Meta-Analysis of Cytokine and Chemokine Genes in Schizophrenia

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Abstract

Introduction: Immune system genes, including cytokines, are associated with schizophrenia risk. Polymorphisms in cytokine genes may also impact on blood levels of cytokines, which are altered in patients with schizophrenia. We performed a meta-analysis of case-control studies of cytokine and chemokine genes in schizophrenia that have not been considered in previous quantitative reviews. **Methods:** We identified articles by systematic searches of PubMed, PsycInfo, and ISI, and the reference lists of identified studies. For each cytokine or chemokine polymorphism, we performed an allele- and genotype-wise meta-analysis, using a random effects model. **Results:** Twenty-one independent studies met the inclusion criteria, comprising polymorphisms for the *IL1B, IL2, IL4, IL6, sIL6R, MCP1,* and *TGFB1* genes. For *IL6*, the A allele (OR=0.95, 95% CI 0.91–0.99) and AA genotype (OR=0.65, 95% CI 0.50–0.85) for the *rs1800795 polymorphism*, and for *sIL6R*, the A allele (OR=0.96 95%, CI 0.92–1.00) and AA genotype (OR=0.72, 95% CI 0.55–0.94) the rs8192284 polymorphism were associated with significantly decreased schizophrenia risk. In the genotype-wise analysis for *IL1B*, homozygosity for either allele (AA: OR=1.91, 95% CI 1.60–2.27; and GG: OR=0.40, 95% CI 0.33–0.49) of the rs1143627 polymorphism was also significantly associated with schizophrenia risk. **Conclusions:** Associations between polymorphisms for the *IL1B, IL6*, and *sIL6R* genes and schizophrenia risk complement and extend previous findings regarding immune dysfunction in this disorder, including genome-wide association studies. Future studies of cytokine expression in schizophrenia should consider the effect of these polymorphisms. The finding of potential "protective" alleles may also be relevant for at-risk populations.

Key Words: Schizophrenia, Genetics, Single Nucleotide Polymorphisms, Cytokines, Meta-Analysis

Introduction

Immunological abnormalities in schizophrenia have been one of the more enduring findings in the field, and a popular area of research over the last decade. This interest has been at least partially stimulated by our increased understanding of the complex interactions that occur between the immune system and the brain in other chronic diseases. Polymorphisms in major histocompatibility complex genes, which are critical to immune function (1), as well as other immune system genes (2), are associated with increased risk of schizophrenia. There is evidence for abnormalities in immune cell numbers (3) and cytokine levels (4-6) in first-episode psychosis, suggesting a role for immune dysfunction that may be independent of antipsychotic medications. Treatment with non-steroidal anti-inflammatory drugs (NSAIDs), in adjunct to antipsychotics, has been associated with significant improvement in psychopathology in schizophrenia (7). These findings provide important empirical support for a pathophysiological role for inflammation in some patients with schizophrenia.

Aberrant blood levels of components of the cytokine network—including blood IL-1β, sIL-2R, IL-6, IL-12, interferon-gamma (IFN-γ), TNF-α, and transforming growth factor-beta (TGF-β) protein levels—have been reported in schizophrenia (4-6). Cytokines are key signaling molecules of the immune system that exert effects in the periphery and the brain. They are produced by both immune and non-

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Clinical Implications

We found that polymorphisms in genes for *IL1B, IL6*, and *sIL6R*—but not *IL2, IL4, MCP1*, or *TGFB1*—were associated with schizophrenia risk. There was significant heterogeneity in these findings; however, the association remained significant in sensitivity analyses.

An important strength of our study is that we considered polymorphisms for a number of different cytokine or chemokine genes that have not been investigated in previous quantitative reviews. For the allele-wise analysis, we were able to include data from the large Psychiatric Genomics Consortium study of schizophrenia (30). Of note, our findings for the *IL6* and *sIL6R* genes remained significant in sensitivity analyses, which excluded this study, supporting the robustness of the association in smaller samples. Blood protein levels IL-1β, IL-6 are also abnormal in schizophrenia, and the sIL-6R enhances the biological activity of IL-6 (4). Thus, our work complements and extends previous findings regarding immune dysfunction in this disorder, including genome-wide association studies. Our findings also directly inform on future studies of blood cytokine expression in schizophrenia, which should consider the effect of these polymorphisms.

Taken together, our results complement and extend previous findings regarding immune dysfunction in schizophrenia, and inform on future studies of cytokine expression in schizophrenia. Future studies of inflammation should consider the effects of cytokine gene polymorphisms on schizophrenia risk, cytokine levels, and other clinical features of the disorder. Replicated findings may contribute to more personalized medicine for patients with schizophrenia.

immune cells, and exert their effects by binding specific cytokine receptors on a variety of target cells. Cytokine receptors also exist in soluble forms, which can inhibit (e.g., soluble interleukin-2 receptor [sIL-2R]) or enhance (e.g., sIL-6R) the biological activity of cytokines. There are also endogenous cytokine receptor antagonists (e.g., IL-1 receptor antagonist [IL-1RA]), which compete with cytokines for membrane receptors. Cytokines are key regulators of acute and chronic inflammation, a complex but vital biological response that impacts all organ systems. Cytokines help coordinate the function of both the innate and adaptive components of the immune system as well as a host of other physiological processes throughout the body.

