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Brief Research Communication

Homozygosity of the Interleukin-10 Receptor 1 G330R Allele Is Associated With Schizophrenia

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Infections of unknown origin and an altered immune response have been hypothesized to play a role in the pathogenesis of schizophrenia. We have previously identified two single nucleotide polymorphisms (SNPs) of the IL-10 receptor 1 (IL-10R1) causing a substitution of glycine 330 to arginine (G330R) and of serine 138 to glycine (S138G). A possible association between these IL-10R1 variants and schizophrenia has been investigated in the present study. DNA of 101 unrelated Austrian patients with a DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders) consensus diagnosis of schizophrenia (n = 70) or schizoaffective disorder (n = 31) and DNA of 121 German schizophrenic patients (DSM-III-R) was analyzed for the presence of S138G and G330R by allele-specific multiplex PCRs. Data from patients were compared with 250 unrelated, psychiatric healthy controls. No difference in allele frequency was detected between patients and controls (G330R: 34.0% vs. 30.0%, *P* = 0.208; S138G: 19.7% vs. 16.6%, *P* = 0.235; by Fisher's exact test). However, there was a significant difference in genotype distribution (wt/wt, wt/mut, mut/mut) for G330R between patients (46.8%, 38.3%, 14.9%) and controls (47.6%, 44.8%, 7.6%; Fisher's test *P* = 0.032). No such difference was seen for S138G. Our results suggest that homozygosity of the IL-10R1 G330R allele is associated with schizophrenia and may contribute to the expression of disease phenotype in susceptible individuals.

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KEY WORDS: schizophrenia; schizoaffective disorder; IL-10R1

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INTRODUCTION

Several studies show a possible role of the immune system in the pathogenesis of schizophrenia, and thereby support the hypothesis that non-specified infections and -associated immune response may lead to schizophrenia [Müller, 2004]. Schizophrenia-associated immune alterations include decreased mitogen-induced lymphocyte proliferation, increased numbers of total T or T-helper cells, and the presence of antibrain antibodies in serum. Changes in cytokines, cytokine receptors, and cytokine activity modifiers have been reported in the serum and cerebrospinal fluid of schizophrenic patients [Zhang et al., 2002].

Interleukin-10 (IL-10) is a cytokine mainly produced by immune cells (monocytes/macrophages, T- and B-lymphocytes) and exhibits diverse activities in various organs. It has been originally described as "cytokine synthesis inhibitory factor," having regulatory function during inflammation and is thought to counteract pro-inflammatory cytokines such as TNF or IL-1. Multiple studies now suggest that IL-10-producing regulatory T-cells exist distinct from Th-1 or Th-2 lymphocytes, which may have an important role in controlling immune response and tolerance in vivo [Moore et al., 2001].

Abbreviations used: cSNPs, coding single nucleotide polymorphisms; IL-10, interleukin 10; IL-10R1, interleukin 10 receptor 1.

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IL-10 has been detected in the brain. Human glia cells are considered a major source of IL-10 [Chabot et al., 1999]. It is involved in the downregulation of proinflammatory cytokines that were found elevated in psychiatric patients (e.g., IL-1β, TNF-α, IL-6, and IFN-γ). IL-10 has been associated with various immunological aspects that might be involved in the etiology of psychiatric disorders including schizophrenia [Eskdale et al., 1997]. In addition, increased serum concentrations of IL-10 have been observed in schizophrenic patients specifically in more severe cases [Rothermundt et al., 1996, Van Kammen et al., 1997; Cazzullo et al., 1998]. In non-psychiatric patients (Crohn's disease), IL-10 treatment was associated with changes in mood (C. Gasche, personal communication).

The human IL-10 receptor (IL-10R) is a heterotetramer composed of two receptor chains (IL-10R1 and IL-10R2), and belongs to the class II cytokine receptor family. The IL-10R1 chain plays a dominant role in mediating high-affinity ligand binding and signal transduction, whereas the IL-10R2 subunit is thought to be required for signaling only. Interaction of IL-10 with the IL-10R complex stabilizes the position of both IL-10R chains, activates phosphorylation of the receptor-associated Janus tyrosine kinases, and induces mainly STAT3-mediated signal transduction [Moore et al., 2001]. Recently, by screening for mutations in the IL-10R1, we identified two coding variants causing a substitution of glycine 330 to arginine (G330R; rs2229113) and of serine 138 to glycine (S138G; rs3135932; Gasche et al., 2003). The allelic frequency in a European cohort was 17% for the S138G and 32% for the G330R substitution, and S138G was in strong linkage disequilibrium with G330R [D′ = 0.94; Gasche et al., 2003]. Structural analysis revealed that the substitution of S138G may interfere with binding of IL-10 to IL-10R1. Homozygous presence of IL-10R1 SNPs seems to render cells IL-10 insensitive. The current study aimed to investigate a possible association between IL-10R1 cSNPs, and schizophrenia.

