad of apparently unrelated diseases including end-stage renal disease, gastric cancer, and febrile seizures (Kira et al., 2005; Manchanda et al., 2006; Starzynska et al., 2006). The diverse associations suggest a key role for this polymorphism in biological processes. The retention of this polymorphism in the population despite its putative role in the common diseases listed above suggest some evolutionary mechanism to conserve it, such as a balancing selection. Neither Laurent et al (Laurent et al., 1997) nor Yang et al (Yang et al., 2003) found associations at a Taq1 polymorphism (rs1143634) in *IL1β*. Katilla et al. (Katila et al., 1999b) investigated SNP rs16944 (*IL1β* –511 C/T) in *IL1β*, an 86bp tandem nucleotide repeat in intron 2 of *IL1RN* (*IL1RN* $(86bp)_n$), and a SNP in IL1alpha and found a significant haplotype association. Six additional association studies have investigated rs16944 (*IL1β* –511 C/T) (Chowdari et al., 2001; Meisenzahl et al., 2001; Papiol et al., 2004; Rosa et al., 2004; Tatsumi et al., 1997; Zanardini et al., 2003). Of these, only one found a significant association

with schizophrenia (Zanardini et al., 2003). Four association studies in addition to Katilla el al. (Katila et al., 1999b) investigated the *IL1RN* $(86bp)_n$ repeat polymorphism. Two of these found no association (Chowdari et al., 2001; Papiol et al., 2004), and the other two found opposite alleles to be associated with schizophrenia (Kim et al., 2004b; Zanardini et al., 2003). Several published studies have investigated *IL1β-IL1RN* haplotypes, but no single haplotype has been consistently associated with schizophrenia. In addition to genetic associations, other researchers have reported that the rs16944 T allele is associated with white and gray matter deficits (Papiol et al., 2005), and the *IL1RN* $(86bp)_2$ allele is associated with ventricular enlargement (Meisenzahl et al., 2001).

Three independent groups have investigated SNPs in the putative promoter of *IL10.* Chiavetto et al (Chiavetto et al., 2002) found that the G allele of rs1800896 (-1082) was associated with schizophrenia. Jun et al (Jun et al., 2003) found no association at rs1800896 or rs1800871 in a Korean sample. Yu et al (Yu et al., 2004) found the A allele of rs1800872 to be associated with schizophrenia, and no significant difference at the rs1800896 allele. In a case-parent trio study (He et al., 2006), the same group later found the rs1800896 G allele to be over-transmitted to cases, but found no preferential transmission of rs1800872 alleles.

Several explanations for discrepancies between association studies of these cytokine candidate genes are possible. First, study design, recruitment, and sample size issues are addressed differently be each study. Second, most previous studies only examined one or two polymorphisms per gene and did not investigate the possibility of linkage disequilibrium with other loci. Thus, population differences in haplotype structure could cause different association patterns. Finally, genetic polymorphisms may only increase schizophrenia risk in the presence of viral factors. Because of their known role in the immune system each of these genes is a

logical target for gene-environment interaction studies aimed at putative infectious agents. We investigated these genes with each of these issues in mind.

We chose to investigate *TNF* because it is localized near D6S2672, and there are numerous published association studies of this gene, some of which are positive (Duan et al., 2004), for review of previous studies). Our studies of *TNF* are summarized in chapter 5. When we began investigations of *IL10, IL-1β*, and *IL1RN* data for these genes was available in the Seattle SNPs database and newer genotyping technology was available. Our studies of these three genes are summarized in chapter 7.

3.3.3. IL-18 Pathway Positional Candidates Involved in Herpes Virus Infection

Previous association studies of cytokine genes have acknowledged linkage data, but have not used linkage data as the primary criteria in a search for related immune system genes. In evaluating schizophrenia linkage regions for related immune system genes we noted that five IL-18 related genes localize to regions that have been linked with schizophrenia. Linkage regions were defined primarily by the largest meta-analysis of multiple whole genome linkage scans in schizophrenia (Lewis et al., 2003). The *IL-18* gene localizes to chromosome 11q22, in the 4th ranked region in this meta-analysis (Lewis et al., 2003). The *IL18R1* and *IL18RAP* gene both localize to chromosome 2q12, the highest ranked region (Lewis et al., 2003) . *IL18BP* localizes to chromosome 11q13, which is not in a highly ranked region in meta-analyses, but has been linked with schizophrenia in a subsequent linkage scan (Yamada et al., 2004). IL12 can act in conjunction with IL-18 to produce inflammatory responses (Robinson et al., 2002). *IL12B* localizes to chromosome 5q31, the 2nd ranked schizophrenia linkage region (Lewis et al., 2003).

As well as being a positional candidate, *IL-18* is a functional candidate for schizophrenia. Serum IL-18 levels are significantly elevated in patients with schizophrenia (Tanaka et al., 2000). Furthermore, treatment with clozapine and risperidone both significantly lower serum IL-18 levels in schizophrenia patients (Lu et al., 2004). IL-18 interacts with many cytokines proteins and their receptors in a myriad of immune functions (Gracie et al., 2003; Reddy, 2004). IL-18 has the unique ability to promote either Th1 or Th2 polarization depending on the context (Akira, 2000; Nakanishi et al., 2001). IL-18 was initially categorized as a proinflammatory cytokine involved in inducing IFN-γ, but elevated IL-18 can also be consistent with Th2 predominance (Saiki et al., 2004; Ueda et al., 2006).

IL-18 has a complex role in cerebral neuroinflammation and neurodegeneration and is involved in the brain's defense against infectious agents (Felderhoff-Mueser et al., 2005a). Cell culture studies have shown that IL-18 is involved in the response to several common CNS infections (Felderhoff-Mueser et al., 2005a). A more specific study found IL-18 activates microglia to clear neurons infected with the influenza A virus (Mori et al., 2001). These infectious and hypoxic pathways in which IL-18 is active are similar to pathways being investigated in schizophrenia pathogenesis (Babulas et al., 2006; Cannon et al., 2002). IL-18 is constitutively expressed by astrocytes, microglia, neurons, and ependymal cells, and must be cleaved by caspase to become active (Conti et al., 1999). In the rodent brain IL-18 is expressed at higher levels in the early postnatal period and then down-regulated (Prinz et al., 1999). Furthermore, knockout mice have demonstrated IL-18 is involved in exacerbating hypoxic injury in the neonatal brain, and after hypoxia it is detectable on apoptotic, immature neurons (Felderhoff-Mueser et al., 2005b; Hedtjarn et al., 2005b). IL-18 can attenuate the Th1 response exacerbating rodent models of CNS autoimmune disease (Shi et al., 2000).

IL-18 is involved in immune-system defense against many infections, including CMV and HSV1. Gracie et al (Gracie et al., 2003) presents an extensive overview of many different infectious diseases influenced by IL-18 expression. Murine CMV infection has been shown to greatly induce IL-18 release in experimental strains of mice (Andrews et al., 2003; Pien et al., 2000). IL-18 interacts with IL-12 to induce interferon-gamma production during HSV2 infection (Malmgaard et al., 2003). There has also been research into IL-18 as an adjuvant to vaccinations against both HSV1 and HSV2 infections (Cui et al., 2005; Lee et al., 2003; Zhu et al., 2003). Interestingly, IL-12 and IL-18 have also been shown to be involved in control of Toxoplasma gondii, another putative schizophrenia risk factor (Vossenkamper et al., 2004).

The location of IL-18 pathway genes in regions implicated in schizophrenia linkage scans, as well as substantial literature demonstrating a role for IL-18 in infection and brain function motivated us to investigate the possibility of genes in this pathway having a role in schizophrenia risk by themselves and in conjunction with HSV1 and CMV infections. These studies are presented in chapter 8.

4. GENERAL EXPERIMENTAL METHODS

This section has been included to avoid redundancy in subsequent sections. Some specific details about methods used and numbers of individuals assayed can be found in the subsequent methods sections for each group of genes (section 5.2, 6.2, 7.2, and 8.2).

All studies were approved by the University of Pittsburgh Institutional Review Board. Written informed consent was obtained from all participants, except the neonatal controls, in accordance with IRB guidelines.

4.1. SAMPLES USED

4.1.1. Pittsburgh Trios and Singleton Cases

Unrelated patients were recruited at Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania and from within a 500-mile radius of Pittsburgh. Board-certified psychiatrists, blind to genotyping results, established a consensus diagnosis of schizophrenia or schizoaffective disorder using DSM-IV criteria. In addition DNA was obtained from parents of patients. Serology was available a subset of patients and their parents. Formal structured diagnostic evaluations of the parents were not conducted, but one father received a clinical diagnosis of schizoaffective disorder, bipolar type.

4.1.2. Pittsburgh Neonatal Controls

Control DNA was collected from the cord blood of unscreened Caucasian neonates born at Magee-Women's Hospital, Pittsburgh, PA. Ancestry was based on maternal report, and no demographic details apart from ethnicity and gender were available.

4.1.3. Baltimore Schizophrenia Cases, Bipolar Cases, and Controls

Serum was obtained from 272 Caucasian individuals with DSM-IV criteria schizophrenia or schizoaffective disorder recruited in and around Baltimore, Maryland. Diagnosis was made by one of two board certified psychiatrists (Dickerson et al., 2004). The average age was 41.9 (SD 11.2), and the sample was 64% male.

Serum was also obtained from 119 Caucasian individuals with DSM-IV criteria bipolar I or bipolar II disorder in and around Baltimore, Maryland. Diagnosis was made by one of two board certified psychiatrists (Dickerson et al., 2004). The average age was 39.5 (SD 12.8), and the sample was 30% male.

For a control sample, serum was also obtained from 108 Caucasian adult individuals screened for current and past psychiatric disorder using a structured interview. The average age was 35.7 (SD 12.6), and the sample was 28% male. None of the patients or control individuals had clinically apparent viral infection or was taking antiviral medications. Detailed descriptive and recruitment criteria can be found in Dickerson et al (Dickerson et al., 2006), where serological information from the majority of individuals in this sample was previously analyzed. DNA for this sample was extracted from serum and amplified using whole genome amplification (Quiagen, Valencia, CA). The study was approved by Sheppard Pratt Institutional Review

Board, and written informed consent was obtained from all participants in accordance with IRB guidelines.

4.1.4. Mental Health Interventional Research Center Samples

Mental Health Interventional Research Center (MHIRC) samples were obtained as part of a study funded through Dr. David Kupfer to optimize the treatment of mood and anxiety disorders. DNA and serum samples were obtained from 399 Caucasian patients visiting the outpatient clinic of Western Psychiatric Institute and Clinic, Pittsburgh, PA. Diagnoses were taken from clinical notes and a limited number of structured examinations. Table 4.1 contains descriptive data about this sample.

Table 4.1 Descriptive statistics for MHIRC sample

Age			
mean	stdev	Q1	Q3
41.1	11.5	31.6	50.8

Sex	
M	F
140	259

Psychiatric diagnoses (not exclusive)	
Bipolar	132
Depression	177
Anxiety or Panic disorder	122
Personality Disorder	55
Eating Disorder	32
Substance Abuse	93
Other	126
no diagnosis recorded	36

4.2. GENOTYPING AND SEQUENCING

4.2.1. SNAPshot Assay

The SNAPSHOT assay is a PCR based single base extension protocol (Applied Biosystems, New Jersey, USA). Genomic DNA flanking the site of interest is amplified using a standard PCR reaction (see Appendix B4). This template is then purified and dNTPs are removed using Exonuclease 1 and Shrimp Alkaline Phosphotase. A target oligonucleotide primer is designed so that the 5' end of the target primer anneals to the nucleotide adjacent to the target SNP. The sizes of these target primers can be varied allowing assays to be multiplexed for cost savings. The SNAPSHOT reaction is a modified PCR reaction that is run with amplified template, target primer, and SNAPSHOT multiplex kit, which contains buffer, polymerase, and fluorescently labeled dideoxynucleotides (see Appendix B4). During the SNAPSHOT run the target primer is extended by one nucleotide that is complementary to the target SNP.

For our experiments SNAPSHOT products were electrophoresed using an ABI PRISM 3100 Genetic Analyzer and the products were analyzed using GeneScan software version 3.7. Genescan–120LIZ standard (Applied Biosystems) was used as an internal electrophoresis size standard. Allele calls were made using Genotyper v3.7 Software (Applied Biosystems). All genotypes were assayed blind to clinical and immunological status

4.2.2. Illumina Golden Gate assay

The Illumina Golden Gate assay is described in detail in Shen et al (Shen et al., 2005). All Illumina reagents and probes were provided by Illumina, Inc. (San Diego, CA). Assays were performed at the University of Pittsburgh Genomics and Proteomics Core Facility. Briefly, the Illumina assay queries genomic DNA with two allele-specific and one locus specific probe. The

3' ends of the allele specific probes are complementary to universal primers and the 5' end is complementary to the 3' end of the locus. These probes are annealed to the genomic DNA and ligated together. The probe fragments are separated from the genomic DNA and used to inoculate a PCR reaction containing specifically coded, fluorescently labeled, biotinylated primers. The PCR product is then hybridized to a Sentrix Bead Array matrix, which contains 50K fiber optic readers and locus specific beads randomly distributed across the matrix. Illumina performs quality control and provides decoding software for each matrix. Each SNP is interrogated at least 6 times on the matrix for each individual sample. The matrix is decoded using an Illumina BeadArray reader, which examines the dye intensity for each specific allele on each bead. Calls are grouped into heterozygote and homozygote clusters using a clustering algorithm. Allele calls are returned with a reliability score for each call.

4.2.3. Pooled DNA Sequencing

Pooled DNA sequencing assays were validated in our lab by Abigail Northup and Kodavali Chowdari. The technique is described in detail in Sham et al (Sham et al., 2002), and Chowdari et al (Chowdari et al., 2006). Briefly, DNA from individuals was quantified by fluorometry using the pico-green method (Molecular Probes, Inc; Eugene, OR) and diluted to 4 ng/ul. A set volume of DNA from pre-specified individuals was combined. Locus specific primers were then used to amplify the sequence of interest in pooled DNA using a standard PCR protocol (see Appendix B1). Amplified products were sequenced using the ABI Prism Big Dye 3.1 Terminator Kit (Applied Biosystems). Similar to the SNAPSHOT reaction, genomic DNA flanking the site of interest is amplified using a standard PCR reaction. This template is then purified and dNTPs are removed using a Exonuclease 1 and Shrimp Alkaline Phosphotase. An

oligonucleotide primer, often one of the template amplification primers, is used as a sequencing primer. The sequencing reaction is a modified PCR reaction that is run with amplified template, sequencing primer, and Big Dye Terminator Kit multiplex kit, which contains buffer, polymerase, and dNTPs combined with fluorescently labeled dideoxynucleotides (see Appendix B1). The product of this reaction then precipitated using isopropanol, and analyzed on an ABI 3700 Genetic Analyzer. The sequence can then be read using ABI Prism Sequencing Analysis software or Sequcencher software (Gene Codes, Ann Arbor, MI).

For pooled sequencing peak heights obtained from ABI Prism Sequencing Analysis software version 3.7 are used to estimate allele frequencies. In these studies SNP allele frequencies were estimated by dividing individual peak heights by the sum of both peak heights. Either both forward and reverse primers were used for sequencing allele frequencies were averaged or only one primer was used and samples were repeated in triplicate. We have empirically found from our previous studies that this method is sensitive enough to detect polymorphisms that are present in >5% of the alleles in a population (Chowdari et al., 2006).

4.3. IMMUNOASSAYS

Immunoassays were performed in the laboratory of Dr. Robert Yolken of the Stanley Division of Neurovirology, Johns Hopkins School of Medicine, Baltimore, USA. Serum samples were obtained from the peripheral venous blood of the subjects, and stored at –80 °C until use. The levels of IgG antibodies to HSV-1, HSV-2, TOX, and CMV were tested with solid phase enzyme immunoassay (Ashley et al., 1998),(Buka et al., 2001). Briefly, wells of microtiter plates coated with target antigens (obtained from KMI Diagnostics Inc, Minneapolis, USA) were reacted with test serum diluted 1:100 in phosphate-buffered saline solution at pH 7.4 containing 0.1 %

polysorbate (Tween) 20. Following incubation for 2 hours at 37 °C, the plates were washed 5 times with phosphate-buffered saline solution containing 0.1 % polysorbate 20, and incubated with peroxidase-labeled anti–human IgG. Following incubation for 1 hour at 37 °C, the plates were washed 5 times and incubated with 2-2'-azino-bis [3-ethylbenzthiazoline-6-sulfonic acid]–hydrogen peroxide peroxidase substrate. Following reaction for 30 minutes, the amount of color generated by reaction between the antigen-bound enzyme and the soluble substrate was quantitated by means of a microplate colorimeter at a wavelength of 450 nm. Assays using different microtiter plates were standardized by the use of curves generated from reference samples run on each assay plate. Antibody titers were quantified as signal/cutoff (S/C) ratios, which were calculated for each sample by dividing the optical density measurements generated in the assay by the optical density of cut-off sera that were provided by the manufacturer of the assays. S/C values of 2.0 or more were taken as abnormal, based on the frequency distribution for S/C values. Immunological analyses were performed only for patients and their parents, as sera were unavailable from the cord blood samples.

4.4. STATISTICAL ANALYSES AND QUALITY CONTROL

4.4.1. Quality Control and Data Management

When using SNAPSHOT assays, we denoted alleles at each polymorphism as '1' or '2' alphabetically for convenience. For Illumina assays alleles are coded 'A' and 'B' and coding documentation was provided by Illumina. For SNAPSHOT assays 4 to 16 samples were sequenced using an ABI 3700 automated DNA sequencer for verification of each SNP. Allele calls were re-checked independently, blind to diagnostic status. SNPs that could not be called for over 5% of the sample were not included in further analysis. Data was formatted for various

analyses using Microsoft Access, Excell, and Word software (Microsoft Corporation, Redmond, WA). Genotypes were evaluated for Hardy-Weinberg equilibrium in individual samples using GenePOP (Raymond et al., 1995), with cases, controls, mothers, and fathers evaluated separately. SNPs not in Hardy-Weinberg equilibrium in controls ($p<0.01$) were evaluated for potential systematic errors in genotype calling. Genotypes that could be systematically corrected were corrected. If no systematic error in genotype calling could be found and genotype calls were still significantly out of Hardy-Weinberg equilibrium these SNPs were not analyzed further. Family based samples were evaluated for Mendelian consistency using PedCheck (O'Connell et al., 1998). Inconsistencies were resolved by repeating the assays or by sequencing where possible. If multiple Mendelian errors could not be resolved, families containing these errors were removed from the analysis.

4.4.2. Power calculations, Tag SNPs, and Linkage Disequilibrium

Power calculations were done using Quanto software, which calculates power for evaluation of genetic, environmental, and gene-environment interaction effects using several study designs (Gauderman, 2002).

Haploview software (Barrett et al., 2005) was used to evaluate linkage disequilibrium (LD) between polymorphisms in our sample. LD is the population level correlation of alleles at nearby loci. LD is commonly calculated in two ways r^2 and D'. The correlation of two SNPs beyond that expected by chance is represented as r^2, which is directly related to Pearson's correlation coefficient 'r'. But if two SNPs have different minor allele frequencies then it is impossible for them to be perfectly correlated in a population, even though alleles are in tight LD. D' is a measurement that takes this into account and expresses observed correlation

proportional to the total possible correlation given differences in minor allele frequency. When the LD information about polymorphisms in a region is know, this enables the selection of 'tag' SNPs.

Tag SNPs for a gene are SNPs that represent other common variants in order to minimize the number of genetic markers evaluated without substantially reducing the amount of information obtained (Stram, 2004). If it is known that two SNPs are highly correlated, than an investigator can genotype only one at a substantial cost savings and a relatively small reduction in information and power. An association at an individual tag SNP indicates that all of the polymorphisms that the tag SNP represents are very likely to be similarly associated.

For this project we used Hclust (Rinaldo et al., 2005) and Haploview (Barrett et al., 2005) software to select and evaluate tag SNPs. Each of these software programs calculates correlation between polymorphisms and groups them into clusters of correlated polymorphisms. The user specifies the minimum correlation acceptable and the minimum minor allele frequency of interest. For the projects presented here we used minimum correlations (r^2) of either 0.8 or 0.9 and minimum minor allele frequencies of either 10% or 5% depending on the project. More aggressive strategies, which use multiple SNPs to predict ungenotyped polymorphisms, can be used to further reduce the number of tag SNPs. These strategies are analogous to using haplotypes to identify associations, and are less straightforward to analyze and interpret than tagging strategies that utilize pairwise correlation between individual SNPs.

4.4.3. Association Analysis for Individual SNPs

We used the χ^2-based Armitage trend test (Devlin et al., 1999) to compare allele and genotype frequencies in cases and controls as well as subgroups defined by gender and specific diagnosis.

Likewise, we evaluated the possibility of association of tag SNPs with infection status by comparing subsets of schizophrenia cases seropositive and seronegative for antibodies to infectious agents using the Armitage trend test.

We used Genehunter software to evaluate the overtransmission of SNPs from parents to children in case-parent trios (Kruglyak et al., 1996). This evaluation of the overtransmission of alleles is known as the transmission disequilibrium test TDT (Spielman et al., 1993a), and is based on the assumption that a child has an equal chance of inheriting either variant of an autosomal polymorphism from a heterozygote parent. Under this assumption, transmission distortion only occurs in the presence of association and linkage.

4.4.4. Genomic Control

Genomic control is a method used for evaluating and correcting for population stratification in a case-control sample (Devlin et al., 1999). Genomic control uses markers not associated with disease to estimate the distribution of allele frequency differences between two populations. The median allele frequency difference can be compared to the expected median difference under the assumption of no population stratification. If the median allele frequency difference is greater than that expected under the null hypothesis then an appropriate correction factor can be applied to test statistics observed while comparing these populations. For studies that were performed using the Illumina assay we selected thirty-one SNPs for genomic control analysis. We selected one SNP from each of the thirty-one genomic bins least likely to be associated with schizophrenia from the Lewis et al (Lewis et al., 2003) meta-analysis. For each of these thirty-one bins, we selected one Hapmap validated SNP located in a gene desert with minor allele frequency greater than 10%. The genomic control SNPs are listed in Table 4.2.

Table 4.2. Genomic Control SNPs

Bin from Lewis et al	dbSNP rs#	Chromosome	Coordinate
120	rs1556691	1p	4403588
119	rs1354616	7p	9444325
118	rs4807030	19p	5340941
117	rs1512779	3q	145949627
116	rs1443146	11p	25553259
115	rs9319189	13q	85516099
114	rs724291	7p	33572377
113	rs4298967	12p	2278455
112	rs10737918	1p	14130441
111	rs9298401	8q	84367357
110	rs2418979	10q	109527084
109	rs1531931	11q	41781138
108	rs167053	13p	21802287
107	rs2169724	2q	194341332
106	rs876644	10p	34218433
105	rs12617498	2p	5961180
104	rs12785159	10q	57584439
103	rs1949514	2p	22031319
102	rs1496180	8q	107043053
101	rs7317328	13q	68971186
100	rs676924	15p	27542278
99	rs1870480	3q	22619924
98	rs2639223	3q	81218291
97	rs7781020	7q	118725121
96	rs2827213	21q	22316388
95	rs924769	8q	134920425
94	rs10851555	15p	52194113
93	rs6464697	7q	144913906
92	rs1471392	19q	23068686
91	rs4900384	14q	97568704
90	rs9459053	6q	164663514

We used the Armitage trend test to calculate test statistics for each of the thirty-one genomic control SNPs. We compared the median test statistics from the genomic control SNPs to the expected median value and assessed the need to correct for population stratification as described in Devlin et al (Devlin et al., 1999). Statistical tests of genomic control SNPs were performed by Joel Wood.

4.4.5. Haplotype Analysis

We estimated haplotype frequencies and tested for haplotype differences between cases and controls using 'Cocaphase' option of the 'Unphased' software (Dudbridge, 2003). This program uses the expectation maximization (EM) algorithm to estimate haplotypes (Excoffier et al., 1995). Briefly, this algorithm uses expected haplotype frequencies as a starting parameter to estimate observed haplotypes. Then it iteratively compares expected and observed haplotypes, updates the expected parameters based on the comparison, and recompares observed and expected haplotypes until they converge. The software then uses likelihood ratios of log-linear models to compare case and control haplotype frequencies estimated using EM (Cordell et al., 2002). For calculation of global p-values during haplotype analysis, haplotypes with estimated frequency below one percent in cases or controls were excluded.

For some genes, we also evaluated the possibility that subsets of SNPs may be associated with schizophrenia using stepwise logistic regression. The regression procedure started with all the SNPs in each gene and eliminated the least significant predictor of schizophrenia status step-wise until only SNPs or combinations of SNPs that predicted schizophrenia risk at a $p < 0.1$

remained. Stepwise logistic regression analysis was performed by Joel Wood and Brian Shirts. We could then use the 'Cocaphase' to estimate haplotypes comprising subsets of these SNPs using EM and compare cases and controls using log-linear models as above.

4.4.6. Analysis of Gene-Environment Interaction

Several definitions of gene-environment interaction exist. Both genetic and environmental factors may contribute to an outcome of interest, but this does not necessarily mean that the factors 'interact.' Interaction implies the effect of genetic and environmental factors considered together is different from the additive main effects of the two factors. The most common interaction model is one where the effect of both factors combined is greater than the additive effects of gene and environment.

4.4.6.1. Standard Methods of Gene-Environment Interaction

The most uncomplicated method for evaluating gene-environment interaction is using a two-by-four table to analyze a case-control sample (Botto et al., 2001). This allows for separate evaluation of genetic, environmental, and interaction factors using simple odds ratios and chi-square tests (or exact tests if cell size is small). The 2 x 4 table can be easily expanded to a 3 x 4 table to allow for all allelic combinations at a SNP (Botto et al., 2001).

When it is necessary to control for other continuous or dichotomous variables, such as gender and age, logistic regression-based modeling can be used, although this can make it more difficult to interpret the outcomes (Gauderman, 2002). Given all the potential study design problems that may arise, including genotyping error and gene-environment correlation, the case-

control design is most often optimal design for studies of gene-environment interaction (Liu et al., 2004).

4.4.6.2. Case-Only Analysis of Gene-Environment Interaction

Case-only analysis has been presented as an alternative to case-control analysis of gene-environment interaction (Khoury et al., 1996b). The key assumption of this analysis is that genetic and environmental factors are independently distributed. If this assumption is true then the presence of association between genetic and environmental factors in the case population is evidence for gene-environment interaction. If genetic and environmental factors are not independent misinterpretation is possible, as there may be distortion of interaction factors or failure to detect interaction (Albert et al., 2001; Schmidt et al., 1999). If the correlation between genetic and environmental factors is known from previous studies it is possible to adjust for this correlation (Gatto et al., 2004).

4.4.6.3. Family-Based Analysis of Gene-Environment Interaction

The growing popularity and availability of family-based genetic samples has led to the development of several family-based methods of evaluation of gene-environment interaction (Cordell et al., 2004; Umbach et al., 2000). These methods generally use log-linear models to perform likelihood ratio tests of the no-interaction null hypothesis. Like the case-only test of gene-environment interaction, a basic assumption of these tests is that genetic and environmental factors are independent; likewise, it is often necessary to assume that the environmental factors are not correlated between parents and controls (Chatterjee et al., 2005). Where additional family members, affected siblings, and controls samples are all available it may be possible to increase the power to find interaction by using all available samples, but in addition to previous assumptions independence of environmental exposure between siblings is necessary (Andrieu et

al., 2004; Goldstein et al., 2006). Besides log-linear model based tests, a multiple dimensionality reduction method has recently been proposed for evaluation of gene-gene and gene-environment interaction; this method is utilizes the same assumptions of non-independence as the other methods (Martin et al., 2006).

5. GENETIC ASSOCIATION AND FUNCTIONAL STUDY OF TNF

5.1. INTRODUCTION

We designed our genetic association study of TNF to address three specific possible reasons for inconsistencies in previous studies (see section 3.3.1). 1) In order to examine the possibility of unidentified polymorphisms in *TNF* being over-represented in Caucasian schizophrenia cases we sequenced the entire *TNF* gene, including all introns and 4kb of 5' and 3kb of 3' UTRs in DNA pooled from 125 Caucasian schizophrenia cases and 200 controls. 2) We evaluated the possibility that exposure to the infectious agents CMV, HSV1, and HSV2 may cause increased risk in combination with *TNF* polymorphisms by measuring the antibody titers to these agents, genotyping selected SNPs, and stratifying our case sample by infectious disease exposure. 3) Lastly, we addressed the possibility of risk caused by one or more common haplotypes increasing *TNF* expression using a dual-luciferase reporter system to assay expression in 3.8kb of genetic context.

5.2. SPECIFIC METHODS

5.2.1. Clinical sample

Cases (n = 244) from the Pittsburgh case sample were used for genetic and immunologic analysis. This subset of the case sample was 65% male and the average age was 38.7 ± 9.7. Unscreened Pittsburgh controls (n = 276) were used for genetic analysis, but serum sample were

unavailable for immunologic analysis of this sample. This subset of the control sample was 50.8% male. Additional details about the recruitment of these samples can be found in sections 4.1.1 and 4.1.2.

5.2.2. Pooled sequencing

DNA from individuals was diluted to 4 ng/ul as recommended (Sham et al., 2002), and combined into pools of 125 cases and 200 controls. Portions of the region of interest were amplified using a standard PCR protocol. Twenty-three overlapping amplicons were designed to cover approximately 10kb including the entire *TNF* gene, 4kb of 5' UTR, and 3kb of 3' UTR. PCR primers and conditions are available in Appendix B. Amplified products were sequenced as described in section 4.2.3.

5.2.3. Immunoassays for antibody titers of sera of subjects

Serum samples were obtained from the peripheral venous blood of the 244 case subjects as described in section 4.3.

5.2.4. DNA markers and genotyping

We selected eight SNPs to genotype individually among cases and controls. Five of these SNPs (rs1799964, rs1800630, rs1799724, rs1800629, rs361525) have been investigated in previous studies. Three additional SNPs were chosen to enable analysis of additional haplotypes (rs2844482, rs3093543, rs1800610). Two pairs of these, rs1800610/rs1799724 and rs1800630/rs2844482) were in near complete LD in our sample, and thus provided an additional check of our genotype assay consistency. There are only two tag SNPs indicated in phase 2 of

the Hapmap project indicating the 6 independent SNPs cover TNF reasonably well (HapMap, 2003). We used Haploview software (Barrett et al., 2005) to evaluate LD and haplotype block structure in this region (Appendix A, Table A1 and Figure A1). SNPs were analyzed using the multiplexed SNAPSHOT method as described in section 4.2.1.

5.2.5. Generation of reporter constructs and transfection studies

Transfection experiments were preformed by Dr. Mikhil Bamne, Ph.D. An extended 5'TNF-alpha promoter region (-3858 to –1) was amplified using sequence specific tagged primers Kpn1 (sense) and Nhe1 (antisense). DNA fragments were taken from 5 different cases homozygous for 5 of the 6 commonly found haplotypes (see Table 5.7). Amplified products were cloned into pGL3 basic promoter-less luciferase vector at Kpn1 and Nhe1 sites. The integrity of each construct was checked by restriction enzyme digestion and sequencing. We were unable to identify an individual homozygous for the remaining common haplotype so site-specific mutation of SNP -308G→A was performed on the haplotype 4 clone, using QuickChange XL Site-Directed Mutagenesis Kit (Stratagene), according to manufacturer's protocol. Transfections into SHSY-5Y (ATCC-CRL-2266) cells were performed in 24-well plates ($1X10^5$ cells/well) using lipofectAMINE (Life Technologies, Inc.) according to manufacturer's instructions. Cells were harvested after 36hrs, and luciferase assays performed using dual luciferase reagents (Promega). Luciferase values were normalized for transfection efficiency using cotransfected promoterless pGL3 (Promega). Each clone was tested in triplicate.

5.2.6 Statistical analysis

We used the Armitage trend test for analysis of case-control allele-wise and genotype-wise differences for individual SNPs (Devlin et al., 1999). We separated cases groups defined by gender, HSV1, HSV2, and CMV seropositivity and compared subgroups of cases with the control sample using the Armitage trends test. Using Quanto Software (Gauderman, 2002) known rs1800629 allele frequencies (minor allele frequency = 0.2) and schizophrenia population prevalence (0.008), we calculated our sample had >95% power to replicate the findings of Boin et al or Meira-Lima et al (relative risk > 2.0) using a log-additive or dominant model . We used the 'Cocaphase' option of UNPHASED software (Dudbridge, 2003) to calculate case and control haplotype frequencies and compare case and control haplotype frequencies for statistical significance. Significance of expression differences was calculated using the unpaired two-tailed students t-test. Statistical methods are described in more detail in section 4.4.

5.3. RESULTS

5.3.1 Pooled sequencing

The primary purpose of our pooled sequencing was SNP discovery in a schizophrenia sample (Sham et al., 2002). We identified 18 SNPs with minor allele frequencies > 5% in patients, all of which were listed in public databases. Estimated allele frequencies for and dbSNP rs numbers for SNPs identified are listed in Appendix B, Table B2. Case and control allele frequencies as estimated by pooled sequencing were correlated with allele frequencies reported in dbSNP (case $r = 0.76$, $p = 0.2 \times 10^{-4}$; control $r = 0.83$, $p = 2 \times 10^{-5}$) (National_Center_for_Biotechnology_Information, 2005).

5.3.2 Genotype analysis of individual samples

None of the 8 individual SNPs we analyzed were associated with schizophrenia (Table 5.1). All of the 8 SNPs genotyped were in Hardy-Weinberg equilibrium in the case and control population. Furthermore, individual SNPs were not associated with subgroups of schizophrenia patients defined by sex or CMV, HSV1, or HSV2 exposure as compared to controls (Table 5.2 – 5.6).

Table 5.1: Association Analysis of SNPs in *TNF* and Its Promoter.

ID#	Marker	Case (n=244)			Control (n= 276)			Genotype-wise p-value	Minor allele frequency		Allele-wise p-value
		1/1	1/2	2/2	1/1	1/2	2/2		case	control	
1	rs2844482 (C/T)	169	69	6	189	74	7	0.896	0.182	0.163	0.896
2	rs3093543 (A/C)	205	38	1	228	44	1	0.892	0.097	0.084	0.894
3	rs1799964 (C/T)	11	82	151	11	93	170	0.897	0.227	0.210	0.898
4	rs1800630 (A/C)	6	67	171	8	84	174	0.268	0.176	0.188	0.274
5	rs1799724 (A/G)	5	39	200	3	55	213	0.538	0.091	0.113	0.529
6	rs1800629 (A/G) (-308)	7	64	173	8	73	195	0.952	0.142	0.161	0.951
7	rs361525 (A/G)	1	22	221	3	24	249	0.721	0.051	0.054	0.708
8	rs1800610 (C/T)	201	38	5	214	55	3	0.482	0.909	0.112	0.472

1/1, 2/2, 1/2: Individuals homozygous for allele1, allele2 and heterozygous, respectively. Nucleotides were labeled 1 or 2 in alphabetical order, nucleotide identity is indicated in the second column.

Table 5.2: Association Analysis of SNPs in *TNF* and Its Promoter Comparing HSV1 Seropositive Schizophrenia Cases with Community Controls.

ID#	Marker	HSV1+ Case (n = 88)			Control (n =276)			Genotype-wise p-value	Minor allele frequency		Allele-wise p-value
		11	12	22	11	12	22		case	control	
1	rs2844482 (C/T)	59	26	3	189	74	7	0.561	0.182	0.163	0.561
2	rs3093543 (A/C)	71	17	0	228	44	1	0.603	0.097	0.084	0.614
3	rs1799964 (C/T)	4	32	52	11	93	170	0.619	0.227	0.210	0.624
4	rs1800630 (A/C)	3	25	60	8	84	174	0.723	0.176	0.188	0.726
5	rs1799724 (A/G)	3	10	75	3	55	213	0.432	0.091	0.113	0.420
6	rs1800629(-G308A) (A/G)	1	23	64	8	73	195	0.542	0.142	0.161	0.542
7	rs361525 (A/G)	0	9	79	3	24	249	0.876	0.051	0.054	0.869
8	rs1800610 (C/T)	75	10	3	214	55	3	0.441	0.909	0.112	0.428

1/1, 2/2, 1/2: Individuals homozygous for allele1, allele2 and heterozygous, respectively. Nucleotides were labeled 1 or 2 in alphabetical order, nucleotide identity is indicated in the second column. Serum was not available for controls, so HSV1 seropositive cases were compared to all controls.

Table 5.3: Association Analysis of SNPs in *TNF* and Its Promoter Comparing HSV2 Seropositive Schizophrenia Cases with Community Controls.

ID#	Marker	HSV2+ Case (n = 29)			Control (n = 276)			Genotype-wise p-value	Minor allele frequency		Allele-wise p-value
		11	12	22	11	12	22		case	control	
1	rs2844482 (C/T)	24	5	0	189	74	7	0.124	0.182	0.163	0.125
2	rs3093543 (A/C)	26	3	0	228	44	1	0.378	0.097	0.084	0.388
3	rs1799964 (C/T)	0	10	19	11	93	170	0.494	0.227	0.210	0.503
4	rs1800630 (A/C)	0	5	24	8	84	174	0.051	0.176	0.188	0.054
5	rs1799724 (A/G)	1	5	23	3	55	213	0.853	0.091	0.113	0.852
6	rs1800629(-G308A) (A/G)	1	5	23	8	73	195	0.428	0.142	0.161	0.420
7	rs361525 (A/G)	0	5	24	3	24	249	0.349	0.051	0.054	0.321
8	rs1800610 (C/T)	23	5	1	214	55	3	0.845	0.909	0.112	0.845

1/1, 2/2, 1/2: Individuals homozygous for allele1, allele2 and heterozygous, respectively. Nucleotides were labeled 1 or 2 in alphabetical order, nucleotide identity is indicated in the second column. Serum was not available for controls, so HSV2 seropositive cases were compared to all controls.

Table 5.4: Association Analysis of SNPs in *TNF* and Its Promoter Comparing CMV Seropositive Schizophrenia Cases with Community Controls.

ID#	Marker	CMV+ case (n = 58)			Control (n = 276)			Genotype-wise p-value	Minor allele frequency		Allele-wise p-value
		11	12	22	11	12	22		case	control	
1	rs2844482 (C/T)	43	15	0	189	74	7	0.360	0.182	0.163	0.366
2	rs3093543 (A/C)	50	8	0	228	44	1	0.576	0.097	0.084	0.585
3	rs1799964 (C/T)	2	21	35	11	93	170	0.890	0.227	0.210	0.892
4	rs1800630 (A/C)	0	15	43	8	84	174	0.125	0.176	0.188	0.134
5	rs1799724 (A/G)	1	8	49	3	55	213	0.408	0.091	0.113	0.407
6	rs1800629(-G308A) (A/G)	0	17	41	8	73	195	0.693	0.142	0.161	0.694
7	rs361525 (A/G)	1	8	49	3	24	249	0.220	0.051	0.054	0.189
8	rs1800610 (C/T)	49	8	1	214	55	3	0.415	0.909	0.112	0.413

1/1, 2/2, 1/2: Individuals homozygous for allele1, allele2 and heterozygous, respectively. Nucleotides were labeled 1 or 2 in alphabetical order, nucleotide identity is indicated in the second column. Serum was not available for controls, so CMV seropositive cases were compared to all controls.