Several recently published meta-analyses investigated polymorphisms in the genes for IL-1beta *(IL1B)* (9, 10), *IL10* (11), and tumor necrosis factor-alpha *(TNFα)* (12) and schizophrenia risk. Across four studies, Xu et al. (10) found a significant genotype-wise association between the rs1143634 polymorphism of the *IL1B* gene and schizophrenia risk in population-based studies and Caucasian subjects. By contrast, they did not find an association between either the rs1800587 polymorphism of the *IL1A* gene or the rs1794068 polymorphism of the *IL1RA* gene and schizophrenia susceptibility. Shibuya et al. (9) did not find an association between the rs16944 polymorphism of the *IL1B* gene and schizophrenia risk across 16 studies. In a meta-analysis of 11 studies, Gao et al. (11) found a significant allele-wise and genotype-wise association between the rs1800872 polymorphism of the *IL10* gene and schizophrenia risk, but no associations with either the rs1800096 or rs1800871 polymorphisms. Across 21 studies, Qin et al. (12) did not find an association between the rs1800629 polymorphism of the *TNFα* gene and schizophrenia risk.

Polymorphisms in cytokine genes may impact not only on schizophrenia risk, but also blood levels of cytokines. For example, Zakharyan et al. (8) found that the rs1800795*C allele of the interleukin-6 *(IL6)* gene was significantly more common in patients with schizophrenia than controls, but that both patients and controls homozygous for the G allele had significantly higher blood IL-6 protein levels. Despite the evidence for cytokine network alterations in schizophrenia, there is tremendous between-study heterogeneity in this research area. Genetic effects on cytokine network alterations in schizophrenia represent an important understudied area.

We performed a meta-analysis of case-control studies of cytokine and chemokine genes in schizophrenia that have not been considered in previous quantitative reviews. The primary aim of the meta-analysis was to identify other cytokine and/or chemokine gene polymorphisms associated with schizophrenia risk that may also contribute to alterations in blood levels of these proteins, and in doing so, inform future studies of cytokines and chemokines in schizophrenia.

Methods

Study Selection

Studies of cytokine (or chemokine) genes and schizophrenia were systematically searched using MEDLINE (National Center for Biotechnology Information, US National Library of Medicine, Bethesda, Maryland), PsycInfo (via Ovid, American Psychological Association, Washington, DC), and Thomson Reuters (formerly Institute for Scientific Information) Web of Knowledge (Science Citation Index and Social Sciences Citation Index, Thomson Reuters, Charlottesville, Virginia) in July 2014. The primary search strategy was "(schizophrenia or psychosis) and (inflammation or cytokine or chemokine or interleukin or interferon or tumor

Table 1 Studies of Cytokine and Chemokine Genes in Schizophrenia

Study	Location	Cytokine/ Chemokine gene(s) studied	Schizophrenia			Control			Included	Comment
			N	Male (%)	Age (years)	N	Male (%)	Age (years)		
Borkowska et al., 2012	Poland	*IL1B*	143	53	42±12	206	64	42±9	Yes	
Debnath et al., 2012	India	*IL6, TNFα*	100	63	N/A	100	N/A	N/A	Yes	
Fila et al., 2012	Poland	*IL4*	182	55	43±12	215	60	44±7	No	HWE deviation for IL-4
Frydecka et al., 2013	Poland	*IL2, IL6, IFNγ, TGFB1*	151	46	38±12	279	70	39±9	Yes	
Jun et al., 2003	Korea	*IL4*	222	60	33±9	165	56	34±9	Yes	
Kalmady et al., 2014	India	*IL6*	28	50	30±6	37	54	27±6	Yes	
Kim et al., 2012	Korea	*IFNγ*	189	58	41±11	383	48	41±14	No	HWE deviation for IFN-γ
Laurent et al., 1997	France	*IL1B*	75	69	40	75	59	49	Yes	
Lee et al., 2010	Korea	*TGFB1*	99	46	35±11	130	56	33±11	Yes	
Mundo et al., 2005	Italy	*MCP1*	191	64	44±12	161	N/A	N/A	Yes	
Pae et al., 2004	Korea	*MCP1*	123	46	32±10	114	45	33±7	Yes	
Paul-Samojedny et al., 2010	Poland	*IL6, IL10*	96	62	45±12	120	67	39±10	Yes	
Paul-Samojedny et al., 2011	Poland	*IFNγ*	179	55	43±12	196	61	47±8	No	HWE deviation for IFN-γ
Paul-Samojedny et al., 2013	Poland	*IL2, IL6, TNFα*	115	58	43±13	135	68	41±9	No	HWE deviation for IL-2, IL-6
Saiz et al., 2006	Spain	*IL1α, IL1B, IL1RA*	228	61	36±12	419	50	41±11	Yes	
Sasayama et al., 2011	Japan	*IL6, sIL6R*	104	53	39±12	112	50	39±13	Yes	
Sasayama et al., 2011b	Japan	*IL1B*	533	57	43±13	1136	34	45±17	Yes	
Schizophrenia PGC, 2011	Multiple	*IL1B, IL4, IL6, sIL6R, MCP1*	17836	62	N/A	33859	49	NA	Yes	
Schwarz et al., 2006	Germany	*IL2, IL4*	230	57	34±12	251	53	40±14	Yes	HWE deviation for IL-2
Shibuya et al., 2014	Japan	*IL1B*	555	53	42±14	674	51	38±11	Yes	
Shirts et al., 2006	USA	*IL1, IL10*	478	65	38±10	501	52	*	Yes	
Sun et al., 2008	China	*IL6*	493	73	N/A	346	65	N/A	Yes	
Watanabe et al., 2008	Japan	*IL2, IL4*	536	52	40±12	510	54	37±10	Yes	
Zakharyan et al., 2012	Armenia	*IL6*	103	51	46±10	105	49	37±11	Yes	
Zakharyan et al., 2012b	Armenia	*MCP1*	103	51	46±10	105	49	37±11	Yes	

*Controls were DNA samples from neonatal cord blood. N/A=not available.

necrosis factor) and gene." Initial search results returned 320 articles from MEDLINE, 154 from PsycInfo, and 311 from Thomson Reuters. From these sources, as well as a manual review of reference lists, we identified 109 potential studies for inclusion. We did not employ formal search software and all publications were retrieved and examined in full text. Based on a preliminary review of these matches, 25 studies presented data on cytokine genes and warranted further consideration (8, 9, 13-35). These studies are summarized in Table 1. The majority of initial matches were excluded as they: 1) were review articles, or 2) did not present data on cytokine genes, or 3) did not study schizophrenia.