METHODS

Two samples of patients, collected in Austria and in Germany, were available for the study. The sample from Austria consisted of 101 unrelated individuals with a DSM-III-R [Diagnostic and Statistical Manual of Mental Disorders; American Psychiatric Association, 1987] consensus diagnosis of schizophrenia (n = 70: 28 females, 42 males) and schizoaffective disorder (n = 31: 19 females, 12 males). The sample from Bonn (Germany) comprised 121 patients from a trio sample (schizophrenic patient and parents). These patients had a family history with psychiatric disorders, that is, presence of at least one additional first- or second-degree member affected with either schizophrenia or bipolar or recurrent unipolar disorder. Altogether, a total of 222 patients with a DSM-III-R diagnosis of either schizophrenia (n = 191) or schizoaffective disorder (n = 31) was available for association studies using a case-control design. Two hundred fifty unrelated individuals (collected in Austria) without DSM-III-R diagnosis of any psychiatric disorder were used as controls. In addition, the sample of 121 trios from Bonn was used for a family-based association design (transmission disequilibrium test) by including the genotypes of the parents. All participants were of similar ethnicity (Caucasians). Written informed consent was obtained from all individuals. The protocols were approved by the local institutional review board. The diagnostic process included a face-to-face interview with all patients/controls using the Schedule for Affective Disorders and Schizophrenia, Lifetime version [SADS-LA; Spitzer and Endicott, 1987]. In addition, an unstructured psychiatric interview and a family history evaluation were completed for each individual. Clinical data were obtained from medical records as well as from the treating psychiatrists. After collection of all available clinical information, a DSM-III-R consensus diagnosis (axes I and II) was conducted by two independent psychiatrists.

Two allele-specific multiplex PCRs were used for detection of S138G and G330R in genomic DNA as described [Gasche et al., 2003]. Fisher's exact test was employed to evaluate possible differences between the groups in the frequencies of diagnoses, alleles and genotypes, the standard χ^2 test was used for Hardy–Weinberg equilibrium. Rate of transmission of alleles from heterozygous parents (Transmission test for linkage disequilibrium, TDT) was estimated as proposed by Spielman et al. [1993], and statistical power of the TDT test was conducted as proposed by Abel and Muller-Myhsok [1998]. All statistical analyses were performed using the statistical computing environment R version 2.2.1. [R Development Core Team, 2005].

RESULTS

The ethnic background in the regions of sampling (Austria and Germany) is similar. Indeed, when comparing patients from Germany (n = 121) with patients from Austria (n = 101), there were no significant differences in allele frequency (G330R: Fisher's test $P = 0.920$) and genotype distribution (G330R: Fisher's test $P = 0.983$) between the two groups. Patients did not show a deviation from Hardy–Weinberg equilibrium for both variants (German cohort: G330R: χ^2 test $P = 0.129$; S138G: χ^2 test $P = 0.896$; Austrian cohort: G330R: χ^2 test $P = 0.113$; S138G: χ^2 test $P = 0.112$), the same holds true for controls (G330R: χ^2 test $P = 0.292$; S138G: χ^2 test $P = 0.960$). The allele frequency of the G330R variant in schizophrenia and

TABLE I. Genetic Association Between Schizophrenia and IL-10R1 S138G and G330R Variants

	S138G alleles		S138G genotypes		
	1	2	1/1	1/2	2/2
Patients	355 (0.803)	87 (0.197)	145 (0.656)	65 (0.294)	11 (0.05)
Controls	417 (0.834)	83 (0.166)	174 (0.696)	69 (0.276)	7 (0.028)
Fisher's test *p*	0.235		0.391		
	G330R alleles		**G330R genotypes**		
	1	2	1/1	1/2	2/2
patients	293 (0.66)	151 (0.34)	104 (0.468)	85 (0.383)	33 (0.149)
Controls	350 (0.70)	150 (0.30)	119 (0.476)	112 (0.448)	19 (0.076)
Fisher's test *p*	0.208		0.032		

G330 is allele1 (wildtype) and S138 is allele 1 (wild type) respectively. Data are absolute (and relative) numbers.