Table 5.5: Association Analysis of SNPs in *TNF* and Its Promoter Comparing Male Schizophrenia Cases with Male Community Controls.

ID#	Marker	Male Case (n = 172)			Male Control (n = 127)			Genotype-wise p-value	Minor allele frequency		Allelewise p-value
		11	12	22	11	12	22		case	control	
1	rs2844482 (C/T)	117	51	4	85	39	4	0.697	0.172	0.184	0.701
2	rs3093543 (A/C)	144	28	0	111	19	0	0.693	0.081	0.073	0.706
3	rs1799964 (C/T)	7	59	106	7	43	77	0.720	0.212	0.224	0.721
4	rs1800630 (A/C)	4	50	119	4	41	83	0.443	0.168	0.191	0.451
5	rs1799724 (A/G)	3	31	138	1	23	102	0.745	0.108	0.099	0.741
6	rs1800629(-G308A) (A/G)	4	48	120	2	37	88	0.963	0.163	0.161	0.964
7	rs361525 (A/G)	1	12	159	2	10	115	0.447	0.041	0.055	0.409
8	rs1800610 (C/T)	138	31	3	100	24	1	0.891	0.108	0.104	0.889

1/1, 2/2, 1/2: Individuals homozygous for allele1, allele2 and heterozygous, respectively. Nucleotides were labeled 1 or 2 in alphabetical order, nucleotide identity is indicated in the second column.

Table 5.6: Association Analysis of SNPs in *TNF* and Its Promoter Comparing Female Schizophrenia Cases with Male Community Controls.

ID#	Marker	Female Case (n = 101)			Female Control (n = 130)			Genotype-wise p-value	Minor allele frequency		Allelewise p-value
		11	12	22	11	12	22		case	control	
1	rs2844482 (C/T)	73	25	3	93	30	2	0.601	0.153	0.136	0.599
2	rs3093543 (A/C)	88	12	1	106	19	1	0.585	0.069	0.083	0.578
3	rs1799964 (C/T)	5	33	63	6	37	84	0.606	0.213	0.193	0.598
4	rs1800630 (A/C)	3	24	74	4	35	85	0.484	0.149	0.173	0.477
5	rs1799724 (A/G)	2	14	85	2	29	96	0.180	0.089	0.130	0.170
6	rs1800629(-G308A) (A/G)	3	24	74	3	32	95	0.944	0.149	0.146	0.943
7	rs361525 (A/G)	0	13	88	1	9	115	0.345	0.064	0.044	0.337
8	rs1800610 (C/T)	86	13	2	94	30	3	0.067	0.084	0.142	0.057

1/1, 2/2, 1/2: Individuals homozygous for allele1, allele2 and heterozygous, respectively. Nucleotides were labeled 1 or 2 in alphabetical order, nucleotide identity is indicated in the second column.

5.3.3. Haplotype analysis

None of the common haplotypes were associated with schizophrenia (Table 5.7). 'Cocaphase' predicted 28 total haplotypes, with 22 rare haplotypes estimated to be present in ≤ 1% of Caucasian schizophrenia cases. Six haplotypes were present at frequencies > 1% in cases. The global p-value for these six haplotypes was not significant ($p = 0.414$). LD and haplotype block structure for our samples is shown in Appendix A1.

5.3.4. Expression analysis of common promoter haplotypes

We identified 6 common haplotypes (frequency > 1%) in our Caucasian case population (Table 5.7). The most common *TNF* promoter haplotype drove the highest levels of luciferase expression (Haplotype IV in Table 5.7). Three haplotypes drove significantly lower levels of expression ($p < 0.05$) in comparison with the most common haplotype (Haplotypes III, V, and VI in Table 5.7). These included the only haplotype that had the -308A allele at SNP rs1800629 (Haplotype III), which showed the lowest expression of any common haplotype.

Table 5.7: Association and Expression Analysis of Common *TNF* Promoter Haplotypes.

ID#	Haplotype 1-2-3-4-5-6-7-8	Case frequency	Control frequency	frequency difference	p-value	Expression level	% maximal Expression
I	C-A-C-C-G-G-A-C	0.047	0.047	-0.001	0.97	5.7+/-1.4	95
II	C-A-T-C-A-G-G-T	0.107	0.134	-0.027	0.20	5.4+/-0.4	90
III	C-A-T-C-G-A-G-C	0.156	0.161	-0.005	0.83	4.1 +/-0.4	68*
IV	C-A-T-C-G-G-G-C	0.533	0.503	0.031	0.35	6.0+/-0.2	100
V	T-A-C-A-G-G-G-C	0.080	0.093	-0.013	0.50	4.7+/-0.6	78*
VI	T-C-C-A-G-G-G-C	0.077	0.051	0.026	0.12	4.5+/-0.2	75*

SNPs nomenclature (#1 – 8) reflects that used in Table 5.1. Expression level is relative to the promoterless pGL3 vector. Percent maximal expression was calculated relative to the most common and most efficient promoter haplotype.

* Significantly lower expression than most common haplotype (IV) ($p < 0.05$).

5.4. DISCUSSION

We evaluated five previously investigated SNPs in the *TNF* gene in an adequately powered sample and found no significant association with schizophrenia. Several explanations have been proposed to explain inconsistencies in previous association studies of *TNF* and schizophrenia. Some groups have suggested that observed associations may be due to rare variants within the *TNF* gene that are novel in schizophrenia (Schwab et al., 2003a) (Handoko et al., 2003) (Tsai et al., 2003). We found no novel polymorphisms that were present in the schizophrenia population at a frequency greater than 5%. Thus, it seems unlikely that the prior associations reflect hitherto undetected SNPs.

Boin et al (2001) postulated that elevated TNF expression might only contribute to risk in the presence of an infectious exposure at a critical stage of neural development; this would cause more significant associations when exposure to infectious agents is taken into account. We quantified antibody titers for four agents that cause latent neural infections and which have been

implicated in schizophrenia. Our assays were not designed to address the precise timing of infection, however, as markers of exposure and latent infection they would increase the power to detect associations if the infectious agents acted in conjunction with *TNF* polymorphisms to increase schizophrenia risk. We find no significant associations with schizophrenia in any of the four subgroups of schizophrenia patients defined by infectious disease exposure.

We have evaluated differential expression at the haplotype level by performing a functional evaluation of all common *TNF* promoter haplotypes in our sample of Caucasian cases. In SHSY-5Y neuroblastoma cells, presence of the A allele at the -308G→A polymorphism in a haplotype is associated with reduced expression. This contrasts a previous report by Wilson et al (1997), who found the –308A allele caused increased expression in a lymphocyte derived B-cell line. These differences are important, as the increased expression due to the –308A allele was invoked as added evidence for a pathogenic role for this variant. Wilson et al (1997) evaluated TNF promoter activity using a construct that contained a 691 bp sequence in a human Raji B-cell line. We used a 3.8kb sequence to evaluate promoter activity in a neuroblastoma cell line. One explanation for observed differences between our results may be translation repressor sequences that are contained in the additional 3kb of sequence we included in our assays. Others have found *TNF* expression to be cell type specific (Barthel et al., 2003). Varying transcriptional activator profiles in B-cell and neuroblastoma cell lines may explain the difference in *TNF* expression. It is not clear which in vitro findings are most applicable to in vivo *TNF* expression in schizophrenia.

6. LOCALIZATION OF CHROMOSOME 6P ASSOCIATIONS AND *MICB*

6.1. INTRODUCTION

Association and linkage with polymorphisms in the MHC region have been reported (Lewis et al., 2003; Wright et al., 2001). We previously found the marker D6S2672 to be associated with schizophrenia in CMV seropositive individuals and other nearby markers may be associated with schizophrenia HSV1 seropositive individuals (Kim et al., 2004a). Here we present a staged study using individuals of Caucasian ancestry designed to localize this association further, to evaluate possible main genetic effects on schizophrenia and viral infection separately, and to investigate possible interactions of the two in schizophrenia risk. Initially, we selected representative or "tag" SNPs spanning a 100kb region flanking D6S2672. We genotyped these polymorphisms in a preliminary sample of schizophrenia cases and unscreened controls. We then selected five SNPs to investigate further. We assayed these SNPs in two additional Caucasian case-control and case-parent trio samples for which serological data on CMV and HSV1 infection were available. The case-control sample was used to confirm previous associations, evaluate genetic associations with viral antibody titers, and investigate the possibility of gene-virus interaction in schizophrenia. We used a family based sample as an additional replicate to evaluate genetic associations while controlling for population stratification. If genetic polymorphisms and environmental exposures are independent, such a sample enables further evaluation of interactions. Simultaneously investigating two independent

samples not only provided replication, but also allowed us to utilize the strengths of both case-control and family based study designs.

6.2. METHODS

6.2.1. Clinical Samples

6.2.1.1. Pittsburgh cases and community controls.

Unrelated patients (n=236) were recruited as described in section 4.4.1. This patient sample had an average age of 38.7 (SD 9.8), and was 64% male. DNA from neonatal cord blood (n=240) was used as a control sample as described in section 4.4.2; the sample was 52% male.

6.2.1.2 Baltimore schizophrenia, cases bipolar cases, and controls

Schizophrenia cases n=272, Bipolar cases n=119, and controls n=108 recruited in Baltimore were used as a replicate case-control sample. These samples are described in section 4.1.3.

6.2.1.3 Pittsburgh cases-parent trios

A sample of 221 unrelated patients diagnosed with schizophrenia and their 442 parents was used for a family based replicate sample. The average age of schizophrenia cases in this sample was 36.1 (SD 9.3), and the cases were 67% male. Serologic information was available for 195 of these cases, and 338 parents. Forty-nine unselected patients included in the trio sample had also been included in the preliminary Pittsburgh case-control sample. This sample is described in more detail in section 4.1.1.

6.2.1.4 MHIRC sample

The MHIRC sample described in section 4.1.4. was used to test the generalizability of associations with antibody titer status and diagnostic specificity of schizophrenia associations.

6.2.2. Immunoassays

Immunoassays were performed on serum samples as described in section 4.3.

6.2.3. Selection of SNPs and genotyping

We used information from dbSNP (National_Center_for_Biotechnology_Information, 2005), phase 1 of the Hapmap project (HapMap, 2003), and our own in-house sequencing using pooled DNA samples (Chowdari 2006 in press) to select SNPs covering a 100kb region centered on D6S2672. This region spans chromosome 6p position 31534000-31634000 (NCBI genome build 36.1), and includes 6 known transcripts: HLA complex P5 (HCP5), MHC class I polypeptide-related sequence B (MICB), mitochondrial coiled-coil domain 1 (MCCD1), HLA-B associated transcript 1 (BAT), ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G2 (ATP6V1), and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1 (NFKBIL1)(see Appendix A, Figure A2 for line diagram of region). In order to estimate how well public databases represented the region and identify SNPs with possible association we sequenced over 20kb (20%) of the region in pools of DNA from 125 cases and 200 controls as described in Chowdari et al (2006 in press). From our sequencing we selected 10 SNPs that showed preliminary evidence for case-control differences ($p < 0.05$). We genotyped these SNPs in the Hapmap CEPH trios (Coriell, New Jersey, USA) to determine linkage disequilibrium (LD), the population-level correlation of alleles at nearby loci, between SNPs selected by exploratory analysis and Hapmap phase 1 SNPs. We then used Hclust software (Rinaldo et al., 2005) to select 26 common SNPs (minor allele frequency > 0.1) that tagged Hapmap phase 1 SNPs in this region at a correlation (r^2) of 0.8. This set of SNPs represent over half of Hapmap

phase 2 SNPs in this 100kb region at an r^2 above 0.8 and over 70% of these SNPs at an r^2 above 0.7. SNPs were assayed using the multiplexed SNAPSHOT method as described in section 4.2.1.

6.2.4. Statistical Analyses

We used the chi-square based Armitage trend test (Devlin et al., 1999) or Fisher's exact test to evaluate allele and genotype frequency differences between schizophrenia case and controls and between seropositive and seronegative individuals. In the trio sample we evaluated the overtransmission of alleles from parents to children using the TDT test with Genehunter software (Kruglyak et al., 1996). In addition we used the SAS software 'proc catmod' to evaluate differences in CMV and HSV1 seropositivity while correcting for age and gender. We used SAS 'proc catmod' to test models of gene-environment interaction. Due to the number of tests necessary to investigate genetic effects on schizophrenia, CMV, and HSV1 antibody titer status as well as gene-virus interactions at five loci we chose not to correct for multiple comparisons. Instead, we chose to evaluate independent samples and interpret the significance of our findings in light of consistency between these samples. Statistical methods are described in more detail in section 4.4.

6.3. RESULTS

Several separate samples were evaluated: a Pittsburgh case-control sample; Baltimore schizophrenia case, bipolar case, and control samples; a Pittsburgh case-parent trio sample; and the MHIRC sample. Results from the Pittsburgh case-control sample was analyzed first, as it was used to identify the SNPs analyzed in the other samples. Subsequently, Baltimore

schizophrenia case-control and Pittsburgh trio samples were analyzed side-by-side to evaluate associations of antibody titer status with schizophrenia, genetic associations with antibody titer status, genetic associations with schizophrenia, and interaction between genes and antibody titer status in schizophrenia. Finally, Baltimore Bipolar case-control and MHIRC were analyzed to evaluate the generalizability of associations with antibody titer status and the specificity of associations with schizophrenia.

6.3.1. Genetic Associations with Schizophrenia in Pittsburgh Case-Control Sample

In the initial sample (Pittsburgh case-control sample, n = 236 cases, 240 controls) we genotyped twenty-six SNPs and evaluated evidence for association with schizophrenia. Five SNPs (rs2523666, rs2523650, rs2516500, rs2855812, and rs2844494) had a failure rate over 5% or were not in Hardy-Weinberg equilibrium ($p<0.01$, see Appendix A, Figure A2 and Table A2 for location and LD estimates). These SNPs were not included in the association analysis. Four of 21 SNPs analyzed were nominally associated with schizophrenia (rs2596453, p =0.026; rs2596536, p = 0.025; rs2523651, p = 0.022, rs2904776, p = 0.021, Table 6.1). Each localized to a 15kb region between the genes *MICB* and *HCP5* (Appendix A, Figure A2). Several of the SNPs were in tight LD and showed similar associations (see rs2523651, rs2904776, and rs2395038 on Table 6.1 and Appendix A, Table A2, for example).

The following SNPs were associated with schizophrenia and were selected for further analysis: rs2596453, rs2596536, and rs2523651. SNPs rs2904776 and rs2395038 were not included because they were in LD with rs2523651, which could be reliably multiplexed for genotype assays with the other SNPs. In addition, we added SNP rs1051788, which causes a non-synonymous change in MICB and is in substantial LD with rs2596536 (r^2 = 0.67) and

rs1055569, which showed a trend towards association and was easily assayed with the other SNPs. We evaluated these five SNPs for associations with schizophrenia, HSV1, CMV, and gene-environment interaction in two other samples (Baltimore case-control sample n= 272 cases, 108 controls and Pittsburgh trio sample, n=221 cases, 442 parents).

Table 6.1. Association Analysis of Chromosome 6p SNPs in Pittsburgh Case-Control Sample.

Marker	Code		Position	Case (n = 236)			Control (n = 240)			Success Rate	Genotype-wise p-value	Allele-wise p-value	case	control
	1	2		11	12	22	11	12	22				freq_1	freq_1
rs2523685	C	G	31534235	138	74	19	123	84	22	0.966	0.224	0.201	0.758	0.721
rs2243621	C	T	31539799	155	60	18	163	60	13	0.985	0.396	0.357	0.794	0.818
rs2523674	A	G	31544768	36	96	103	27	108	90	0.966	0.937	0.936	0.357	0.360
rs2596453	A	G	31544881	123	91	22	148	72	16	0.992	**0.026**	**0.020**	0.714	0.780
rs2244839	C	T	31546347	127	94	14	112	109	18	0.996	0.123	0.134	0.740	0.697
rs14597	G	T	31547993	84	111	36	76	130	34	0.989	0.597	0.608	0.604	0.588
rs1055569	C	T	31548061	113	100	21	98	109	28	0.985	0.118	0.120	0.697	0.649
rs2248372	A	G	31554445	33	97	105	24	112	99	0.987	0.838	0.837	0.347	0.340
rs2248462	C	T	31554775	146	84	6	152	80	5	0.994	0.582	0.601	0.797	0.810
rs2596536	C	T	31555827	106	110	18	85	117	29	0.977	**0.025**	**0.032**	0.688	0.621
rs2523651	A	G	31556133	43	114	79	28	105	97	0.979	**0.022**	**0.021**	0.424	0.350
rs2904776	C	G	31557060	43	112	81	27	111	100	0.996	**0.021**	**0.021**	0.419	0.347
rs3094009	C	T	31565157	144	86	4	139	90	6	0.985	0.513	0.543	0.799	0.783
rs2395038	A	C	31568430	39	113	78	29	107	97	0.973	0.055	0.056	0.415	0.354
rs2242955	A	G	31582259	2	57	177	5	53	180	0.996	0.886	0.887	0.129	0.132
rs3131635	A	G	31584113	18	81	134	13	89	137	0.992	0.712	0.708	0.251	0.241
rs3130637	C	T	31596124	135	80	18	139	73	20	0.977	0.856	0.849	0.751	0.756
rs3130055	A	G	31605378	135	87	10	149	75	15	0.989	0.690	0.688	0.769	0.780
rs2071594	C	G	31620699	25	114	97	29	103	104	0.992	0.836	0.837	0.347	0.341
rs2255798	C	G	31629281	1	62	169	2	57	170	0.968	0.824	0.833	0.138	0.133
rs3130062	C	T	31633891	1	36	196	2	23	214	0.992	0.133	0.128	0.082	0.056

6.3.2. Associations of Antibody Titer Status with Schizophrenia in Baltimore Case-Control and Pittsburgh Trio Samples

Based on established cutoff values the samples were classified as "seropositive" or "seronegative" with respect to HSV1 and CMV antibodies. Detailed serologic analyses of the Baltimore schizophrenia and bipolar case-control sample have been published (Dickerson et al., 2004), Torrey et al 2006(submitted)). In brief, there was no significant difference between either schizophrenia or bipolar cases and controls from Baltimore in regard to HSV1 status, but there was a significant association with cognitive deficits in HSV1 seropositive individuals (Dickerson et al., 2004). CMV seropositivity was significantly associated with schizophrenia and specifically deficit schizophrenia (Torrey et al 2006(submitted)(Dickerson et al., 2006).

Among cases from Pittsburgh and their parents, 34.9 % and 61.8% were HSV1 seropositive respectively and 22.2 % and 43.1% were CMV seropositive respectively (see Tables 6.2 and 6.3). These rates are similar to other studies, with the prevalence of HSV1 and CMV infection correlated with age (Marshall et al., 2005; Yolken, 2004). Associations of antibody status with schizophrenia were not investigated in the Pittsburgh trio sample because suitable control samples were not available.

6.3.3. Associations of SNPs with CMV and HSV1 Antibody Titer Status

In the Baltimore control sample the rs1051788 'G' allele was associated with HSV1 seropositivity even after adjusting for age and gender ($p = 0.006$, OR=2.7, 95% CI = 0.7-4.7, see Table 6.2). The rs2523651 'A' allele was significantly associated with CMV antibody seropositivity after adjusting for age and gender ($p=0.001$, OR = 3.6, 95% CI 1.5-5.8, Table 6.3).

Neither of the SNPs was significantly associated with infection in the Baltimore schizophrenia case sample or bipolar case sample (Tables 6.2 and 6.3).

In parents of Pittsburgh trios the same allele of SNP rs1051788 was also associated with HSV1 antibody titer after adjusting for age and gender (p = 0.024, OR = 1.6, 95% CI 0.1-3.1 Table 6.2. No SNPs were associated with HSV1 antibody titer status in the Pittsburgh case sample. In the Pittsburgh trio sample SNP rs2523651 was not significantly associated with CMV antibody titer status in either parents or cases (Table 6.3).

Table 6.2: Association of rs1051788 with HSV1.

rs1051788	HSV1+			HSV1-			HSV1+		HSV1-		OR[a]	95% CI[a]	pval[a]
	AA	AG	GG	AA	AG	GG	freq A	Freq G	freq A	freq G	G vs A		
Baltimore control	2	13	34	6	27	26	0.173	0.827	0.331	0.669	2.7	[1.3,5.5]	**0.006**
Baltimore case	10	41	43	13	65	61	0.324	0.676	0.327	0.673	1.0	[0.7,1.5]	0.991
Pitt trio case	7	28	45	14	42	59	0.263	0.738	0.304	0.696	1.0	[0.6,1.6]	0.981
Pitt parent	11	76	120	11	57	60	0.237	0.763	0.309	0.691	1.6	[1.1,2.4]	**0.024**
Baltimore Bipolar	3	23	21	7	28	35	0.309	0.691	0.300	0.700	0.9	[1.3,5.5]	0.817
MHIRC Caucasian	18	72	75	22	107	104	0.327	0.673	0.324	0.676	1.3	[0.7,1.5]	0.201

a- Odds ratios, 95% confidence intervals, and p-values calculated using SAS proc genmod to correct for age and gender.

Table 6.3: Association of rs2523651 with CMV

rs2523651	CMV+			CMV-			CMV+		CMV-		OR[a]	95% CI[a]	pval[a]
	AA	AG	GG	AA	AG	GG	freq A	freq G	freq A	freq G	A vs G		
Baltimore control	5	13	1	11	29	49	0.605	0.395	0.287	0.713	3.6	[1.7,7.8]	**0.001**
Baltimore case	14	37	39	26	82	68	0.361	0.639	0.381	0.619	0.9	[0.6,1.4]	0.791
Pitt trio case	7	15	17	24	55	58	0.372	0.628	0.376	0.624	1.1	[0.6,2.0]	0.798
Pitt Parent	21	52	56	50	137	126	0.418	0.582	0.378	0.622	1.2	[0.8,1.7]	0.425
Baltimore Bipolar	2	21	9	14	66	35	0.391	0.609	0.409	0.591	1.1	[1.7,7.8]	0.766
MHIRC Caucasian	16	72	55	29	123	103	0.364	0.636	0.355	0.645	1.1	[0.6,1.4]	0.703

a- Odds ratios, 95% confidence intervals, and p-values calculated using SAS proc genmod to correct for age and gender.

Neither rs1051788 nor rs2523651 was associated with HSV2 status in Baltimore schizophrenia case or control samples, but the number of HSV2 seropositive control individuals was small (Table 6.4). SNPs rs2596453, rs1055569 and rs2596536 were not significantly associated with HSV1 or CMV antibody titer status in any of the Pittsburgh or Baltimore samples. None of the five SNPs evaluated was significantly associated with HSV1 or CMV in Caucasians from the Baltimore bipolar case or Caucasian MHIRC samples (data not shown).

Table 6.4. Evaluation of rs2523651 and rs1051788 and HSV2 Status in Baltimore Controls and Schizophrenia Cases.

HSV2 Baltimore Control Marker	HSV2+ (N=15) allele 1 frequency	HSV2 - (N=86) Allele 1 frequency	X^2	p-value
rs2523651 [a]	0.46667	0.31977	2.14706	0.14284
rs1051788 [a]	0.16667	0.27326	1.42196	0.23308

HSV2 Baltimore Case Marker	HSV2+ (N=51) allele 1 frequency	HSV2 - (N=210) Allele 1 frequency	X^2	p-value
rs2523651 [a]	0.37255	0.37799	0.01	0.92035
rs1051788 [a]	0.26471	0.33333	1.86568	0.17197

a- At rs2523651 and rs1051788 allele 1 = A and allele 2 = G.

6.3.4. Genetic Associations with Schizophrenia in Baltimore Case-Control and Pittsburgh Trio Samples

In the Baltimore schizophrenia case-control sample, none of the SNPs was associated with schizophrenia. Likewise, when comparing Baltimore bipolar cases with controls, none of the SNPs showed significant associations. The rs1051788 'A' allele frequency was elevated in schizophrenia cases compared to controls, consistent with the Pittsburgh trio sample (see

following paragraph), but the difference was not quite significant (p = 0.101, OR = 1.357, CI 0.943-1.955, Table 6.5).

In the Pittsburgh trio sample there were overtransmissions of the rs1051788 'A' allele (Transmitted (T)= 107, not transmitted (NT) = 74, p = 0.014) and the rs1055569 'C' allele (T = 90, NT = 64, p = 0.036) in the entire trio sample (Table 6.5). The association at 1055569 was consistent with the association at this SNP in the Pittsburgh case-control sample. No other allele was significantly overtransmitted to schizophrenia cases.

Table 6.5: Associations with Schizophrenia in Pittsburgh Trio Sample and Baltimore Case-Control Sample.

Pittsburgh Trios (n = 221 trios)	Transmitted Allele			
Marker	1	2	X^2	p-value
rs2596453	69	68	0.01	0.932
rs1055569	90	64	4.39	**0.036**
rs2596536	92	107	1.13	0.287
rs2523651	116	103	0.77	0.380
rs1051788 [a]	107	74	6.02	**0.014**

Baltimore case-control (n = 272 cases, 108 controls)	Allele Count			
Marker	case allele 1 frequency	Control allele 1 frequency	X^2	p-value
rs2596453	0.718	0.728	0.063	0.802
rs1055569	0.638	0.673	0.809	0.367
rs2596536	0.653	0.693	1.045	0.307
rs2523651	0.377	0.342	0.782	0.377
rs1051788 [a]	0.320	0.257	2.696	0.101

a- At rs1051788 allele 1 = A and allele 2 = G. The coding system for all other SNPs is given in Table 6.1.

6.3.5. Interaction Between Antibody Titer Status and Genotype in Schizophrenia Risk in Baltimore Case-Control and Pittsburgh Trio Samples

We used logistic regression analyses to assess possible interactions of rs1051788 with HSV1 and rs2523651 with CMV in schizophrenia pathogenesis in the Baltimore case-control sample while

adjusting for gender, age, and main effects. When modeling main effects and interaction neither HSV1, rs1051788, nor the interaction of HSV1 and rs1051788 was a significant predictor of schizophrenia, but there was suggestive evidence for separate interaction (p=0.06) and genetic main effects (p = 0.08, Table 6.6 Model B). Using a similar model for CMV, rs2523651, and CMV-rs2523651 interaction, the interaction was the only significant factor (p = 0.003, Table 6.6 Model D). Age and gender were significantly associated with case status, but controlling for age and gender made little impact in estimates of main effects or interaction effects for either model (Compare Table 6.6 Models A and C with Table 6.6 Model B and C).

Table 6.6: Interaction Analysis: Maximum Likelihood Estimates Using SAS "proc catmod" for Models of Associations.

Model A: Diagnosis = HSV1 + rs1051788 + rs1051788*HSV1

Parameter	Estimate	Std Error	Chi-Square	p-value
Intercept	-1.45	0.32	21.21	<.0001
Hsv1	0.51	0.32	2.57	0.11
r1051788	0.37	0.20	3.65	0.06
r1051788*hsv1	-0.42	0.20	4.62	**0.03**

Model B: Diagnosis = HSV1 + rs1051788 + rs1051788*HSV1 + age + gender

Parameter	Estimate	Std Error	Chi-Square	p-value
Intercept	0.91	0.56	2.67	0.10
hsv1	0.48	0.35	1.91	0.17
r1051788	0.38	0.22	2.99	0.08
r1051788*hsv1	-0.41	0.22	3.45	0.06
age	-0.06	0.01	26.44	**<.0001**
gender	-0.90	0.14	40.80	**<.0001**

Model C: Diagnosis = CMV + rs2523651 + rs2523651*CMV

Parameter	Estimate	Std Error	Chi-Square	p-value
Intercept	-0.87	0.26	11.40	0.0007
Cmv	-0.34	0.26	1.71	0.19
r2523651	-0.29	0.21	1.92	0.17
r2523651*cmv	0.69	0.21	10.65	**0.001**

Model D: Diagnosis = CMV + rs2523651 + rs2523651*CMV + age + gender

Parameter	Estimate	Std Error	Chi-Square	p-value
Intercept	1.47	0.53	7.61	0.006
cmv	-0.28	0.30	0.85	0.36
r2523651	-0.32	0.25	1.65	0.20
r2523651*cmv	0.75	0.25	8.84	**0.003**
age	-0.06	0.01	24.96	**<.0001**
gender	-0.94	0.15	41.52	**<.0001**

Formal evaluation of interaction in the case-parent trio are complicated by the constraints of family based tests of gene-environment interaction (Chatterjee et al., 2005); however, the

association of rs1051788 in parents, but not in cases, is consistent with an interaction (See Table 6.2). Antibody titers were unavailable for the Pittsburgh controls.

6.4. DISCUSSION AND CONCLUSIONS

Although our staged design targeted localization of associations with schizophrenia on chromosome 6p in CMV and HSV1 exposed patients, we were also able to query the genetic basis of risk for HSV1 and CMV seroposivity. We identified separate polymorphisms in and near the *MICB* gene that appear to modulate risk for HSV1 and CMV seropositivity. Interestingly, these polymorphisms could also contribute to schizophrenia risk.

The evidence for association with HSV1 is strongest in *MICB* at the non-synonymous polymorphism, rs1051788, as this association is seen in more than one non-schizophrenia sample (see Table 6.2). This association was not seen in either schizophrenia case sample, MHIRC sample, or the bipolar case sample. This could be interpreted as sample heterogeneity, or it could suggest that this SNP is involved in both schizophrenia and bipolar disorder. This possibility is discussed below. Though the MHIRC sample is diagnostically variable, after adjusting for age and gender there is a trend similar to that seen in controls and parents although it is not significant, possibly because of a large number of bipolar cases in the sample. The 'G' to 'A' change in rs1051788 causes an aspartic acid to asparagine change in the MICB protein on one of the alpha-helical folds presented to the NKG2D receptor on NK cells or gamma/delta T cells, so this polymorphism seems likely to have functional effects.

We also find some evidence for association of rs2523651 with CMV seropositivity. This association is very significant in one control sample, but the sample is small (only 19 CMV seropositive individuals), and the other samples are not as consistent (see Table 6.3). There are

no known functional changes caused by rs2523651, and there is extensive linkage disequilibrium in this region, so rs2523651 may be a genetic marker for a nearby functional polymorphism.

One of the difficulties in interpreting these studies is the inconsistency between samples. Some inconsistencies could be due to stochastic variation and differences in power across samples. It is likely that the true relative risk is smaller than the risk estimated by the most significant sample, so it would not be unusual for other small samples to fail to detect significant associations. Another possibility is that associations of these SNPs with schizophrenia are obscuring associations with HSV1 and CMV in schizophrenia samples.

Intriguingly, the two SNPs that show associations with HSV1 and CMV are also the SNPs most consistently associated with schizophrenia. The 'A' allele of SNP rs1051788 is associated with schizophrenia in the Pittsburgh Trio sample and shows a similar trend in the Baltimore case-control sample that is consistent with the association in the trio sample (see Table 6.5). The association of rs2523651 is only significant in the initial Pittsburgh case-control sample (see Table 6.1, but both additional samples do show a similar trend consistent with a small effect (OR~1.2, see Table 6.5). The association of these SNPs with both viral infection and schizophrenia could indicate pleiotropic effects (unrelated phenotypes produced by a single gene) or an interaction between viruses and *MICB* in schizophrenia.

Interactions between genetic polymorphisms and viral infection in schizophrenia pathogenesis have long been hypothesized (Kirch, 1993), and could account for variability in previous studies of HSV1 and CMV in schizophrenia (Pearce, 2003; Rapoport et al., 2005). We formally evaluated the possibility of interaction for rs1051788 with HSV1 and rs2523651 with CMV. We found significant evidence for interaction of rs2523651 and CMV in schizophrenia in the Baltimore case-control sample, but as noted above, these associations are based on one

relatively small case-control sample and are not consistent with the trio sample. We also found suggestive evidence for interaction of HSV1 with rs1051788 in schizophrenia pathogenesis in the Baltimore case-control sample. Though not statistically significant, evidence for this interaction is more consistent with the Pittsburgh trio sample. One interpretation of the statistical interaction between HSV1, rs1051788, and schizophrenia is that the genetic variant has an impact on risk for HSV1 and schizophrenia; however, other models are also consistent with these data, and only future studies will be able to draw compelling conclusions.

Further studies of associated polymorphisms are necessary to confirm functional effects of genetic variants. While these functional studies are forthcoming, hypotheses of likely outcomes can be built from previous studies of MICB and the related protein MICA. MICA and MICB are highly polymorphic MHC-class-1-related proteins (Elsner et al., 2001). Both MIC proteins interact with and activate NKG2D receptor on NK cells and gamma/delta T cells (Groh et al., 2001). Although constitutively expressed only in the gut, MICB expression can be induced in many other cell types including CD4+ and CD8+ T cells and glial cells in the brain (Azimi et al., 2006; Friese et al., 2003). MICA and MICB are thought to be involved in the activation of the innate immune system to control viral infection and recognize tumor cells (Collins, 2004; Groh et al., 2001). A common polymorphism in MICA has been shown to modify NK cell mediated apoptosis in CMV infected cells (Zou et al., 2005), and MICB translocation to the cell surface is inhibited by CMV infection (Dunn et al., 2003). *MICB* alleles associated with lower antibody titers could cause more efficient activation of NK cells in response to HSV1 resulting in fewer latently infected cells or less frequent viral reactivation. This in turn would allow viral antibody titers to decrease below the seropositivity-threshold over time. Given that cytokines released by NK cells have known effects in neurodevelopment and

neuromodulation (Alsharifi et al., 2006; Ashdown et al., 2006; Stellwagen et al., 2006; Stoll et al., 2002), it is conceivable that MICB activation of the innate immune system could also impair neurodevelopment through neuromodulatory cytokine release.

The association of MICB polymorphisms with HSV1 and possibly CMV seropositivity reported here suggest the need for functional studies to explore the exact mechanism of MICB action in HSV1 infection. Additional genetic associations studies will be necessary to confirm intriguing findings of associations of MICB with viral immune response, and to evaluate the possible interaction of herpes viruses and MICB in schizophrenia pathogenesis.

7. ASSOCIATION STUDY OF *IL10*, *IL1B*, AND *IL1RN*.

7.1. INTRODUCTION

In our investigation of the genes *IL1β*, *IL1RN*, and *IL10* we have attempted to address some of the concerns about discrepancies in previous association studies that were discussed in section 3.3.2. We used a publicly accessible database of sequenced genes to evaluate LD between common polymorphisms of *IL1β*, *IL1RN*, and *IL10* among individuals reporting Caucasian ancestry. We then genotyped representative 'tag SNPs' in a moderately sized sample. In addition, we typed genomic control SNPs in both case and control samples to evaluate and control for population stratification. We also examined associations in subgroups of our population defined by gender, diagnosis (schizophrenia / schizoaffective disorder), and infectious disease exposure as these variables might impact on the associations. Finally, we conducted a meta-analysis of rs16944, which is localized to *IL1β,* because findings at this SNP appeared remarkably consistent between studies.

7.2. MATERIALS AND METHODS

7.2.1. Sample

Unrelated patients from the Pittsburgh case sample were used for this study (n = 478). This case sample was 64.9% male, with a mean age of 38.2 years (standard deviation 9.6). Antibody titers for cytomegalovirus, herpes simplex virus 1 and 2, and *Toxoplasma gondii* were available for 352 cases. Unscreened Pittsburgh controls (n = 501) were used as a comparison group. This

control sample was 51.9% male. Additional details about the recruitment of these samples can be found in sections 4.1.1 and 4.4.2.

7.2.2. SNP Selection

The Seattle SNPs Program for Genetic Applications (Nickerson et al., 2005) has made information available from completely sequenced *IL10*, *IL1β*, and *IL1RN* genes in a set of 23 individuals of European descent. LD estimates based on D' and r^2 are available for these SNPs (Devlin et al., 1995). We used the hierarchical clustering procedure 'Hclust' (Rinaldo et al., 2005) to select representative "tag" SNPs using a minor allele frequency cutoff of five percent and a minimum correlation (r^2) of 0.9. This criterion was intended to ensure that the selected SNPs would have over 90% relative power to detect associations at the SNPs they tag, based on LD estimates from the Seattle SNPs database. Small insertion/deletion polymorphisms were included in Hclust analysis, and where possible tag SNPs were chosen to represent such polymorphisms. The *IL1RN* $(86bp)_n$ variable tandem repeat polymorphism was not included in the Seattle SNPs database. To determine LD between this polymorphism and other SNPs, we genotyped it among 47 Caucasian cases; historically studies label *IL1RN* $(86bp)_4$ as allele 1 and *IL1RN* $(86bp)_2$ as allele 2 (Tarlow et al., 1993). The analyzed tag SNPs are listed in Table 7.1 and LD between the tag SNPs in our sample is shown in Appendix A, Figures A3-A4 and Tables A3-A4. We selected a total of thirty-four polymorphisms: eleven polymorphisms from 17.4kb at *IL1β* including 1.5 kb 5' and 9kb 3' flanking sequence, fifteen SNPs from 19.7kb at *IL1RN* including 2kb of 5' and 3' flanking sequence, and eight SNPs from 7.9kb at *IL10* including 1kb 5' and 2kb 3' flanking sequence (Appendix A, Figure A3-A4 for location of individual SNPs).

The average coverage was one SNP per 1.3kb. We used genomic control as described in section 4.4.4 to correct for population stratification.

7.2.3. Genotyping

Polymorphisms were genotyped using highly accurate, multiplexed, hybridization based Illumina Golden Gate assays (Shen et al., 2005) as described in section 4.2.2. Candidate polymorphisms are first evaluated for assay designability with a primer design algorithm. Four tag polymorphisms were not included in Illumina assays: two because the assays were not feasible (rs1143625, rs3917354), and two were excluded due to errors (rs1143640, rs3021094). These SNPs were not re-genotyped using other techniques resulting in some gaps in coverage as noted in the results section. Nevertheless, all common SNPs except one were represented at r^2 greater than 0.6.

7.2.4 Statistical Analysis

7.2.4.1. SNP and haplotype analysis

We used the χ^2-based Armitage trend test (Devlin et al., 1999) to compare allele and genotype frequencies in cases and controls and subgroups defined by gender and specific diagnosis. We evaluated the possibility of association of tag SNPs with infection status by comparing subsets of schizophrenia cases seropositive and seronegative for antibodies to CMV, HSV1, and HSV2 using the Armitage trend test. We controlled for multiple testing using Bonferroni correction. We did not statistically control for genotyping error-rate. We estimated haplotype frequencies for haplotypes comprising all SNPs in each gene and haplotypes identified from stepwise logistic regression using 'Cocaphase' software as described in section 4.4.5. We

also used 'Cocaphase' to estimate frequencies of the *IL1β-IL1RN* haplotype reported in published studies. Statistical methods are described in more detail in section 4.4.

7.2.4.2. Meta-analysis

We performed a meta-analysis of published case-control studies at rs16944 (*IL1β* -511) (Table 7.9). We considered meta-analyses of *IL1RN* $(86bp)_n$ and rs1800896 (*IL10* –1081), but three or fewer studies have been published among the same ethnic group for each polymorphism, making meta-analysis less meaningful for these polymorphisms.