The inclusion criteria were: 1) case-control studies with patients with schizophrenia compared to healthy controls, 2) studies in English, 3) specific cytokine genetic polymorphisms were determined, and 4) original allelotype or genotype data available. Studies were excluded if they: 1) used a family-based design, 2) did not include controls (i.e., cases only), or 3) genotype frequencies for control subjects were not in Hardy-Weinberg Equilibrium. We did not include cytokines investigated in recently published meta-analysis, including selected polymorphisms for *IL1B* (8, 9), *IL10* (10), and *TNFα* (11) genes. After independent searches, detailed review of study methods by two authors (ZH and BJM), 21 studies met the inclusion criteria, which are summarized in Table 1. Three studies were excluded from the final analysis due to genotype frequencies for control subjects not being in Hardy-Weinberg Equilibrium (15, 19, 25, 26). Data repository access to findings from the Schizophrenia Psychiatric Genomics Consortium (30) was obtained. This study had available data for allelotypes only, and was included. There was universal agreement on the included studies. A flow

Figure 1 Flowchart of the Study Selection Process

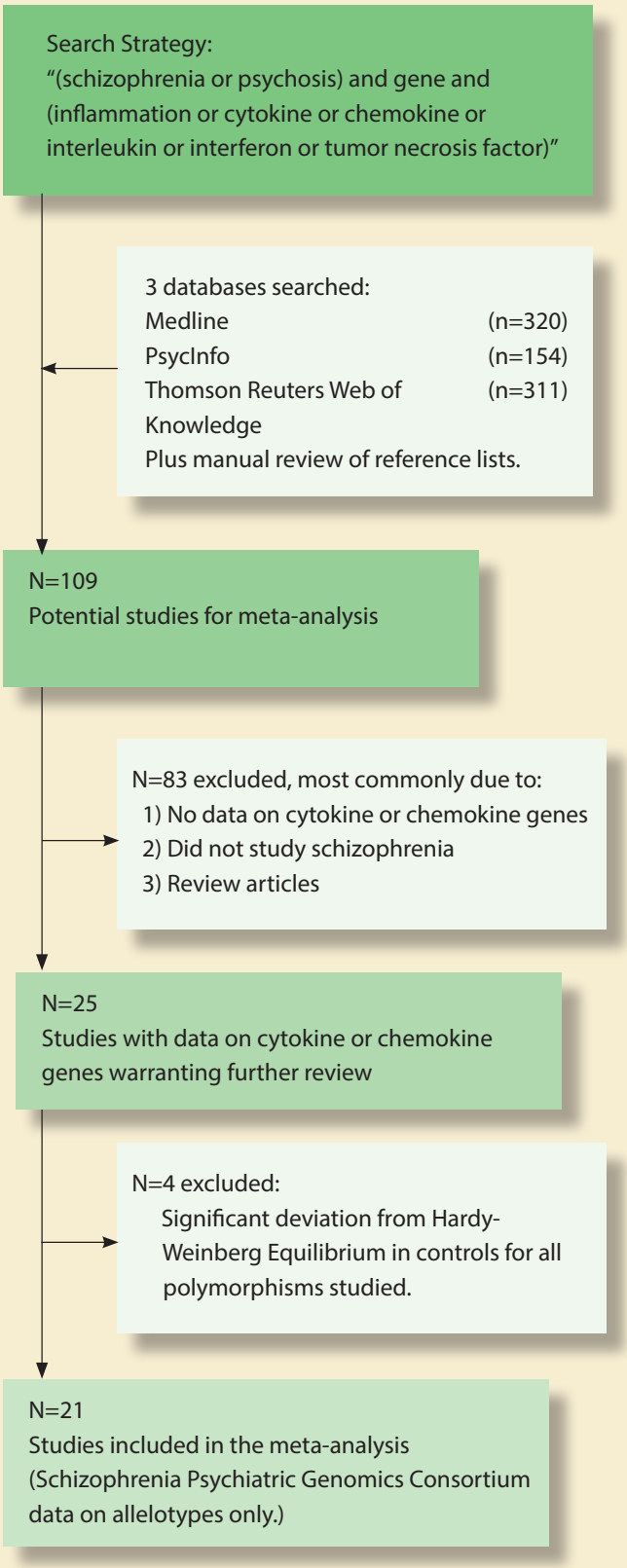


chart summarizing the study selection process is presented in Figure 1.

Data Extraction and Meta-Analysis

Data were extracted (diagnostic criteria, total number of cases and controls, region and race of participants, and allele and genotype number and frequency) for each cytokine polymorphism in each study. The Schizophrenia Psychiatric Genomics Consortium study (30) did not have data on raw numbers for alleles, but included an odds ratio and standard error for allelotype data, from which 95% confidence intervals (95% CIs) could be calculated, thus facilitating inclusion of the data. One author (ZH) extracted all data, which were independently verified by another author (BJM). Hardy-Weinberg Equilibrium (HWE) was assessed in control groups using a chi-square goodness-of-fit test (with a p-value <0.05 representing significant deviation from HWE). For each polymorphism for each cytokine or chemokine, we performed both an allele-wise and a genotype-wise analysis. Allele 1 represented the major allele, and allele 2 represented the minor allele. The allele-wise analysis compared the frequency of allele 1 versus allele 2. The two genotype-wise analyses were 1) genotype 11 versus (genotypes 12+22), and 2) genotype 22 versus (genotypes 11+12). Effect size estimates (odds ratios [ORs] and 95% confidence intervals [95% CIs]) were aggregated utilizing the random effects method of DerSimonian and Laird (36). Random effects methods are considered to be more representative of real-world data in comparison to the alternative fixed-effect approach (37) and provide a more conservative estimate of the average weighted effect size (38). Random effects models also yield their actual first-error rate while fixed-effect models tend to inflate their first-error rate. Confidence intervals obtained by fixed-effect models are also biased and their actual coverage rate is smaller than their nominal coverage rate (39). One study of the G913C polymorphism of the *TGFB1* gene that otherwise met the inclusion criteria could not be analyzed in the random effects meta-analysis because all patients and controls were homozygous for the same genotype (16), which results in an OR and 95% CI that is undefined. The main statistical hypothesis was that the ORs for the allelotypes and genotypes of patients with schizophrenia versus controls equal 1.00.