TABLE II. Disease Penetrance Models (A, B, C) for Estimation of TDT-Power (Table III)

	f_{11}	f_{12}	f_{22}
Model A	1.0%	1.7%	3.0%
Model B	1.5%	2.3%	3.5%
Model C	2.0%	2.8%	4.0%

Assumptive probability (f_{11}, f_{12}, and f_{22}) of schizophrenia phenotype expression for genotypes 1/1 (wt/wt), 1/2 (wt/mut), and 2/2 (mut/mut). The TDT-power was evaluated following the muliplicative model by Abel and Muller-Myhsok [1998] with genotype relative risks of g and $g \times g$ for the 1/2 and 2/2 subjects, respectively. We considered the three parameter settings shown above to get a range of penetrance for 1/1 and disease-allele effects.

controls was 34.0% and 30.0%, allele frequency of the S138G variant was 19.7% and 16.6% respectively. Allele frequencies between groups were not statistically significant (G330R: $P = 0.208$, S138G: $P = 0.235$; Table I). No difference was seen between cases and controls within haplotype analysis (data not shown). However, a statistically significant difference in genotype distribution of the G330R variant between schizophrenic patients and controls (Fisher's test $P = 0.032$) was detected. The disease group contained a higher number of homozygous mutant G330R carriers as compared to the control group (Table I). No genotype difference was seen for S138G. There was no favorable transmission of any of these alleles when the trio sample was analyzed by TDT (data not shown), but given our sample size, the power of the TDT test was rather low (Table II and III).

DISCUSSION

Our results are in line with previous studies suggesting that the investigated IL-10R1 variants are loss-of-function alleles that may be associated with certain disease phenotypes when homozygously present [Hofer et al., 2005]. It is assumed that the variants analyzed in this study may potentially alter IL-10 signaling during immune stimulation. While IL-10R1 G330R- and S138G alleles were found to be similarly distributed in schizophrenic patients and controls, we obtained evidence for association of the homozygous genotype of IL-10R1 G330R with schizophrenia.

The human IL10R1 gene is located on chromosome 11q23.3 [Liu et al., 1994; Taniyama et al., 1995], a region that shows some but inconsistent linkage with schizophrenia. Nanko et al. [1992] discovered LOD scores of 1.00–1.50 with marker D11S35 (located at chromosome 11q22.2) within a Japanese population, and Maziade et al. [1995] reported a LOD score of 3.41 (chromosome 11q21-22) in one Canadian pedigree. Gurling et al. [2001] found an overall maximum admixture LOD score of 3.2 at D11S934 (located at chromosome 11q24.2). When testing trios, we did not detect preferred transmission. There are several possible explanations for this paradoxon. Most likely, as only 121 trios were available for testing a lack of power is the reason for this (Table II and III). Besides, the

TABLE III. Estimated Power for TDT

	S138G	G330R
Model A	0.83	0.90
Model B	0.63	0.71
Model C	0.48	0.56

Maximum power (full linkage between marker and disease) of the TDT test for models A–C in Table II. The significance level is alpha = 0.05, the sample size is 120, and the SNP allele 2 frequency is 0.2 for S138G, and is 0.3 for G330R. Only model A shows acceptable power.

Austrian patients and controls did not necessarily have a positive family history as the German patients did. Last, we need to keep in mind that the association that was found with the IL-10R1 genotype could be false positive (type I error) as we did not perform correction for multiple testing.

In summary, our data indicate a possible role of IL-10R1 signaling in the pathogenesis of schizophrenia. A speculative model involves a yet unidentified viral infection in the pathogenesis of schizophrenia [Müller, 2004]. In line with this hypothesis, IL-10 mediates viral persistence by suppressing a Th1-immune response. Several herpes viruses (e.g., CMV, EBV) take advantage of IL-10 downstream effects since they carry an IL-10 gene homolog in their genome [Kotenko et al., 2000, Liu et al., 1997]. During infection and viral replication, this IL-10 homolog is expressed, interacts with the human IL-10R1, and induces signal transduction similar to human IL-10 [Jones et al., 2002]. Again, the functional role of the human IL-10R1 variants is incompletely understood, however, we suspect that they alter viral IL-10 signaling and thereby the host's immune response to viral infection. Binding and signaling studies between the viral IL-10 homologs and IL-10R1 variants are on their way.

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