We used χ^2 tests and descriptive statistics to evaluate heterogeneity between samples (Munafo et al., 2004). Genotype information was available for all case-control studies, so we initially summed, unweighted case and control genotype counts separately for Caucasian and Asian samples (Caucasian sample: n = 819 cases, 1292 controls; Asian sample: n = 235 cases 239 controls). Differences in proportion of cases and controls between studies could inflate artifacts due to population stratification when analyzing genotype counts. Therefore, as a second method of analysis average unweighted effect sizes were calculated. Averaging effect sizes is less powerful than summing genotypes counts, but it is independent of sample size and does not risk introducing stratification (Rosenthal et al., 2001).

7.3. RESULTS

7.3.1 LD Between Selected SNPs

We selected thirty-four tag polymorphisms in *IL10*, *IL1β*, and *IL1RN* that represent all 120 common polymorphisms present in the Caucasian sample from the Seattle SNPs database. We genotyped 478 cases and 501 unscreened controls for 30 SNPs. Of these SNPs, rs3024494 and rs3087269 were later excluded from analysis due to quality control considerations (cluster

scoring below cutoff values). In view of these exclusions, LD was re-evaluated in our sample. The analyzed SNPs represented all common polymorphisms satisfactorily (mean r^2 = 0.961, n = 28 selected SNPs, total reported SNPs, n=120, see Appendix A, Table A3-A4). Of these, 87% (n=104) polymorphisms were represented at r^2 greater than 0.9 by the analyzed tag SNPs, and 99% (n=119) were represented at r^2 greater than 0.6. Most tag SNPs were in relatively low LD with each other, as would be expected. We found the common alleles $(86bp)_2$ and $(86bp)_4$ of *IL1RN* $(86bp)_n$ were perfectly correlated with rs1794068 A and G alleles respectively. Hence this SNP was investigated *in lieu* of *IL1RN* $(86bp)_n$.

7.3.2 Associations at Individual SNPs

No significant case-control differences were detected at any individual SNP in the entire sample (Table 7.1). For twenty-one SNPs with minor allele frequency greater than 20%, including tags for all SNPs reported in previous schizophrenia association studies, our sample had greater than 80% power at α=0.05 to detect a relative risk of 1.35 using a log-additive model and 1.45 using a dominant model. For the remaining seven SNPs our sample had over 80% power to detect a relative risk greater than 1.8 using either dominant or log-additive models.

Furthermore, we found no significant associations among sub-groups based on gender (Table 7.2). When patients were divided into schizophrenia and schizoaffective subgroups, the “A” allele of rs4252041 was associated with schizophrenia (p = 0.024, χ^2 =5.1, 1df, Table 7.2). This association did not remain significant after correcting for multiple comparisons. Sub-groups of cases seropositive and seronegative for CMV, HSV1, or HSV2 did not differ significantly from each other except at rs3917356 for HSV1 (p = 0.037, χ^2 =4.4, 1df), and

rs2853550 and rs3917365 for CMV (p=0.018, χ^2 =5.6, 1df for each SNP)(Table 7.3). These associations did not remain significant after correcting for twenty-eight comparisons.

Table 7.1. Distribution of Genotypes for Tag SNPs Among Cases and Controls.

Chr	Genomic Location	Gene	SNP	Allele code		Case (n = 478[a])			Control (n = 501[a])			allele 1 frequency		Genotype-wise p-value	Allele-wise p-value
				1	2	11	12	22	11	12	22	case	control		
1	203330808	*IL10*	rs3024495	T	C	11	131	336	12	114	375	0.160	0.138	0.168	0.165
1	203332507	*IL10*	rs3024492	T	A	21	176	280	39	167	295	0.229	0.245	0.412	0.405
1	203333256	*IL10*	rs1518110	T	G	30	151	294	18	198	285	0.222	0.234	0.544	0.547
1	203333441	*IL10*	rs3024491[b]	A	C	110	222	146	101	238	162	0.462	0.439	0.314	0.302
1	203334802	*IL10*	rs1800872[b]	A	C	41	157	279	23	208	269	0.251	0.254	0.860	0.860
1	203335061	*IL10*	rs1800894	T	C	1	33	444	2	31	468	0.037	0.035	0.845	0.841
2	113302700	*IL1β*	rs3917365	T	C	3	72	403	3	75	423	0.082	0.081	0.951	0.951
2	113303352	*IL1β*	rs2853550	A	G	4	72	402	4	83	414	0.084	0.091	0.577	0.576
2	113306621	*IL1β*	rs1143634[b]	A	G	17	175	286	28	179	294	0.219	0.235	0.393	0.401
2	113306698	*IL1β*	rs1143633	T	C	74	203	199	68	220	212	0.369	0.356	0.571	0.560
2	113307506	*IL1β*	rs3136558	T	C	298	162	17	303	172	26	0.795	0.776	0.327	0.330
2	113308594	*IL1β*	rs3917356	A	G	112	222	144	95	250	156	0.467	0.439	0.229	0.223
2	113310618	*IL1β*	rs1143627	A	G	204	220	53	205	233	63	0.658	0.642	0.439	0.443
2	113311098	*IL1β*	rs16944[b]	T	C	53	220	205	63	231	207	0.341	0.356	0.475	0.478
2	113590698	*IL1RN*	rs4251961	A	G	194	215	68	204	233	64	0.632	0.640	0.727	0.725
2	113592309	*IL1RN*	rs4251974	C	G	256	183	39	279	184	38	0.727	0.741	0.507	0.498
2	113593010	*IL1RN*	rs2637988	T	C	180	221	77	188	238	75	0.608	0.613	0.821	0.819
2	113599937	*IL1RN*	rs315934	A	G	300	158	20	312	163	26	0.793	0.785	0.688	0.686
2	113600433	*IL1RN*	rs3087262	G	C	3	108	367	5	115	379	0.119	0.125	0.676	0.686
2	113601999	*IL1RN*	rs3087263	T	C	6	86	384	3	80	418	0.103	0.086	0.196	0.195
2	113602581	*IL1RN*	rs1794066	T	C	176	225	77	186	236	79	0.604	0.607	0.885	0.884
2	113602734	*IL1RN*	rs1794068[b]	T	C	40	187	250	40	185	276	0.280	0.264	0.453	0.444
2	113603566	*IL1RN*	rs2232354	T	G	291	160	25	331	151	19	0.779	0.811	0.083	0.080
2	113604788	*IL1RN*	rs451578	T	C	46	190	242	44	194	263	0.295	0.281	0.517	0.508
2	113605131	*IL1RN*	rs380092	A	T	40	218	220	55	226	220	0.312	0.335	0.256	0.264
2	113606535	*IL1RN*	rs315952	A	G	232	209	37	250	204	47	0.704	0.703	0.946	0.947
2	113606841	*IL1RN*	rs4252041	A	G	0	51	427	1	37	463	0.053	0.039	0.123	0.128
2	113609005	*IL1RN*	rs315949	T	C	83	222	173	75	237	189	0.406	0.386	0.379	0.375

a – Missing genotype counts for less than three individuals for some SNPs due to uncertain genotype calls.

b- Represents a polymorphism investigated in a published study: rs3024491 (A/C) represents *IL10* –1082 (G/A), r^2 = .965; rs1800872 is *IL10* –592; rs1143634 is *IL1β* 105 Taq1; rs16944 is *IL1β* –511 C/T; rs1794068 (T/C) represents *IL1RN* $(86bp)_n$ or IL1RA(VNTR), r^2 = 1.00, rs1794068 T = *IL1RN* $(86bp)_2$ = IL1RA allele 2, rs1794068 C = *IL1RN* $(86bp)_4$ = IL1RA allele 1.

Table 7.2 Association Analysis by Gender and Diagnostic Category, IL-10, IL1β, and IL1RN.

				Female case vs Female control			Male case vs Male control			Schizophrenia vs Control		
Chr	Coordinate	Gene	SNP	case[a]	control[a]	pval[b]	case[a]	control[a]	pval[b]	case[a]	control[a]	pval[b]
1	203330808	IL-10	rs3024495	0.161	0.145	0.549	0.160	0.131	0.168	0.145	0.138	0.693
1	203332507	IL-10	rs3024492	0.250	0.255	0.871	0.217	0.235	0.475	0.218	0.245	0.243
1	203333256	IL-10	rs1518110	0.223	0.239	0.606	0.222	0.229	0.766	0.234	0.234	0.976
1	203333441	IL-10	rs3024491	0.491	0.444	0.187	0.447	0.435	0.692	0.434	0.439	0.844
1	203334802	IL-10	rs1800872	0.256	0.260	0.886	0.248	0.248	0.985	0.267	0.254	0.589
1	203335061	IL-10	rs1800894	0.048	0.029	0.185	0.031	0.040	0.377	0.031	0.035	0.708
2	113302700	IL1β	rs3917365	0.065	0.075	0.599	0.090	0.087	0.826	0.097	0.081	0.265
2	113303352	IL1β	rs2853550	0.065	0.083	0.331	0.094	0.098	0.801	0.101	0.091	0.509
2	113304897	IL1β	rs1143641	0.000	0.000	0.000	0.000	0.002	0.275	0.000	0.001	0.462
2	113306621	IL1β	rs1143634	0.211	0.222	0.715	0.223	0.246	0.336	0.232	0.235	0.896
2	113306698	IL1β	rs1143633	0.377	0.353	0.492	0.364	0.359	0.864	0.382	0.356	0.330
2	113307506	IL1β	rs3136558	0.793	0.780	0.649	0.795	0.773	0.360	0.788	0.776	0.606
2	113308594	IL1β	rs3917356	0.464	0.429	0.339	0.468	0.448	0.507	0.480	0.439	0.132
2	113310618	IL1β	rs1143627	0.652	0.629	0.497	0.662	0.654	0.775	0.683	0.642	0.107
2	113311098	IL1β	rs16944	0.345	0.369	0.481	0.339	0.344	0.843	0.318	0.356	0.131
2	113590698	IL1RN	rs4251961	0.628	0.631	0.937	0.634	0.648	0.632	0.609	0.640	0.230
2	113592309	IL1RN	rs4251974	0.711	0.730	0.564	0.735	0.750	0.580	0.761	0.741	0.385
2	113593010	IL1RN	rs2637988	0.604	0.589	0.679	0.610	0.635	0.380	0.651	0.613	0.138
2	113599937	IL1RN	rs315934	0.783	0.813	0.292	0.798	0.760	0.114	0.787	0.785	0.952
2	113600433	IL1RN	rs3087262	0.107	0.135	0.226	0.126	0.116	0.587	0.108	0.125	0.316
2	113601999	IL1RN	rs3087263	0.126	0.100	0.247	0.091	0.073	0.279	0.089	0.086	0.839
2	113602581	IL1RN	rs1794066	0.610	0.587	0.521	0.600	0.625	0.385	0.645	0.607	0.138
2	113602734	IL1RN	rs1794068	0.290	0.272	0.571	0.274	0.258	0.535	0.245	0.264	0.427
2	113604788	IL1RN	rs451578	0.301	0.282	0.578	0.292	0.281	0.683	0.259	0.281	0.361
2	113605131	IL1RN	rs380092	0.295	0.322	0.398	0.321	0.348	0.329	0.329	0.335	0.800
2	113606535	IL1RN	rs315952	0.711	0.718	0.838	0.700	0.688	0.671	0.706	0.703	0.893
2	113606841	IL1RN	rs4252041	0.048	0.041	0.676	0.056	0.037	0.106	0.064	0.039	**0.024**[c]
2	113609005	IL1RN	rs315949	0.411	0.398	0.723	0.403	0.375	0.338	0.417	0.386	0.239

a- See Table 7.1 for Allele coding. Case and control frequencies for Allele 1 are shown.
b- Genotypewise p-values.
c- The "A" allele of rs4252041 was associated with schizophrenia ($p = 0.024$, $\chi 2 = 5.1$, 1 d.f.). This association did not remain significant after correcting for multiple comparisons.

Table 7.3 Associations with Infections Agents, IL-10, IL1β, and IL1RN

Gene	SNP	HSV1+ [a]	HSV1- [a]	pval [b]	CMV+ [a]	CMV- [a]	pval[b]	HSV2+ [a]	HSV2- [a]	pval[b]
IL-10	rs3024495	0.171	0.147	0.391	0.176	0.152	0.399	0.143	0.163	0.593
IL-10	rs3024492	0.259	0.225	0.264	0.252	0.235	0.590	0.286	0.232	0.196
IL-10	rs1518110	0.213	0.203	0.752	0.199	0.214	0.665	0.173	0.216	0.332
IL-10	rs3024491	0.500	0.451	0.206	0.513	0.457	0.170	0.491	0.473	0.728
IL-10	rs1800872	0.256	0.222	0.315	0.223	0.247	0.508	0.214	0.243	0.538
IL-10	rs1800894	0.035	0.039	0.792	0.042	0.035	0.632	0.027	0.039	0.535
IL1β	rs3917365	0.098	0.067	0.138	0.118	0.065	**0.018**[c]	0.071	0.085	0.636
IL1β	rs2853550	0.098	0.067	0.138	0.118	0.065	**0.018**[c]	0.071	0.085	0.636
IL1β	rs1143634	0.228	0.216	0.706	0.202	0.234	0.311	0.205	0.226	0.611
IL1β	rs1143633	0.345	0.399	0.152	0.387	0.372	0.722	0.393	0.374	0.718
IL1β	rs3136558	0.799	0.781	0.535	0.797	0.786	0.728	0.786	0.790	0.913
IL1β	rs3917356	0.424	0.505	**0.037**[c]	0.466	0.470	0.936	0.438	0.474	0.485
IL1β	rs1143627	0.639	0.694	0.110	0.660	0.674	0.696	0.634	0.676	0.375
IL1β	rs16944	0.361	0.304	0.100	0.340	0.325	0.668	0.357	0.325	0.493
IL1RN	rs4251961	0.627	0.624	0.921	0.627	0.626	0.968	0.625	0.626	0.980
IL1RN	rs4251974	0.728	0.735	0.845	0.752	0.721	0.388	0.714	0.735	0.664
IL1RN	rs2637988	0.604	0.613	0.811	0.609	0.608	0.980	0.554	0.619	0.202
IL1RN	rs315934	0.794	0.789	0.853	0.782	0.799	0.592	0.848	0.782	0.112
IL1RN	rs3087262	0.130	0.121	0.719	0.151	0.113	0.125	0.161	0.119	0.201
IL1RN	rs3087263	0.111	0.080	0.153	0.081	0.102	0.372	0.107	0.092	0.624
IL1RN	rs1794066	0.601	0.611	0.798	0.613	0.602	0.767	0.554	0.616	0.225
IL1RN	rs1794068	0.275	0.268	0.832	0.240	0.288	0.183	0.286	0.269	0.717
IL1RN	rs2232354	0.775	0.772	0.917	0.769	0.776	0.830	0.750	0.778	0.515
IL1RN	rs451578	0.291	0.281	0.772	0.248	0.305	0.125	0.304	0.282	0.660
IL1RN	rs380092	0.329	0.312	0.614	0.345	0.307	0.305	0.321	0.320	0.971
IL1RN	rs315952	0.668	0.696	0.424	0.660	0.699	0.288	0.652	0.692	0.400
IL1RN	rs4252041	0.066	0.041	0.125	0.046	0.056	0.562	0.063	0.051	0.609
IL1RN	rs315949	0.386	0.412	0.490	0.403	0.400	0.942	0.357	0.410	0.310

a- See Table 7.1 for Allele coding. Frequencies for Allele 1 are shown.
b- Genotypewise p-values.
c- For HSV1 rs3917356, p = 0.037, χ^2 =4.4, 1df; for CMV rs2853550 and rs3917365, p=0.018, χ^2 =5.6, 1df. These association did not remain significant after correcting for multiple comparisons.

7.3.3 Genomic control analysis

In addition to tag SNPs, thirty-one genomic control SNPs were assayed (see Table 4.2). The median χ^2 for the genomic control SNPs was 0.336 when comparing cases and controls. This value is below the expected median χ^2 of 0.456 under the null hypothesis, indicating insufficient evidence for population stratification between cases and controls due. Hence corrections for population sub-structure were not applied.

7.3.4 Haplotype associations

We used ‘Cocaphase’ to estimate haplotype frequency differences and compare common haplotype distributions in cases and controls. When all tag SNPs were used to determine haplotypes for each gene, there were seven common (frequency > 1%) haplotypes at *IL10* (n = 7 SNPs), seven common haplotypes in *IL1β* (9 SNPs), and thirteen common haplotypes at *IL1RN* (14 SNPs). No individual haplotypes were associated with schizophrenia; the respective gene-based global p-values were 0.196 for *IL10*, 0.819 for *IL1β*, and 0.627 for *IL1RN* (Tables 7.4-7.6). We next used stepwise logistic regression to evaluate the possibility that smaller subsets of SNPs might be associated with schizophrenia. Logistic regression showed no individual SNPs or sets of SNPs in *IL1β* or *IL10* predicted schizophrenia status. Logistic regression of *IL1RN* SNPs revealed three SNPs (rs1794068, rs315952, and rs315949) that remained significant at < 0.05 level after the last iteration of the logistic regression. Notably, the A allele of rs1794068 was completely correlated with *IL1RN* $(86bp)_4$. ‘Cocaphase’ was used to estimate haplotypes spanning these three SNPs. Case-control comparisons suggested that the A-A-C haplotype was over-represented among the controls (case frequency 4.8%, control frequency 7.2%, p = 0.011), and there was a trend for case-control differences in the overall distribution of all haplotypes

(global p-value = 0.058, Table 7.7). Since we conducted statistical comparisons for 36 common haplotypes this is likely to be a chance finding. Published studies reported associations with haplotypes comprising the rs16944 (*IL1β* –511)-*IL1RN*$(86bp)_n$ haplotype (Katila et al., 1999b; Papiol et al., 2004; Zanardini et al., 2003), so we evaluated the rs16944-rs1794088 haplotype, which corresponds to this haplotype in our sample. None of the four haplotypes defined by these markers were associated with schizophrenia (global p-value = 0.62, Table 7.8).

Table 7.4. *IL10* Haplotype Analysis Using 'Cocaphase'.

Markers						
1	2	3	4	5	6	7
3024495	3024494	3024492	1518110	3024491	1800872	1800894

Haplotype	Case[a]	Freq	Control[a]	Freq	OR[b]	chisq	P
1-2-2-2-1-2-2	151	0.158	136.8	0.137	1.24	1.77	0.18
2-2-1-2-1-2-2	218	0.228	245	0.245	1.00	0.74	0.39
2-2-2-1-2-1-2	213.7	0.224	234	0.234	1.02	0.27	0.60
2-2-2-2-1-2-1	35	0.037	35	0.035	1.12	0.04	0.84
2-2-2-2-1-2-2	36	0.038	22	0.022	1.83	4.23	0.04
2-2-2-2-2-1-2	25.27	0.026	19.75	0.020	1.43	0.90	0.34
2-2-2-2-2-2-2	275	0.288	308.2	0.308	1.00	0.92	0.34

Likelihood ratio test: null = -2684.62 alternative = -2678.46
LRS = 12.3239 DF = 9 p = 0.195658

a- Estimated case and control haplotype counts.
b- Odds Ratio compared to most common haplotype.

Table 7.5. *IL1β* Haplotype Analysis Using 'Cocaphase'.

Markers							
1	2	3	4	5	6	7	8
rs3917365	rs2853550	rs1143634	Rs1143633	rs3136558	rs3917356	rs1143627	rs16944

Haplotype	Case[a]	freq	Control[a]	freq	OR[b]	chisq	p
1-1-2-2-1-1-1-2	23.94	0.02504	16.75	0.01672	1.435521	1.326	0.2496
1-1-2-2-1-2-2-1	52.26	0.05467	62.1	0.06198	0.84556	0.3233	0.5696
2-2-1-2-1-2-2-1	26.69	0.02792	29.16	0.0291	0.918919	0.03739	0.8467
2-2-1-2-2-2-1-2	160.6	0.168	182.1	0.1818	0.885714	0.6636	0.4153
2-2-2-1-1-1-1-2	323.3	0.3381	324.7	0.3241	1	0.4279	0.513
2-2-2-2-1-1-1-2	73	0.07636	70.27	0.07013	1.043243	0.418	0.518
2-2-2-2-1-2-2-1	227.9	0.2384	227.9	0.2275	1.004633	0.2595	0.6104

Likelihood ratio test: null = -3244.83 alternative = -3243.37
LRS = 2.92081 DF = 6 p = 0.818719

a- Estimated case and control haplotype counts.
b- Odds Ratio compared to most common haplotype

Table 7.6. *IL1RN* Haplotype Analysis Using 'Cocaphase'.

Markers						
1	2	3	4	5	6	7
rs4251961	rs4251974	rs2637988	rs315934	rs3087262	rs3087263	rs1794066
8	9	10	11	12	13	14
rs1794068	rs2232354	rs451578	rs380092	rs315952	rs4252041	rs315949

Haplotype	Case[a]	freq	Control[a]	freq	OR[b]	chisq	P
1-1-1-1-2-2-1-2-1-2-1-1-2-2	17.91	0.01873	18.99	0.01896	1.03533	0.001682	0.9673
1-1-1-2-2-2-1-2-1-1-1-2-2-2	13	0.01359	18	0.01796	0.793395	0.6028	0.4375
1-1-1-2-2-2-1-2-1-2-1-1-2-1	13.65	0.01428	14.64	0.01461	1.02381	0.002137	0.9631
1-1-1-2-2-2-1-2-1-2-1-2-2-2	129	0.1349	130.1	0.1298	1.089094	0.1111	0.7389
1-1-1-2-2-2-1-2-1-2-2-1-2-1	37.56	0.03929	34.86	0.03479	1.183564	0.2556	0.6131
1-1-2-1-1-2-2-2-1-2-1-2-2-2	105.5	0.1103	116.5	0.1162	0.994624	0.1733	0.6772
1-2-2-1-2-1-2-1-1-1-2-1-2-2	80.96	0.08469	73.97	0.07382	1.201997	0.7812	0.3768
1-2-2-1-2-2-2-1-1-1-2-1-2-2	147.9	0.1547	162.4	0.1621	1	0.1824	0.6694
1-2-2-1-2-2-2-1-1-1-2-2-2-2	19.1	0.01998	11.58	0.01156	1.811828	2.203	0.1377
2-1-1-1-2-2-1-2-1-2-2-1-2-1	137.5	0.1439	164.8	0.1645	0.917051	1.57	0.2102
2-1-1-1-2-2-1-2-2-2-2-1-1-1	48	0.05021	35.99	0.03592	1.465438	2.438	0.1184
2-1-1-1-2-2-1-2-2-2-2-1-2-1	141.2	0.1477	127.4	0.1271	1.218126	1.722	0.1894
2-1-1-1-2-2-1-2-2-2-2-1-2-2	13.03	0.01363	18.88	0.01885	0.758065	0.865	0.3524

Likelihood ratio test: null = -4342.59 alternative = -4337.67
LRS = 9.84425 DF = 12 p = 0.629622

a- Estimated case and control haplotype counts.
b- Odds Ratio compared to most common haplotype

Table 7.7. Analysis of *IL1RN* Associated Haplotype Using 'Cocaphase'.

Markers		
1	2	3
rs1794068	rs315952	rs315949

Haplotype	Case[a]	Freq	Control[a]	Freq	OR[b]	chisq	p
1-1-2	240.7	0.2504	243.7	0.2444	0.976514	0.1934	0.6601
1-2-2	25.88	0.02806	19.87	0.01888	1.416873	1.215	0.2704
2-1-1	386.5	0.404	385.5	0.3851	1	0.7821	0.3765
2-1-2	44.35	0.0477	73.33	0.07194	0.631953	6.446	0.01112
2-2-2	257.1	0.268	278.1	0.2785	0.917027	0.1845	0.6675

Likelihood ratio test: null = -2122.35 alternative = -2118.61
LRS = 7.4818 DF = 3 p = 0.0580279

a- Estimated case and control haplotype counts.
b- Odds Ratio compared to most common haplotype.

Table 7.8. Analysis of *IL1β-IL1RN* Previously Reported Haplotype Using 'Cocaphase'.

Markers	
1[c]	2[c]
rs16944	rs1794068

Haplotype	Case[a]	Freq	Control[a]	freq	OR[b]	chisq	p
1-1	158	0.1652	158.3	0.1579	1.033378	0.1318	0.7165
1-2	168	0.1758	198.7	0.1983	0.875168	1.581	0.2087
2-1	110.1	0.1151	106.7	0.1065	1.06748	0.6242	0.4295
2-2	519.9	0.5439	538.3	0.5372	1	0.05334	0.8173

Likelihood ratio test: null = -1788.51 alternative = -1787.62
LRS = 1.783 DF = 3 p = 0.618642

a- Estimated case and control haplotype counts.
b- Odds Ratio compared to most common haplotype.
c- Alleles in tables are opposite those reported in published literature for rs16944: rs16944 1 represents the T allele in this table and C in published literature, likewise rs1794068 allele 1 represents the IL1RN(86bp)$_2$ variant in this table, which is the IL1RN 2 allele in published literature.

7.3.5 Meta-Analysis of Association Studies at rs16944

We found evidence for heterogeneity between studies of Asian and Caucasian samples with respect to allele frequency differences ($\chi^2 = 6.16$, 1df, $p = 0.013$). Among samples of Caucasian ancestry, heterogeneity was apparent with regard to the ratio of cases to controls in each sample (range = 0.125-1). In view of these sources of heterogeneity, we used two methods to analyze the published case-control studies (Table 7.9). First, we summed allele counts for Caucasian and Asian sub-samples separately. The C allele was significantly associated with schizophrenia in the Caucasian samples (n = 819 cases, 1292 controls, p = 0.0013, OR = 1.24, 95% CI 1.09,1.41), but not in the Asian samples (n = 235 cases, 239 controls, p = 0.828, OR = 1.03, 95% CI 0.80,1.33). We then averaged unadjusted effect sizes for Caucasian and Asian studies separately and together. This method is more robust to sample size and allele frequency heterogeneity (Rosenthal et al., 2001). We found similar results using this method; the C allele was significantly associated with schizophrenia in the Caucasian samples (effect size = 0.056, 95% CI 0.014, 0.098), but not in the Asian samples. An effect size of 0.056 corresponds to an odds ratio of 1.25, which is very similar to the odds ratio estimate from the summed samples. Assuming an odds ratio of 1.24 and log additive risk, a replicate sample of 1,350 Caucasian cases and controls would be needed to attain 80% power to detect this association ($\alpha = 0.05$). In retrospect it is likely that even this study, which is the largest association study of this marker conducted to date, was underpowered.

Table 7.9. Meta-Analysis of IL1β -511 (rs16944) Association Studies in Schizophrenia.

Study	population	DC [c]	CC [d]	case C	case T	control C	control T	pval [e]	OR	95% CI	r [f]	
Tatsumi 1997[a]	Japanese	1	+	64	44	64	44	1	1.00	0.58,1.72	0.000	
Katilla 1999[b]	Finnish	2	+	71	29	472	328	0.021	1.70	1.08,2.68	0.077	
Chowdari 2001[a]	Chinese	2	+/-	198	164	198	172	0.809	1.05	0.78,1.40	0.009	
Meisenzhal 2001[b]	American	2	++	60	28	60	36	0.419	1.29	0.70,2.37	0.060	
Zanardini 2003[b]	Italy	3	++	227	111	212	142	0.047	1.37	1.00,1.87	0.075	
Papiol 2004[b]	Spanish	2	+	111	45	219	113	0.253	1.27	0.84,1.93	0.052	
Shirts 2006[b]	American	4	-	630	326	645	357	0.478	1.07	0.89,1.29	0.016	
Totals											mean r	95% CI
Sub-samples	Asian			262	208	262	216	0.828	1.04	0.80,1.33	0.004	-0.006,0.096
	Caucasian			1099	539	1608	976	0.001	1.24	1.09,1.41	0.056	0.014,0.098
Total											0.041	-0.013,0.096

a- Included in Asian subsample.
b- Included in Caucasian subsample.
c- Diagnostic Criteria: 1= DSM-III-R schizophrenia, 2 = DSM-IV or ICD10 schizophrenia, 3 = DSM-IV schizophrenia or schizophrenia spectrum, 4 = DSM-IV schizophrenia or schizoaffective.
d- Control Criteria: ++ = controls screened for personal and family psychiatric illness, + = controls screened for general health, +/- = reports two control samples one screened for general health and another portion unscreened, - = unscreened controls.
e- p-values calculated from χ^2 test of allele counts.
f- *phi* calculated from case and control allele counts.

7.4. DISCUSSION

Previous genetic association studies of schizophrenia and *IL10*, *IL1β*, and *IL1RN* generally focused on single polymorphisms. We used a tag SNP approach to comprehensively evaluate common polymorphisms at these genes in a moderately sized Caucasian sample. We did not find significant associations at twenty-eight tag SNPs when we evaluated the entire sample. Following corrections for multiple comparisons, significant associations with haplotypes were also not detected. We did find one suggestive association at rs4252041 in *IL1RN* among the sub-group of patients with schizophrenia. We also found suggestive associations with certain sub-groups at SNPs in *IL1β*. The G allele of rs3917356 was over-represented in herpes simplex virus 1 seropositive cases ($p = 0.037$). The A and T alleles of rs2853550 and rs3917365, which are in tight LD, were over-represented in cytomegalovirus seropositive patients ($p=0.018$ for both SNPs). More focused investigations of these polymorphisms in unscreened populations will be necessary to determine if they are associated with the immune response to viral infection.

Our meta-analysis suggests that the C allele of rs16944 (*IL1β* –511) is associated with a modest increase in schizophrenia risk in Caucasian populations. Consistent with our results, several investigators reported that haplotypes comprising the C allele at rs16944 was significantly associated with increased risk (Katila et al., 1999b; Papiol et al., 2004). In support of the association, other investigators reported that haplotypes comprising the T allele at this SNP were less frequent among cases (Zanardini et al., 2003). Interestingly, the C allele at rs16844 has also been associated with psychosis in Alzheimer's disease (Craig et al., 2004). In contrast, significant association with schizophrenia was not detected in papers reporting on samples with Asian participants. This may reflect a difference in risk etiology among Asian

populations. It may also be attributable to the smaller number of Asian participants analyzed (Table 7.9).

Publication bias was not evaluated in our meta-analysis as six of the seven association studies included in the meta-analyses report no association at the individual SNP level. We observed three common features among the studies with larger effect sizes (see Table 7.9). First, these studies involved Caucasian individuals. Second, control individuals had been screened for physical health. Two studies reported that the control individuals were also screened for personal and family history of psychiatric illness. Finally, these studies investigated patients with schizophrenia, but not those with schizoaffective disorder. Due to the small numbers of studies and the qualitative nature of inclusion criterion we did not calculate the magnitude or significance of these factors. For reference, if our study had been limited to cases with schizophrenia the odds ratio at rs16944 in our sample would have been 1.19 instead of 1.07 (see Table 7.2). The schizophrenia-schizoaffective disorder dichotomy in this context, is supported by a family based association study of *IL1β* (Rosa et al., 2004).

As they are located in the putative promoter of *IL1β,* variations at rs16944 or rs1143627, which is also located in the promoter of *IL1β* and is in LD with rs16944, may impact on *IL1β* expression. In support, Barak et al (Barak et al., 1995) report decreased *IL1β* levels in CSF from patients with schizophrenia, and the rs16944-rs1143627 C-T promoter haplotype causes 2-3 fold lower *IL1β* secretion in LPS stimulated blood (Hall et al., 2004). On the other hand, other investigators have found evidence for an increase in *IL1β* expression in monocytes and serum from schizophrenia cases (Katila et al., 1994a; Kowalski et al., 2001). The majority of studies of *IL1β* expression in schizophrenia have found no significant change (Baker et al., 1996; Bessler et al., 1995; Erbagci et al., 2001; Katila et al., 1994b; Kim et al., 1998). The relationship of these

results to brain function is uncertain, though it has been suggested that exposure to elevated *IL1β* levels leads to a dose dependent reduction in neuronal survival (Marx et al., 2001).

In conclusion, comprehensive evaluation of common polymorphisms of three positional candidate genes did not reveal convincing evidence for association in a moderately large case-control sample. Meta analysis suggests modest genetic risk due to polymorphisms in the promoter region of *IL1β*. Future replicate studies are needed to confirm this modest risk, and additional cytokine expression studies may benefit from evaluation of genetic effects.

8. IL-18 PATHWAY POSITIONAL CANDIDATE GENES

8.1. INTRODUCTION

In light of localization of many IL-18 related genes to schizophrenia linkage regions and recent literature on the role of IL-18 in the CNS we investigated polymorphisms in four IL-18 pathway genes (IL-18, IL18BP, IL18R1, and IL18RAP) as well as two IL12 genes (IL12A and IL12B), which can act synergistically with IL-18, for associations with schizophrenia. Our primary experiments were designed to evaluate all common polymorphisms in these genes using a tag SNP approach. We utilized permutation testing to evaluate the significance level of associations while adjusting for multiple comparisons and we used genomic control (Bacanu et al., 2000) to control for population stratification. Subsequently we performed exploratory analysis to investigate the possibility of gene-gene interaction among these six genes. Additionally we used a case-only design to explore potential interactions of associated SNPs with three infections agents thought to be involved in schizophrenia HSV1, HSV2 , and CMV.

8.2. MATERIALS AND METHODS

8.2.1. Sample

The sample used for evaluation of IL-18 pathway genes was identical to the sample used to evaluate *IL1β*, *IL1RN*, and *IL10* (see section 7.2.1, 4.1.1, and 4.1.2).

8.2.2. SNP Selection

The Innate Immunity Program for Genetic Applications (PGA) (Martinez et al., 2005) has made information available from *IL-18, IL18BP, IL18R1,* and *IL18RAP* genes, and the Seattle SNPs PGA (Nickerson et al., 2005) has made information available from *IL12A* and *IL12B* genes. The PGA initiatives have sequenced exons, introns, UTRs and several kb flanking sequence for these genes in a set of twenty-three individuals of European descent and twenty-four individuals of African descent. Linkage disequilibrium (LD) estimates based on D' and r^2 were available for these SNPs. We used the hierarchical clustering procedure 'Hclust' (Rinaldo et al., 2005) to select representative "tag" SNPs using a minor allele frequency (MAF) cutoff of five percent and a minimum correlation (r^2) of 0.9. This criterion was intended to ensure that the selected SNPs would have over 90% relative power to detect associations at the SNPs they tag, based on LD estimates from the Program for Genetic Applications databases. All small sequenced insertion/deletion polymorphisms were included in Hclust analysis, and where possible tag SNPs were chosen to represent such polymorphisms. In addition, several non-synonymous polymorphisms with MAF slightly below five percent were included. The analyzed tag SNPs are listed in Table 8.1 and LD between the tag SNPs in our sample is shown in AppendixA, Tables A5-8 and Figures A5-A8. We selected a total of sixty-five tag SNPs: twelve at *IL-18*, two at *IL18BP*, seventeen at *IL18R1,* sixteen at *IL18RAP,* seven at *IL12A*, and eleven at *IL12B*. The average coverage was one SNP per 1.85 kb. We also utilized genomic control SNPs described in section 4.4.4.

8.2.3. Genotyping and Immunology

Polymorphisms were genotyped using highly accurate, multiplexed, hybridization based Illumina Golden Gate assays (Shen et al., 2005)(see section 4.2.2). Candidate polymorphisms are first evaluated for assay designability with a primer design algorithm. Five polymorphisms were not included in Illumina assays because the assays were not feasible and no alternative tag SNPs were available (rs3771167, rs582054, rs5744237, rs5744241, rs675614). This resulted in some decrease in coverage (see results, section 8.3).

Specific enzyme based immunoassays for HSV1, HSV2, and CMV were performed on serum samples from 352 cases as described in section 4.3. Serological analysis was not feasible for the controls as IgG antibodies are not produced in the neonatal period.

8.2.4. Statistical Analysis

8.2.4.1. Evaluation of associations at SNPs

We used the Armitage trend test (Devlin et al., 1999) to compare allele and genotype frequencies in cases and controls. We ran 20,000 random permutations of case and controls status using the 'Cocaphase' option of 'Unphased' software to generate empiric p-values (Dudbridge, 2003). Genomic control was used to correct for population stratification as described in section 4.4.4.

8.2.4.2. Exploratory Analysis.

We evaluated the possibility that combinations of SNPs within genes may be associated with schizophrenia using backwards stepwise logistic regression. We then used the 'Cocaphase' option of 'Unphased' software (Dudbridge, 2003) to estimate haplotype frequencies as

described in section 4.4.5.

We used Multifactor Dimensionality Reduction (MDR) (Hahn et al., 2003) to explore the possibility of gene-gene interaction. We ran 1000 permutations of MDR to estimate significance. To better assess the nature, magnitude, and significance of main effects and interaction effects we performed chi-square analysis comparing expected frequencies compared to observed marker combinations calculated by 'Cocaphase'.

We used the Armitage trend test to compare allele and genotype frequencies in the subset of 352 cases classified as seropositive and seronegative for three infectious agents, CMV, HSV1, and HSV2. This test is similar in theory to standard case-only analysis of gene-environment interaction, and assumes independence of genetic and environmental factors for correct interpretation of interaction effects (Gatto et al., 2004).

8.3. RESULTS

8.3.1. Analysis of associations at individual SNPs

8.3.1.1. LD between selected SNPs

A total of sixty tag SNPs were genotyped among 478 schizophrenia cases and 501 unscreened controls. Of these SNPs five (rs11465672, rs11465648, rs2058623, rs5744257, and rs2243131) were later excluded from analysis due to quality control considerations (cluster scoring on Illumina assays below cutoff values). For most marker pairs the LD in our sample was similar to that reported in the PGA databases, but for some marker pairs the LD was different. This resulted in some markers being in greater LD than expected and greater redundancy in genotyping results (see Appendix A, Figures A5-A8). In view of exclusions and redundancies, gene coverage was re-evaluated comparing our sample with the PGA samples.

The fifty-five analyzed tag SNPs captured 230 of 263 polymorphisms with MAF over 5% in the PGA databases at r^2 greater than 0.9, and 215 of 231 polymorphisms with MAF over 10% at r^2 greater than 0.8.

8.3.1.2. Individual SNP associations

Nominally significant case-control differences ($p<0.05$) were detected at seven individual SNPs (Table 8.1): four including the most significant in *IL18RAP* (rs2272127, rs1146702, rs1146723, and rs6543135), one in *IL18R1* (rs7558013), one in *IL-18* (rs5744247), and one in *IL12B* (rs2853694). Only one association remained significant after empirically adjusting for multiple comparisons (rs2272127, $p = 0.028$). All other associated SNPs in *IL18R1* and *IL18RAP*, which are contiguous on chromosome 2p, were in LD with rs2272127.

Table 8.1. Associations of SNPs in IL-18 Pathway Candidate Genes with Schizophrenia.