The meta-analysis procedure also calculates an χ^2 value for the heterogeneity in effect size estimates, which is based on Cochran's Q-statistic (40), and I^2, the proportion of the variation in effect size attributable to between-study heterogeneity. Between-study heterogeneity χ^2 was considered significant for $p<0.10$ (41). For any polymorphism with three or more studies, a significant odds ratio, and significant between-study heterogeneity we performed a sensitivity analysis. This was done by removing one study at a time

and repeating the meta-analysis procedure to examine its impact on the odds ratio and between-study heterogeneity (42). When possible, for a given cytokine polymorphism, the potential for publication bias was examined by means of Sterne's funnel plot analysis (43) and Egger's regression intercept (44). The statistical analyses were performed in Stata 10.0 (StataCorp LP, College Station, TX) (45). All tests were two-sided, and p-values were considered statistically significant at the α=0.05 level. Given the exploratory nature of this meta-analysis, we did not correct for multiple comparisons.

Results

As described in Table 1, search procedures yielded twenty-one independent studies. These studies included polymorphisms for *IL1B, IL2, IL4, IL6, sIL6R*, monocyte chemoattractant protein-1 *(MCP1)*, and *TGFB1* genes. We included selected polymorphisms for the *IL1B* gene due to the availability of new studies and/or polymorphisms not considered in the previous meta-analysis (10). We excluded the following studies due to significant deviations from HWE: Paul-Samojedny et al. (2013) (26) and Schwarz et al. (31) (both for the *IL2* gene); Fila-Danilow et al. (15) (for the *IL4* gene); Paul-Samojedny et al. (26) (for the *IL6* gene); and, Kim et al. (19) and Paul-Samojedny et al. (25) (for the *IFNγ* gene).

There were seven studies and four different polymorphisms (rs1143627, rs1143630, rs1143633, and rs1143634) for the *IL1B* gene, two studies of one polymorphism (rs2069762) for the *IL2* gene, four studies of one polymorphism (rs2243250) for the *IL4* gene, six studies of one polymorphism (rs1800795) for the *IL6* gene, three studies of one polymorphism (rs8192284) for the *sIL6R* gene, four studies for one polymorphism (rs1024611) for the *MCP1* gene, and two studies of two different polymorphisms (rs1800470 and rs1800471) for the *TGFB1* gene. We were not able to perform meta-analyses for the *IL12B* and *IFNγ* gene because only one study for each gene met the inclusion criteria.

Table 2 presents the results of meta-analyses. In the allele-wise analysis, the rs1800795 polymorphism for the *IL6* gene (OR=0.95, 95% CI=0.91–0.99) and the rs8192284 polymorphism for the sIL6R gene (OR=0.96, 95% CI=0.92–1.00) were associated with significantly decreased odds of schizophrenia. Between-study heterogeneity was significant for both of these polymorphisms (p≤0.01 for each). In sensitivity analyses, the odds ratio remained significant and the between-study heterogeneity was no longer significant following removal of one study (30) for both the *IL6* (OR=0.78, 95% CI=0.65–0.94, p<0.01) and *sIL6R* (OR=0.76, 95% CI=0.64–0.91, p<0.01) genes. No other polymorphisms were significantly associated with schizophrenia in the allele-wise analysis.

In the genotype-wise analysis for the rs1143627 polymorphism for the *IL1B* gene, homozygosity for the major allele was associated with significantly increased odds of schizophrenia (OR=1.91, 95% CI 1.60–2.27, p<0.01), and homozygosity for the minor allele was associated with a significantly decreased odds of schizophrenia (OR=0.40, 95% CI 0.33–0.49, p<0.01), with significant between-study heterogeneity. In a sensitivity analyses, the odds ratio remained significant and the between-study heterogeneity was no longer significant following removal of one study (Shibuya et al., 2014) (9). The genotype for the rs1800795 polymorphism for the *IL6* gene was associated with significantly decreased odds of schizophrenia (GG vs. CC+GC, OR=0.65, 95% CI=0.50–0.85, p<0.01), and between-study heterogeneity was not significant. Genotype for the rs8192284 polymorphism for the *sIL6R* gene was also associated with significantly decreased odds of schizophrenia (AA vs. AC+CC, OR=0.72, 95% CI=0.55–0.94, p=0.02), and between-study heterogeneity was not significant. All other polymorphisms showed no significant genotype-wise associations. Forest plots for the rs1800795 polymorphism for the *IL6* gene, the rs8192284 polymorphism for the *sIL6R* gene, and the rs1143627 polymorphism for the *IL1B* gene are shown in Figure 2. There were an insufficient number of studies to assess for publication bias for the *sIL6R* and *IL1B* polymorphisms (42). However, for the IL6 gene, the funnel plot (see Figure 3) and results of Egger's test (intercept -0.03, 95% CI -4.72–4.67, p=0.99) showed no evidence for potential publication bias.

Discussion

We found that polymorphisms in genes for *IL1B, IL6,* and *sIL6R*—but not *IL2, IL4, MCP1*, or *TGFB1*—were associated with schizophrenia risk. There was significant heterogeneity in these findings; however, the association remained significant in sensitivity analyses.

An important strength of our study is that we considered polymorphisms for a number of different cytokine or chemokine genes that have not been investigated in previous quantitative reviews. For the allele-wise analysis, we were able to include data from the large Psychiatric Genomics Consortium study of schizophrenia (30). Of note, our findings for the *IL6* and *sIL6R* genes remained significant in sensitivity analyses, which excluded this study, supporting the robustness of the association in smaller samples. Blood protein levels IL-1β, IL-6 are also abnormal in schizophrenia, and the sIL-6R enhances the biological activity of IL-6 (4). Thus, our work complements and extends previous findings regarding immune dysfunction in this disorder, including genome-wide association studies. Our findings also directly inform on future studies of blood cytokine expression in schizophrenia, which should consider the effect of these

Table 2 Meta-Analysis of Cytokine Gene Polymorphisms in Schizophrenia

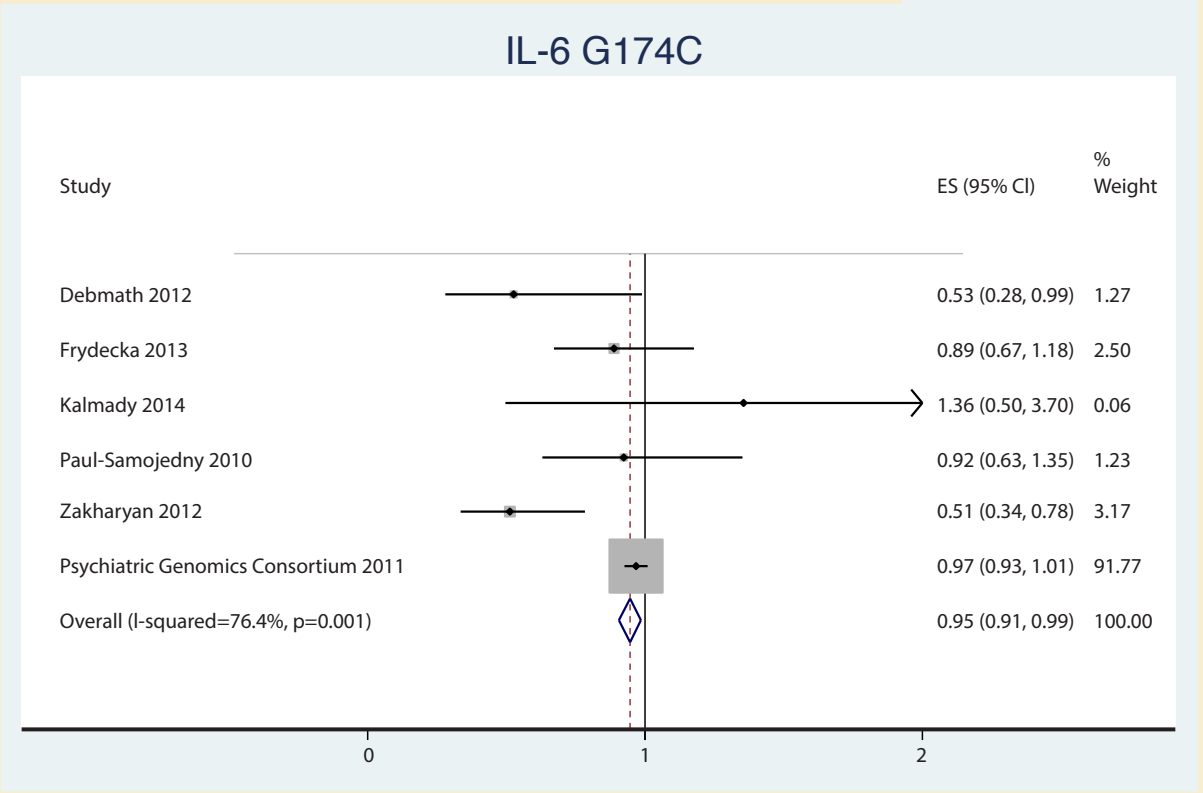
Gene	N Studies	Subject Group	Allele-wise Analysis n	OR	95% CI		Genotype-wise Analysis Frequency (n)			OR	95% CI	p-value	OR	95% CI	p-value	References	
IL1B; rs1143627	4*				A vs G		AA	AG	GG	AA vs (GA or GG)			GG vs (GA or AA)			Borkowska 2012	Schizophrenia PGC 2011
		Schizophrenia	20185	1.03	0.98	1.07	436	587	152	**1.91**	**1.60**	**2.27**	**<0.01**	**0.40**	**0.33**	**0.49**	**<0.01**	Shibuya 2014	
		Control	20542				305	691	357									Shirts 2006	
IL1B; rs1143630	2				T vs G		TT	TG	GG	TT vs (TG or GG)			GG vs (TG or TT)			Sasayama 2011	
		Schizophrenia	2150	1.05	0.90	1.23	784	272	19	1.01	0.79	1.29	0.96	0.91	0.73	1.13	0.38	Shibuya 2014	
		Control	3580				1305	443	42										
IL1B; rs1143633	4*				T vs C		TT	TC	CC	TT vs (TC or CC)			CC vs (TC or TT)			Sasayama 2011	Schizophrenia PGC 2011
		Schizophrenia	20938	1.03	1.00	1.07	371	735	445	1.13	0.97	1.33	0.12	0.91	0.79	1.06	0.22	Shibuya 2014	
		Control	22424				486	1080	728									Shirts 2006	
IL1B; rs1143634	6*				A vs G		AA	AG	GG	AA vs (GA or GG)			GG vs (GA or AA)			Laurent 1997	Schizophrenia PGC 2011
		Schizophrenia	20936	1.00	0.96	1.05	33	383	1440	0.82	0.61	1.10	0.18	0.98	0.83	1.16	0.81	Saiz 2006	Shibuya 2014
		Control	23416				67	501	2222									Sasayama 2011	Shirts 2006
IL2; rs2069762	2				T vs G		TT	TG	GG	TT vs (TG or GG)			GG vs (TG or TT)			Frydecka 2013	
		Schizophrenia	2060	0.98	0.84	1.14	433	498	99	0.93	0.76	1.15	0.51	0.92	0.69	1.22	0.55	Watanabe 2008	
		Control	2340				460	589	121										
IL4; rs2243250	4*				C vs T		CC	CT	TT	CC vs (CT or TT)			TT vs (CT or CC)			Jun 2003	Schizophrenia PGC 2011
		Schizophrenia	20176	0.98	0.93	1.04	341	438	391	1.12	0.85	1.48	0.42	0.96	0.78	1.17	0.66	Schwarz 2006	
		Control	20116				352	434	354									Watanabe 2008	
IL6; rs1800795	6*				G vs C		CC	CG	GG	GG vs (CC or CG)			CC vs (CG or GG)			Debmath 2012	Paul-Samojedny 2010
		Schizophrenia	19022	0.95	0.91	0.99	218	294	81	**0.65**	**0.50**	**0.85**	**<0.01**	1.12	0.81	1.54	0.51	Frydecka 2013	Schizophrenia PGC 2011
		Control	19378				333	329	109									Kalmady 2014	Zakharyan 2012
sIL6R; rs8192284	3*				A vs C		AA	AC	CC	AA vs (CA or CC)			CC vs (CA or AA)			Sasayama 2011	Schizophrenia PGC 2011
		Schizophrenia	19030	**0.96**	**0.92**	**1.00**	163	305	129	**0.72**	**0.55**	**0.94**	**0.02**	1.35	0.98	1.85	0.06	Sun 2008	
		Control	18752				160	226	72										
MCP1; rs1024611	4*				A vs G		AA	AG	GG	AA vs (GA or GG)			GG vs (GA or AA)			Mundo 2005	Schizophrenia PGC 2011
		Schizophrenia	18670	1.00	0.96	1.04	157	183	77	0.80	0.59	1.09	0.16	0.88	0.64	1.20	0.42	Pae 2004	
		Control	18596				159	143	78									Zakharyan 2012	
TGFB1; rs1800470	2				C vs T		CC	CT	TT	CC vs (CT or TT)			TT vs (CT or CC)			Frydecka 2013	
		Schizophrenia	498	0.96	0.77	1.21	60	124	66	0.86	0.59	1.25	0.42	1.03	0.72	1.49	0.86	Lee 2010	
		Control	812				103	192	111										