Gene	Chr	Coordinate	SNP	Allele code		Case			Control			Genotype-wise	Allele 1 frequency		Allele-wise
				1	2	11	12	22	11	12	22	pval	case	control	pval
IL-18	11	111519971	rs3882891	T	G	161	233	84	168	240	93	0.799	0.581	0.575	0.800
IL-18	11	111521724	rs5744280	T	C	50	212	216	57	212	232	0.962	0.326	0.325	0.962
IL-18	11	111526126	rs549908	T	G	231	206	41	262	193	45	0.375	0.699	0.717	0.378
IL-18	11	111529037	rs1834481	C	G	32	177	269	37	173	291	0.775	0.252	0.247	0.780
IL-18	11	111531366	rs5744247	C	G	5	81	392	8	108	385	**0.043**	0.095	0.124	**0.045**
IL-18	11	111531913	rs360722	T	A	8	96	374	11	122	368	0.081	0.117	0.144	0.085
IL-18	11	111536834	rs2043055	A	G	193	224	61	214	219	68	0.725	0.638	0.646	0.727
IL-18	11	111540198	rs187238	G	C	259	184	35	284	183	34	0.442	0.734	0.750	0.447
IL-18	11	111540717	rs1946519	A	C	79	234	165	87	239	175	0.924	0.410	0.412	0.924
IL18BP	11	71386920	rs3814721	T	C	424	52	2	441	58	2	0.759	0.941	0.938	0.760
IL18R1	2	102436918	rs11465567	A	G	395	79	4	391	103	6	0.081	0.909	0.885	0.081
IL18R1	2	102438142	rs9308857	T	C	74	207	197	73	238	190	0.587	0.371	0.383	0.593
IL18R1	2	102438741	rs11465572	T	G	4	79	394	6	104	391	0.075	0.091	0.116	0.074
IL18R1	2	102439495	rs11465576	T	C	420	56	2	442	57	2	0.863	0.937	0.939	0.863
IL18R1	2	102443202	rs1558627	A	G	276	165	37	293	183	25	0.366	0.750	0.767	0.373
IL18R1	2	102445731	rs11465597	T	C	389	85	4	394	100	7	0.235	0.903	0.886	0.236
IL18R1	2	102445879	rs7579737	A	G	239	192	47	253	203	45	0.744	0.701	0.708	0.747
IL18R1	2	102445977	rs6543124	A	T	72	204	201	67	244	189	0.546	0.365	0.378	0.549
IL18R1	2	102448203	rs11465608	G	C	476	2	0	498	3	0	0.693	0.998	0.997	0.692
IL18R1	2	102451324	rs7558013	T	G	22	169	287	18	143	340	**0.015**	0.223	0.179	**0.015**
IL18R1	2	102461783	rs2241116	T	G	32	139	306	21	156	324	0.406	0.213	0.198	0.423
IL18R1	2	102464905	rs4851570	T	C	257	167	54	268	195	38	0.396	0.712	0.730	0.415
IL18R1	2	102471420	rs1420095	A	G	396	80	2	427	73	1	0.292	0.912	0.925	0.280
IL18RAP	2	102493267	rs4851581	A	G	389	86	2	426	73	2	0.166	0.906	0.923	0.158
IL18RAP	2	102493637	rs1420105	A	G	153	209	116	127	253	121	0.147	0.539	0.506	0.158
IL18RAP	2	102493893	rs11465673	T	C	403	72	3	406	90	5	0.161	0.918	0.900	0.161
IL18RAP	2	102494407	rs2293225	A	G	32	139	306	21	156	324	0.406	0.213	0.198	0.423
IL18RAP	2	102495520	rs1420100	A	C	116	209	153	121	253	127	0.147	0.461	0.494	0.158
IL18RAP	2	102497045	rs3755268	C	G	275	166	37	290	185	26	0.454	0.749	0.763	0.460
IL18RAP	2	102498391	rs2272127	G	C	18	114	346	26	165	310	**0.0007**	0.157	0.217	**0.001**
IL18RAP	2	102499463	rs887972	A	G	59	182	236	45	199	257	0.209	0.314	0.288	0.226
IL18RAP	2	102512967	rs2058660	A	G	268	172	36	292	183	26	0.263	0.744	0.765	0.268
IL18RAP	2	102516410	rs11465702	A	G	367	99	11	346	138	17	**0.005**	0.873	0.828	**0.007**
IL18RAP	2	102520693	rs6748390	T	C	236	194	48	263	196	42	0.244	0.697	0.721	0.253
IL18RAP	2	102520924	rs6543135	A	G	22	169	286	19	142	340	**0.016**	0.223	0.180	**0.017**
IL18RAP	2	102522038	rs11465723	T	C	11	99	367	17	138	346	**0.005**	0.127	0.172	**0.007**
IL18RAP	2	102522373	rs11465724	A	G	0	31	447	0	28	473	0.562	0.032	0.028	0.556
IL18RAP	2	102526341	rs11465732	A	G	5	61	412	5	61	435	0.771	0.074	0.071	0.779

Table 8.1 (continued)

Gene	Chr	Coordinate	SNP	Allele code 1	2	Case 11	12	22	Control 11	12	22	Genotype-wise pval	Allele 1 Frequency case	control	Allele-wise pval
IL12A	3	161192353	rs2243123	T	C	232	217	29	255	211	35	0.723	0.712	0.720	0.713
IL12A	3	161193092	rs583911	T	C	146	245	87	151	258	92	0.900	0.562	0.559	0.897
IL12A	3	161196548	rs640039	A	G	364	107	7	359	129	13	0.074	0.873	0.845	0.075
IL12A	3	161198088	rs2243147	A	G	167	240	71	189	235	77	0.607	0.600	0.612	0.604
IL12A	3	161198944	rs2243154	A	G	7	81	390	5	78	418	0.380	0.099	0.088	0.390
IL12B	5	158674592	rs1368439	A	C	330	130	18	358	128	15	0.342	0.826	0.842	0.353
IL12B	5	158675981	rs2853697	T	G	329	134	14	347	136	18	0.914	0.830	0.828	0.915
IL12B	5	158676366	rs3213119	A	C	0	24	454	0	28	473	0.696	0.025	0.028	0.692
IL12B	5	158680142	rs919766	T	G	381	90	7	388	102	11	0.302	0.891	0.876	0.313
IL12B	5	158681666	rs2853694	A	C	113	248	117	144	258	99	**0.030**	0.496	0.545	**0.027**
IL12B	5	158682907	rs3213096	T	C	0	6	472	0	9	492	0.492	0.006	0.009	0.491
IL12B	5	158683347	rs3213094	T	C	22	162	294	29	188	284	0.115	0.215	0.246	0.114
IL12B	5	158683571	rs2569253	A	G	121	237	119	152	233	116	0.134	0.502	0.536	0.142
IL12B	5	158684724	rs3181218	T	C	22	162	294	29	187	284	0.121	0.215	0.245	0.120
IL12B	5	158685556	rs3181216	A	T	44	228	206	41	209	250	0.059	0.331	0.291	0.053
IL12B	5	158687708	rs3212217	G	C	294	161	22	284	188	29	0.108	0.785	0.754	0.107

8.3.1.3. Genomic control analysis

The results of genomic control analysis are describe in section 7.3.3.

8.3.2. Exploratory Analysis

8.3.2.1. Haplotype analysis

Several related haplotypes in a large haplotype block are associated with schizophrenia (see Table 8.2 for extended haplotype). The rs7558013-rs1420100 haplotype was the best predictor of schizophrenia (global p-value = 0.0003, Table 8.3). Of these, rs7558013 was significantly associated with schizophrenia; rs1420100 was in tight LD, but was not highly correlated with rs7558013 (D' = 1.0, r^2 = 0.27, see Table 8.1 and Appendix A, Figures A5-A8). All other associated SNPs in *L18R1-IL18RAP* region are in tight LD with the rs7558013-rs1420100 haplotype and do not significantly predict schizophrenia when controlling for these

markers. At the rs7558013 –rs1420100 haplotype the frequency of the (T-A) haplotype is 4.4% higher in cases where the (T-C) haplotype is 7.7% higher in controls. The third (G-C) haplotype is 3.2 % higher in cases (see Table 8.3). Stepwise logistic regression identified a combination of three markers in *IL12A* that was associated with schizophrenia (rs583911, rs640039, and rs2243147). From 'Cocaphase', the global p-value associated with this haplotype was 0.015 (Table 8.4). There were no haplotypes in *IL-18, IL12B*, or *IL18BP* that were significantly associated with schizophrenia.

Table 8.2 Extended Haplotypes associated with schizophrenia in IL18R1-IL18RAP.

rs11465567	rs9308857	rs7558013	rs1420105	rs2293225	rs1420100
1	2	3	4	5	6
rs2272127	rs2058660	rs11465702	rs22465723	rs6543135	
7	8	9	10	11	

Haplotype	Case	freq	Control	freq	OR	chisq	p
1-1-1-2-2-1-2-1-1-2-1	208.4	0.218	170.9	0.171	1.28	6.98	0.008
1-1-2-2-2-1-1-1-2-1-2	118.4	0.124	168.5	0.168	0.74	7.69	0.006
1-1-2-2-2-1-2-1-1-2-2	18.6	0.019	29.6	0.029	0.66	1.93	0.165
1-2-2-1-1-2-2-1-1-2-2	196.8	0.206	194.5	0.194	1.06	0.41	0.520
1-2-2-1-2-2-2-1-1-2-2	72.5	0.076	73.8	0.074	1.03	0.03	0.853
1-2-2-1-2-2-2-2-1-2-2	237.5	0.248	230.4	0.230	1.08	0.91	0.341
2-2-2-2-2-1-1-1-1-2-2	19.4	0.020	34.7	0.035	0.59	3.74	0.053
2-2-2-2-2-1-2-1-1-2-2	61.4	0.064	67.7	0.068	0.95	0.11	0.742

Likelihood ratio test: null = -3335.44, alternative = -3326.03,
LRS = 18.82, DF = 7, **p = 0.0088**

Table 8.3: Most Significantly Associated Two-Marker Haplotype in IL18R1-IL18RAP.

1	2
7558013	1420100

Haplotype	Case	freq	Control	freq	OR	chisq	p
1-1	213	0.223	179	0.179	1.25	5.96	0.0146
2-1	228	0.239	316	0.315	0.76	14.46	0.0001
2-2	515	0.539	507	0.506	1.06	2.10	0.1474

Likelihood ratio test: null = -1591.74 alternative = -1583.62,
LRS = 16.23 DF = 2 **p = 0.0003**

Table 8.4: Three Marker Associated Haplotype in *IL12A*

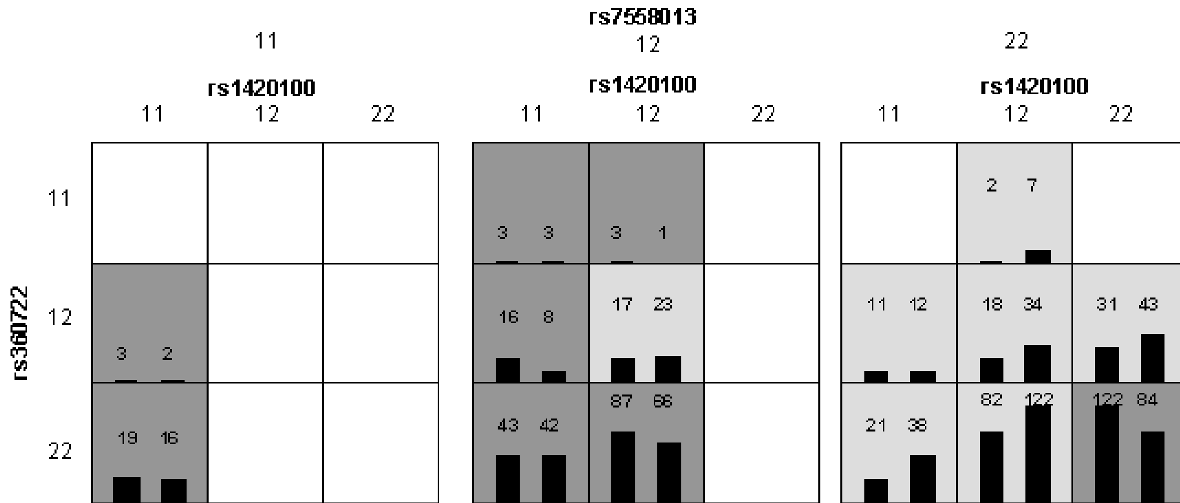
583911	640039	2243147
1	2	3

Haplotype	Case	freq	Control	freq	OR	chisq	p
1-1-1	34	0.036	16	0.016	2.23	7.694	0.006
1-2-1	121	0.127	155	0.155	0.82	3.204	0.073
2-1-1	419	0.438	442	0.441	0.99	0.016	0.900
1-1-2	382	0.400	389	0.388	1.03	0.264	0.607

Likelihood ratio test: null = -1713.41, alternative = -1708.2,
LRS = 10.4241, DF = 3, **p = 0.0152849**

8.3.2.2. Interaction analysis

MDR was used as a hypothesis free test of gene-gene interactions. MDR analysis confirmed that the rs7558013-rs1420100 haplotype is the best two-marker predictor of schizophrenia risk (p=0.005 after 1000 permutations). MDR also suggests that rs360722 in IL-18 may interact with the rs7558013-rs142100 haplotype (p=0.04 after 1000 permutations)(Figure 8.1). Chi-square analysis of expected frequencies compared to observed three-marker combinations estimated by 'Cocaphase' confirms that the rs7558013-rs142100 haplotype and rs360722 do not segregate independently in cases or controls (p=0.04, Table 8.5). The model of interaction is not simple; the rs360722 (2) allele preferentially segregates to cases in the presence of the rs7558013-rs1420100 (G-C) or (T-C) haplotype, but it distributes as expected in combination with the risk (T-A) haplotype (Table 8.5).



IL18R1-RAP genotypes at rs7558013 and rs1420100 are indicated by column headings and IL18 rs360722 genotypes are indicated by row headings. In each cell case counts are represented in the left bar and control counts in the right bar. Cells where the case count is equal or greater than the control count are highlighted in dark gray. The empiric p-value for model from 1000 MDR permutations is 0.04.

Figure 8.1: MDR Analysis of rs7558013-rs1420100-rs360722 interaction.

Table 8.5: Chi-test for IL18R1-IL18RAP, IL-18 gene-gene interaction .

		rs7558013-rs1420100 haplotype		
	observed	TA	TC	GC
Case	360722-T	24.8	37.1	50.1
	360722-C	188.2	190.9	464.9
Control	360722-T	17.2	41.1	85.7
	360722-C	161.8	274.9	421.3

	expected	11	12	22
Case	360722-T	24.9	26.7	60.3
	360722-C	188.1	201.2	454.7
Control	360722-T	25.8	45.5	73.0
	360722-C	153.2	270.5	434.0

Chi-square analysis was performed to evaluate the significance of interaction after taking into account main effects of the IL18R1-IL18RAP haplotype and rs360722. The 'observed' table shows frequencies of observed 3 marker haplotypes estimated by 'Cocaphase'. The 'expected' table shows expected frequencies given genotype and haplotype frequency differences between cases and controls. Chi-square for interaction = 13.4, 6 DF, p = 0.04.

8.3.2.3. Evaluation of gene-environment interaction

Among cases with serological data we compared allele frequencies SNPs in subsets defined by infectious disease serum antibody titer. Significant associations with HSV1 antibody titer and putative gene-environment interaction were seen at three correlated SNPs in *IL18RAP* (rs11465702, p = 0.006; rs11465723, p = 0.006; rs2272127 p = 0.04), each of which were also associated with schizophrenia (see Table 8.6). HSV2 antibody titer status was associated with rs2043055 in *IL-18*, rs3814721 in *IL18BP,* and rs9308857, rs11465597, and 6543124 in *IL18RAP*. There are similar trends for HSV2 and HSV1 antibody status at rs11465702, rs11465723, rs2272127, rs6543124, and rs9308857 suggesting that effects may not be specific (Table 8.6). CMV antibody titer status was associated with rs11465576 in *IL18R1*.

Table 8.6. Associaitons of IL-18 Pathway Genes with Viral Antibody Status in Schizophrenia Cases.

Gene	SNP	HSV1+ [a]	HSV1- [a]	pval [b]	CMV+ [a]	CMV- [a]	pval[b]	HSV2+ [a]	HSV2- [a]	pval[b]
IL-18	rs3882891	0.589	0.585	0.924	0.580	0.589	0.821	0.625	0.578	0.357
IL-18	rs5744280	0.332	0.330	0.945	0.315	0.342	0.461	0.393	0.321	0.129
IL-18	rs549908	0.703	0.696	0.847	0.702	0.699	0.944	0.714	0.697	0.718
IL-18	rs1834481	0.256	0.250	0.849	0.256	0.247	0.786	0.223	0.255	0.482
IL-18	rs5744247	0.089	0.103	0.516	0.109	0.093	0.494	0.080	0.102	0.478
IL-18	rs360722	0.114	0.111	0.898	0.122	0.110	0.654	0.089	0.119	0.367
IL-18	rs2043055	0.623	0.629	0.877	0.634	0.619	0.680	0.545	0.639	**0.049**
IL-18	rs187238	0.753	0.735	0.579	0.752	0.740	0.738	0.777	0.738	0.397
IL-18	rs1946519	0.383	0.415	0.381	0.399	0.403	0.929	0.348	0.412	0.206
IL18BP	rs3814721	0.934	0.943	0.597	0.954	0.931	0.237	0.982	0.930	**0.039**
IL18R1	rs11465567	0.902	0.918	0.471	0.916	0.905	0.627	0.946	0.901	0.130
IL18R1	rs9308857	0.396	0.345	0.181	0.353	0.381	0.477	0.455	0.355	**0.050**
IL18R1	rs11465572	0.099	0.082	0.455	0.085	0.095	0.650	0.054	0.099	0.128
IL18R1	rs11465576	0.924	0.938	0.464	0.903	0.948	**0.027**	0.938	0.932	0.832
IL18R1	rs1558627	0.769	0.753	0.619	0.735	0.777	0.227	0.750	0.765	0.732
IL18R1	rs11465597	0.899	0.907	0.703	0.903	0.900	0.901	0.955	0.891	**0.036**
IL18R1	rs7579737	0.693	0.711	0.607	0.718	0.690	0.454	0.723	0.696	0.568
IL18R1	rs6543124	0.392	0.337	0.137	0.349	0.374	0.521	0.464	0.346	**0.020**
IL18R1	rs11465608	0.997	0.997	0.884	0.996	0.998	0.632	1.000	0.997	0.536
IL18R1	rs7558013	0.228	0.219	0.776	0.214	0.232	0.595	0.277	0.216	0.149
IL18R1	rs2241116	0.191	0.250	0.083	0.185	0.241	0.112	0.179	0.230	0.259
IL18R1	rs4851570	0.725	0.673	0.165	0.702	0.697	0.905	0.759	0.687	0.157
IL18R1	rs1420095	0.934	0.907	0.191	0.941	0.905	0.089	0.884	0.923	0.153
IL18RAP	rs4851581	0.921	0.904	0.422	0.936	0.896	0.069	0.873	0.917	0.127
IL18RAP	rs1420105	0.506	0.575	0.084	0.563	0.526	0.374	0.491	0.548	0.294
IL18RAP	rs11465673	0.918	0.920	0.909	0.916	0.918	0.936	0.955	0.910	0.113
IL18RAP	rs2293225	0.193	0.249	0.101	0.185	0.241	0.112	0.179	0.230	0.259
IL18RAP	rs1420100	0.494	0.425	0.084	0.437	0.474	0.374	0.509	0.452	0.294
IL18RAP	rs3755268	0.769	0.753	0.619	0.735	0.777	0.227	0.750	0.765	0.732
IL18RAP	rs2272127	0.185	0.126	**0.041**	0.139	0.158	0.521	0.188	0.145	0.270
IL18RAP	rs887972	0.291	0.352	0.103	0.319	0.324	0.907	0.268	0.333	0.202
IL18RAP	rs2058660	0.766	0.740	0.422	0.727	0.769	0.222	0.750	0.755	0.907
IL18RAP	rs11465702	0.832	0.904	**0.006**	0.874	0.870	0.875	0.821	0.881	0.100
IL18RAP	rs6748390	0.690	0.704	0.700	0.702	0.688	0.723	0.679	0.696	0.728
IL18RAP	rs6543135	0.231	0.218	0.665	0.218	0.230	0.714	0.277	0.217	0.154
IL18RAP	rs11465723	0.168	0.096	**0.006**	0.126	0.130	0.875	0.179	0.119	0.100
IL18RAP	rs11465724	0.041	0.034	0.586	0.038	0.039	0.939	0.027	0.041	0.471
IL18RAP	rs11465732	0.082	0.077	0.818	0.113	0.061	**0.020**	0.063	0.082	0.513

a- See Table 8.1 for Allele coding. Frequencies for Allele 1 are shown.
b- Genotypewise p-values.

Table 8.6 (continued)

Gene	SNP	HSV1+ [a]	HSV1- [a]	pval [b]	CMV+ [a]	CMV- [a]	pval[b]	HSV2+ [a]	HSV2- [a]	pval[b]
IL12A	rs2243123	0.706	0.704	0.949	0.702	0.712	0.761	0.723	0.706	0.694
IL12A	rs583911	0.547	0.580	0.372	0.571	0.554	0.651	0.554	0.561	0.877
IL12A	rs640039	0.886	0.874	0.616	0.891	0.872	0.478	0.911	0.872	0.255
IL12A	rs2243147	0.611	0.582	0.442	0.567	0.617	0.201	0.598	0.600	0.966
IL12A	rs2243154	0.098	0.103	0.835	0.122	0.093	0.258	0.089	0.105	0.623
IL12B	rs1368439	0.845	0.827	0.545	0.811	0.848	0.220	0.875	0.828	0.237
IL12B	rs2853697	0.816	0.830	0.642	0.861	0.805	0.065	0.786	0.832	0.243
IL12B	rs3213119	0.032	0.021	0.350	0.017	0.030	0.279	0.009	0.029	0.215
IL12B	rs919766	0.880	0.894	0.551	0.887	0.885	0.961	0.902	0.883	0.567
IL12B	rs2853694	0.500	0.497	0.945	0.466	0.522	0.157	0.500	0.503	0.946
IL12B	rs3213096	0.003	0.008	0.421	0.013	0.004	0.216	0.000	0.009	0.326
IL12B	rs3213094	0.196	0.216	0.489	0.206	0.212	0.840	0.188	0.214	0.503
IL12B	rs2569253	0.472	0.505	0.379	0.517	0.483	0.396	0.411	0.510	0.056
IL12B	rs3181218	0.196	0.216	0.489	0.206	0.212	0.840	0.188	0.214	0.503
IL12B	rs3181216	0.345	0.330	0.669	0.345	0.327	0.632	0.375	0.325	0.293
IL12B	rs3212217	0.804	0.785	0.521	0.794	0.789	0.872	0.813	0.787	0.519

a- See Table 8.1 for Allele coding. Frequencies for Allele 1 are shown.
b- Genotypewise p-values.

8.4 DISCUSSION

Many previous studies of candidate genes for immune dysfunction in schizophrenia have focused on candidate genes derived from serological studies. Prompted by more successful positional candidate gene studies we investigated schizophrenia linkage regions for candidate genes that could contribute to immune dysfunction (Norton et al., 2006). We found it remarkable that many of the genes most closely related to IL-18 function localized to prominent schizophrenia linkage regions. Our analysis of six IL-18 pathway genes in schizophrenia revealed rs2272127 allele 'C' in *IL18RAP* to be significantly associated with schizophrenia after empirically correcting for multiple comparisons and population stratification.

Further analysis revealed that several haplotypes in the same haplotype block as rs2272127 were associated with schizophrenia. The association was clearest in this sample when

evaluating the rs7558013-rs1420100 haplotype, which spans *IL18R1* and *IL18RAP*. Hapmap data indicates that in Caucasian populations there is extensive LD in this region that extends well beyond the *IL18R1* and *IL18RAP* genes. Other genes in the region of extensive LD include several interleukin 1 receptor-like genes and sodium/hydrogen solute carrier family genes. Due to the extensive LD, we cannot rule out the possibility of other risk polymorphisms in these genes.

Exploratory analysis suggests the rs7558013-rs1420100 haplotype may interact with rs360722 in intron 1 of *IL-18*. We were unable to identify alleles with known functional relevance of any of these SNPs, so the biological implication of both single SNP and gene-gene interaction effects is not clear. Further investigation of this interaction in additional samples would be advisable.

We found that rs22721217 allele 'T' and other SNPs in the same haplotype block were associated with HSV1 seropositivity. Case-only analysis of gene-environment interaction shows a significant interaction between HSV1 and rs2272127 in schizophrenia, but this assumes that there is no association between these alleles and HSV1 serology in controls. Prior studies indicate that IL-18 is involved in the immune response to HSV1 (Fujioka et al., 1999; Lee et al., 2003; Zhu et al., 2003), so it is possible that the assumption of gene-environment independence is false. However, the association of a schizophrenia risk allele with HSV1 is important regardless of the presence of association in control individuals. If the same allele is associated with HSV1 seroposivity in controls and in cases there may not be a significant statistical interaction effect, but the main effects of *IL18R1-IL18RAP* alleles on HSV1 would be an important factor in modeling schizophrenia risk.

It is intriguing that the allele associated with lower HSV1 antibody titers, with a similar trend for HSV2 antibody titers, is also associated with schizophrenia. Could an immune response responsible for controlling primary HSV infection or reactivation from latency also play a role in schizophrenia pathogenesis? If serum HSV antibody titer is associated with genetic variations, how good of a measure is it of HSV exposure? We are currently investigating additional case and control groups to evaluate these possibilities.

Our findings should be investigated in other samples. Haplotype patterns may not be identical in additional populations of different ethnicities; therefore, replicate studies in non-Caucasian populations may need to investigate multiple SNPs to identify associated haplotypes. If replicated, these findings could lead to greater understanding of the immune imbalance that is observed in schizophrenia patients, and may establish a link between genetic and environmental factors influencing schizophrenia risk.

9. SUMMARY AND CONCLUSIONS: MODELS OF GENE-ENVIRONMENT INTERACTION IN SCHIZOPHRENIA

Our studies were designed to address the hypothesis that immune-related positional candidate genes interact with common infectious agents to influence schizophrenia risk. We evaluated the possibility of gene-virus interaction at 11 loci, namely: *TNF, MICB* and contiguous genes, *IL10, IL1β, IL1RN, IL-18, IL-18BP, IL-18R1, IL-18RAP, IL12A*, and *IL12B*. We also evaluated these genes for associations with schizophrenia. We found evidence for associations of individual SNPs with schizophrenia at *MICB* and *IL-181-IL18RAP*. We also identified a significant association with schizophrenia at *IL1β* upon meta-analysis of our data with other published studies. We found some evidence for interaction of SNPs in *MICB* with HSV1 and CMV and SNPs in *IL-18RAP* with HSV1 and possibly HSV2. Detailed discussion of these specific findings is available in chapters 5-8.

This section will address difficulties and concerns about the implementation of this study as well as unique strengths of this project. This will be followed by a discussion of models of gene-infection interaction and future experiments suggested by our results that may help to clarify these models

9.1. DIFFICULTIES AND CONCERNS

Despite widespread acknowledgement of the potential importance of gene-environment interaction, there is a paucity of genetic association studies that incorporate evaluation of environmental interactions (Clark et al., 2005). Some of the reasons that similar studies have not

previously been performed are challenges inherent in investigating gene-infection interactions identified in section 1.6. Though we have attempted to address these concerns, our studies have not been free from difficulties. Our studies have highlighted several expected and unexpected difficulties; these include: 1) Difficulties evaluating exposure to infectious agents of interest, 2) Difficulties obtaining appropriate control samples, and 3) non-independence of genetic and environmental factors.

9.1.1. Difficulties in Measuring Infection

Common difficulties in measuring infectious disease involvement in schizophrenia were addressed in section 3.1. In the study of schizophrenia these concerns revolve around the possible delay between infectious disease exposure and onset of psychiatric symptoms. One reason our studies focused on herpes viruses, which cause latent infections, is that periodic reactivation of latent viruses can cause long term elevation in antibody titers.

Our studies revealed another potential concern about antibody measurements as markers of infectious disease exposure. We found several genetic variants were associated with viral antibody status. Significant genetic variability in measures of infectious status indicates a potential form of bias in the categorization of exposed and unexposed individuals if these genetic markers are unevenly distributed between compared groups. To further complicate matters, several of the alleles we found to be associated with viral antibody status were also associated with schizophrenia. Our findings at *MICB* and *IL18R1-IL18RAP* suggest that schizophrenia samples may be enriched for variants that are associated with lower herpes virus antibody titers. If this is the case, previous studies investigating the association of herpes viruses with schizophrenia using antibody titers as markers of infection could be skewed. Observed infection

rates as measured by antibody titer may be underestimated in schizophrenia samples. This concern about antibody titer as a marker of exposure to CMV, HSV1, and HSV2 may not be resolved until genetic variability in virus specific antibody production is understood, and researchers can correct for these biases in their investigations of potential viral risk factors.

9.1.2. Difficulties Obtaining Control Samples

Another issue that has repeatedly come up in our studies is the difficulty in obtaining appropriate control samples for the investigation of gene-virus interactions in schizophrenia. An ideal control sample would be one that has been recruited in a similar manner from a similar area and characterized in similar manner as the case sample. This would hopefully yield a sample that is well matched in terms of age, ethnicity, sex, socioeconomic status, and genetic background. Avoiding both genetic and environmental bias, the non-random assortment of a trait, other than the trait of interest, in case and control samples, is a major concern. Population stratification is a form of genetic bias. Concerns about population stratification have lead our group and many others analyze populations with separate ethnic ancestry separately, as it has been documented that disparate proportions of case and control samples from varying ethnic groups can lead to spurious associations due to population stratification (Devlin et al., 2001). Similar issues arise in case-control studies of infectious agents. A number of factors, including age, gender, location of residence, and socio-economic status are known to be associated with infectious disease exposure. These could lead to segregation artifacts in case-control studies. Since the development of the transmission disequilibrium test (TDT)(Spielman et al., 1993a) family based tests of genetic linkage and association in case-parent trios have become popular, as they are robust to population stratification (Cardon et al., 2003). We used a Caucasian case-parent trio

sample to identify and confirm schizophrenia risk at *MICB*. Although trio samples are useful for genetic association studies, they are not ideal for studies of gene-environment interaction because of familial correlation of many environmental factors. New methods are being developed to evaluate gene-environment interaction in family samples, but these often require assumptions of gene-environment independence (Cordell et al., 2004; Umbach et al., 2000)(See section 4.4.6.2). So, trio samples cannot replace case-control samples for analysis interaction between genes and viral infection.

The only matched case-control sample with both DNA and serum available that we have had access to is the Baltimore schizophrenia case-control sample used in the study of *MICB*. This contains a well-categorized sample of 108 Caucasian control individuals. Despite the quality of this sample the small size leads to concerns about power (Only 19 Baltimore controls are CMV seropositive). For several genetic studies we have used DNA from the cord blood of unscreened neonates as a control sample. The genetic background of neonatal control samples collected in a specific region will theoretically be the similar to that of the general population in that region if there is not substantial immigration, selection, or selective breeding. Genomic control analysis of the Pittsburgh case-control sample confirms that the neonatal control sample is similar in genetic background to the cases at non-disease loci

For a period of time we had considered using the MHIRC samples discussed in section 5.2 as 'non-schizophrenia controls.' The MHIRC samples are a collection of samples from patients gathered at the outpatient clinics of Western Psychiatric Institute and Clinic. Some patients with psychosis and those with a diagnosis of schizophrenia were generally excluded from the sample, but the sample still has a substantial portion of individuals diagnosed with major depression or bipolar disorder that have a history of psychosis. Furthermore, about one

third of the sample has a diagnosis of bipolar disorder (see Table 4.1), which may have overlapping genetic etiology with schizophrenia (Cardno et al., 2002). Major depression, is the other major diagnostic group represented in the MHIRC sample. Unfortunately, putative infectious risk factors for both bipolar disorder and depression are similar to those for schizophrenia (Hinze-Selch, 2002; Marques-Deak et al., 2005). The MHIRC sample may eventually be an ideal sample to identify risk factors that are specific to schizophrenia and not related with other psychiatric diseases. But, until greater understanding of general schizophrenia risk factors is achieved, this sample has less utility. For these reasons we decided against large-scale genotyping of the MHIRC sample at most loci.

9.1.3. Non-Independence of Genetic and Environmental Factors

Related to concerns about control selection are concerns about non-independence of genetic and environmental factors. Owing to the lack of availability of a large, matched control sample we have resorted to using case-only analysis of gene-environment interaction for all genes except *MICB*. Similarly, we considered using family based analysis of gene-environment interaction. Both case-only and family based analyses of gene-environment interaction depend on the assumption that genetic and environmental risk factors are acquired independently (see section 4.4.6).

It seems intuitive that inheritance of genes and infection with viruses are likely to be independent, because they happen through unrelated mechanisms. However, evidence that serum antibody titer levels are correlated with genetic variants indicates that measures of infection and the presence genetic variants are certainly not independent, even if exposure to infection may be independent of genetic risk. Moreover, genes involved in infectious disease

response could modify transmission of disease from parents to offspring. Correlation of infectious disease measures and genes is especially likely in this project because we used evidence that a gene is involved in controlling HSV and CMV as a selection criterion for candidate genes.

The consequences of using case-only estimates of gene-environment interaction can be illustrated by comparing our findings at rs2272127 in *IL-18RAP* and rs1051788 in *MICB*. As noted in the discussion of the IL-18 pathway chapter (section 5.5), case-only analysis limits the interpretation of the interaction noted at rs2272127 because gene-environment independence has not been established at this locus. It is impossible to tell if the association we see represents interaction of HSV1 and rs2272127 in schizophrenia risk or separate genetic effects on HSV1 and schizophrenia. On the other hand, SNP rs1051788 is associated with HSV1 status in two non-schizophrenia samples, but it is not associated with HSV1 in two schizophrenia samples. This apparent interaction would not have been detected by case-only analysis because there is no association of rs1051788 with HSV1 in schizophrenia cases. It is impossible to determine if other potential interactions were missed due to case-only analysis of correlated genetic and environmental factors.

9.1.4. A More Positive View: Unique Strengths

These studies have attempted to overcome the difficulties inherent in studies of gene-environment interaction. Despite difficulties, we found preliminary evidence of gene-environment interaction that appears to be consistent across multiple independent samples. The importance of investigations of gene-environment interaction in schizophrenia has been reiterated for decades (Clark et al., 2005; Murray et al., 1986). Several reasons for the shortage

of gene-environment investigations are listed in section 1.6. Our studies were made possible by unique well-characterized samples collected with the foresight to obtain both serological and genetic data. Our results highlight the need to design future studies of schizophrenia with the analysis of both serological and genetic data in mind.

Our studies address a reasonable, testable hypothesis of schizophrenia pathogenesis. In light of the controversy that surrounds previously reported genetic associations with schizophrenia (Shirts et al., 2004), our results should be considered preliminary. Though these data are preliminary, they can be used to refine current models of schizophrenia. A discussion gene-virus interaction models is presented in the next section.

9.2. DEVELOPING EVIDENCE BASED MODELS OF GENE-VIRUS INTERACTION IN SCHIZOPHRENIA

The hypothesis investigated in this dissertation was that immune-related positional candidate genes interact with common infectious agents to influence schizophrenia risk. We have found evidence that polymorphisms in two genes could interact with herpes viruses in schizophrenia risk. Additional studies will be necessary to confirm these findings and explore the exact mechanism of interactions.

For the remainder of this general discussion I will discuss how information from this project combined with published literature can be used to evaluate models of gene-virus interaction in schizophrenia pathogenesis. The goal of this discussion is not to comprehensively evaluate the vast space of possible schizophrenia models, but to evaluate a few general models with the intention of suggesting a model that will facilitate future hypothesis driven research.

Other models may also be consistent with this data, and other interpretations of these results may prove to be more consistent with future data.

9.2.1. A Statistical Model of Gene-Infection Interaction

A simple model of general interaction is presented in Figure 9.1.

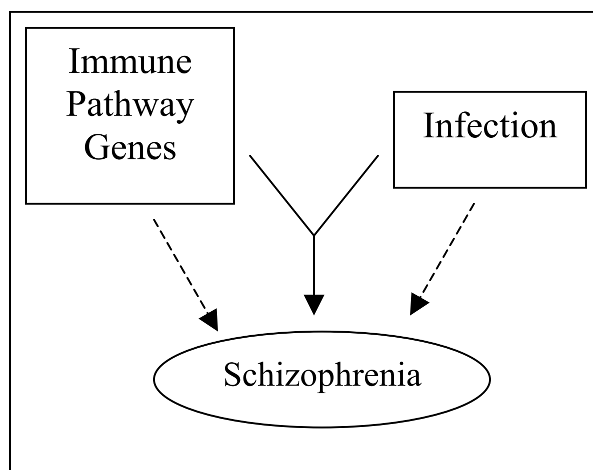


Figure 9.1: General Model of Gene-Infection Interaction.

In this figure, solid arrows represent interaction effects. Dashed arrow lines represent marginal effects apparent because of the interaction effects.

This general model represents statistical relationships. There may also be main genetic and environmental effects in addition to interaction effects. Marginal effects may only be apparent if the interaction is not modeled. As we have seen there may also be associations between immune pathway genes and infection (not diagrammed in Figure 9.1). This model does not represent biological mechanisms of infection and genes interacting or how this interaction may cause schizophrenia.

9.2.2. Evaluating models of gene-infection interaction

Several biological models of interaction are shown in Figure 9.2.

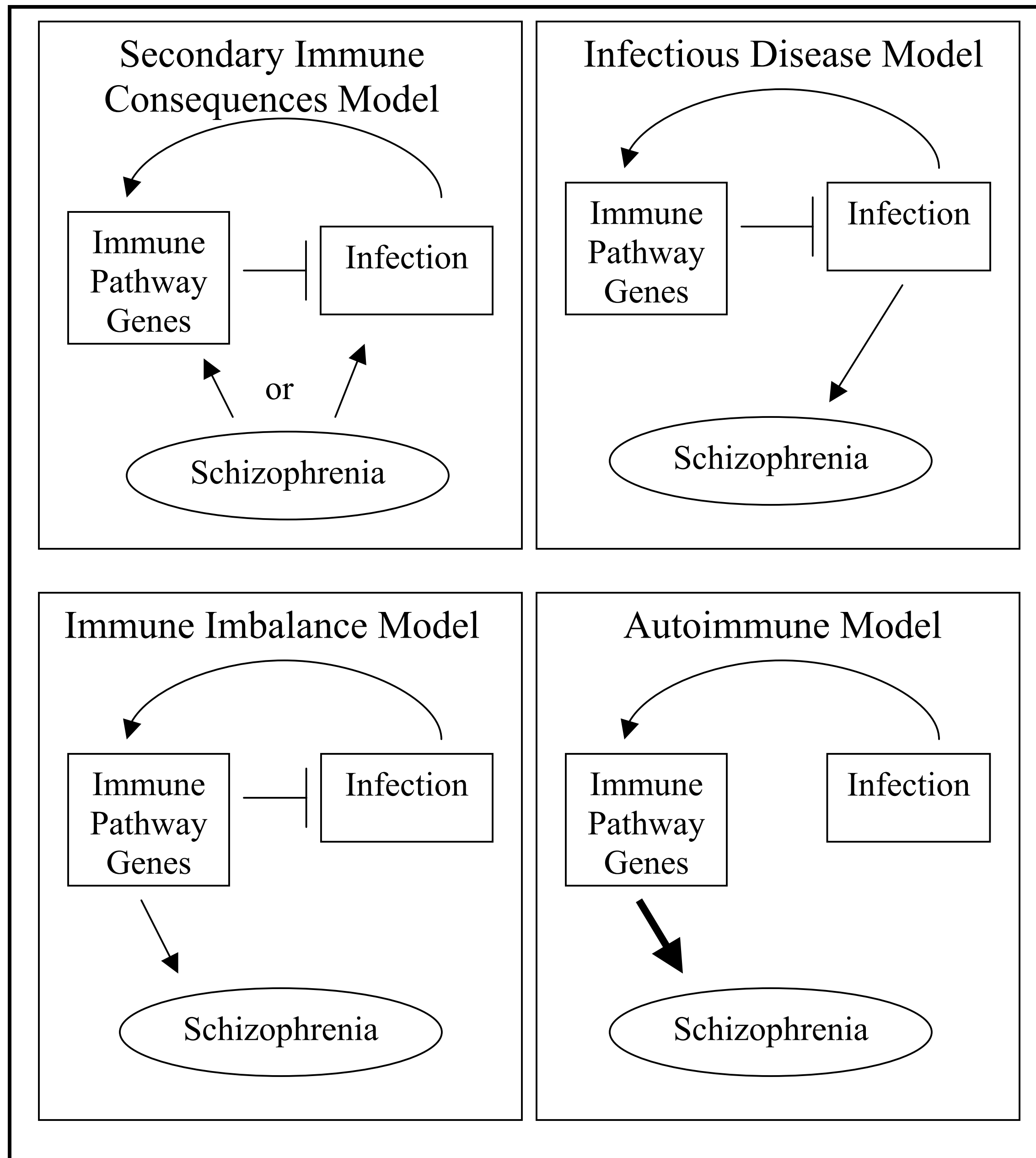


Figure 9.2: Several Biological Models Explaining Gene-Infection Interaction in Schizophrenia Pathogenesis.