* Denotes number of studies for allele-wise analysis, as genotype-wise data not available for the Schizophrenia PGC 2011 study.

Gene	N Studies	Subject Group	Allele-wise Analysis n	Frequency (n)		OR	95% CI		p-value	Genotype-wise Analysis Frequency (n)			OR	95% CI		p-value	OR	95% CI		p-value	References
IL1A; rs1800587	5			T	C		T vs C			TT	TC	CC	TT vs (TC or CC)				CC vs (TC or TT)				Lu 2010
		Schizophrenia	20185	299	1451	1.30	0.91	1.86		41	217	617	1.24	0.52	2.94		0.72	0.32	1.59		
		Control	20542	616	2270					82	452	909									
IL1RA; rs1794068	10			T	C		T vs C			TT	TC	CC	TT vs (TC or CC)				CC vs (TC or TT)				Lu 2010
		Schizophrenia	2150	653	3033	0.98	0.82	1.18		80	493	1270	0.94	0.70	1.26		1.00	0.82	1.22		
		Control	3580	1028	4054					144	740	1657									
IL1B; rs1143634	5			T	C		T vs C			TT	TC	CC	TT vs (TC or CC)				CC vs (TC or TT)				Lu 2010
		Schizophrenia	20938	392	2002	0.95	0.82	1.10		33	326	838	**0.64**	**0.41**	**0.99**		1.17	0.88	1.57		
		Control	22424	518	2352					65	388	982									
IL1B; rs16944	16			A	G		A vs G			AA	AG	GG	AA vs (GA or GG)				GG vs (GA or AA)				Shibuya 2014
		Schizophrenia	20936	3055	4052	1.03	0.97	1.10													
		Control	23416	4282	5785																
IL10; rs1800096	18			G	A		G vs A			GG	AG	AA	AA vs (GA or GG)				GG vs (GA or AA)				Gao 2010
		Schizophrenia	2060			1.02	0.76	1.38					0.95	0.68	1.32		1.35	0.62	2.94		
		Control	2340																		
IL10; rs1800871	4			C	T		C vs T			CC	CT	TT	TT vs (CC or CT)				CC vs (TC or TT)				Gao 2010
		Schizophrenia	20176			0.98	0.87	1.11					0.97	0.82	1.14		1.00	0.90	1.15		
		Control	20116																		
IL10; rs1800872	7			A	C		A vs C			AA	AC	CC	CC vs (AA or AC)				AA vs (CA or CC)				Gao 2010
		Schizophrenia	19022			**1.12**	**1.02**	**1.23**					1.02	0.90	1.15		1.35	1.15	1.58		
		Control	19378																		
TNFα; rs1800629	21			A	G		A vs G			AA	AG	GG	AA vs (GA or GG)				GG vs (GA or AA)				Qin 2014
		Schizophrenia	19030			1.05	0.88	1.25													
		Control	18752																		

Figure 2 Meta-Analyses of Gene Polymorphisms in Schizophrenia

2A ***IL6*** **gene-allelotype analysis**



2B ***sIL6R*** **gene-allelotype analysis**

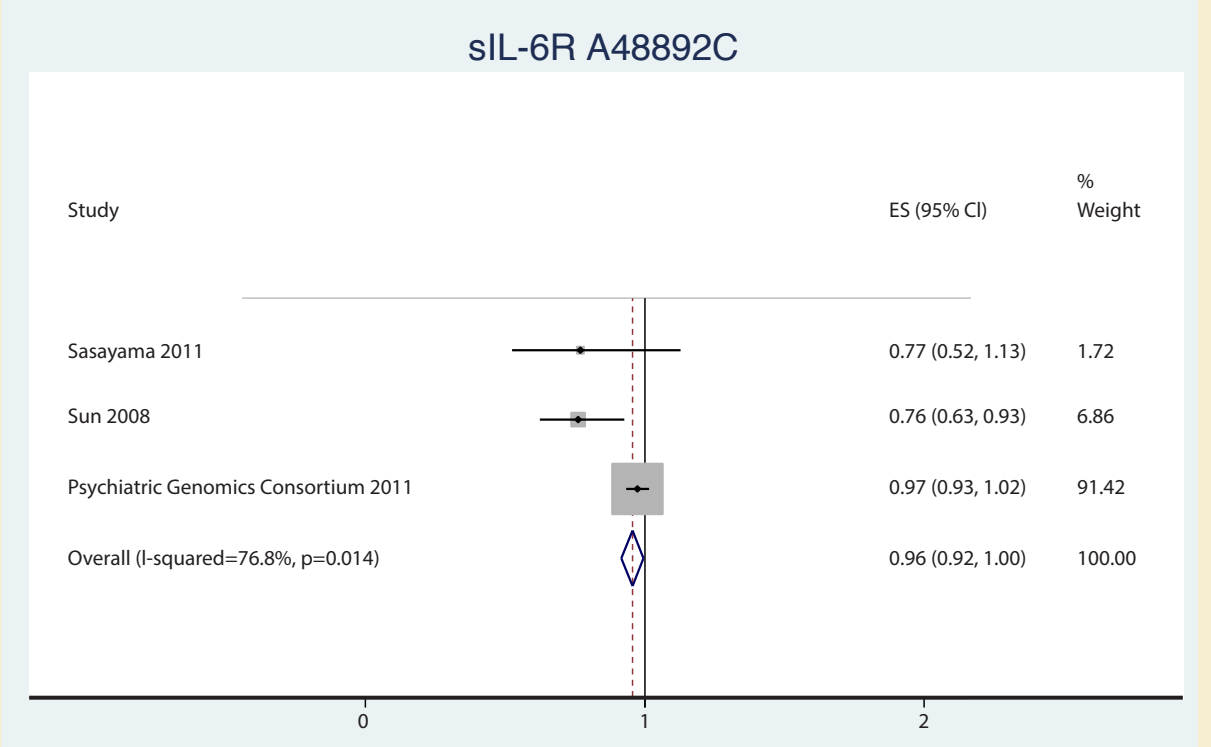


Figure 2 Meta-Analyses of Gene Polymorphisms in Schizophrenia

2C ***IL1B*** **(rs1143627 polymorphism) gene-genotype analysis**

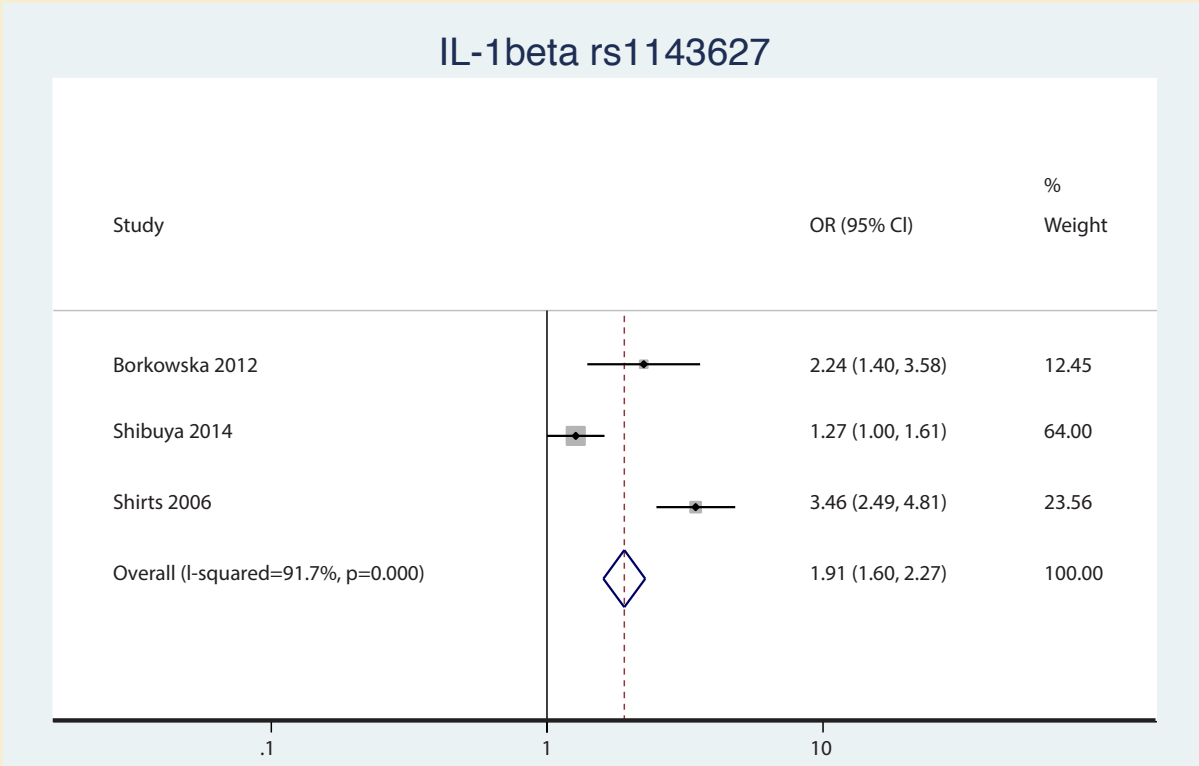
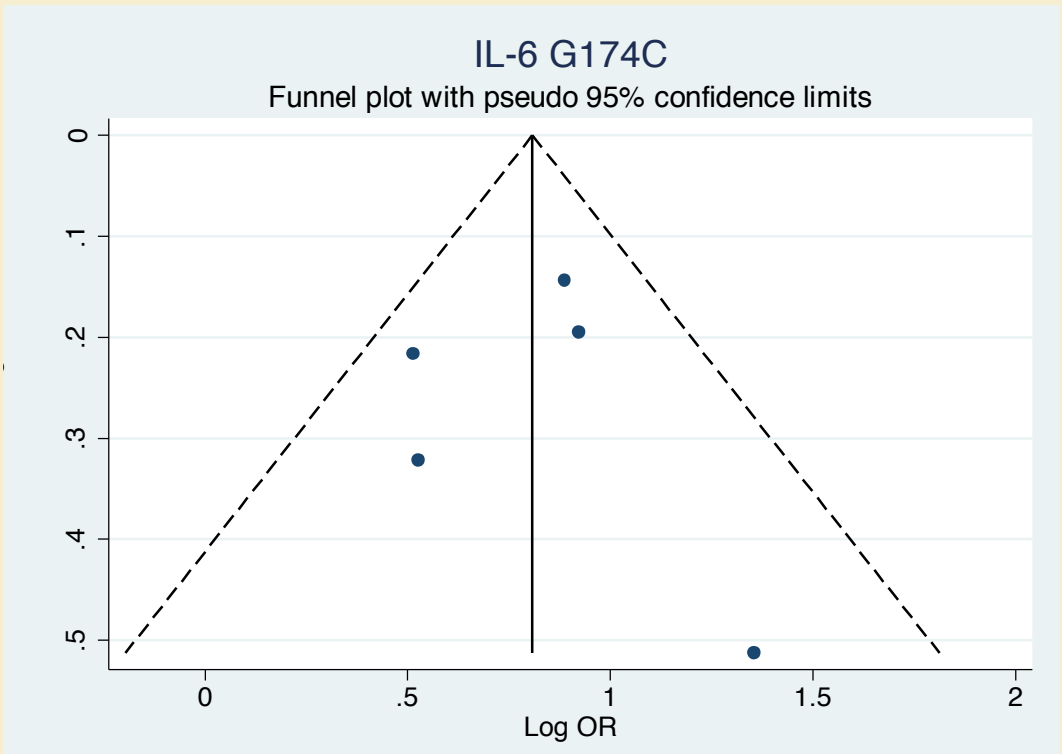