Models presented in Figure 9.2 show a feedback loop between infection and the immune response; infection stimulates immune responses, which act to control infection. The relationship between infection, the immune response, and schizophrenia varies from model to model. The first model is a model where immune imbalance and infection are not causal, but are secondary effects of schizophrenia or schizophrenia treatment. Next, in the infections disease model infection is the main factor that contributes directly to schizophrenia risk and immune genes modify this risk by controlling the infection. In the immune imbalance model imbalance of the immune system is the main factor that contributes directly to schizophrenia risk and infection modifies risk by activating immune pathways and triggering imbalance. Finally, the autoimmune pathway is a specific case of the immune imbalance pathway where immune cells are activated to attack 'self' cells instead of infection; this response can be, but is not necessarily, triggered by infection. The developmental timing of infection and genetic susceptibility in other non-related pathways could influence all of these models. Evidence from published literature and from our studies supporting each model is presented below.

9.2.2.1 The Secondary Immune Consequences Model

In this model immune and infectious observations are secondary to schizophrenia. For example: psychiatric hospitalization could cause increased infection rates, and observed immune system changes could be due to antipsychotic medications.

Our studies and published literature do not support this model. Recent onset schizophrenia patients have antibody titers to CMV that are significantly higher than controls and also higher than patients with chronic schizophrenia (Leweke et al., 2004). Several studies cytokine studies have found imbalance in medicated schizophrenia patients (O'Donnell et al.,

1996; Sperner-Unterweger et al., 1999; Theodoropoulou et al., 2001), and others have found cytokine levels normalize with antipsychotic treatment (Kowalski et al., 2001; McAllister et al., 1995; Zhang et al., 2004b).

Furthermore, our findings of gene-virus interaction are not consistent with this model, because specific genetic polymorphisms necessarily precede the onset of schizophrenia. Similarly, findings of immune gene associations with schizophrenia are not consistent with this model. We found several instances of immune gene associations with schizophrenia and possible gene-virus interaction. If our associations are replicated they will present a strong argument against the secondary immune consequences model.

9.2.2.2. The infectious disease model

Initially, proponents of the infectious disease hypothesis suggested specific infections are likely to be associated with schizophrenia, but newer versions of this hypothesis are consistent with multiple infections (Crow, 1983; Kirch, 1993). The presence of general immune dysfunction does not rule out the infectious disease model, as immune dysfunction may be induced by viral infection (Schwarz et al., 2006). Decades of schizophrenia investigations have failed to identify a specific infectious agent or groups of infectious agents that contribute a large portion to schizophrenia attributable risk (Koponen et al., 2004; Rantakallio et al., 1997). However, the inability to find evidence of an infection does not rule out this model if the timing or methods of detecting the infections agents are not ideal; there is also the possibility that the putative risk infection has yet to be discovered. In support of this hypothesis, several infectious agents have been shown to be associated with schizophrenia risk especially if infection happens in the prenatal-neonatal time period (Brown, 2006).

The infectious disease model is consistent with alleles that confer schizophrenia risk also being associated with an immune response that is more permissive to infection; if infection causes schizophrenia, then an immune response that allows the infection to propagate should be associated with schizophrenia as well. On the other hand, the infectious disease model is not consistent with schizophrenia risk alleles being associated with a strong protective immune response to infection. The schizophrenia associated alleles we see at *MICB* and *IL-18* are also associated with lower HSV1 antibody levels, or HSV1 seronegativity. These findings would be consistent with the infectious model of schizophrenia if these alleles are markers for a weaker antibody and more permissive immune response to HSV1.

9.2.2.3. The immune imbalance model

In the immune imbalance model dysfunction in the immune system causes schizophrenia risk. These immune processes could be initiated or exacerbated by infectious disease. The immune pathways are often stimulated by various infections, but this hypothesis could also be consistent with one or a few specific infections if the pathway is only activated in response to a few types of infection. The immune imbalance pathway is consistent with findings of general immune dysfunction and little evidence of active infection. Studies of immune dysfunction in schizophrenia routinely use active infection as an exclusion criterion, but few of have evaluated antibody titers to specific infections agents. The presence of immune imbalance with no evidence of concurrent infection, as well as the recent identification of genetic polymorphisms that may contribute to this imbalance suggests that immune-imbalance in itself may be the main cause of schizophrenia (Schwarz et al., 2006). There is growing evidence for cytokine imbalance in schizophrenia, which is the primary evidence for the immune imbalance model (see

section 1.3). Unfortunately, because cytokines are involved in complex interconnected immune networks it may be difficult to identify the root causes of immune imbalance. Genetics presents one method of parsing cause from effect.

In contrast to the infectious disease model, the immune imbalance model is most consistent with schizophrenia associated alleles also being associated with a strong protective immune response to infection; the strong immune response would prevent or quickly neutralize infection, but would also cause increased schizophrenia risk. This balancing mechanism would explain the persistence of schizophrenia risk alleles through evolutionary time. The schizophrenia associated alleles we see at *MICB* and *IL-18* are also associated with lower HSV1 antibody levels. These findings would be consistent with the immune imbalance model of schizophrenia if observed lower HSV1 antibody titers are caused indirectly by a robust innate immune response to HSV1. A robust innate immune response may prevent HSV1 infection from establishing latency or reactivation from latently infected cells allowing antibody levels to drop below threshold for seropositivity.

9.2.2.4. The autoimmune model

The autoimmune model is a specific case of the immune imbalance model. Autoimmune disease happens when an immune response is mounted when an adaptive immune response is mounted against self-antigen (Janeway et al., 2005). As in the immune imbalance model, autoimmune processes can be initiated or exacerbated by infectious disease (Obermayer-Straub et al., 2001; Thacker et al., 2006). The accepted hypothesis is that an infectious agent with an antigen similar to a self-antigen may initially stimulate T-cells, which can then kept active as

they attack self-antigens. This hypothesis is most consistent with involvement of one or a few specific infections.

Several lines of evidence suggest that schizophrenia may be an autoimmune disease including high serum levels of autoantibodies, association with other autoimmune diseases, and possible associations of MHC haplotypes with schizophrenia (Ganguli et al., 1993; Jones et al., 2005; Wright et al., 2001). The Th2 predominance that is seen in schizophrenia is consistent with the stimulation of antibody producing cells (Gaughran, 2002). Nevertheless, inconsistencies between studies investigating the autoimmune hypothesis of schizophrenia have been common, and several unanswered questions remain, such as: How do autoantibodies cross the blood brain barrier? Or, why are antigen-antibody complexes not detectable in the brain (Jones et al., 2005)?

The presence of gene-virus interaction could be consistent with autoimmune disease. Specific to our work, IL-18 has been associated with rheumatoid arthritis, autoimmune diabetes, and lupus nephritis (Calvani et al., 2005; Gracie et al., 2005; Novota et al., 2005). If the MICB protein is involved in autoimmunity it is likely to do so in an indirect or non-traditional manner. MICB is known to activating natural killer T-cells (NKT cells) and NK cells, which can protect against autoimmune disease (Cardell, 2006; Liu et al., 2006). *MICB* polymorphisms associated with schizophrenia that cause decreased or deficient activation of NK cells would be consistent with this hypothesis.

9.2.3 Biological Pathways Suggested by Observed Associations

We identified alleles of rs1051788 in *MICB* and rs2272127 in *IL-18RAP* that are associated with schizophrenia and are also associated with HSV1 antibody seronegativity. It is intriguing that

we found alleles associated with schizophrenia and lower HSV1 antibody titers in two independent genes. The similarity between the patterns of association suggests the two genes may act via a common pathway. As presented in section 5.2.4 MICB interacts through the NKG2D pathway, which activates NK and NKT cells to recognize and kill virus infected and tumor cells. Interestingly, NK cell activation and IFN-gamma production via NKG2D mechanisms has been shown to be dependent on IL-18 stimulation (Andoniou et al., 2005; Ortaldo et al., 2006; Smyth et al., 2004). Furthermore, NK cell cytotoxic activity to murine CMV infected cells is greatly enhanced by IL-18 (Andoniou et al., 2005). This literature suggests a mechanism whereby alleles in *MICB* and *IL-18* pathway genes could logically be associated with decreased HSV1 antibody titers. If associated alleles in both *IL-18RAP* and *MICB* genes cause increased NK activation, activated NK could directly act to kill infected cells and prevent the spread of HSV1, resulting in less reactivation and lower antibody titers. This mechanism leading to decreased HSV1 is consistent with the immune imbalance model of schizophrenia presented in section 9.2.3.3. A more specific diagram of this model is shown in Figure 9.3.

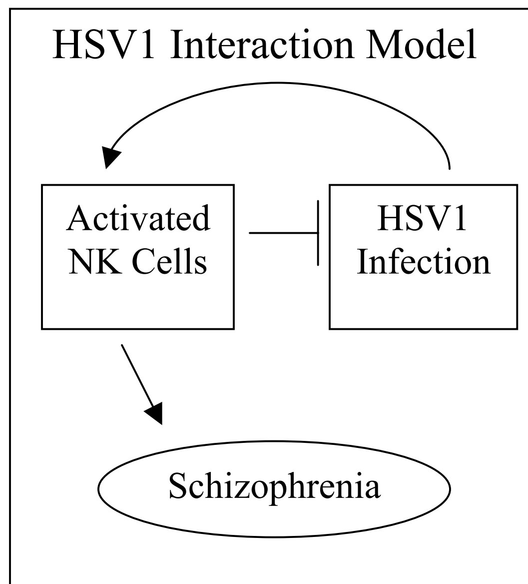


Figure 9.3. Specific Model of Immune Imbalance and Schizophrenia Risk.

This model shows HSV1 infection because it was also found to be associated with schizophrenia risk alleles, but this model is consistent with a wide array of infections being involved in schizophrenia, as many infections could activate CNS NK cells.

Published studies of NK cell activity in schizophrenia patients have shown mixed results, with some articles showing significantly higher NK cell activity (Theodoropoulou et al., 2001; Yovel et al., 2000), and others finding significantly lower (Vasil'eva et al., 2002) or unchanged NK cell activity (Caldwell et al., 1991; McDaniel et al., 1992; Mizruchin et al., 1999). Each of these studies has been small and only a few have controlled for antipsychotic treatment and smoking, which are known to influence NK cell activity (Yovel et al., 2000; Zeidel et al., 2002).

NK cell populations are heterogeneous (Peritt et al., 1998). If NK cells are involved in schizophrenia pathogenesis it is possible that one of several recently identified specific subsets of NK cells is responsible. For example: NK2 cells can be distinguished from NK1 cells by their expression of predominantly cytokines associated with a Th2 response(Peritt et al., 1998);

cytokine imbalance findings in schizophrenia discussed in section 1.3 would be consistent with selective activation of this population of NK cells. Another population of NK cells, adherent natural killer (A-NK) cells, are defined by their expression of a specific isoform of N-CAM, can be stimulated by IL-2, and are present in solid tissues more frequently than in circulation (Li et al journal of leukocyte biology 2004); activation of this subset of cells could be consistent with findings of elevated IL-2 and with N-CAM disregulation has been observed in schizophrenia (Vawter, 2000).

Further research will be necessary to identify the mechanism by which NK cells could cause schizophrenia. If replicated, the associations identified by this project could become a starting point for additional research.

9.3 ONGOING AND PLANNED STUDIES TO FURTHER REFINE MODELS OF GENE-VIRUS INTERACTION

9.3.1. Replicate Studies

The importance of replicate studies in complex genetics cannot be understated. The first goal of replicate studies is confirming or rejecting initial findings in additional samples. In addition well-designed replicate studies can also refine estimates of the magnitude of risk and location of risk alleles. We are in the process of investigating polymorphisms in *MICB* and *IL-18RAP* in a large schizophrenia trio sample collected in India. This investigation was designed to confirm the presence of schizophrenia risk loci in these genes. In addition a study of *MICB* and *IL-18RAP* polymorphisms associated with HSV1 and CMV risk are being investigated in a sample of normal control individuals obtained from the United States military.

9.3.2. Refining Associations

With the exception of rs1051788 in *MICB* and perhaps rs16944 in *IL1β,* none of the alleles we found to be associated with schizophrenia has a known functional effect. These alleles could be markers of schizophrenia risk that are in LD with functional variants. There is extensive LD on chromosome 6p that spans for hundreds of kilobases, so SNP rs2523651 could be in LD with functional variants in MICB, MICA, or more distant genes. Associated SNPs in the *IL-18R1-LI18RAP* region are all part of an extended haplotype that covers almost 200kb, polymorphisms anywhere in this haplotype block could cause the functional changes involved in schizophrenia risk. Further work will be necessary to identify functional variants. If the genes are sufficiently covered, information from other populations with different LD structure may be useful in identifying functional variants.

9.3.3. Functional Assays of Associated Polymorphisms

Steinle et al (Steinle et al., 2001) showed how non-synonymous variations in MICA had different binding affinities for the NKG2D receptor, and thus activate NK cells to at different levels. Similar studies of non-synonymous polymorphisms in MICB would indicate whether schizophrenia associated alleles are those that cause more or less activation of NK cells. Understanding the nature of the *MICB* associations would clarify models of schizophrenia pathogenesis, and may help identify risk pathways for additional candidate genes.

APPENDIX A: PAIRWISE LINKAGE DISEQUILIBRIUM AT EVALUATED GENES

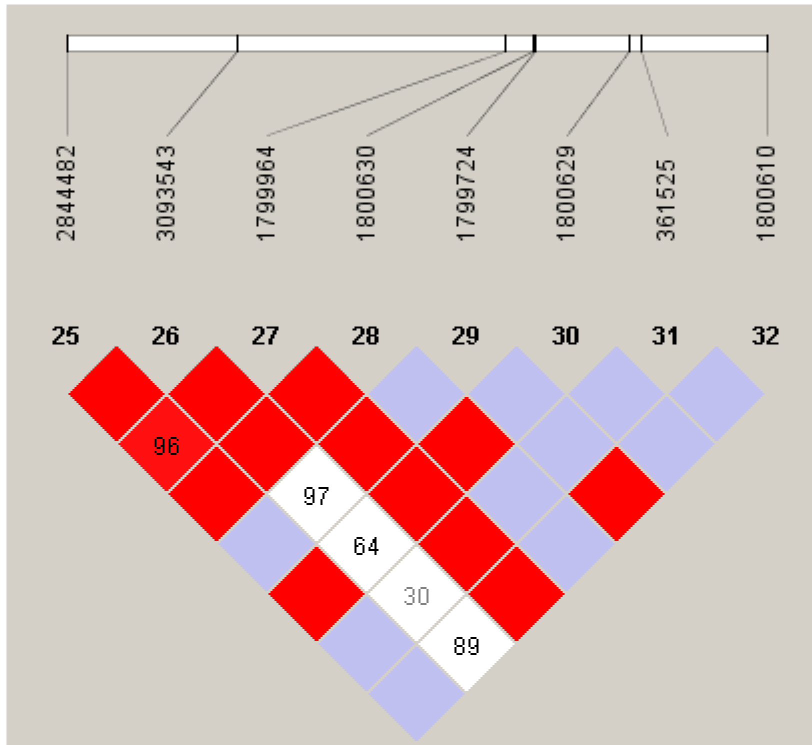


Figure A1: Haploview Diagram of Pair-wise D' Between Genotyped *TNF* SNPs

Table A1: Calculated Pairwise r^2 and D' Values for *TNF*

		r2							
		rs2844482	**rs3093543**	**rs1799964**	**rs1800630**	**rs1799724**	**rs1800629**	**rs361525**	**rs1800610**
	rs2844482	-	0.423	0.707	0.955	0.024	0.037	0.010	0.023
	rs3093543	1.000	-	0.320	0.443	0.410	0.006	0.020	0.008
	rs1799964	0.966	1.000	-	0.723	0.032	0.049	0.184	0.031
D'	**rs1800630**	1.000	1.000	1.000	-	0.023	0.036	0.009	0.022
	rs1799724	1.000	0.971	1.000	1.000	-	0.023	0.006	0.956
	rs1800629	1.000	0.641	1.000	1.000	1.000	-	0.009	0.022
	rs361525	1.000	0.303	1.000	1.000	1.000	1.000	-	0.006
	rs1800610	1.000	0.897	1.000	1.000	1.000	1.000	1.000	-

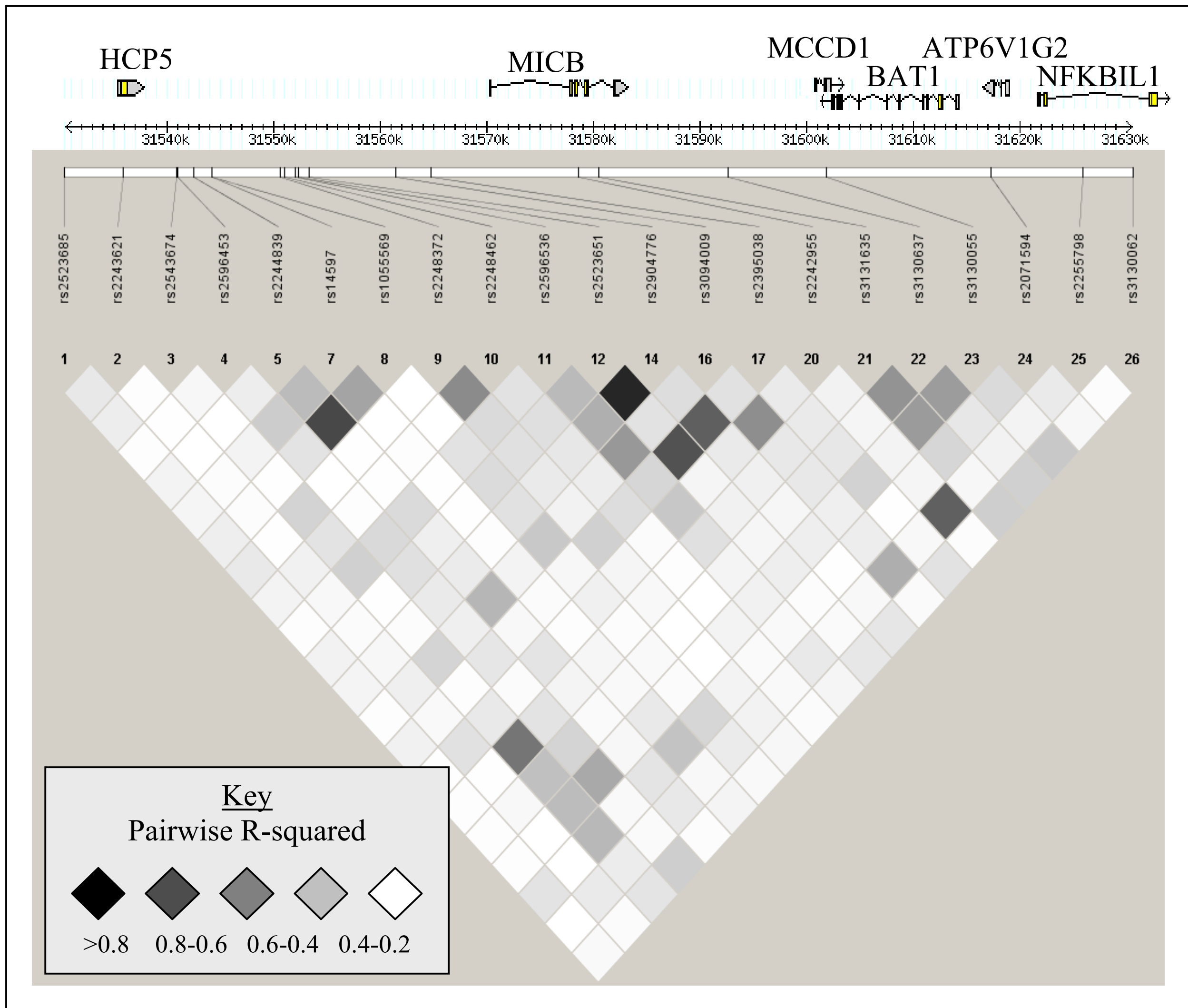


Figure A2: Haploview Diagram of Pairwise r^2 of 100kb on Chromosome 6p Region Flanking D6S2672.

Table A2: Calculated Pairwise r^2 and D' Values for Chromosome 6p Region Flanking D6S2672.

D' (above diagonal); r^2 (below diagonal)

	rs2523685	rs2243621	rs2543674	rs2596453	rs2244839	rs14597	rs1055569	rs2248372	rs2248462	rs2596536	rs2523651	rs2904776	rs3094009	rs2395038	rs2242955	rs3131635	rs3130637	rs3130055	rs2071594	rs2255798	rs3130062
rs2523685	-	0.40	0.32	0.22	0.23	0.37	0.30	0.34	0.28	0.15	0.49	0.44	0.07	0.51	0.05	0.09	0.20	0.08	0.73	0.04	1.00
rs2243621	0.09	-	0.19	0.18	0.06	0.24	0.15	0.15	1.00	0.26	0.36	0.49	0.27	0.41	0.41	0.09	0.60	0.05	0.06	0.34	1.00
rs2543674	0.07	0.01	-	0.24	0.02	0.33	0.03	0.44	0.52	0.72	0.04	0.11	1.00	0.07	1.00	1.00	0.70	0.73	0.97	0.89	1.00
rs2596453	0.01	0.00	0.03	-	0.76	1.00	0.85	0.02	0.08	0.91	0.48	0.39	0.87	0.43	0.59	0.35	0.43	0.58	0.37	0.64	0.94
rs2244839	0.05	0.00	0.00	0.07	-	0.92	0.94	0.07	0.06	0.45	0.52	0.57	0.66	0.34	0.55	0.54	0.34	0.48	0.68	0.49	0.59
rs14597	0.04	0.01	0.04	0.20	0.26	-	0.96	0.04	0.18	0.03	0.05	0.07	0.48	0.18	0.26	0.40	0.31	0.66	0.56	0.48	1.00
rs1055569	0.07	0.01	0.00	0.11	0.72	0.35	-	0.02	0.01	0.36	0.70	0.63	0.64	0.33	0.45	0.23	0.36	0.29	0.77	0.51	0.77
rs2248372	0.08	0.00	0.17	0.00	0.00	0.00	0.00	-	1.00	0.66	0.66	0.54	0.58	0.79	0.67	0.21	0.01	0.02	0.42	0.78	1.00
rs2248462	0.05	0.05	0.11	0.01	0.00	0.01	0.00	0.45	-	0.88	1.00	0.86	1.00	1.00	0.58	0.31	0.01	0.83	0.38	0.75	1.00
rs2596536	0.01	0.03	0.18	0.15	0.15	0.00	0.11	0.14	0.11	-	0.91	0.98	0.92	0.70	0.93	0.77	0.46	0.55	0.16	0.58	1.00
rs2523651	0.05	0.02	0.00	0.12	0.06	0.00	0.14	0.12	0.12	0.27	-	0.92	0.84	0.83	0.69	0.31	0.25	0.36	0.09	0.48	0.92
rs2904776	0.04	0.03	0.01	0.08	0.08	0.00	0.12	0.08	0.09	0.31	0.85	-	0.94	0.80	1.00	0.31	0.23	0.35	0.05	0.57	0.93
rs3094009	0.00	0.06	0.17	0.06	0.29	0.04	0.22	0.05	0.07	0.40	0.11	0.13	-	0.89	0.91	1.00	1.00	0.88	0.69	0.74	1.00
rs2395038	0.05	0.02	0.01	0.10	0.03	0.01	0.03	0.18	0.13	0.16	0.68	0.63	0.12	-	1.00	0.37	0.40	0.58	0.03	0.53	1.00
rs2242955	0.00	0.12	0.09	0.02	0.10	0.01	0.06	0.04	0.01	0.22	0.04	0.08	0.44	0.08	-	1.00	0.89	1.00	0.68	0.81	1.00
rs3131635	0.01	0.00	0.54	0.11	0.04	0.04	0.01	0.03	0.01	0.12	0.05	0.06	0.09	0.08	0.05	-	0.66	0.66	1.00	0.80	1.00
rs3130637	0.01	0.03	0.25	0.17	0.02	0.02	0.02	0.00	0.00	0.04	0.03	0.03	0.09	0.09	0.04	0.42	-	0.66	0.77	1.00	1.00
rs3130055	0.00	0.00	0.26	0.34	0.03	0.09	0.01	0.00	0.05	0.05	0.07	0.07	0.06	0.17	0.04	0.39	0.39	-	1.00	1.00	1.00
rs2071594	0.11	0.00	0.28	0.02	0.10	0.23	0.16	0.05	0.02	0.02	0.01	0.00	0.07	0.00	0.04	0.16	0.09	0.14	-	1.00	1.00
rs2255798	0.00	0.08	0.07	0.02	0.09	0.03	0.08	0.05	0.02	0.09	0.02	0.03	0.32	0.02	0.62	0.03	0.05	0.04	0.08	-	1.00
rs3130062	0.02	0.01	0.10	0.19	0.01	0.04	0.02	0.03	0.01	0.04	0.09	0.10	0.02	0.11	0.01	0.19	0.18	0.21	0.03	0.01	-

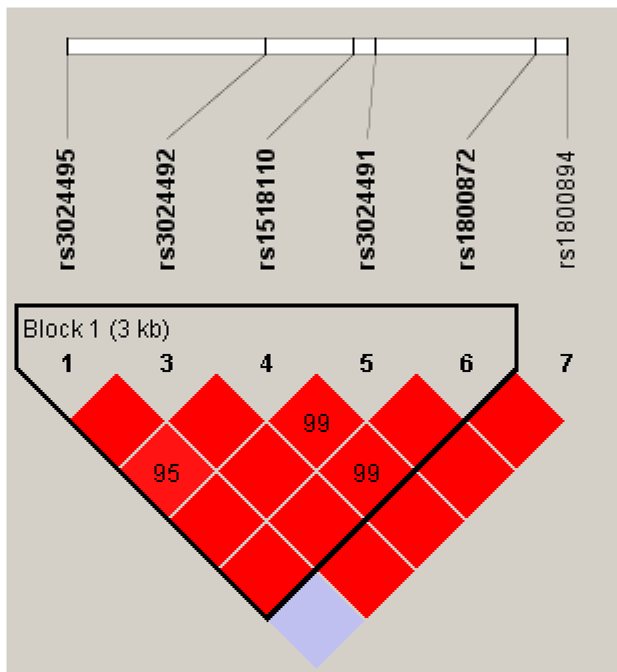


Figure A3: Haploview Diagram of Linkage Disequilibrium (D') Between SNPs at *IL10* .

Table A3. Linkage Disequilibrium Between Genotyped SNPs at *IL10.*

D' (upper triangle), r^2 (lower triangle)

	rs3024495	rs3024492	rs1518110	rs3024491	rs1800872	rs1800894
rs3024495	-	1	0.957	1	1	1
rs3024492	0.054	-	1	1	1	1
rs1518110	0.047	0.092	-	0.993	0.997	1
rs3024491	0.213	0.378	0.24	-	1	1
rs1800872	0.059	0.104	0.88	0.276	-	1
rs1800894	0.006	0.012	0.011	0.045	0.013	-

D' and r^2 were calculated using 979 combined cases and controls for all markers.

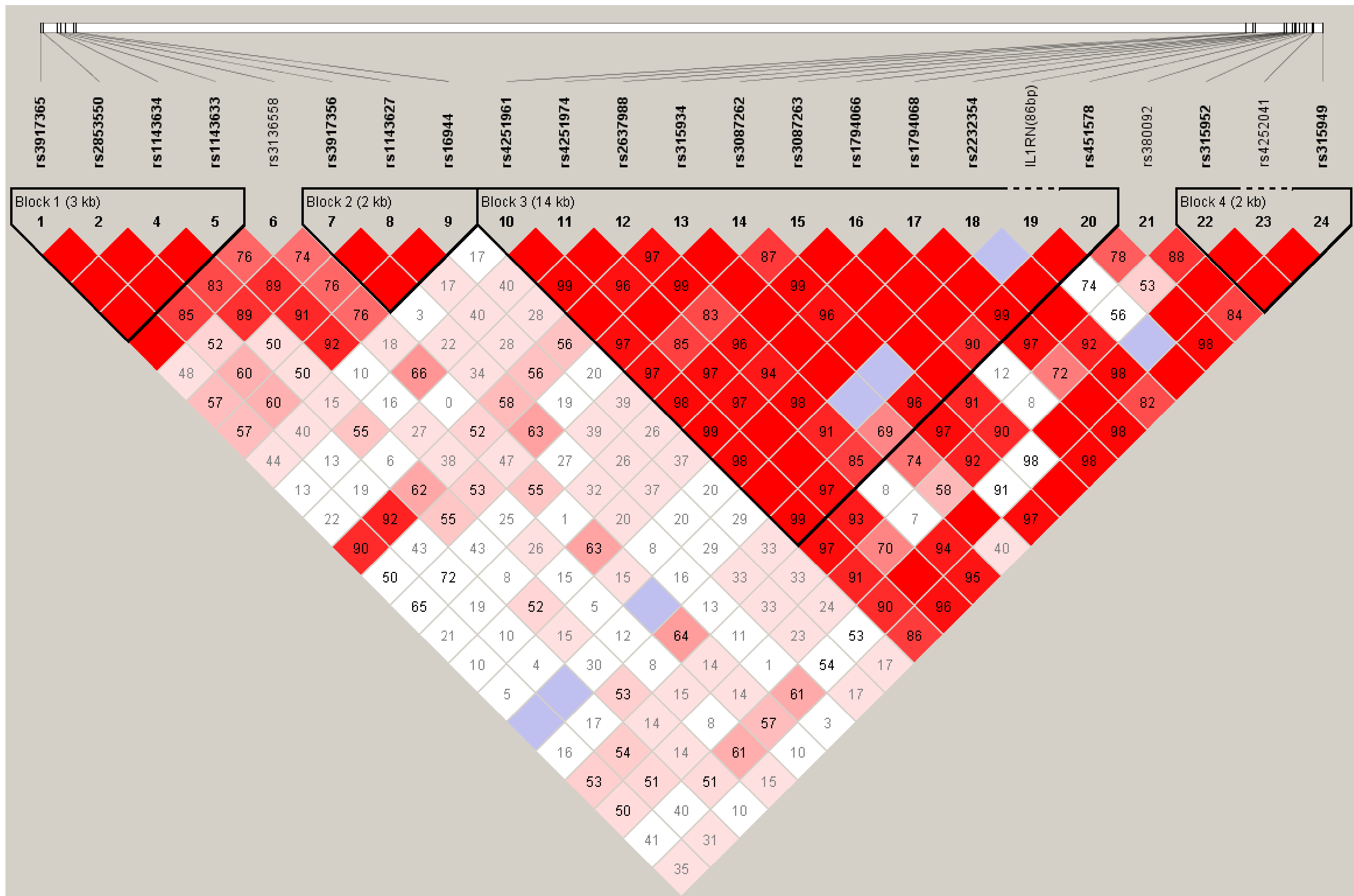


Figure A4: Haploview Diagram of Linkage Disequilibrium (D') Between SNPs in *IL1β* and *IL1RN*.

Table A4: Linkage Disequilibrium at *IL1β* and *IL1RN*.

	D' / r^2	rs3917365	rs2853550	rs1143634	rs1143633	rs3136558	rs3917356	rs1143627	rs16944	rs4251961	rs4251974	rs2637988	rs315934	rs3087262	rs3087263	rs1794066	rs1794068	rs2232354	IL1RN(86bp)	rs451578	rs380092	rs315952	rs4252041	rs315949
		IL1-beta								IL1RN														
IL1-beta	rs3917365	-	1.00	1.00	1.00	1.00	0.49	0.57	0.58	0.44	0.13	0.22	0.90	0.51	0.66	0.22	0.10	0.06	1.00	0.16	0.54	0.51	0.42	0.35
	rs2853550	0.92	-	1.00	1.00	0.86	0.53	0.60	0.61	0.40	0.13	0.19	0.92	0.43	0.72	0.20	0.11	0.05	1.00	0.17	0.54	0.52	0.41	0.32
	rs1143634	0.03	0.03	-	1.00	0.83	0.89	0.51	0.50	0.16	0.56	0.07	0.62	0.56	0.44	0.08	0.52	0.16	0.30	0.54	0.14	0.15	0.51	0.10
	rs1143633	0.05	0.06	0.17	-	0.77	0.90	0.91	0.92	0.10	0.17	0.28	0.39	0.54	0.25	0.26	0.15	0.05	0.13	0.08	0.16	0.09	0.61	0.16
	rs3136558	0.02	0.02	0.65	0.09	-	0.75	0.77	0.76	0.18	0.67	0.01	0.53	0.48	0.56	0.02	0.63	0.15	1.00	0.65	0.14	0.15	0.58	0.11
	rs3917356	0.02	0.02	0.19	0.56	0.13	-	1.00	1.00	0.03	0.22	0.34	0.58	0.64	0.27	0.33	0.21	0.09	0.16	0.14	0.11	0.02	0.62	0.03
	rs1143627	0.05	0.07	0.04	0.25	0.09	0.45	-	1.00	0.17	0.40	0.29	0.57	0.20	0.40	0.27	0.38	0.21	0.29	0.33	0.33	0.23	0.54	0.17
	rs16944	0.06	0.07	0.04	0.26	0.09	0.44	0.99	-	0.17	0.40	0.29	0.57	0.20	0.39	0.27	0.37	0.21	0.29	0.33	0.34	0.24	0.54	0.17
IL1RN	rs4251961	0.03	0.03	0.01	0.00	0.02	0.00	0.01	0.01	-	1.00	1.00	1.00	0.97	0.97	0.98	0.99	0.99	1.00	0.99	0.97	0.92	0.90	0.86
	rs4251974	0.00	0.00	0.03	0.01	0.04	0.02	0.11	0.11	0.21	-	1.00	0.96	1.00	0.86	0.98	0.98	1.00	1.00	0.98	0.94	0.70	1.00	0.96
	rs2637988	0.00	0.00	0.00	0.03	0.00	0.06	0.07	0.07	0.36	0.57	-	0.97	0.99	0.83	0.97	0.94	0.99	0.92	0.86	0.09	0.08	0.94	0.95
	rs315934	0.02	0.02	0.03	0.07	0.02	0.11	0.05	0.05	0.15	0.09	0.16	-	1.00	1.00	1.00	1.00	1.00	1.00	0.70	0.74	0.59	1.00	0.41
	rs3087262	0.00	0.00	0.15	0.02	0.12	0.05	0.00	0.00	0.08	0.05	0.22	0.04	-	0.87	0.99	0.96	1.00	1.00	0.96	0.98	0.93	0.91	0.98
	rs3087263	0.00	0.01	0.01	0.00	0.01	0.01	0.03	0.03	0.06	0.21	0.11	0.03	0.01	-	1.00	1.00	1.00	1.00	1.00	0.92	0.91	0.98	1.00
	rs1794066	0.00	0.00	0.00	0.03	0.00	0.06	0.06	0.06	0.36	0.53	0.92	0.17	0.21	0.16	-	1.00	1.00	1.00	0.91	0.13	0.08	1.00	0.99
	rs1794068	0.00	0.00	0.03	0.01	0.04	0.01	0.10	0.10	0.21	0.92	0.52	0.10	0.05	0.28	0.57	-	1.00	1.00	1.00	0.97	0.72	1.00	0.99
	rs2232354	0.00	0.00	0.02	0.00	0.02	0.00	0.01	0.01	0.44	0.09	0.16	0.07	0.04	0.03	0.17	0.10	-	1.00	1.00	1.00	0.93	0.98	0.83
	IL1RN(86bp)	0.00	0.00	0.01	0.00	0.11	0.01	0.08	0.08	0.20	0.95	0.42	0.10	0.08	0.29	0.48	1.00	0.10	-	1.00	0.75	0.56	1.00	1.00
	rs451578	0.00	0.00	0.03	0.00	0.05	0.01	0.08	0.08	0.23	0.85	0.47	0.05	0.05	0.26	0.51	0.92	0.10	0.90	-	0.79	0.54	1.00	0.99
	rs380092	0.01	0.01	0.01	0.02	0.01	0.01	0.03	0.03	0.26	0.15	0.00	0.31	0.28	0.04	0.01	0.17	0.12	0.11	0.12	-	0.88	1.00	0.85
	rs315952	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.01	0.20	0.08	0.00	0.22	0.28	0.04	0.00	0.08	0.09	0.06	0.05	0.69	-	1.00	1.00
	rs4252041	0.00	0.00	0.04	0.01	0.06	0.02	0.01	0.01	0.07	0.02	0.03	0.01	0.01	0.01	0.03	0.02	0.18	0.02	0.02	0.02	0.02	-	1.00
	rs315949	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.65	0.22	0.38	0.03	0.09	0.07	0.42	0.24	0.27	0.22	0.26	0.23	0.28	0.07	-

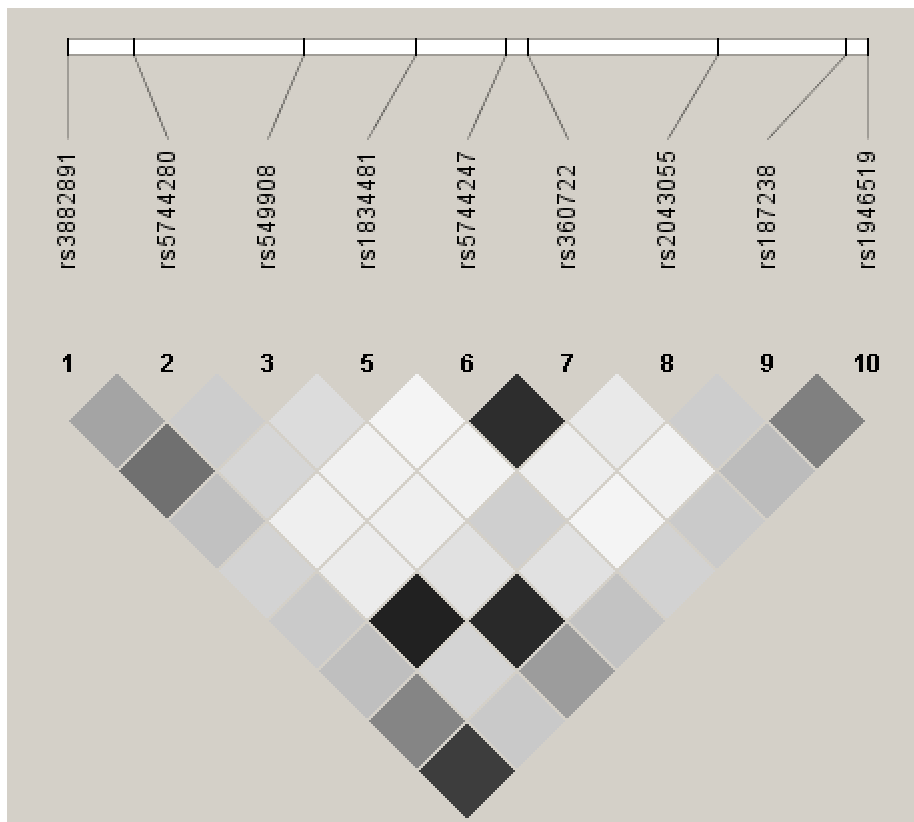


Figure A5: Haploview Diagram of Pairwise r^2 Between Genotyped SNPs at *IL-18*

Table A5: Linkage Disequilibrium at IL-18.