Figure 3 Funnel Plot for IL-6 G174C Polymorphism



polymorphisms. We also excluded studies that had significant deviations from HWE in controls, which may bias effect size estimates for genes in meta-analysis (46).

There are always limitations to studies. Our findings must be interpreted with some caution in light of the small number of studies for some cytokine or chemokine genes. Due to the small number of studies, we were not able to perform meta-regression analyses to explore possible moderating variables (e.g., sex, race, geographic location, and genotyping method), which warrant investigation in future studies. We were not able to control for other potential confounding factors such as the uniformity of diagnosis of schizophrenia across studies (and the absence of diagnosis in controls). The impact of these factors on the associations is unclear.

Additional studies are needed to investigate polymorphisms in other cytokine genes that may be associated with schizophrenia risk and cytokine network expression. We previously found alterations in blood sIL-2R, IL-12, and IFN-γ protein levels in schizophrenia (Miller et al., 2011) (4). Sufficient data for meta-analysis were not available for polymorphisms in these cytokine genes, which should be explored. Relationships between cytokine gene polymorphisms and other aspects of schizophrenia have not been investigated. One previous study found that an *IL6* gene polymorphism also impacts on blood *IL-6* protein levels (8). Another study found that an *sIL6R* gene polymorphism is associated with higher blood protein levels of both *sIL-6R* and *IL-6* in patients with schizophrenia (28). Given evidence for alterations in blood cytokine levels in schizophrenia (4-6), future studies should investigate the effects of these polymorphisms on protein expression in the peripheral blood and CNS. Whether cytokine gene polymorphisms are associated with other clinical features in schizophrenia, such as psychopathology, cognition, brain volumes, treatment response, or illness course, is also unknown. Outside of schizophrenia, polymorphisms in genes for *IL1B* and *TNFα* may impact on risk of depression, and polymorphisms for *IL1B* and *IL6* genes may impact on antidepressant response (47). Future studies in schizophrenia could investigate relationships between cytokine gene polymorphisms and response to antipsychotics or other adjunctive treatments.

Taken together, our results complement and extend previous findings regarding immune dysfunction in schizophrenia, and inform on future studies of cytokine expression in schizophrenia. Future studies of inflammation should consider the effects of cytokine gene polymorphisms on schizophrenia risk, cytokine levels, and other clinical features of the disorder. Replicated findings may contribute to more personalized medicine for patients with schizophrenia.

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None.

Conflict of Interest

Dr. Hudson has nothing to disclose. Dr. Miller has nothing to disclose for the work under consideration. In the past 12 months, Dr. Miller reports employment at Georgia Regents University, grant support from the National Institute of Mental Health (K23MH098014), the American Psychiatric Association (Kempf Fund Award) and Georgia Regents University; Research support from the National Institutes of Health Clinical Loan Repayment Program; Honoraria from *Psychiatric Times;* and Speaker fees for lectures from the University of Nevada-Reno.

Contributors

Dr. Miller designed the study. Drs. Miller and Hudson managed the literature searches. Dr. Miller managed the analyses. Drs. Miller and Hudson wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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