D'

		rs3882891	rs5744280	rs549908	rs1834481	rs5744247	rs360722	rs2043055	rs187238	rs1946519
	rs3882891	-	1	0.997	1	1	1	0.783	1	0.891
	rs5744280	0.353	-	0.991	1	1	1	1	1	0.79
	rs549908	0.56	0.196	-	1	1	1	0.703	0.997	0.811
	rs1834481	0.243	0.16	0.137	-	1	1	1	1	1
r^2	rs5744247	0.169	0.06	0.051	0.041	-	1	1	1	0.989
	rs360722	0.206	0.073	0.062	0.05	0.82	-	1	1	0.972
	rs2043055	0.25	0.867	0.114	0.185	0.069	0.084	-	1	0.817
	rs187238	0.475	0.168	0.839	0.115	0.043	0.052	0.194	-	1
	rs1946519	0.757	0.211	0.388	0.232	0.173	0.204	0.26	0.498	-

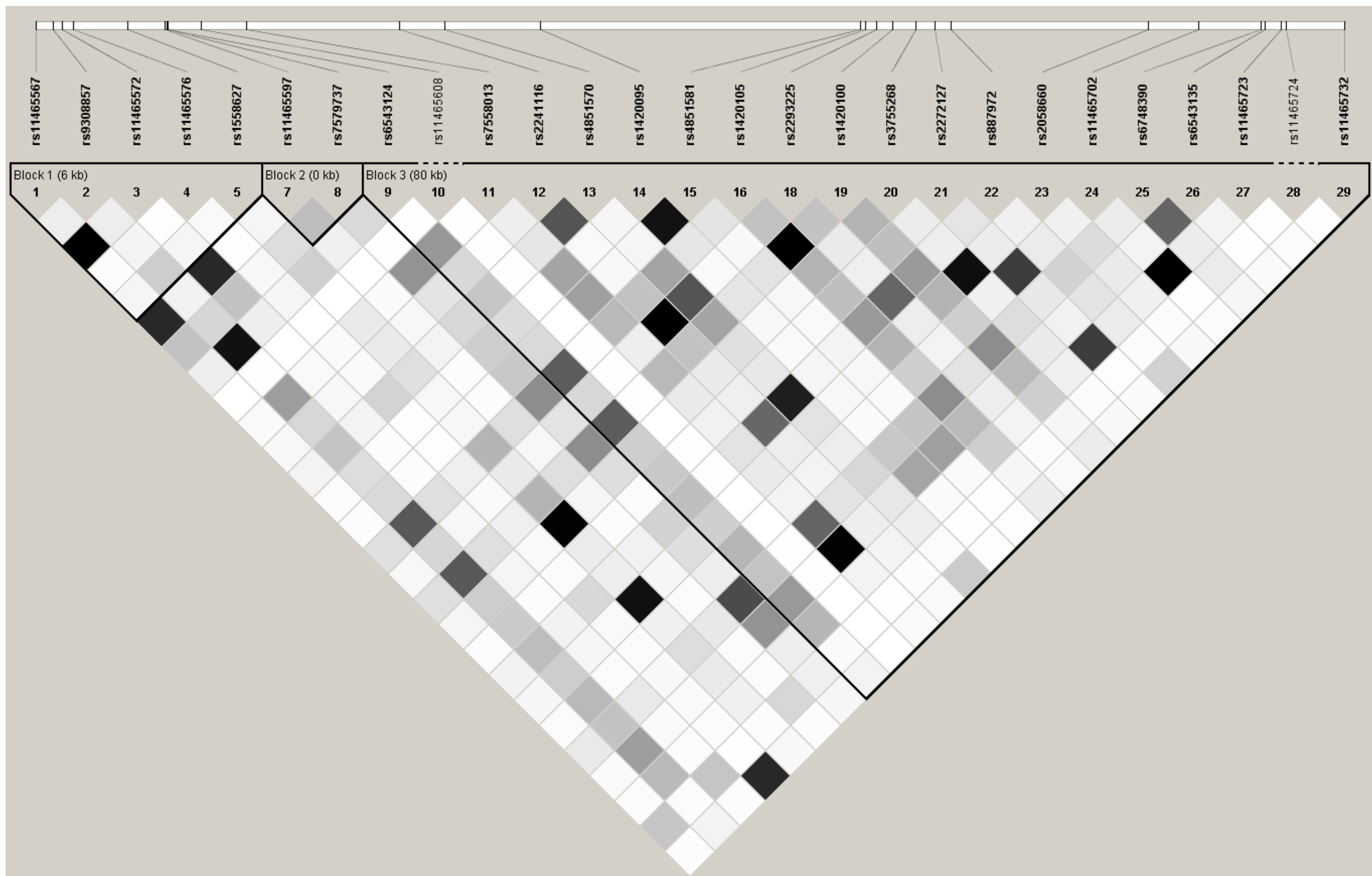


Figure A6: Haploview Diagram of Pairwise r^2 Between *IL18R1* and *IL18RAP* SNPs

Table A6: Linkage Disequilibrium at IL-181-IL18RAP

	r^2 \ D'	IL18R1 rs11465567	IL18R1 rs9308857	IL18R1 rs11465572	IL18R1 rs11465576	IL18R1 rs1558627	IL18R1 rs11465597	IL18R1 rs7579737	IL18R1 rs6543124	IL18R1 rs11465608	IL18R1 rs7558013	IL18R1 rs2241116	IL18R1 rs4851570	IL18R1 rs1420095
IL18R1	rs11465567	-	1.00	1.00	1.00	1.00	0.93	0.93	0.97	1.00	0.85	1.00	1.00	1.00
IL18R1	rs9308857	0.07	-	1.00	1.00	1.00	0.82	0.48	0.98	1.00	0.97	1.00	0.99	0.96
IL18R1	rs11465572	1.00	0.07	-	0.99	1.00	0.93	0.93	0.97	1.00	0.86	1.00	1.00	1.00
IL18R1	rs11465576	0.01	0.04	0.01	-	1.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IL18R1	rs1558627	0.04	0.19	0.04	0.02	-	1.00	1.00	1.00	0.23	1.00	1.00	1.00	1.00
IL18R1	rs11465597	0.84	0.05	0.84	0.01	0.04	-	0.96	0.90	1.00	0.74	1.00	1.00	1.00
IL18R1	rs7579737	0.24	0.16	0.24	0.03	0.13	0.26	-	0.46	1.00	0.84	0.98	0.98	0.97
IL18R1	rs6543124	0.06	0.93	0.06	0.04	0.19	0.06	0.15	-	1.00	0.98	1.00	0.99	0.96
IL18R1	rs11465608	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	-	1.00	1.00	1.00	1.00
IL18R1	rs7558013	0.02	0.38	0.02	0.02	0.08	0.02	0.42	0.40	0.00	-	1.00	1.00	1.00
IL18R1	rs2241116	0.03	0.16	0.03	0.02	0.08	0.03	0.11	0.15	0.00	0.06	-	1.00	1.00
IL18R1	rs4851570	0.05	0.23	0.05	0.17	0.12	0.05	0.16	0.22	0.00	0.10	0.67	-	1.00
IL18R1	rs1420095	0.01	0.13	0.01	0.01	0.03	0.01	0.20	0.14	0.00	0.35		0.03	-
IL18RAP	rs4851581	0.01	0.14	0.01	0.01	0.03	0.01	0.21	0.15	0.00	0.38	0.02	0.04	0.93
IL18RAP	rs1420105	0.12	0.65	0.12	0.06	0.29	0.13	0.44	0.64	0.00	0.27	0.24	0.35	0.10
IL18RAP	rs2293225	0.03	0.16	0.03	0.02	0.08	0.03	0.11	0.15	0.00	0.07	1.00	0.67	0.02
IL18RAP	rs1420100	0.12	0.65	0.12	0.06	0.29	0.13	0.44	0.64	0.00	0.27	0.24	0.35	0.10
IL18RAP	rs3755268	0.04	0.20	0.04	0.02	0.99	0.04	0.13	0.19	0.00	0.08	0.08	0.12	0.03
IL18RAP	rs2272127	0.01	0.21	0.01	0.02	0.07	0.01	0.01	0.21	0.00	0.05	0.06	0.09	0.02
IL18RAP	rs887972	0.05	0.26	0.05	0.15	0.07	0.05	0.17	0.25	0.00	0.11	0.60	0.88	0.04
IL18RAP	rs2058660	0.03	0.19	0.03	0.02	0.93	0.03	0.13	0.19	0.00	0.08	0.07	0.11	0.03
IL18RAP	rs11465702	0.01	0.27	0.01	0.01	0.06	0.01	0.06	0.29	0.00	0.04	0.05	0.07	0.02
IL18RAP	rs6748390	0.09	0.24	0.09	0.03	0.13	0.09	0.70	0.23	0.01	0.61	0.10	0.16	0.22
IL18RAP	rs6543135	0.02	0.38	0.02	0.02	0.08	0.01	0.42	0.40	0.00	0.99	0.07	0.09	0.35
IL18RAP	rs11465723	0.01	0.27	0.01	0.01	0.06	0.01	0.06	0.29	0.00	0.04	0.05	0.07	0.02
IL18RAP	rs11465724	0.23	0.01	0.23	0.00	0.01	0.16	0.06	0.02	0.00	0.00	0.01	0.01	0.00
IL18RAP	rs11465732	0.01	0.05	0.01	0.84	0.03	0.01	0.03	0.05	0.00	0.02	0.02	0.20	0.01

Table A6 (continued)

	D' / r^2	IL18RAP													
		rs4851581	rs1420105	rs2293225	rs1420100	rs3755268	rs2272127	rs887972	rs2058660	rs11465702	rs6748390	rs6543135	rs11465723	rs11465724	rs11465732
IL18R1	rs11465567	1.00	0.99	1.00	0.99	1.00	0.13	1.00	0.93	0.79	0.55	0.78	0.80	0.92	1.00
	rs9308857	0.96	0.99	1.00	0.99	1.00	0.74	0.99	0.99	0.97	0.59	0.96	0.97	0.82	1.00
	rs11465572	1.00	0.99	1.00	0.99	1.00	0.13	1.00	0.94	0.80	0.55	0.78	0.80	0.92	1.00
	rs11465576	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	rs1558627	1.00	1.00	1.00	1.00	1.00	1.00	0.69	0.98	1.00	1.00	1.00	1.00	1.00	1.00
	rs11465597	1.00	0.99	1.00	0.99	1.00	0.12	1.00	0.93	0.82	0.55	0.69	0.82	0.77	1.00
	rs7579737	0.98	0.98	0.98	0.98	0.97	0.37	0.98	0.96	0.92	0.85	0.84	0.92	0.90	1.00
	rs6543124	0.96	1.00	1.00	1.00	1.00	0.74	1.00	1.00	0.98	0.58	0.97	0.98	1.00	1.00
	rs11465608	1.00	1.00	1.00	1.00	0.22	1.00	1.00	0.22	1.00	1.00	1.00	1.00	1.00	1.00
	rs7558013	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	0.56	1.00
	rs2241116	1.00	1.00	1.00	1.00	0.98	1.00	1.00	0.91	1.00	0.98	1.00	1.00	1.00	1.00
	rs4851570	1.00	1.00	1.00	1.00	0.99	1.00	0.99	0.93	1.00	0.99	0.98	1.00	1.00	1.00
	rs1420095	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IL18RAP	rs4851581	-	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00
	rs1420105	0.10	-	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	1.00
	rs2293225	0.02	0.24	-	1.00	0.98	1.00	1.00	0.91	1.00	0.98	1.00	1.00	1.00	1.00
	rs1420100	0.10	1.00	0.24	-	1.00	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	1.00
	rs3755268	0.03	0.30	0.08	0.30	-	1.00	0.69	0.98	1.00	1.00	1.00	1.00	1.00	1.00
	rs2272127	0.02	0.25	0.06	0.25	0.07	-	1.00	0.97	1.00	0.96	0.97	1.00	1.00	1.00
	rs887972	0.04	0.40	0.60	0.40	0.07	0.10	-	0.64	1.00	0.99	0.98	1.00	1.00	1.00
	rs2058660	0.03	0.29	0.07	0.29	0.94	0.07	0.06	-	1.00	1.00	1.00	1.00	1.00	1.00
	rs11465702	0.02	0.19	0.05	0.19	0.06	0.76	0.08	0.06	-	1.00	1.00	1.00	1.00	1.00
	rs6748390	0.23	0.45	0.10	0.45	0.13	0.09	0.17	0.13	0.07	-	1.00	1.00	1.00	1.00
	rs6543135	0.37	0.27	0.07	0.27	0.08	0.05	0.10	0.08	0.04	0.61	-	1.00		1.00
	rs11465723	0.02	0.19	0.05	0.19	0.06	0.76	0.08	0.06	1.00	0.07	0.04	-	1.00	1.00
	rs11465724	0.00	0.03	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.08	0.00	0.01	-	1.00
	rs11465732	0.01	0.07	0.02	0.07	0.03	0.02	0.18	0.03	0.01	0.03	0.02	0.01	0.00	-

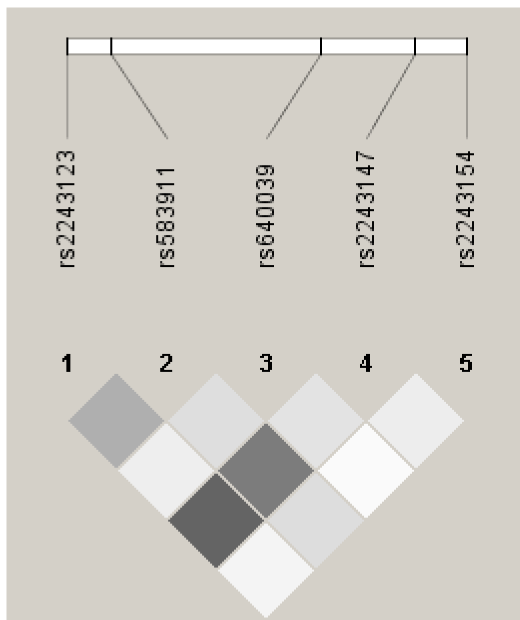


Figure A7: Haploview Diagram of Pairwise r^2 Between SNPs at *IL12A*.

Table A7: Linkage Disequilibrium at *IL12A*.

		D'				
		rs2243123	rs583911	rs640039	rs2243147	rs2243154
	rs2243123	-	1	1	0.997	1
	rs583911	0.311	-	1	1	1
r^2	rs640039	0.065	0.129	-	1	1
	rs2243147	0.606	0.51	0.107	-	1
	rs2243154	0.041	0.131	0.017	0.067	-

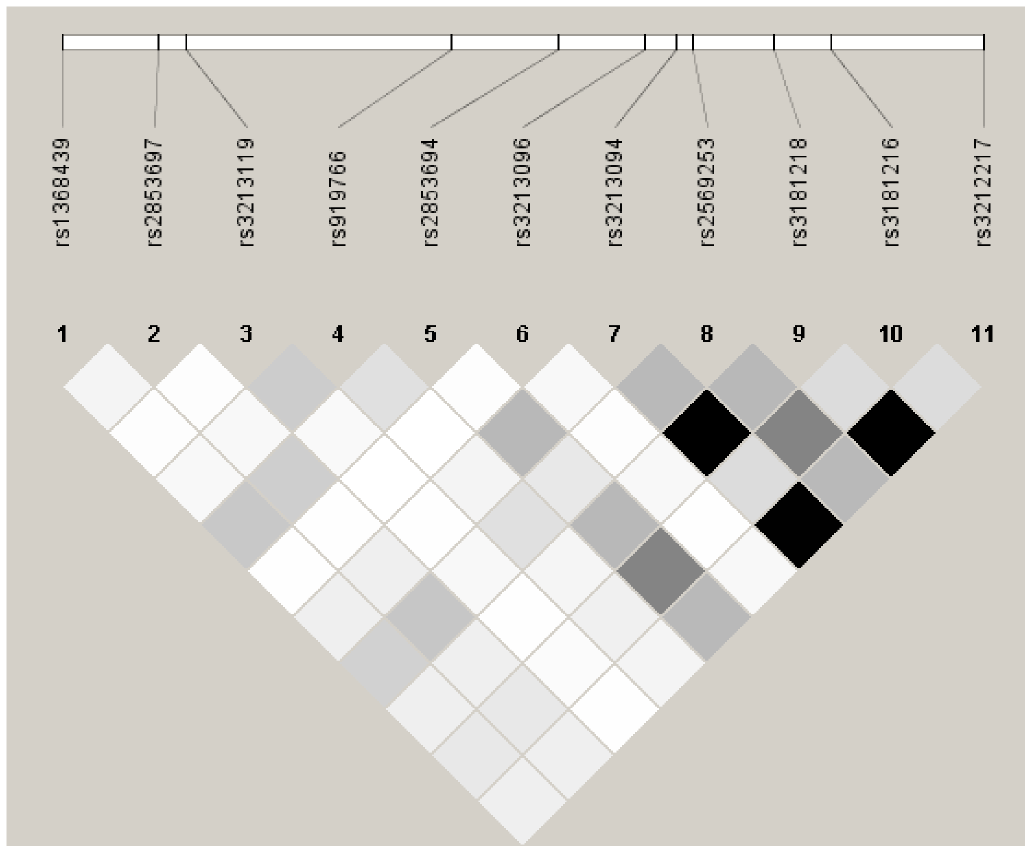


Figure A8: Haploview Diagram of Pairwise r^2 Between SNPs at *IL12B*.

Table A8: Linkage Disequilibrium at *IL12B*.

		D'										
		rs1368439	rs2853697	rs3213119	rs919766	rs2853694	rs3213096	rs3213094	rs2569253	rs3181218	rs3181216	rs3212217
	rs1368439	-	1	1	1	0.991	0.998	1	0.99	1	1	1
	rs2853697	0.041	-	1	1	1	1	0.973	1	1	0.977	1
	rs3213119	0.005	0.006	-	0.976	0.931	1	0.635	1	0.631	1	0.631
	rs919766	0.026	0.027	0.197	-	1	0.329	1	0.985	1	0.965	1
	rs2853694	0.212	0.19	0.022	0.121	-	1	1	0.301	1	0.988	0.993
r^2	rs3213096	0.002	0.002	0	0	0.007	-	1	1	1	1	1
	rs3213094	0.06	0.059	0.003	0.04	0.276	0.026	-	0.994	1	1	0.997
	rs2569253	0.18	0.223	0.025	0.118	0.09	0.007	0.274	-	1	0.992	0.994
	rs3181218	0.059	0.062	0.003	0.039	0.276	0.026	0.997	0.277	-	1	1
	rs3181216	0.089	0.089	0.012	0.055	0.479	0.003	0.135	0.479	0.135	-	1
	rs3212217	0.059	0.062	0.003	0.04	0.272	0.026	0.994	0.274	0.997	0.135	-

APPENDIX B: PROTOCOLS, PRIMERS, AND LABORATORY METHODS

Sequencing Protocol

1x PCR Mix

dH2O	9.7 µL
10X PCR buffer	2.0 µL
dNTP (5µm)	0.8 µL
MgCl2 (25µm)	2.4 µL
F-primer (5µm)	1.0 µL
R-primer (5µm)	1.0 µL
Taq polymerase	0.1µL
DNA (10ng/µL)	3.0 µL
Total Volume	20.0 µL

PCR thermocycler conditions

1. 95°C	10 min
2. 94°C	45 sec
3. 55, 60, or 65 °C	45 sec
4. 72°C	1 min
5. repeat 2-4	34 times
6. 72°C	10 min
7. 4°C	forever

1x SAP Treatment

PCR product	2.5µL
SAPH	1.25µL
Exo I	0.05µL
dH2O	1.2µL
Total Volume	5.0µL

Incubate at 37°C for 1hr, then at 75°C for 15 min

1x Sequencing Reaction

SAP treated product	3.0µL
Big Dye 3.1 Terminator Mix	1.0µL
Sequencing primer (5µm)	0.7µL
dH2O	5.3µL
Total Volume	10.0µL

Sequencing Thermocycler Conditions

1. 96°C 10 sec
2. 50°C 5 sec
3. 60°C 4 min
4. repeat 1-3. 24times
5. 4°C forever

Precipitate sequencing reaction products using isopropanol precipitation.

Run ABI 3100 or 3300 automated sequencer with 50 or 85 cm capillaries using corresponding settings.

Table B1: Primers Used for Pooled Sequencing and Preparation of *TNF* Promoter Expression Constructs.

Primers used for pooled TNF sequencing			
F Primer	**Sequence**	**R Primer**	**Sequence**
TNFSeq01_F	CCAGGCTGGAATACAGTGGT	TNFSeq01_R	GATGCCCTCCATCTCTTCCT
TNFSeq02_F	TCACCCATGTGGAATTCTGA	TNFSeq02_R	GCGACTGAGTTCTGGGAAAG
TNFSeq03_F	CGCCTCCTCTCTGAATTGAC	TNFSeq03_R	AAGGTGAGCAGAGGGAGACA
TNFSeq04_F	GGGTTTGGTTTTGGTTTCCT	TNFSeq04_R	GACATGTCTGGGAGGTCAGG
TNFSeq05_F	CCCCCTCAACTCTGTTCTCC	TNFSeq05_R	GGAGGAGAAGAGCTGGACCT
TNFSeq06_F	CCCACCAGTGGCATCTACTT	TNFSeq06_R	TGCTCTTCCTCTGTGTGTGG
TNFSeq07_F	TGATGTCTGTCTGGCTGAGG	TNFSeq07_R	TCAGCTTCTCCTTTGCTTCC
TNFSeq08_F	GTGAGAACTTCCCAGTCTATCTAAGG	TNFSeq08_R	GGGAATTCACAGACCCCACT
TNFSeq09_F	GAAGATATGGCCACACACTGG	TNFSeq09_R	AAAGTTGGGGACACACAAGC
TNFSeq10_F	ACCTGGTCCCCAAAAGAAAT	TNFSeq10_R	AAGAGGCTGAGGAACAAGCA
TNFSeq11_F	CTCTCTCCCCTGGAAAGGAC	TNFSeq11_R	TCTTCTGTGTGCCAGACACC
TNFSeq12_F	GAGACAGATGTGGGGTGTGA	TNFSeq12_R	TAGCCCTCCAAGTTCCAAGA
TNFSeq13_F	GAGGATGGATGGAGGTGAAA	TNFSeq13_R	TTGATGGCAGAGAGGAGGTT
TNFSeq14_F	ATCAGAGGGCCTGTACCTCA	TNFSeq14_R	CAGGGATCAAAGCTGTAGGC
TNFSeq15_F	TCGGAACCCAAGCTTAGAAC	TNFSeq15_R	CAGAGGCTCAGCAATGAGTG
TNFSeq16_F	TAGGCTGTTCCCATGTAGCC	TNFSeq16_R	TCACATGCCCTATGTGTGGT
TNFSeq17_F	CTTCCAGAGATTCGGGTGTC	TNFSeq17_R	GCCCCACCCTTATTTGTCTT
TNFSeq18_F	GTCCCACTGGTCTTTGTGGT	TNFSeq18_R	CCCCATTACTTCATGGCTGA
TNFSeq19_F	AACCATGTGGTAGGCTGAGG	TNFSeq19_R	ACATGGTCCCAACCAGACTC
TNFSeq20_F	GCAGCTCTAGGGGGAGAAGT	TNFSeq20_R	AGGGAATATGAGTGCGTGGT
TNFSeq5b_F	AACTTCCCCCACGCTAAAAA	TNFSeq5b_R	CGAAGGCTCCAAAGAAGACA
TNFSeq11b_F	GAGACAGGATGTCTGGCACA	TNFSeq11b_R	TTTCACCTCCATCCATCCTC
TNFSeq19b_F	AAGCCCAGGAGTTTGAGGTT	TNFSeq19b_R	GGAGGAGGCTGAAGGAGAAA
Primers used for preparation of expression constructs			
TNF_Kpn1	*ggggtacccc* tatgattgctcttcagggaac		
TNF_Nhe1	*ctagctagctag* gtgtcctttccaggggagag		

Table B2. SNPs Estimated to be Present with Minor Allele rFequency Greater than 5% in Caucasian Case and Control by Pooled Sequencing.

SNP	Amplicon used	Sample	Estimated MAF
rs2844482	TNFseq01	control	0.15
	TNFseq02	case	0.15
rs2071290	TNFseq01	control	0.30
	TNFseq02	case	0.29
rs1800683	TNFseq02	control	0.48
	TNFseq03	case	0.50
rs2239704	TNFseq02	control	0.24
	TNFseq03	case	0.20
rs3093546	TNFseq02	control	0.09
	TNFseq03	case	0.14
rs909253	TNFseq03	control	0.27
	TNFseq04	case	0.35
rs746868	TNFseq04	control	0.35
		case	0.35
rs2857713	TNFseq04	control	0.36
		case	0.29
rs3093543	TNFseq05	control	0.16
		case	0.12
rs1041981	TNFseq05	control	0.40
		case	0.47
rs1799964	TNFseq08	control	0.20
		case	0.19
rs1800630	TNFseq08	control	0.15
		case	0.13
rs1799724	TNFseq08	control	0.16
		case	0.16
rs1800629	TNFseq09	control	0.21
		case	0.26
rs1800610	TNFseq11	control	0.16
		case	0.15
rs3091257	TNFseq17	control	0.16
		case	0.19
rs769178	TNFseq18	control	0.19
	TNFseq19b	case	0.16
rs3093559	TNFseq19b	control	0.08
		case	0.09

The dbSNP identification number is shown in the first column, the primer from Table B1 used for sequencing is indicated in the second column. Minor allele frequency was estimated by averaging calculated allele frequencies from sequencing with forward and reverse primers.

SNAPSHOT protocol

1x PCR Mix

dH2O	3.9 µL
10X PCR buffer	1.0 µL
dNTP (5µm)	0.5 µL
MgCl2 (25µm)	1.2 µL
pooed primers	1.3 µL
Taq polymerase	0.13µL
DNA (10ng/µL)	2.0 µL
Total Volume	10.0 µL

PCR thermocycler conditions

1. 95°C 10 min
2. 94°C 45 sec
3. 60°C 45 sec
4. 72°C 1 min
5. repeat 2-4 34 times
6. 72°C 10 min
7. 4°C forever

1x SAP Treatment

PCR product	2.5µL
SAPH	1.25µL
Exo I	0.05µL
dH2O	1.2µL
Total Volume	5.0µL

Incubate at 37°C for 1hr, then at 75°C for 15 min

1x SNaPshot Extension

SAP treated product	1.5µL
SNaPshot Multiplex Kit	1.5µL
Pooled internal primers	0.5µL
dH2O	1.5µL
Total Volume	5.0µL

SNaPshot Thermocycler Conditions

1. 96°C 10 sec
2. 50°C 5 sec
3. 60°C 30 sec
4. repeat 1-3. 24times
5. 4°C forever

1x Post Extension

SnapShop product	2.0μL
SAPH	1.0μL
dH2O	7.0μL
Total Volume	10.0μL

Incubate at 37°C for 1hr, then at 75°C for 15 min

1x SNaPshot for ABI

Formamide	8.85μL
LIZ120 Size Standard	0.15μL
Product	1.0 μL
Total Volume	10.0μL

Denature at 95°C for 5 min before placing in ABI machine. Run on 25 or 35 cm capillaries using corresponding settings.

APPENDIX C: ASSOCIATIONS OF ANALYZED GENES WITH *TOXOPLASMA GONDII*

Table C1: Association Analysis of SNPs in *TNF* Comparing *Toxoplasma gondii* Seropositive Schizophrenia Cases with Community Based Controls.

ID#	Marker	TOX+ Case (n = 31)			Control (n = 276)			Genotype-wise p-value	Minor allele frequency		Allele-wise p-value
		11	12	22	11	12	22		case	control	
1	rs2844482 (C/T)	24	6	1	189	74	7	0.491	0.182	0.163	0.489
2	rs3093543 (A/C)	26	5	0	228	44	1	0.921	0.097	0.084	0.923
3	rs1799964 (C/T)	2	6	23	11	93	170	0.370	0.227	0.210	0.369
4	rs1800630 (A/C)	1	6	24	8	84	174	0.250	0.176	0.188	0.255
5	rs1799724 (A/G)	1	7	23	3	55	213	0.447	0.091	0.113	0.447
6	rs1800629(-G308A) (A/G)	0	7	24	8	73	195	0.323	0.142	0.161	0.320
7	rs361525 (A/G)	0	2	29	3	24	249	0.488	0.051	0.054	0.458
8	rs1800610 (C/T)	24	6	1	214	55	3	0.692	0.909	0.112	0.691

1/1, 2/2, 1/2: Individuals homozygous for allele1, allele2 and heterozygous, respectively. Nucleotides were labeled 1 or 2 in alphabetical order, nucleotide identity is indicated in the second column. Serum was not available for controls, so TOX seropositive cases were compared to all controls.

Table C2: Association of *MICB* SNPs with *Toxoplasma gondii* in Baltimore Controls and Schizophrenia Cases.

Baltimore controls	TOX positive			TOX negative			Genotype -wise pval	TOX +	TOX -	Allele-wise pval
	11	12	22	11	12	22		freq_1	freq_1	
rs2523651	0	1	5	15	38	42	0.05	0.08	0.36	0.07
rs1055569	1	5	0	43	43	9	0.49	0.58	0.68	0.48
rs2596536	2	4	0	49	34	12	0.84	0.67	0.69	0.85
rs2596453	3	3	0	50	38	7	0.86	0.75	0.73	0.86
rs1051788	0	4	2	8	32	55	0.54	0.33	0.25	0.55
Baltimore SZ cases										
rs2523651	9	18	22	30	100	81	0.83	0.37	0.38	0.83
rs1055569	18	23	9	94	82	32	0.27	0.59	0.65	0.30
rs2596536	19	24	6	93	85	28	0.64	0.63	0.66	0.65
rs2596453	32	14	4	108	81	22	0.13	0.78	0.70	0.15
rs1051788	5	28	17	19	91	101	0.15	0.38	0.31	0.14

Table C3. Associations of IL10, IL1β, and IL1RN SNPs with *Toxoplasma gondii* in Schizophrenia Cases.

SNP	Gene	Toxo + (N=45)		Toxo – (N=305)		Genotype-wise pval
		freq_1	freq_2	freq_1	freq_2	
rs1800894	IL-10	0.0667	0.9333	0.0328	0.9672	0.1200
rs3024491	IL-10	0.5667	0.4333	0.4623	0.5377	0.0700
rs1518110	IL-10	0.1556	0.8444	0.2169	0.7831	0.2034
rs1800872	IL-10	0.1778	0.8222	0.2475	0.7525	0.1751
rs3024492	IL-10	0.3000	0.7000	0.2319	0.7681	0.1343
rs3024495	IL-10	0.1444	0.8556	0.1623	0.8377	0.6697
rs1143627	IL1β	0.6111	0.3889	0.6776	0.3224	0.1980
rs1143633	IL1β	0.4222	0.5778	0.3705	0.6295	0.3621
rs1143634	IL1β	0.1667	0.8333	0.2312	0.7689	0.1506
rs16944	IL1β	0.3778	0.6222	0.3230	0.6771	0.2886
rs2853550	IL1β	0.0889	0.9111	0.0820	0.9180	0.8259
rs3136558	IL1β	0.8444	0.1556	0.7813	0.2188	0.1531
rs3917356	IL1β	0.4667	0.5333	0.4689	0.5312	0.9699
rs3917365	IL1β	0.0889	0.9111	0.0820	0.9180	0.8259
rs1794066	IL1RN	0.6000	0.4000	0.6066	0.3934	0.9068
rs1794068	IL1RN	0.2889	0.7111	0.2689	0.7312	0.6965
rs2232354	IL1RN	0.7667	0.2333	0.7747	0.2253	0.8656
rs2637988	IL1RN	0.6111	0.3889	0.6082	0.3918	0.9587
rs3087262	IL1RN	0.1111	0.8889	0.1279	0.8721	0.6386
rs3087263	IL1RN	0.1444	0.8556	0.0872	0.9128	0.0876
rs315934	IL1RN	0.7667	0.2333	0.7967	0.2033	0.5075
rs315949	IL1RN	0.4111	0.5889	0.4000	0.6000	0.8450
rs315952	IL1RN	0.6778	0.3222	0.6869	0.3131	0.8625
rs380092	IL1RN	0.3333	0.6667	0.3180	0.6820	0.7656
rs4251961	IL1RN	0.6444	0.3556	0.6234	0.3766	0.7033
rs4251974	IL1RN	0.7111	0.2889	0.7344	0.2656	0.6501
rs4252041	IL1RN	0.0778	0.9222	0.0492	0.9508	0.2441
rs451578	IL1RN	0.2889	0.7111	0.2853	0.7148	0.9451

Coding for individual SNPs is shown in Table 7.1

Table C4. Associations of IL-18 Pathway SNPs with *Toxoplasma gondii* in Schizophrenia Cases.

SNP	Gene	Toxo + (N=45) freq_1	Toxo + (N=45) freq_2	Toxo - (N=305) freq_1	Toxo - (N=305) freq_2	Genotype-wise pval
rs1834481	IL-18	0.300	0.700	0.243	0.757	0.248
rs187238	IL-18	0.656	0.344	0.757	0.243	**0.042**
rs1946519	IL-18	0.422	0.578	0.398	0.602	0.664
rs2043055	IL-18	0.689	0.311	0.615	0.385	0.161
rs360722	IL-18	0.044	0.956	0.125	0.875	**0.027**
rs3882891	IL-18	0.589	0.411	0.585	0.415	0.948
rs549908	IL-18	0.633	0.367	0.710	0.290	0.138
rs5744247	IL-18	0.044	0.956	0.107	0.893	0.063
rs5744280	IL-18	0.289	0.711	0.339	0.661	0.328
rs3814721	IL18BP	0.944	0.056	0.938	0.062	0.807
rs11465567	IL18R1	0.889	0.111	0.911	0.089	0.488
rs11465572	IL18R1	0.111	0.889	0.089	0.911	0.494
rs11465576	IL18R1	0.956	0.044	0.930	0.070	0.361
rs11465597	IL18R1	0.856	0.144	0.908	0.092	0.115
rs11465608	IL18R1	1.000	0.000	0.997	0.003	0.586
rs1420095	IL18R1	0.967	0.033	0.910	0.090	0.061
rs1558627	IL18R1	0.722	0.278	0.769	0.231	0.340
rs2058623	IL18R1	0.667	0.333	0.696	0.304	0.640
rs2241116	IL18R1	0.200	0.800	0.225	0.775	0.614
rs4851570	IL18R1	0.733	0.267	0.693	0.307	0.473
rs6543124	IL18R1	0.311	0.689	0.373	0.627	0.262
rs7558013	IL18R1	0.222	0.778	0.226	0.774	0.931
rs7579737	IL18R1	0.667	0.333	0.705	0.295	0.470
rs9308857	IL18R1	0.344	0.656	0.375	0.625	0.579
rs11465673	IL18RAP	0.856	0.144	0.926	0.074	**0.025**
rs11465702	IL18RAP	0.911	0.089	0.865	0.135	0.243
rs11465723	IL18RAP	0.089	0.911	0.135	0.865	0.243
rs11465724	IL18RAP	0.089	0.911	0.031	0.969	**0.007**
rs11465732	IL18RAP	0.067	0.933	0.080	0.920	0.670
rs1420100	IL18RAP	0.456	0.544	0.462	0.538	0.909
rs1420105	IL18RAP	0.544	0.456	0.538	0.462	0.909
rs2058660	IL18RAP	0.727	0.273	0.758	0.242	0.525
rs2272127	IL18RAP	0.089	0.911	0.161	0.839	0.092
rs2293225	IL18RAP	0.193	0.807	0.226	0.774	0.515
rs3755268	IL18RAP	0.722	0.278	0.769	0.231	0.340
rs4851581	IL18RAP	0.944	0.056	0.905	0.095	0.203
rs6543135	IL18RAP	0.222	0.778	0.227	0.773	0.918
rs6748390	IL18RAP	0.633	0.367	0.702	0.298	0.201
rs887972	IL18RAP	0.322	0.678	0.322	0.678	0.998

Table C4 (continued)

SNP	Gene	Toxo + (N=45) freq_1	Toxo + (N=45) freq_2	Toxo - (N=305) freq_1	Toxo - (N=305) freq_2	Genotype-wise pval
rs2243123	IL12A	0.711	0.289	0.708	0.292	0.952
rs2243131	IL12A	0.778	0.222	0.762	0.238	0.803
rs2243147	IL12A	0.567	0.433	0.605	0.395	0.487
rs2243154	IL12A	0.089	0.911	0.105	0.895	0.656
rs583911	IL12A	0.600	0.400	0.554	0.446	0.397
rs640039	IL12A	0.878	0.122	0.879	0.121	0.980
rs1368439	IL12B	0.811	0.189	0.839	0.161	0.515
rs2569253	IL12B	0.544	0.456	0.487	0.513	0.312
rs2853694	IL12B	0.511	0.489	0.502	0.498	0.864
rs2853697	IL12B	0.844	0.156	0.821	0.179	0.591
rs3181216	IL12B	0.300	0.700	0.338	0.662	0.471
rs3181218	IL12B	0.289	0.711	0.198	0.802	**0.039**
rs3212217	IL12B	0.711	0.289	0.803	0.197	**0.037**
rs3213094	IL12B	0.289	0.711	0.198	0.802	**0.039**
rs3213096	IL12B	0.022	0.978	0.005	0.995	0.068
rs3213119	IL12B	0.011	0.989	0.028	0.972	0.342
rs919766	IL12B	0.944	0.056	0.877	0.123	0.066

For allele coding see Table 8.1.

BIBLIOGRAPHY

A.P.A., 1994. Diagnostic and Statistical Manual of Mental Disorders -- DSM-IV.

Akira, S., 2000. The role of IL-18 in innate immunity. *Curr Opin Immunol* 12, 59-63.

Akiyama, K., 1999. Serum levels of soluble IL-2 receptor alpha, IL-6 and IL-1 receptor antagonist in schizophrenia before and during neuroleptic administration. *Schizophr Res* 37, 97-106.

Albert, P. S., Ratnasinghe, D., Tangrea, J., Wacholder, S., 2001. Limitations of the case-only design for identifying gene-environment interactions. *Am J Epidemiol* 154, 687-93.

Albrecht, P., Torrey, E. F., Boone, E., Hicks, J. T., Daniel, N., 1980. Raised cytomegalovirus-antibody level in cerebrospinal fluid of schizophrenic patients. *Lancet* 2, 769-72.

Allan, S. M., Tyrrell, P. J., Rothwell, N. J., 2005. Interleukin-1 and neuronal injury. *Nat Rev Immunol* 5, 629-40.

Almanzar, G., Schwaiger, S., Jenewein, B., Keller, M., Herndler-Brandstetter, D., Wurzner, R., Schonitzer, D., Grubeck-Loebenstein, B., 2005. Long-term cytomegalovirus infection leads to significant changes in the composition of the CD8+ T-cell repertoire, which may be the basis for an imbalance in the cytokine production profile in elderly persons. *J Virol* 79, 3675-83.

Alsharifi, M., Lobigs, M., Simon, M. M., Kersten, A., Muller, K., Koskinen, A., Lee, E., Mullbacher, A., 2006. NK cell-mediated immunopathology during an acute viral infection of the CNS. *Eur J Immunol* 36, 887-96.

Andoniou, C. E., van Dommelen, S. L., Voigt, V., Andrews, D. M., Brizard, G., Asselin-Paturel, C., Delale, T., Stacey, K. J., Trinchieri, G., Degli-Esposti, M. A., 2005. Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity. *Nat Immunol* 6, 1011-9.

Andrews, D. M., Scalzo, A. A., Yokoyama, W. M., Smyth, M. J., Degli-Esposti, M. A., 2003. Functional interactions between dendritic cells and NK cells during viral infection. *Nat Immunol* 4, 175-81.

Andrieu, N., Goldstein, A. M., 2004. The case-combined-control design was efficient in detecting gene-environment interactions. *J Clin Epidemiol* 57, 662-71.

Arolt, V., Rothermundt, M., Peters, M., Leonard, B., 2002. Immunological research in clinical psychiatry: report on the consensus debate during the 7th Expert Meeting on Psychiatry and Immunology. *Mol Psychiatry* 7, 822-6.

Ashdown, H., Dumont, Y., Ng, M., Poole, S., Boksa, P., Luheshi, G. N., 2006. The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. *Mol Psychiatry* 11, 47-55.

Ashley, R. L., Wu, L., Pickering, J. W., Tu, M. C., Schnorenberg, L., 1998. Premarket evaluation of a commercial glycoprotein G-based enzyme immunoassay for herpes simplex virus type-specific antibodies. *J Clin Microbiol* 36, 294-5.

Azimi, N., Jacobson, S., Tanaka, Y., Corey, L., Groh, V., Spies, T., 2006. Immunostimulation by induced expression of NKG2D and its MIC ligands in HTLV-1-associated neurologic disease. *Immunogenetics* 28, 28.

Babulas, V., Factor-Litvak, P., Goetz, R., Schaefer, C. A., Brown, A. S., 2006. Prenatal exposure to maternal genital and reproductive infections and adult schizophrenia. *Am J Psychiatry* 163, 927-9.

Bacanu, S. A., Devlin, B., Roeder, K., 2000. The Power of Genomic Control. *Am J Hum Genet* 66, 1933-1944.

Badner, J. A., Gershon, E. S., 2002. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 7, 405-11.

Baker, I., Masserano, J., Wyatt, R. J., 1996. Serum cytokine concentrations in patients with schizophrenia. *Schizophr Res* 20, 199-203.

Barak, V., Barak, Y., Levine, J., Nisman, B., Roisman, I., 1995. Changes in interleukin-1 beta and soluble interleukin-2 receptor levels in CSF and serum of schizophrenic patients. *J Basic Clin Physiol Pharmacol* 6, 61-9.

Baron, M., 1985. The genetics of schizophrenia: new perspectives. *Acta Psychiatrica Scandinavica, Supplementum* 319, 85-92.

Baron, M., 2001. Genetics of schizophrenia and the new millennium: progress and pitfalls. *Am J Hum Genet* 68, 299-312.

Barrett, J. C., Fry, B., Maller, J., Daly, M. J., 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21, 263-5. Epub 2004 Aug 05.

Barthel, R., Goldfeld, A. E., 2003. T cell-specific expression of the human TNF-alpha gene involves a functional and highly conserved chromatin signature in intron 3. *J Immunol* 171, 3612-9.

Beattie, E. C., Stellwagen, D., Morishita, W., Bresnahan, J. C., Ha, B. K., Von Zastrow, M., Beattie, M. S., Malenka, R. C., 2002. Control of synaptic strength by glial TNFalpha. *Science* 295, 2282-5.

Bessler, H., Levental, Z., Karp, L., Modai, I., Djaldetti, M., Weizman, A., 1995. Cytokine production in drug-free and neuroleptic-treated schizophrenic patients. *Biol Psychiatry* 38, 297-302.

Beutler, B., Crozat, K., Koziol, J. A., Georgel, P., 2005. Genetic dissection of innate immunity to infection: the mouse cytomegalovirus model. *Curr Opin Immunol* 17, 36-43.

Bird, S., Zou, J., Wang, T., Munday, B., Cunningham, C., Secombes, C. J., 2002. Evolution of interleukin-1beta. *Cytokine Growth Factor Rev* 13, 483-502.

Boin, F., Zanardini, R., Pioli, R., Altamura, C. A., Maes, M., Gennarelli, M., 2001. Association between -G308A tumor necrosis factor alpha gene polymorphism and schizophrenia. *Mol Psychiatry* 6, 79-82.

Botto, L. D., Khoury, M. J., 2001. Commentary: facing the challenge of gene-environment interaction: the two-by-four table and beyond. *Am J Epidemiol* 153, 1016-20.

Boydell, J., Murray, R. M., 2003. Urbanization, migration and risk of schizophrenia. In R. M. Murray, P. B. Jones, E. S. Susser, J. van Os, M. Cannon (Eds.), *The Epidemiology of Schizophrenia* (pp. 49-70). Cambridge, UK: Cambridge University Press.

Brown, A. S., 2006. Prenatal Infection as a Risk Factor for Schizophrenia. *Schizophr Bull* 9, 9.

Brown, A. S., Begg, M. D., Gravenstein, S., Schaefer, C. A., Wyatt, R. J., Bresnahan, M., Babulas, V. P., Susser, E. S., 2005a. Serologic Evidence of Prenatal Influenza in the Etiology of Schizophrenia. *Obstet Gynecol Surv* 60, 77-78.

Brown, A. S., Cohen, P., Harkavy-Friedman, J., Babulas, V., Malaspina, D., Gorman, J. M., Susser, E. S., 2001. A.E. Bennett Research Award. Prenatal rubella, premorbid abnormalities, and adult schizophrenia. *Biol Psychiatry* 49, 473-86.

Brown, A. S., Schaefer, C. A., Quesenberry, C. P., Jr., Liu, L., Babulas, V. P., Susser, E. S., 2005b. Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. *Am J Psychiatry* 162, 767-73.

Buka, S. L., Tsuang, M. T., Torrey, E. F., Klebanoff, M. A., Bernstein, D., Yolken, R. H., 2001. Maternal infections and subsequent psychosis among offspring. *Arch Gen Psychiatry* 58, 1032-7.

Bureau, A., Dupuis, J., Falls, K., Lunetta, K. L., Hayward, B., Keith, T. P., Van Eerdewegh, P., 2005. Identifying SNPs predictive of phenotype using random forests. *Genet Epidemiol* 28, 171-82.

Caldwell, C. L., Irwin, M., Lohr, J., 1991. Reduced natural killer cell cytotoxicity in depression but not in schizophrenia. *Biol Psychiatry* 30, 1131-8.

Calvani, N., Tucci, M., Richards, H. B., Tartaglia, P., Silvestris, F., 2005. Th1 cytokines in the pathogenesis of lupus nephritis: the role of IL-18. *Autoimmun Rev* 4, 542-8.

Cannon, M., Clarke, M. C., 2005. Risk for schizophrenia--broadening the concepts, pushing back the boundaries. *Schizophr Res* 79, 5-13.

Cannon, M., Kendell, R., Susser, E. S., Jones, P. B., 2003. Prenatal and perinatal risk factors for schizophrenia. In R. Murray, P. B. Jones, E. S. Susser, J. van Os, M. Cannon (Eds.), *The epidemiology of schizophrenia* (pp. 454). Cambridge: Cambridge University Press.

Cannon, T. D., van Erp, T. G., Rosso, I. M., Huttunen, M., Lonnqvist, J., Pirkola, T., Salonen, O., Valanne, L., Poutanen, V. P., Standertskjold-Nordenstam, C. G., 2002. Fetal hypoxia and structural brain abnormalities in schizophrenic patients, their siblings, and controls. *Arch Gen Psychiatry* 59, 35-41.

Cardell, S. L., 2006. The natural killer T lymphocyte: a player in the complex regulation of autoimmune diabetes in non-obese diabetic mice. *Clin Exp Immunol* 143, 194-202.

Cardno, A. G., Rijsdijk, F. V., Sham, P. C., Murray, R. M., McGuffin, P., 2002. A twin study of genetic relationships between psychotic symptoms. *Am J Psychiatry* 159, 539-45.

Cardon, L. R., Palmer, L. J., 2003. Population stratification and spurious allelic association. *Lancet* 361, 598-604.

Carlson, C. S., Eberle, M. A., Kruglyak, L., Nickerson, D. A., 2004. Mapping complex disease loci in whole-genome association studies. *Nature* 429, 446-52.

Carlsson, A., Lindqvist, M., 1963. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacology Toxicology* 20, 140-44.

Carpenter, J., W.T.; Buchanan, R.W., 1994. Medical Progress - Schizophrenia. *The New England Journal of Medicine* 330, 681-690.

Carter, C. L., Chung, C. S., 1980. Segregation analysis of schizophrenia under a mixed genetic model. *Human Heredity* 30, 350-6.

Cazzullo, C. L., Scarone, S., Grassi, B., Vismara, C., Trabattoni, D., Clerici, M., 1998. Cytokines production in chronic schizophrenia patients with or without paranoid behaviour. *Prog Neuropsychopharmacol Biol Psychiatry* 22, 947-57.

Chatterjee, N., Kalaylioglu, Z., Carroll, R. J., 2005. Exploiting gene-environment independence in family-based case-control studies: increased power for detecting associations, interactions and joint effects. *Genet Epidemiol* 28, 138-56.

Cheeran, M. C., Hu, S., Yager, S. L., Gekker, G., Peterson, P. K., Lokensgard, J. R., 2001. Cytomegalovirus induces cytokine and chemokine production differentially in microglia and astrocytes: antiviral implications. *J Neurovirol* 7, 135-47.

Chiavetto, L. B., Boin, F., Zanardini, R., Popoli, M., Michelato, A., Bignotti, S., Tura, G. B., Gennarelli, M., 2002. Association between promoter polymorphic haplotypes of interleukin-10 gene and schizophrenia. *Biol Psychiatry* 51, 480-4.
Chowdari, K. V., Northup, A., Pless, L., Wood, J., Joo, Y. H., Mirnics, K., Lewis, D. A., Levitt, P. R., Bacanu, S. A., Nimgaonkar, V. L., 2006. DNA pooling: a comprehensive, multi-stage association analysis of ACSL6 and SIRT5 polymorphisms in schizophrenia. *Genes Brain Behav* 10, 10.
Chowdari, K. V., Xu, K., Zhang, F., Ma, C., Li, T., Xie, B. Y., Wood, J., Trucco, M., Tsoi, W. F., Saha, N., Rudert, W. A., Nimgaonkar, V. L., 2001. Immune related genetic polymorphisms and schizophrenia among the Chinese. *Hum Immunol* 62, 714-24.
Clark, A. G., Boerwinkle, E., Hixson, J., Sing, C. F., 2005. Determinants of the success of whole-genome association testing. *Genome Res* 15, 1463-7.
Cleator, G. M., Klapper, P. E., 2004. The Herpesviridae. In A. J. Zuckerman, J. E. Bantavala, J. R. Pattison, P. D. Griffiths, B. D. Schoub (Eds.), *Priniciples and Practice of Clinical Virology* (pp. 23-26). Hoboken, NJ: J. Wiley & Sons.
Collier, D. A., Li, T., 2003. The genetics of schizophrenia: glutamate not dopamine? *Eur J Pharmacol* 480, 177-84.
Collins, R. W., 2004. Human MHC class I chain related (MIC) genes: their biological function and relevance to disease and transplantation. *Eur J Immunogenet* 31, 105-14.
Conti, B., Park, L. C., Calingasan, N. Y., Kim, Y., Kim, H., Bae, Y., Gibson, G. E., Joh, T. H., 1999. Cultures of astrocytes and microglia express interleukin 18. *Brain Res Mol Brain Res* 67, 46-52.
Cooke, G. S., Hill, A. V., 2001. Genetics of susceptibility to human infectious disease. *Nat Rev Genet* 2, 967-77.
Cordell, H. J., Barratt, B. J., Clayton, D. G., 2004. Case/pseudocontrol analysis in genetic association studies: A unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. *Genet Epidemiol* 26, 167-85.
Cordell, H. J., Clayton, D. G., 2002. A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: application to HLA in type 1 diabetes. *Am J Hum Genet* 70, 124-41.
Cosman, D., Mullberg, J., Sutherland, C. L., Chin, W., Armitage, R., Fanslow, W., Kubin, M., Chalupny, N. J., 2001. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* 14, 123-33.
Cotter, D. R., Pariante, C. M., Everall, I. P., 2001. Glial cell abnormalities in major psychiatric disorders: the evidence and implications. *Brain Res Bull* 55, 585-95.
Craig, D., Hart, D. J., McCool, K., McIlroy, S. P., Passmore, A. P., 2004. The interleukin 1beta gene promoter polymorphism (-511) acts as a risk factor for psychosis in Alzheimer's dementia. *Ann Neurol* 56, 121-4.
Crow, T. J., 1983. Is schizophrenia an infectious disease? *Lancet* 1, 173-5.
Cui, F. D., Asada, H., Jin, M. L., Kishida, T., Shin-Ya, M., Nakaya, T., Kita, M., Ishii, M., Iwai, M., Okanoue, T., Imanishi, J., Mazda, O., 2005. Cytokine genetic adjuvant facilitates prophylactic intravascular DNA vaccine against acute and latent herpes simplex virus infection in mice. *Gene Ther* 12, 160-8.

Davies, G., Welham, J., Chant, D., Torrey, E. F., McGrath, J., 2003. A systematic review and meta-analysis of Northern Hemisphere season of birth studies in schizophrenia. *Schizophr Bull* 29, 587-93.

Davis, K. L., Kahn, R. S., Ko, G., Davidson, M., 1991. Dopamine in schizophrenia: a review and reconceptualization. *American Journal of Psychiatry* 148, 1474-86.

DeBiasi, R. L., Kleinschmidt-DeMasters, B. K., Richardson-Burns, S., Tyler, K. L., 2002. Central nervous system apoptosis in human herpes simplex virus and cytomegalovirus encephalitis. *J Infect Dis* 186, 1547-57.

DeLisi, L. E., Goldin, L. R., Hamovit, J. R., Maxwell, M. E., Kurtz, D., Gershon, E. S., 1986. A family study of the association of increased ventricular size with schizophrenia. *Archives of General Psychiatry* 43, 148-53.

DeLisi, L. E., Shaw, S. H., Crow, T. J., Shields, G., Smith, A. B., Larach, V. W., Wellman, N., Loftus, J., Nanthakumar, B., Razi, K., Comazzi, M., Vita, A., Heffner, T., Sherrington, R., 2002. A genome-wide scan for linkage to schimosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. *American Journal of Psychiatry* in press.

Devlin, B., Bacanu, S. A., Roeder, K., 2004. Genomic Control to the extreme. *Nat Genet* 36, 1129-30; author reply 1131.

Devlin, B., Risch, N., 1995. A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* 29, 311-22.

Devlin, B., Roeder, K., 1999. Genomic control for association studies. *Biometrics* 55.

Devlin, B., Roeder, K., Bacanu, S. A., 2001. Unbiased methods for population-based association studies. *Genet Epidemiol* 21, 273-84.

Dickerson, F., Boronow, J. J., Stallings, C., Origoni, A. E., Cole, S. K., Yolken, R. H., 2004. Cognitive functioning in schizophrenia and bipolar disorder: comparison of performance on the Repeatable Battery for the Assessment of Neuropsychological Status. *Psychiatry Res* 129, 45-53.

Dickerson, F., Kirkpatrick, B., Boronow, J., Stallings, C., Origoni, A., Yolken, R., 2006. Deficit schizophrenia: association with serum antibodies to cytomegalovirus. *Schizophr Bull* 32, 396-400.

Dickerson, F. B., Boronow, J. J., Stallings, C., Origoni, A. E., Ruslanova, I., Yolken, R. H., 2003a. Association of serum antibodies to herpes simplex virus 1 with cognitive deficits in individuals with schizophrenia. *Arch Gen Psychiatry* 60, 466-72.

Dickerson, F. B., Boronow, J. J., Stallings, C. R., Origoni, A. E., Yolken, R. H., 2003b. Reduction of symptoms by valacyclovir in cytomegalovirus-seropositive individuals with schizophrenia. *Am J Psychiatry* 160, 2234-6.

Duan, S., Xu, Y., Chen, W., Liu, Z., Guo, T., Gao, J., Bian, L., Zhen, Y., Li, X., Zhang, X., Pan, Y., Gu, N., Feng, G., He, L., 2004. No association between the promoter variants of tumor necrosis factor alpha (TNF-alpha) and schizophrenia in Chinese Han population. *Neurosci Lett* 366, 139-43.

Dudbridge, F., 2003. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 25, 115-21.

Dunn, C., Chalupny, N. J., Sutherland, C. L., Dosch, S., Sivakumar, P. V., Johnson, D. C., Cosman, D., 2003. Human cytomegalovirus glycoprotein UL16 causes intracellular sequestration of NKG2D ligands, protecting against natural killer cell cytotoxicity. *J Exp Med* 197, 1427-39.

Eaton, W. W., Byrne, M., Ewald, H., Mors, O., Chen, C. Y., Agerbo, E., Mortensen, P. B., 2006. Association of schizophrenia and autoimmune diseases: linkage of danish national registers. *Am J Psychiatry* 163, 521-8.
Elsner, H. A., Schroeder, M., Blasczyk, R., 2001. The nucleotide diversity of MICA and MICB suggests the effect of overdominant selection. *Tissue Antigens* 58, 419-21.
Erbagci, A. B., Herken, H., Koyluoglu, O., Yilmaz, N., Tarakcioglu, M., 2001. Serum IL-1beta, sIL-2R, IL-6, IL-8 and TNF-alpha in schizophrenic patients, relation with symptomatology and responsiveness to risperidone treatment. *Mediators Inflamm* 10, 109-15.
Evans, D. M., Cardon, L. R., 2006. Genome-wide association: a promising start to a long race. *Trends Genet* 18, 18.
Excoffier, L., Slatkin, M., 1995. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 12, 921-7.
Falk, C. T., Rubinstein, P., 1987. Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. *Annals of Human Genetics* 51, 227-33.
Felderhoff-Mueser, U., Schmidt, O. I., Oberholzer, A., Buhrer, C., Stahel, P. F., 2005a. IL-18: a key player in neuroinflammation and neurodegeneration? *Trends Neurosci* 28, 487-93.
Felderhoff-Mueser, U., Sifringer, M., Polley, O., Dzietko, M., Leineweber, B., Mahler, L., Baier, M., Bittigau, P., Obladen, M., Ikonomidou, C., Buhrer, C., 2005b. Caspase-1-processed interleukins in hyperoxia-induced cell death in the developing brain. *Ann Neurol* 57, 50-9.
Friese, M. A., Platten, M., Lutz, S. Z., Naumann, U., Aulwurm, S., Bischof, F., Buhring, H. J., Dichgans, J., Rammensee, H. G., Steinle, A., Weller, M., 2003. MICA/NKG2D-mediated immunogene therapy of experimental gliomas. *Cancer Res* 63, 8996-9006.
Fujioka, N., Akazawa, R., Ohashi, K., Fujii, M., Ikeda, M., Kurimoto, M., 1999. Interleukin-18 protects mice against acute herpes simplex virus type 1 infection. *J Virol* 73, 2401-9.
Fux, M., Sarov, I., Ginot, Y., Sarov, B., 1992. Herpes simplex virus and cytomegalovirus in the serum of schizophrenic patients versus other psychosis and normal controls. *Isr J Psychiatry Relat Sci* 29, 33-5.
Ganguli, R., Brar, J. S., Chengappa, K. N., Yang, Z. W., Nimgaonkar, V. L., Rabin, B. S., 1993. Autoimmunity in schizophrenia: a review of recent findings. *Annals of Medicine* 25, 489-96.
Ganguli, R., Brar, J. S., Rabin, B. S., 1994. Immune abnormalities in schizophrenia: evidence for the autoimmune hypothesis. *Harvard Review of Psychiatry* 2, 2-70.
Garver, D. L., Holcomb, J., Mapua, F. M., Wilson, R., Barnes, B., 2001. Schizophrenia spectrum disorders: an autosomal-wide scan in multiplex pedigrees. *Schizophr Res* 52, 145-60.
Gatto, N. M., Campbell, U. B., Rundle, A. G., Ahsan, H., 2004. Further development of the case-only design for assessing gene-environment interaction: evaluation of and adjustment for bias. *Int J Epidemiol* 33, 1014-24.
Gauderman, W. J., 2002. Sample size requirements for matched case-control studies of gene-environment interaction. *Stat Med* 21, 35-50.
Gaughran, F., 2002. Immunity and schizophrenia: autoimmunity, cytokines, and immune responses. *Int Rev Neurobiol* 52, 275-302.
Gilmore, J. H., Fredrik Jarskog, L., Vadlamudi, S., Lauder, J. M., 2004. Prenatal Infection and Risk for Schizophrenia: IL-1beta, IL-6, and TNFalpha Inhibit Cortical Neuron Dendrite Development. *Neuropsychopharmacology* 29, 1221-9.

Goldstein, A. M., Dondon, M. G., Andrieu, N., 2006. Unconditional analyses can increase efficiency in assessing gene-environment interaction of the case-combined-control design. *Int J Epidemiol* 23, 23.

Gottesman, I., 1991. *Schizophrenia Genesis: The Origins of Madness*. New York: WH Freeman.

Gracie, J. A., Koyama, N., Murdoch, J., Field, M., McGarry, F., Crilly, A., Schobel, A., Madhok, R., Pons-Kuhnemann, J., McInnes, I. B., Moller, B., 2005. Disease association of two distinct interleukin-18 promoter polymorphisms in Caucasian rheumatoid arthritis patients. *Genes Immun* 6, 211-6.

Gracie, J. A., Robertson, S. E., McInnes, I. B., 2003. Interleukin-18. *J Leukoc Biol* 73, 213-24.

Groh, V., Rhinehart, R., Randolph-Habecker, J., Topp, M. S., Riddell, S. R., Spies, T., 2001. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat Immunol* 2, 255-60.

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

Hafner, H., 2003. Prodrome, onset and early course of schizophrenia. In R. Murray, P. B. Jones, E. S. Susser, J. van Os, M. Cannon (Eds.), *The Epidemiology of Schizophrenia* (pp. 454). Cambridge: Cambridge University Press.

Hahn, L. W., Ritchie, M. D., Moore, J. H., 2003. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. *Bioinformatics* 19, 376-82.

Halford, W. P., Gebhardt, B. M., Carr, D. J., 1996. Persistent cytokine expression in trigeminal ganglion latently infected with herpes simplex virus type 1. *J Immunol* 157, 3542-9.

Hall, S. K., Perregaux, D. G., Gabel, C. A., Woodworth, T., Durham, L. K., Huizinga, T. W., Breedveld, F. C., Seymour, A. B., 2004. Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. *Arthritis Rheum* 50, 1976-83.

Handoko, H. Y., Nancarrow, D. J., Hayward, N. K., Ohaeri, J. U., Aghanwa, H., McGrath, J. J., Levinson, D. F., Johns, C., Walters, M. K., Nertney, D. A., Srinivasan, T. N., Thara, R., Mowry, B. J., 2003. Tumor necrosis factor haplotype analysis amongst schizophrenia probands from four distinct populations in the Asia-Pacific region. *Am J Med Genet* 121B, 1-6.

Hanninen, K., Katila, H., Rontu, R., Mattila, K. M., Hurme, M., Lehtimaki, T., 2005. Tumor necrosis factor-alpha -G308A polymorphism in schizophrenia in a Finnish population. *Neurosci Lett* 385, 76-81.

HapMap, 2003. The International HapMap Project. *Nature* 426, 789-96.

Harrison, P. J., Owen, M. J., 2003. Genes for schizophrenia? Recent findings and their pathophysiological implications. *Lancet* 361, 417-9.

Hashimoto, R., Yoshida, M., Ozaki, N., Yamanouchi, Y., Iwata, N., Suzuki, T., Kitajima, T., Tatsumi, M., Kamijima, K., Kunugi, H., 2004. Association analysis of the -308G>A promoter polymorphism of the tumor necrosis factor alpha (TNF-alpha) gene in Japanese patients with schizophrenia. *J Neural Transm* 111, 217-21.

He, G., Zhang, J., Li, X. W., Chen, W. Y., Pan, Y. X., Yang, F. P., Gu, N. F., Feng, G. Y., Yang, S. L., He, J. Y., Liu, B. X., Peng, Y. W., Liu, J., He, L., 2006. Interleukin-10 -1082

promoter polymorphism is associated with schizophrenia in a Han Chinese sib-pair study. *Neurosci Lett* 394, 1-4.

Hedtjarn, M., Mallard, C., Arvidsson, P., Hagberg, H., 2005a. White matter injury in the immature brain: role of interleukin-18. *Neurosci Lett* 373, 16-20.

Hedtjarn, M., Mallard, C., Iwakura, Y., Hagberg, H., 2005b. Combined deficiency of IL-1beta18, but not IL-1alphabeta, reduces susceptibility to hypoxia-ischemia in the immature brain. *Dev Neurosci* 27, 143-8.

Heidema, G. A., Boer, J. M., Nagelkerke, N., Mariman, E. C., van der, A. D., Feskens, E. J., 2006. The challenge for genetic epidemiologists: how to analyze large numbers of SNPs in relation to complex diseases. *BMC Genet* 7, 23.

Heila, H., Lonnqvist, J., 2003. The clinical epidemiology of suicide in schizophrenia. In R. M. Murray, P. B. Jones, E. S. Susser, J. van Os, M. Cannon (Eds.), *The Epidemiology of Schizophrenia* (pp. 288-316). Cambridge: Cambridge University Press.

Hensley, K., Robinson, K. A., Pye, Q. N., Floyd, R. A., Cheng, I., Garland, W. A., Irwin, I., 2000. CPI-1189 inhibits interleukin 1beta-induced p38-mitogen-activated protein kinase phosphorylation: an explanation for its neuroprotective properties? *Neurosci Lett* 281, 179-82.

Heston, L. L., 1966. Psychiatric Disorders in Foster-home-reared Children of Schizophrenic Mothers. *British Journal of Psychiatry* 112, 819-825.

Hill, A. V., 2001. The genomics and genetics of human infectious disease susceptibility. 373-400.

Hinze-Selch, D., 2002. Infection, treatment and immune response in patients with bipolar disorder versus patients with major depression, schizophrenia or healthy controls. *Bipolar Disord* 4, 81-3.

Hurme, M., Haanpaa, M., Nurmikko, T., Wang, X. Y., Virta, M., Pessi, T., Kilpinen, S., Hulkkonen, J., Helminen, M., 2003. IL-10 gene polymorphism and herpesvirus infections. *J Med Virol* 70, S48-50.

Hurme, M., Helminen, M., 1998. Resistance to human cytomegalovirus infection may be influenced by genetic polymorphisms of the tumour necrosis factor-alpha and interleukin-1 receptor antagonist genes. *Scand J Infect Dis* 30, 447-9.

Hurst, L. A., 1972. Hypothesis of a Single-locus Recessive Genotype for Schizophrenia. In A. R. Kaplan (Ed.), *Genetic Factors in "Schizophrenia"* (pp. 219-245). Springfield: Charles C. Thomas.

Irie, H., Shiga, J., 2005. Pathogenesis of herpes simplex hepatitis in macrophage-depleted mice: possible involvement of tumor necrosis factor-alpha and inducible nitric oxide synthase in massive apoptosis. *Anat Sci Int* 80, 199-211.

Jablensky, A., 1993. The epidemiology of schizophrenia. *Current Opinion in Psychiatry* 6, 43-52.

Jablensky, A., 1997. The 100-year epidemiology of schizophrenia. *Shizophrenia Research* 28, 111-125.

Jablensky, A., 2000. Epidemiology of schizophrenia: the global burden of disease and disability. *Eur Arch Psychiatry Clin Neurosci* 250, 274-85.

Jablensky, A., Sartorius, N., Ernberg, G., Anker, M., Korten, K., Cooper, J. E., Day, R., Bertelson, A., 1992. Schizophrenia: Manifestations, Incidence and Course in Different Cultures. A World Health Organization Ten-Country Study. *Psychological Medicine Monograph* Supplement 20.

Janeway, C. A., Travers, P., Walport, M., Shlomchik, M., 2005. *Immunobiology: the immune system in health and disease* (6th ed.). New York: Garland Science.

John, S., Shephard, N., Liu, G., Zeggini, E., Cao, M., Chen, W., Vasavda, N., Mills, T., Barton, A., Hinks, A., Eyre, S., Jones, K. W., Ollier, W., Silman, A., Gibson, N., Worthington, J., Kennedy, G. C., 2004. Whole-genome scan, in a complex disease, using 11,245 single-nucleotide polymorphisms: comparison with microsatellites. *Am J Hum Genet* 75, 54-64.

Jones, A. L., Mowry, B. J., Pender, M. P., Greer, J. M., 2005. Immune dysregulation and self-reactivity in schizophrenia: do some cases of schizophrenia have an autoimmune basis? *Immunol Cell Biol* 83, 9-17.

Jones, P. B., Rantakallio, P., Hartikainen, A. L., Isohanni, M., Sipila, P., 1998. Schizophrenia as a long-term outcome of pregnancy, delivery, and perinatal complications: a 28-year follow-up of the 1966 north Finland general population birth cohort. *Am J Psychiatry* 155, 355-64.

Jorde, L. B., 2000. Linkage disequilibrium and the search for complex disease genes. *Genome Res* 10, 1435-44.

Jun, T. Y., Pae, C. U., Kim, K. S., Han, H., Serretti, A., 2003. Interleukin-10 gene promoter polymorphism is not associated with schizophrenia in the Korean population. *Psychiatry Clin Neurosci* 57, 153-9.

Kaminska, T., Wysocka, A., Marmurowska-Michalowska, H., Dubas-Slemp, H., Kandefer-Szerszen, M., 2001. Investigation of serum cytokine levels and cytokine production in whole blood cultures of paranoid schizophrenic patients. *Arch Immunol Ther Exp* 49, 439-45.

Kampman, O., Anttila, S., Illi, A., Mattila, K. M., Rontu, R., Leinonen, E., Lehtimaki, T., 2004. Interaction of tumor necrosis alpha - G308A and epidermal growth factor gene polymorphisms in early-onset schizophrenia. *Eur Arch Psychiatry Clin Neurosci* Epub ahead of print.

Kanno, T., Nagata, T., Yamamoto, S., Okamura, H., Nishizaki, T., 2004. Interleukin-18 stimulates synaptically released glutamate and enhances postsynaptic AMPA receptor responses in the CA1 region of mouse hippocampal slices. *Brain Res* 1012, 190-3.

Kastrukoff, L. F., Kim, S. U., 2002. Oligodendrocytes from human donors differ in resistance to herpes simplex virus 1 (HSV-1). *Glia* 38, 87-92.

Katila, H., Appelberg, B., Hurme, M., Rimon, R., 1994a. Plasma levels of interleukin-1 beta and interleukin-6 in schizophrenia, other psychoses, and affective disorders. *Schizophrenia Research* 12, 29-34.

Katila, H., Hanninen, K., Hurme, m., 1999a. Polymorphisms of the interleukin-1 gene complex in schizophrenia. *Molecular Psychiatry* 4, 179-181.

Katila, H., Hanninen, K., Hurme, M., 1999b. Polymorphisms of the interleukin-1 gene complex in schizophrenia. *Mol Psychiatry* 4, 179-81.

Katila, H., Hurme, M., Wahlbeck, K., Appelberg, B., Rimon, R., 1994b. Plasma and cerebrospinal fluid interleukin-1 beta and interleukin-6 in hospitalized schizophrenic patients. *Neuropsychobiology* 30, 20-3.

Kety, S. S., Ingraham, L. J., 1992. Genetic transmission and improved diagnosis of schizophrenia from pedigrees of adoptees. *Journal of Psychiatric Research* 26, 247-55.

Khoury, M. J., Flanders, W. D., 1996a. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! *Am J Epidemiol* 144, 207-13.

Khoury, M. J., Flanders, W. D., 1996b. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls:. *American Journal of Epidemiology* 144, 207-13.

Kim, J. J., Dayal, M., Bacanu, S.-A., Shirts, B. H., Wood, J., Xie, W., Zhang, X., Chowdari, K., Yolken, R., Devlin, B., Nimagaonkar, V. L., 2004a. Exposure to cytomegalovirus and polymorphisms in two genes on chromosome 6p21-23 as joint risk factors for schizophrenia. *American Journal of Medical Genetics* 130B, 19.

Kim, S. J., Lee, H. J., Koo, H. G., Kim, J. W., Song, J. Y., Kim, M. K., Shin, D. H., Jin, S. Y., Hong, M. S., Park, H. J., Yoon, S. H., Park, H. K., Chung, J. H., 2004b. Impact of IL-1 receptor antagonist gene polymorphism on schizophrenia and bipolar disorder. *Psychiatr Genet* 14, 165-7.

Kim, Y. K., Lee, M. S., Suh, K. Y., 1998. Decreased interleukin-2 production in Korean schizophrenic patients. *Biol Psychiatry* 43, 701-4.

Kim, Y. K., Myint, A. M., Lee, B. H., Han, C. S., Lee, H. J., Kim, D. J., Leonard, B. E., 2004c. Th1, Th2 and Th3 cytokine alteration in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 28, 1129-34.

Kim, Y. K., Suh, I. B., Kim, H., Han, C. S., Lim, C. S., Choi, S. H., Licinio, J., 2002. The plasma levels of interleukin-12 in schizophrenia, major depression, and bipolar mania: effects of psychotropic drugs. *Mol Psychiatry* 7, 1107-14.

Kipnis, J., Cohen, H., Cardon, M., Ziv, Y., Schwartz, M., 2004. T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. *Proc Natl Acad Sci U S A* 101, 8180-5.

Kira, R., Torisu, H., Takemoto, M., Nomura, A., Sakai, Y., Sanefuji, M., Sakamoto, K., Matsumoto, S., Gondo, K., Hara, T., 2005. Genetic susceptibility to simple febrile seizures: interleukin-1beta promoter polymorphisms are associated with sporadic cases. *Neurosci Lett* 384, 239-44.

Kirch, D. G., 1993. Infection and autoimmunity as etiologic factors in schizophrenia: a review and reappraisal. *Schizophrenia Bulletin* 19, 355-70.

Koponen, H., Rantakallio, P., Veijola, J., Jones, P., Jokelainen, J., Isohanni, M., 2004. Childhood central nervous system infections and risk for schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 254, 9-13.

Kowalski, J., Blada, P., Kucia, K., Madej, A., Herman, Z. S., 2001. Neuroleptics normalize increased release of interleukin- 1 beta and tumor necrosis factor-alpha from monocytes in schizophrenia. *Schizophr Res* 50, 169-75.

Kowalski, J., Labuzek, K., Herman, Z. S., 2003. Flupentixol and trifluperidol reduce secretion of tumor necrosis factor-alpha and nitric oxide by rat microglial cells. *Neurochem Int* 43, 173-8.

Krabbendam, L., van Os, J., 2005. Schizophrenia and urbanicity: a major environmental influence--conditional on genetic risk. *Schizophr Bull* 31, 795-9.

Kraft, P., Hunter, D., 2005. Integrating epidemiology and genetic association: the challenge of gene-environment interaction. *Philos Trans R Soc Lond B Biol Sci* 360, 1609-16.

Kruglyak, L., Daly, M. J., Reeve-Daly, M. P., Lander, E. S., 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. *American Journal of Human Genetics* 58, 1347-63.

Kumaraguru, U., Rouse, B. T., 2002. The IL-12 response to herpes simplex virus is mainly a paracrine response of reactive inflammatory cells. *J Leukoc Biol* 72, 564-70.

Lander, E. S., Schork, N. J., 1994. Genetic dissection of complex traits [published erratum appears in Science 1994 Oct 21;266(5184):353]. *Science* 265, 2037-48.
Laurent, C., Thibaut, F., Ravassard, P., Campion, D., Samolyk, D., Lafargue, C., Petit, M., Martinez, M., Mallet, J., 1997. Detection of two new polymorphic sites in the human interleukin-1 beta gene: lack of association with schizophrenia in a French population. *Psychiatric Genetics* 7, 103-5.
Lee, S., Gierynska, M., Eo, S. K., Kuklin, N., Rouse, B. T., 2003. Influence of DNA encoding cytokines on systemic and mucosal immunity following genetic vaccination against herpes simplex virus. *Microbes Infect* 5, 571-8.
Lee, S. H., Girard, S., Macina, D., Busa, M., Zafer, A., Belouchi, A., Gros, P., Vidal, S. M., 2001. Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nat Genet* 28, 42-5.
Levinson, D. F., Mahtani, M. M., Nancarrow, D. J., Brown, D. M., Kruglyak, L., Kirby, A., Hayward, N. K., Crowe, R. R., Andreasen, N. C., Black, D. W., Silverman, J. M., Endicott, J., Sharpe, L., Mohs, R. C., Siever, L. J., Walters, M. K., Lennon, D. P., Jones, H. L., Nertney, D. A., Daly, M. J., Gladis, M., Mowry, B. J., 1998. Genome scan of schizophrenia. *Am J Psychiatry* 155, 741-50.
Leweke, F. M., Gerth, C. W., Koethe, D., Klosterkotter, J., Ruslanova, I., Krivogorsky, B., Torrey, E. F., Yolken, R. H., 2004. Antibodies to infectious agents in individuals with recent onset schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 254, 4-8.
Lewis, C. M., Levinson, D. F., Wise, L. H., DeLisi, L. E., Straub, R. E., Hovatta, I., Williams, N. M., Schwab, S. G., Pulver, A. E., Faraone, S. V., Brzustowicz, L. M., Kaufmann, C. A., Garver, D. L., Gurling, H. M., Lindholm, E., Coon, H., Moises, H. W., Byerley, W., Shaw, S. H., Mesen, A., Sherrington, R., O'Neill, F. A., Walsh, D., Kendler, K. S., Ekelund, J., Paunio, T., Lonnqvist, J., Peltonen, L., O'Donovan, M. C., Owen, M. J., Wildenauer, D. B., Maier, W., Nestadt, G., Blouin, J. L., Antonarakis, S. E., Mowry, B. J., Silverman, J. M., Crowe, R. R., Cloninger, C. R., Tsuang, M. T., Malaspina, D., Harkavy-Friedman, J. M., Svrakic, D. M., Bassett, A. S., Holcomb, J., Kalsi, G., McQuillin, A., Brynjolfson, J., Sigmundsson, T., Petursson, H., Jazin, E., Zoega, T., Helgason, T., 2003. Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73, 34-48.
Lewis, D. A., Lieberman, J. A., 2000. Catching up on schizophrenia: natural history and neurobiology. *Neuron* 28, 325-34.
Lewis, D. A., Pierri, J. N., Volk, D. W., Melchitzky, D. S., Woo, T. U., 1999. Altered GABA neurotransmission and prefrontal cortical dysfunction in schizophrenia. *Biol Psychiatry* 46, 616-26.
Lipoldova, M., Demant, P., 2006. Genetic susceptibility to infectious disease: lessons from mouse models of leishmaniasis. *Nat Rev Genet* 7, 294-305.
Liu, R., Van Kaer, L., La Cava, A., Price, M., Campagnolo, D. I., Collins, M., Young, D. A., Vollmer, T. L., Shi, F. D., 2006. Autoreactive T cells mediate NK cell degeneration in autoimmune disease. *J Immunol* 176, 5247-54.
Liu, X., Fallin, M. D., Kao, W. H., 2004. Genetic dissection methods: designs used for tests of gene-environment interaction. *Curr Opin Genet Dev* 14, 241-5.
Lokensgard, J. R., Cheeran, M. C., Hu, S., Gekker, G., Peterson, P. K., 2002. Glial cell responses to herpesvirus infections: role in defense and immunopathogenesis. *J Infect Dis* 1, S171-9.

Lokensgard, J. R., Hu, S., Sheng, W., vanOijen, M., Cox, D., Cheeran, M. C., Peterson, P. K., 2001. Robust expression of TNF-alpha, IL-1beta, RANTES, and IP-10 by human microglial cells during nonproductive infection with herpes simplex virus. *J Neurovirol* 7, 208-19.

Lopez, A. D., Murray, C. C., 1998. The global burden of disease, 1990-2020. *Nat Med* 4, 1241-3.

Lu, L. X., Guo, S. Q., Chen, W., Li, Q., Cheng, J., Guo, J. H., 2004. [Effect of clozapine and risperidone on serum cytokine levels in patients with first-episode paranoid schizophrenia.]. *Di Yi Jun Yi Da Xue Xue Bao* 24, 1251-4.

Lunetta, K. L., Hayward, L. B., Segal, J., Van Eerdewegh, P., 2004. Screening large-scale association study data: exploiting interactions using random forests. *BMC Genet* 5, 32.

Maes, M., Bocchio Chiavetto, L., Bignotti, S., Battisa Tura, G., Pioli, R., Boin, F., Kenis, G., Bosmans, E., de Jongh, R., Lin, A., Racagni, G., Altamura, C. A., 2000. Effects of atypical antipsychotics on the inflammatory response system in schizophrenic patients resistant to treatment with typical neuroleptics. *Eur Neuropsychopharmacol* 10, 119-24.

Maes, M., Bosmans, E., Ranjan, R., Vandoolaeghe, E., Meltzer, H. Y., De Ley, M., Berghmans, R., Stans, G., Desnyder, R., 1996. Lower plasma CC16, a natural anti-inflammatory protein, and increased plasma interleukin-1 receptor antagonist in schizophrenia: effects of antipsychotic drugs. *Schizophr Res* 21, 39-50.

Malmgaard, L., Paludan, S. R., 2003. Interferon (IFN)-alpha/beta, interleukin (IL)-12 and IL-18 coordinately induce production of IFN-gamma during infection with herpes simplex virus type 2. *J Gen Virol* 84, 2497-500.

Manchanda, P. K., Kumar, A., Bid, H. K., Mittal, R. D., 2006. Interleukin-1beta and receptor antagonist (IL-1Ra) gene polymorphisms and the prediction of the risk of end-stage renal disease. *Biomarkers* 11, 164-73.

Marques, C. P., Hu, S., Sheng, W., Cheeran, M. C., Cox, D., Lokensgard, J. R., 2004. Interleukin-10 attenuates production of HSV-induced inflammatory mediators by human microglia. *Glia* 47, 358-66.

Marques-Deak, A., Cizza, G., Sternberg, E., 2005. Brain-immune interactions and disease susceptibility. *Mol Psychiatry* 10, 239-50.

Marshall, G. S., Stout, G. G., 2005. Cytomegalovirus seroprevalence among women of childbearing age during a 10-year period. *Am J Perinatol* 22, 371-6.

Martin, E. R., Ritchie, M. D., Hahn, L., Kang, S., Moore, J. H., 2006. A novel method to identify gene-gene effects in nuclear families: the MDR-PDT. *Genet Epidemiol* 30, 111-23.

Martinez, F., Weiss, S. T., 2005. Innate Immunity in Heart, Blood, and Lung Diseases, Program for Genetic Applications (pp. Innage Immunity Program for Genetic Applications). Tuscon, Arizona; Boston, MA: Supported by National Heart, Lung, and Blood Institute.

Marx, C. E., Jarskog, L. F., Lauder, J. M., Lieberman, J. A., Gilmore, J. H., 2001. Cytokine effects on cortical neuron MAP-2 immunoreactivity: implications for schizophrenia. *Biol Psychiatry* 50, 743-9.

McAllister, C. G., van Kammen, D. P., Rehn, T. J., Miller, A. L., Gurklis, J., Kelley, M. E., Yao, J., Peters, J. L., 1995. Increases in CSF levels of interleukin-2 in schizophrenia: effects of recurrence of psychosis and medication status. *Am J Psychiatry* 152, 1291-7.

McDaniel, J. S., Jewart, R. D., Eccard, M. B., Pollard, W. E., Caudle, J., Stipetic, M., Risby, E. D., Lewine, R., Risch, S. C., 1992. Natural killer cell activity in schizophrenia and schizoaffective disorder: a pilot study. *Schizophr Res* 8, 125-8.

McGrath, N., Anderson, N. E., Croxson, M. C., Powell, K. F., 1997. Herpes simplex encephalitis treated with acyclovir: diagnosis and long term outcome. *J Neurol Neurosurg Psychiatry* 63, 321-6.

McGue, M., Gottesman, I. I., Rao, D. C., 1983. The transmission of schizophrenia under a multifactorial threshold model. *American Journal of Human Genetics* 35, 1161-78.

Meira-Lima, I. V., Pereira, A. C., Mota, G. F., Floriano, M., Araujo, F., Mansur, A. J., Krieger, J. E., Vallada, H., 2003. Analysis of a polymorphism in the promoter region of the tumor necrosis factor alpha gene in schizophrenia and bipolar disorder: further support for an association with schizophrenia. *Mol Psychiatry* 8, 718-20.

Meisenzahl, E. M., Rujescu, D., Kirner, A., Giegling, I., Kathmann, N., Leinsinger, G., Maag, K., Hegerl, U., Hahn, K., Moller, H. J., 2001. Association of an interleukin-1beta genetic polymorphism with altered brain structure in patients with schizophrenia. *Am J Psychiatry* 158, 1316-9.

Merikangas, K. R., Chakravarti, A., Moldin, S. O., Araj, H., Blangero, J. C., Burmeister, M., Crabbe, J., Jr., Depaulo, J. R., Jr., Foulks, E., Freimer, N. B., Koretz, D. S., Lichtenstein, W., Mignot, E., Reiss, A. L., Risch, N. J., Takahashi, J. S., 2002. Future of genetics of mood disorders research. *Biol Psychiatry* 52, 457-77.

Mettenleiter, T. C., 2003. Pathogenesis of neurotropic herpesviruses: role of viral glycoproteins in neuroinvasion and transneuronal spread. *Virus Res* 92, 197-206.

Mirnics, K., Levitt, P., Lewis, D. A., 2006. Critical Appraisal of DNA Microarrays in Psychiatric Genomics. *Biol Psychiatry* 14, 14.

Mizruchin, A., Gold, I., Krasnov, I., Livshitz, G., Shahin, R., Kook, A. I., 1999. Comparison of the effects of dopaminergic and serotonergic activity in the CNS on the activity of the immune system. *J Neuroimmunol* 101, 201-4.

Moghaddam, B., 2003. Bringing order to the glutamate chaos in schizophrenia. *Neuron* 40, 881-4.

Monteleone, P., Natale, M., La Rocca, A., Maj, M., 1997. Decreased nocturnal secretion of melatonin in drug-free schizophrenics: no change after subchronic treatment with antipsychotics. *Neuropsychobiology* 36, 159-63.

Mori, I., Hossain, M. J., Takeda, K., Okamura, H., Imai, Y., Kohsaka, S., Kimura, Y., 2001. Impaired microglial activation in the brain of IL-18-gene-disrupted mice after neurovirulent influenza A virus infection. *Virology* 287, 163-70.

Mortensen, P. B., 2003. Mortality and physical illness in schizophrenia. In R. M. Murray, P. B. Jones, E. S. Susser, J. van Os, M. Cannon (Eds.), *The Epidemiology of Schizophrenia* (pp. 275-287). Cambridge: Cambridge University Press.

Muller, D. J., Schulz, T. G., Macciardi, F., Ohlraun, S., Gross, M., Bauer, I., Scherk, H., Kischkel, E., Neidt, H., Syagailo, Y., Grassle, M., Nothen, M., Maier, W., Lesch, K., Rietschel, M., 2000. Moclobemide response in depressed patients: association study with a functional polymorphism in the monoamine oxidase-A promoter. *A J Med Genet* 96, 537 - 537.

Muller, N., Riedel, M., Ackenheil, M., Schwarz, M. J., 1999. The role of immune function in schizophrenia: an overview. *Eur Arch Psychiatry Clin Neurosci* 4, 62-8.

Munafo, M. R., Flint, J., 2004. Meta-analysis of genetic association studies. *Trends Genet* 20, 439-44.

Murray, R. M., Jones, P. B., Susser, E., Van Os, J., Cannon, M., 2003. The Epidemiology of Schizophrenia (pp. 454). Cambridge, UK: Cambridge University Press.

Murray, R. M., Reveley, A. M., McGuffin, P., 1986. Genetic vulnerability to schizophrenia. *Psychiatr Clin North Am* 9, 3-16.

Nakanishi, K., Yoshimoto, T., Tsutsui, H., Okamura, H., 2001. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 19, 423-74.

National_Center_for_Biotechnology_Information, 2005. dbSNP. Bethesda, MD: National Center for Biotechnology Information.

Naudin, J., Capo, C., Giusano, B., Mege, J. L., Azorin, J. M., 1997. A differential role for interleukin-6 and tumor necrosis factor-alpha in schizophrenia? *Schizophr Res* 26, 227-33.

Nickerson, D., Rieder, M. J., 2005. Seattle SNPs Variation Discovery Resource (pp. Seattle SNPs Program for Genetics Applications). Seattle: Supported by National Heart, Lung, and Blood Institute.

Nimgaonkar, V., 1997. In defense of genetic association studies. *Molecular Psychiatry* 2, 275-277.

Norose, K., Yano, A., Zhang, X. M., Blankenhorn, E., Heber-Katz, E., 2002. Mapping of genes involved in murine herpes simplex virus keratitis: identification of genes and their modifiers. *J Virol* 76, 3502-10.

Norton, N., Williams, H. J., Owen, M. J., 2006. An update on the genetics of schizophrenia. *Curr Opin Psychiatry* 19, 158-64.

Novota, P., Kolostova, K., Pinterova, D., Novak, J., Treslova, L., Andel, M., Cerna, M., 2005. Interleukin IL-18 gene promoter polymorphisms in adult patients with type 1 diabetes mellitus and latent autoimmune diabetes in adults. *Immunol Lett* 96, 247-51.

Obermayer-Straub, P., Manns, M. P., 2001. Hepatitis C and D, retroviruses and autoimmune manifestations. *J Autoimmun* 16, 275-85.

O'Connell, J. R., Weeks, D. E., 1998. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63, 259-66.

O'Donnell, M. C., Catts, S. V., Ward, P. B., Liebert, B., Lloyd, A., Wakefield, D., McConaghy, N., 1996. Increased production of interleukin-2 (IL-2) but not soluble interleukin-2 receptors (sIL-2R) in unmedicated patients with schizophrenia and schizophreniform disorder. *Psychiatry Res* 65, 171-8.

O'Donovan, M. C., Owen, M. J., 1999. Candidate-gene association studies of schizophrenia. *Am J Hum Genet* 65, 587-92.

Ohashi, J., Clark, A. G., 2005. Application of the stepwise focusing method to optimize the cost-effectiveness of genome-wide association studies with limited research budgets for genotyping and phenotyping. *Ann Hum Genet* 69, 323-8.

Ortaldo, J. R., Winkler-Pickett, R., Wigginton, J., Horner, M., Bere, E. W., Mason, A. T., Bhat, N., Cherry, J., Sanford, M., Hodge, D. L., Young, H. A., 2006. Regulation of ITAM-positive receptors: role of IL-12 and IL-18. *Blood* 107, 1468-75.

Ott, J., Bhat, A., 1999. Linkage analysis in heterogeneous and complex traits. *Eur Child Adolesc Psychiatry* 8, 43-6.

Ottman, R., 1996. Gene-environment interaction: definitions and study designs. *Prev Med* 25, 764-70.

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

Pae, C. U., Chae, J. H., Bahk, W. M., Han, H., Jun, T. Y., Kim, K. S., Kwon, Y. S., Serretti, A., 2003. Tumor necrosis factor-alpha gene polymorphism at position -308 and schizophrenia in the Korean population. *Psychiatry Clin Neurosci* 57, 399-403.

Papiol, S., Molina, V., Desco, M., Rosa, A., Reig, S., Gispert, J. D., Sanz, J., Palomo, T., Fananas, L., 2005. Ventricular enlargement in schizophrenia is associated with a genetic polymorphism at the interleukin-1 receptor antagonist gene. *Neuroimage* 27, 1002-6.

Papiol, S., Rosa, A., Gutierrez, B., Martin, B., Salgado, P., Catalan, R., Arias, B., Fananas, L., 2004. Interleukin-1 cluster is associated with genetic risk for schizophrenia and bipolar disorder. *J Med Genet* 41, 219-23.

Pearce, B. D., 2003. *Can a virus cause schizophrenia? : facts and hypotheses*. Boston: Kluwer Academic.

Peritt, D., Robertson, S., Gri, G., Showe, L., Aste-Amezaga, M., Trinchieri, G., 1998. Differentiation of human NK cells into NK1 and NK2 subsets. *J Immunol* 161, 5821-4.

Peuskens, J., Demily, C., Thibaut, F., 2005. Treatment of cognitive dysfunction in schizophrenia. *Clin Ther* 27 Suppl A, S25-37.

Pien, G. C., Satoskar, A. R., Takeda, K., Akira, S., Biron, C. A., 2000. Cutting edge: selective IL-18 requirements for induction of compartmental IFN-gamma responses during viral infection. *J Immunol* 165, 4787-91.

Prasad, K. M., Shirts, B. H., Yolken, R., Keshavan, M. S., Nimagaonkar, V. L., in press. Brain Morphological Changes associated with exposure to HSV1 in First Episode Schizophrenia. *Molecular Psychiatry*.

Prinz, M., Hanisch, U. K., 1999. Murine microglial cells produce and respond to interleukin-18. *J Neurochem* 72, 2215-8.

Pritchard, J. K., Rosenberg, N. A., 1999. Use of unlinked genetic markers to detect population stratification in association studies. *American Journal of Human Genetics* 65, 220-8.

Raftery, M. J., Wieland, D., Gronewald, S., Kraus, A. A., Giese, T., Schonrich, G., 2004. Shaping phenotype, function, and survival of dendritic cells by cytomegalovirus-encoded IL-10. *J Immunol* 173, 3383-91.

Randolph-Habecker, J., Iwata, M., Geballe, A. P., Jarrahian, S., Torok-Storb, B., 2002. Interleukin-1-mediated inhibition of cytomegalovirus replication is due to increased IFN-beta production. *J Interferon Cytokine Res* 22, 765-72.

Rantakallio, P., Jones, P., Moring, J., Von Wendt, L., 1997. Association between central nervous system infections during childhood and adult onset schizophrenia and other psychoses: a 28-year follow-up. *Int J Epidemiol* 26, 837-43.

Rao, D. C., Morton, N. E., Gottesman, I. I., Lew, R., 1981. Path analysis of qualitative data on pairs of relatives: application to schizophrenia. *Human Heredity* 31, 325-33.

Rapaport, M. H., Delrahim, K. K., 2001. An abbreviated review of immune abnormalities in schizophrenia. *CNS Spectr* 6, 392-7.

Rapoport, J. L., Addington, A. M., Frangou, S., Psych, M. R., 2005. The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry* 10, 434-49.

Ray, N., Enquist, L. W., 2004. Transcriptional response of a common permissive cell type to infection by two diverse alphaherpesviruses. *J Virol* 78, 3489-501.

Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Heredity* 86, 248-249.

Reddy, P., 2004. Interleukin-18: recent advances. *Curr Opin Hematol* 11, 405-10.

Riedel, M., Kronig, H., Schwarz, M. J., Engel, R. R., Kuhn, K. U., Sikorski, C., Sokullu, S., Ackenheil, M., Moller, H. J., Muller, N., 2002. No association between the G308A polymorphism of the tumor necrosis factor-alpha gene and schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 252, 232-4.

Riedel, M., Spellmann, I., Schwarz, M. J., Strassnig, M., Sikorski, C., Moller, H. J., Muller, N., 2006. Decreased T cellular immune response in schizophrenic patients. *J Psychiatr Res* 21, 21.

Rimon, R., Ahokas, A., Palo, J., 1986. Serum and cerebrospinal fluid antibodies to cytomegalovirus in schizophrenia. *Acta Psychiatr Scand* 73, 642-4.

Rinaldo, A., Bacanu, S. A., Devlin, B., Sonpar, V., Wasserman, L., Roeder, K., 2005. Characterization of multilocus linkage disequilibrium. *Genet Epidemiol* 28, 193-206.

Risch, N., 1990. Linkage strategies for genetically complex traits. I. Multilocus models. *American Journal of Human Genetics* 46, 222-8.

Robinson, D. S., O'Garra, A., 2002. Further checkpoints in Th1 development. *Immunity* 16, 755-8.

Rosa, A., Peralta, V., Papiol, S., Cuesta, M. J., Serrano, F., Martinez-Larrea, A., Fananas, L., 2004. Interleukin-1beta (IL-1beta) gene and increased risk for the depressive symptom-dimension in schizophrenia spectrum disorders. *Am J Med Genet B Neuropsychiatr Genet* 124, 10-4.

Rosenthal, R., DiMatteo, M. R., 2001. Meta-analysis: recent developments in quantitative methods for literature reviews. *Annu Rev Psychol* 52, 59-82.

Rothermundt, M., Arolt, V., Bayer, T. A., 2001. Review of immunological and immunopathological findings in schizophrenia. *Brain Behav Immun* 15, 319-39.

Rothermundt, M., Arolt, V., Weitzsch, C., Eckhoff, D., Kirchner, H., 1996. Production of cytokines in acute schizophrenic psychosis. *Biol Psychiatry* 40, 1294-7.

Saiki, O., Uda, H., Nishimoto, N., Miwa, T., Mima, T., Ogawara, T., Azuma, N., Katada, Y., Sawaki, J., Tsutsui, H., Matsui, K., Maeda, A., Nakanishi, K., 2004. Adult Still's disease reflects a Th2 rather than a Th1 cytokine profile. *Clin Immunol* 112, 120-5.

Schmidt, S., Schaid, D. J., 1999. Potential misinterpretation of the case-only study to assess gene-environment interaction. *Am J Epidemiol* 150, 878-85.

Schwab, S. G., Hallmayer, J., Albus, M., Lerer, B., Eckstein, G. N., Borrmann, M., Segman, R. H., Hanses, C., Freymann, J., Yakir, A., Trixler, M., Falkai, P., Rietschel, M., Maier, W., Wildenauer, D. B., 2000. A genome-wide autosomal screen for schizophrenia susceptibility loci in 71 families with affected siblings: support for loci on chromosome 10p and 6. *Molecular Psychiatry* 5, 638-49.

Schwab, S. G., Knapp, M., Mondabon, S., Hallmayer, J., Borrmann-Hassenbach, M., Albus, M., Lerer, B., Rietschel, M., Trixler, M., Maier, W., Wildenauer, D. B., 2003a. Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. *Am J Hum Genet* 72, 185-90.

Schwab, S. G., Mondabon, S., Knapp, M., Albus, M., Hallmayer, J., Borrmann-Hassenbach, M., Trixler, M., Gross, M., Schulze, T. G., Rietschel, M., Lerer, B., Maier, W., Wildenauer, D. B., 2003b. Association of tumor necrosis factor alpha gene -G308A polymorphism with schizophrenia. *Schizophr Res* 65, 19-25.

Schwarz, M. J., Chiang, S., Muller, N., Ackenheil, M., 2001. T-helper-1 and T-helper-2 responses in psychiatric disorders. *Brain Behav Immun* 15, 340-70.

Schwarz, M. J., Kronig, H., Riedel, M., Dehning, S., Douhet, A., Spellmann, I., Ackenheil, M., Moller, H. J., Muller, N., 2006. IL-2 and IL-4 polymorphisms as candidate genes in schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 256, 72-6.

Segal, S., Hill, A. V., 2003. Genetic susceptibility to infectious disease. *Trends Microbiol* 11, 445-8.

Sham, P., Bader, J. S., Craig, I., O'Donovan, M., Owen, M., 2002. DNA Pooling: a tool for large-scale association studies. *Nat Rev Genet* 3, 862-71.

Sharma, T., Antonova, L., 2003. Cognitive function in schizophrenia. Deficits, functional consequences, and future treatment. *Psychiatr Clin North Am* 26, 25-40.

Shen, R., Fan, J. B., Campbell, D., Chang, W., Chen, J., Doucet, D., Yeakley, J., Bibikova, M., Wickham Garcia, E., McBride, C., Steemers, F., Garcia, F., Kermani, B. G., Gunderson, K., Oliphant, A., 2005. High-throughput SNP genotyping on universal bead arrays. *Mutat Res* 573, 70-82.

Shi, F. D., Takeda, K., Akira, S., Sarvetnick, N., Ljunggren, H. G., 2000. IL-18 directs autoreactive T cells and promotes autodestruction in the central nervous system via induction of IFN-gamma by NK cells. *J Immunol* 165, 3099-104.

Shirts, B. H., Nimgaonkar, V., 2004. The genes for schizophrenia: finally a breakthrough? *Curr Psychiatry Rep* 6, 303-12.

Sierra-Honigmann, A. M., Carbone, K. M., Yolken, R. H., 1995. Polymerase chain reaction (PCR) search for viral nucleic acid sequences in schizophrenia. *Br J Psychiatry* 166, 55-60.

Sinsheimer, J. S., McKenzie, C. A., Keavney, B., Lange, K., 2001. SNPs and snails and puppy dogs' tails: analysis of SNP haplotype data using the gamete competition model. *Ann Hum Genet* 65, 483-90.

Sitaraman, S., Metzger, D. W., Belloto, R. J., Infante, A. J., Wall, K. A., 2000. Interleukin-12 enhances clinical experimental autoimmune myasthenia gravis in susceptible but not resistant mice. *J Neuroimmunol* 107, 73-82.

Slater, E., 1958. The Monogenic Theory of Schizophrenia. *Acta Genetics and Statistics in Medicine* 8, 50-60.

Smyth, M. J., Swann, J., Kelly, J. M., Cretney, E., Yokoyama, W. M., Diefenbach, A., Sayers, T. J., Hayakawa, Y., 2004. NKG2D recognition and perforin effector function mediate effective cytokine immunotherapy of cancer. *J Exp Med* 200, 1325-35.

Somech, R., Amariglio, N., Spirer, Z., Rechavi, G., 2003. Genetic predisposition to infectious pathogens: a review of less familiar variants. *Pediatr Infect Dis J* 22, 457-61.

Song, Y. J., Stinski, M. F., 2002. Effect of the human cytomegalovirus IE86 protein on expression of E2F-responsive genes: a DNA microarray analysis. *Proc Natl Acad Sci U S A* 99, 2836-41.

Sperner-Unterweger, B., Whitworth, A., Kemmler, G., Hilbe, W., Thaler, J., Weiss, G., Fleischhacker, W. W., 1999. T-cell subsets in schizophrenia: a comparison between drug-naive first episode patients and chronic schizophrenic patients. *Schizophr Res* 38, 61-70.

Spielman, R. S., Ewens, W. J., 1993a. Transmission/disequilibrium test (TDT) for linkage and linkage disequilibrium between disease and marker. *American Journal of Human Genetics* 53a, 863.

Spielman, R. S., Ewens, W. J., 1996. The TDT and other family-based tests for linkage disequilibrium and association [editorial]. *American Journal of Human Genetics* 59, 983-9.

Spielman, R. S., McGinnis, R. E., Ewens, W. J., 1993b. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *American Journal of Human Genetics* 52, 506-16.
Starzynska, T., Ferenc, K., Wex, T., Kahne, T., Lubinski, J., Lawniczak, M., Marlicz, K., Malfertheiner, P., 2006. The association between the interleukin-1 polymorphisms and gastric cancer risk depends on the family history of gastric carcinoma in the study population. *Am J Gastroenterol* 101, 248-54.
Steen, R. G., Mull, C., McClure, R., Hamer, R. M., Lieberman, J. A., 2006. Brain volume in first-episode schizophrenia: systematic review and meta-analysis of magnetic resonance imaging studies. *Br J Psychiatry* 188, 510-8.
Steinle, A., Li, P., Morris, D. L., Groh, V., Lanier, L. L., Strong, R. K., Spies, T., 2001. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. *Immunogenetics* 53, 279-87.
Stellwagen, D., Malenka, R. C., 2006. Synaptic scaling mediated by glial TNF-alpha. *Nature* 440, 1054-9.
Stevens, J. R., Langloss, J. M., Albrecht, P., Yolken, R., Wang, Y. N., 1984. A search for cytomegalovirus and herpes viral antigen in brains of schizophrenic patients. *Arch Gen Psychiatry* 41, 795-801.
Stoll, G., Jander, S., Schroeter, M., 2002. Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. *Adv Exp Med Biol* 513, 87-113.
Stram, D. O., 2004. Tag SNP selection for association studies. *Genet Epidemiol* 27, 365-74.
Straub, R. E., MacLean, C. J., Ma, Y., Webb, B. T., Myakishev, M. V., Harris-Kerr, C., Wormley, B., Sadek, H., Kadambi, B., O'Neill, F. A., Walsh, D., Kendler, K. S., 2002. Genome-wide scans of three independent sets of 90 Irish multiplex schizophrenia families and follow-up of selected regions in all families provides evidence for multiple susceptibility genes. *Mol Psychiatry* 7, 542-59.
Taller, A. M., Asher, D. M., Pomeroy, K. L., Eldadah, B. A., Godec, M. S., Falkai, P. G., Bogert, B., Kleinman, J. E., Stevens, J. R., Torrey, E. F., 1996. Search for viral nucleic acid sequences in brain tissues of patients with schizophrenia using nested polymerase chain reaction. *Arch Gen Psychiatry* 53, 32-40.
Tan, E. C., Chong, S. A., Tan, C. H., Teo, Y. Y., Peng, K., Mahendran, R., 2003. Tumor necrosis factor-alpha gene promoter polymorphisms in chronic schizophrenia. *Biol Psychiatry* 54, 1205-11.
Tanaka, K. F., Shintani, F., Fujii, Y., Yagi, G., Asai, M., 2000. Serum interleukin-18 levels are elevated in schizophrenia. *Psychiatry Res* 96, 75-80.
Tarlow, J. K., Blakemore, A. I., Lennard, A., Solari, R., Hughes, H. N., Steinkasserer, A., Duff, G. W., 1993. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Human Genetics* 91, 403-4.
Tatsumi, M., Sasaki, T., Sakai, T., Kamijima, K., Fukuda, R., Kunugi, H., Hattori, M., Nanko, S., 1997. Genes for interleukin-2 receptor beta chain, interleukin-1 beta, and schizophrenia: no evidence for the association or linkage. *Am J Med Genet* 74, 338-41.
Terwilliger, J. D., Goring, H. H., 2000. Gene mapping in the 20th and 21st centuries: statistical methods, data analysis, and experimental design. *Hum Biol* 72, 63-132.
Thacker, E. L., Mirzaei, F., Ascherio, A., 2006. Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. *Ann Neurol* 59, 499-503.

The Chinese National Human Genome Center, B. C., 2003. Database for Schizophrenia candidate genes focusing on Variations(VSD): Tsinghua Bioinfomatics Group.

Theodoropoulou, S., Spanakos, G., Baxevanis, C. N., Economou, M., Gritzapis, A. D., Papamichail, M. P., Stefanis, C. N., 2001. Cytokine serum levels, autologous mixed lymphocyte reaction and surface marker analysis in never medicated and chronically medicated schizophrenic patients. *Schizophr Res* 47, 13-25.

Torrey, E. F., Yolken, R. H., 2000. Familial and genetic mechanisms in schizophrenia. *Brain Res Brain Res Rev* 31, 113-7.

Torrey, E. F., Yolken, R. H., 2003. Toxoplasma gondii and schizophrenia. *Emerg Infect Dis* 9, 1375-80.

Torrey, E. F., Yolken, R. H., Winfrey, C. J., 1982. Cytomegalovirus antibody in cerebrospinal fluid of schizophrenic patients detected by enzyme immunoassay. *Science* 216, 892-4.

Tsai, S. J., Hong, C. J., Yu, Y. W., Lin, C. H., Liu, L. L., 2003. No association of tumor necrosis factor alpha gene polymorphisms with schizophrenia or response to clozapine. *Schizophr Res* 65, 27-32.

Ueda, T., Takeyama, Y., Yasuda, T., Matsumura, N., Sawa, H., Nakajima, T., Ajiki, T., Fujino, Y., Suzuki, Y., Kuroda, Y., 2006. Significant elevation of serum interleukin-18 levels in patients with acute pancreatitis. *J Gastroenterol* 41, 158-65.

Umbach, D. M., Weinberg, C. R., 2000. The use of case-parent triads to study joint effects of genotype and exposure. *Am J Hum Genet* 66, 251-61.

Urakubo, A., Jarskog, L. F., Lieberman, J. A., Gilmore, J. H., 2001. Prenatal exposure to maternal infection alters cytokine expression in the placenta, amniotic fluid, and fetal brain. *Schizophr Res* 47, 27-36.

Vasil'eva, E. F., Kushner, S. G., Abramova, L. I., Kaleda, V. G., Tsutsul'kovskaia, M., 2002. [The changes of the function of lymphocytes natural killers in schizophrenics]. *Zh Nevrol Psikhiatr Im S S Korsakova* 102, 30-6.

Vawter, M. P., 2000. Dysregulation of the neural cell adhesion molecule and neuropsychiatric disorders. *Eur J Pharmacol* 405, 385-95.

Verdoux, H., 2004. Perinatal risk factors for schizophrenia: how specific are they? *Curr Psychiatry Rep* 6, 162-7.

Viviani, B., Bartesaghi, S., Corsini, E., Galli, C. L., Marinovich, M., 2004. Cytokines role in neurodegenerative events. *Toxicol Lett* 149, 85-9.

Vliegen, I., Duijvestijn, A., Stassen, F., Bruggeman, C., 2004. Murine cytomegalovirus infection directs macrophage differentiation into a pro-inflammatory immune phenotype: implications for atherogenesis. *Microbes Infect* 6, 1056-62.

Vossenkamper, A., Struck, D., Alvarado-Esquivel, C., Went, T., Takeda, K., Akira, S., Pfeffer, K., Alber, G., Lochner, M., Forster, I., Liesenfeld, O., 2004. Both IL-12 and IL-18 contribute to small intestinal Th1-type immunopathology following oral infection with Toxoplasma gondii, but IL-12 is dominant over IL-18 in parasite control. *Eur J Immunol* 34, 3197-207.

Wang, H. L., Wang, G. H., Li, Q. Y., Shu, C., Jiang, M. S., Guo, Y., 2006. Prevalence of Toxoplasma infection in first-episode schizophrenia and comparison between Toxoplasma-seropositive and Toxoplasma-seronegative schizophrenia. *Acta Psychiatr Scand* 114, 40-8.

Wang, W. Y., Barratt, B. J., Clayton, D. G., Todd, J. A., 2005. Genome-wide association studies: theoretical and practical concerns. *Nat Rev Genet* 6, 109-18.

Wara-aswapati, N., Yang, Z., Waterman, W. R., Koyama, Y., Tetradis, S., Choy, B. K., Webb, A. C., Auron, P. E., 1999. Cytomegalovirus IE2 protein stimulates interleukin 1beta gene transcription via tethering to Spi-1/PU.1. *Mol Cell Biol* 19, 6803-14.
Weiss, K. M., Terwilliger, J. D., 2000. How many diseases does it take to map a gene with SNPs? *Nature Genetics* 26, 151-7.
Whitley, R. J., 2004. Viral Infections of the Central Nervous System. In J. Cohen, W. G. Powderly (Eds.), *Infectious Diseases* (pp. 267-278). Edinburgh; New York: Mosby.
Williams, N. M., Norton, N., Williams, H., Ekholm, B., Hamshere, M. L., Lindblom, Y., Chowdari, K. V., Cardno, A. G., Zammit, S., Jones, L. A., Murphy, K. C., Sanders, R. D., McCarthy, G., Gray, M. Y., Jones, G., Holmans, P., Nimgaonkar, V., Adolfson, R., Osby, U., Terenius, L., Sedvall, G., O'Donovan, M. C., Owen, M. J., 2003. A systematic genomewide linkage study in 353 sib pairs with schizophrenia. *Am J Hum Genet* 73, 1355-67. Epub 2003 Nov 18.
Williams, N. M., O'Donovan, M. C., Owen, M. J., 2002. Genome scans and microarrays: converging on genes for schizophrenia? *Genome Biol* 3, REVIEWS1011.
Wilson, A. G., Symons, J. A., McDowell, T. L., McDevitt, H. O., Duff, G. W., 1997. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci U S A* 94, 3195-9.
Wilson, L. G., 1976. Viral encephalopathy mimicking functional psychosis. *Am J Psychiatry* 133, 165-70.
Witkin, S. S., Gerber, S., Ledger, W. J., 2002. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. *Clin Infect Dis* 34, 204-9.
Wong, M. Y., Day, N. E., Luan, J. A., Chan, K. P., Wareham, N. J., 2003. The detection of gene-environment interaction for continuous traits: should we deal with measurement error by bigger studies or better measurement? *Int J Epidemiol* 32, 51-7.
WorldHealthOrganization, 1989. International Classification of Diseases (ICD) 10 (pp. Online ICD-10). Geneva: World Health Organization.
Wright, P., Nimgaonkar, V. L., Donaldson, P. T., Murray, R. M., 2001. Schizophrenia and HLA: a review. *Schizophrenia Research* 47, 1-12.
Wu, J., Chalupny, N. J., Manley, T. J., Riddell, S. R., Cosman, D., Spies, T., 2003. Intracellular retention of the MHC class I-related chain B ligand of NKG2D by the human cytomegalovirus UL16 glycoprotein. *J Immunol* 170, 4196-200.
Yamada, K., Iwayama-Shigeno, Y., Yoshida, Y., Toyota, T., Itokawa, M., Hattori, E., Shimizu, H., Yoshikawa, T., 2004. Family-based association study of schizophrenia with 444 markers and analysis of a new susceptibility locus mapped to 11q13.3. *Am J Med Genet B Neuropsychiatr Genet* 127, 11-9.
Yang, J., Si, T., Ling, Y., Ruan, Y., Han, Y., Wang, X., Zhou, M., Zhang, D., Zhang, H., Kong, Q., Liu, C., Li, X., Yu, Y., Liu, S., Shu, L., Ma, D., Wei, J., 2003. Association study between interleukin-1beta gene (IL-1beta) and schizophrenia. *Life Sci* 72, 3017-21.
Yolken, R., 2004. Viruses and schizophrenia: a focus on herpes simplex virus. *Herpes* 11, 83A-88A.
Yolken, R. H., Torrey, E. F., 1995. Viruses, schizophrenia, and bipolar disorder. *Clinical Microbiology Reviews* 8, 131-45.
Yovel, G., Sirota, P., Mazeh, D., Shakhar, G., Rosenne, E., Ben-Eliyahu, S., 2000. Higher natural killer cell activity in schizophrenic patients: the impact of serum factors, medication, and smoking. *Brain Behav Immun* 14, 153-69.

Yu, L., Yang, M. S., Zhao, J., Shi, Y. Y., Zhao, X. Z., Yang, J. D., Liu, Z. J., Gu, N. F., Feng, G. Y., He, L., 2004. An association between polymorphisms of the interleukin-10 gene promoter and schizophrenia in the Chinese population. *Schizophr Res* 71, 179-83.

Zanardini, R., Bocchio-Chiavetto, L., Scassellati, C., Bonvicini, C., Tura, G. B., Rossi, G., Perez, J., Gennarelli, M., 2003. Association between IL-1beta -511C/T and IL-1RA (86bp)n repeats polymorphisms and schizophrenia. *J Psychiatr Res* 37, 457-62.

Zeidel, A., Beilin, B., Yardeni, I., Mayburd, E., Smirnov, G., Bessler, H., 2002. Immune response in asymptomatic smokers. *Acta Anaesthesiol Scand* 46, 959-64.

Zhang, X., Wei, J., Yu, Y. Q., Liu, S. Z., Shi, J. P., Liu, L. L., Ju, G. Z., Yang, J. Z., Zhang, D., Xu, Q., Shen, Y., Hemmings, G. P., 2004a. Is NOTCH4 associated with schizophrenia? *Psychiatr Genet* 14, 43-6.

Zhang, X. Y., Zhou, D. F., Cao, L. Y., Zhang, P. Y., Wu, G. Y., Shen, Y. C., 2004b. Changes in serum interleukin-2, -6, and -8 levels before and during treatment with risperidone and haloperidol: relationship to outcome in schizophrenia. *J Clin Psychiatry* 65, 940-7.

Zhu, M., Xu, X., Liu, H., Liu, X., Wang, S., Dong, F., Yang, B., Song, G., 2003. Enhancement of DNA vaccine potency against herpes simplex virus 1 by co-administration of an interleukin-18 expression plasmid as a genetic adjuvant. *J Med Microbiol* 52, 223-8.

Zintzaras, E., Ioannidis, J. P., 2005. Heterogeneity testing in meta-analysis of genome searches. *Genet Epidemiol* 28, 123-37.

Zou, Y., Bresnahan, W., Taylor, R. T., Stastny, P., 2005. Effect of human cytomegalovirus on expression of MHC class I-related chains A. *J Immunol* 174, 3098-104.