



Brief report

Tourette's syndrome is not associated with interleukin-10 receptor 1 variants on chromosome 11q23.3

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Abstract

Interleukin-10 receptor 1 (IL-10R1) single nucleotide polymorphisms, located on chromosome 11q23 – a strong candidate for linkage with Tourette's syndrome (TS) – have been investigated for association with TS. DNA of 77 patients with a DSM-IV (Diagnostic and Statistical Manual IV) diagnosis of TS and 250 healthy controls was genotyped. IL-10R1 was not associated with TS.

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1. Introduction

Several reports (Hoekstra et al., 2004) indicate a possible role of the immune response system in the pathogenesis of Tourette's syndrome (TS), and thereby support the long existing hypothesis that infection and immunity may be involved in the pathogenesis of TS. The concept of PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) (Swedo et al., 1998) highlights the temporal association of streptococcal infection and symptom exacerbation in a subgroup of TS patients and has stimulated research regarding the immune mechanisms

of this disease. Recent investigations focused on group A β -haemolytic streptococci (Luo et al., 2004; Murphy et al., 2004), cross-reacting autoantibodies (Morshed et al., 2001), and the D8/17 overexpression and Fc- μ receptors on B lymphocytes (Murphy et al., 1997; Hoekstra et al., 2001). Most results, however, were inconsistent. In fact, only one study has examined the possible role of cytokines and T cell function in TS (Leckman et al., 2005). The authors reported elevated levels of IL-12 and TNF- α at baseline and an increase during symptom exacerbation in paediatric patients with TS.

A strong genetic background for TS has been shown in family studies, linkage analyses, and association analyses (Pauls, 2003). The majority of studies provide strong evidence for the effect of major genes in the expression of the TS phenotype. Genome-wide scans

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have been carried out (Barr et al., 1999; TSAICG, 1999). Only the involvement of the 11q23 region has been confirmed in independent studies within different ethnicities and research groups (Simonic et al., 1998; Merette et al., 2000; Simonic et al., 2001).

Interleukin-10 (IL-10) is a cytokine produced by B cells, T helper cells, and cells of the monocyte/macrophage lineage, and it has regulatory function on inflammatory and cell-mediated immunological mechanisms. IL-10 is a Th2 cytokine that exerts its anti-inflammatory activity by inhibiting Th1-type cytokine responses, such as IL-12, IFN- γ , and TNF- α production. Another important IL-10 function is the limitation and termination of inflammatory responses after infection. Furthermore, the IL-10 signaling pathway is utilized by a subgroup of herpes viruses (incl. EBV and CMV) that express an IL-10 homolog gene to interfere with the host's immune response. IL-10 signaling may also be involved in immunological aspects of other psychiatric disorders, e.g. schizophrenia and major depression (Rothermundt et al., 1996; Cazzullo et al., 1998; Kubera et al., 2000; Maes, 2001; Schosser et al., 2007).

The human IL-10 receptor (IL-10R) is a heterotrimer composed of two of each of the receptor chains (IL-10R1 and IL-10R2). The human IL10R1 gene is located on chromosome 11q23.3 (Liu et al., 1994; Taniyama et al., 1995) within the TS candidate region. The IL-10R1 chain plays a predominant role in mediating high affinity ligand binding and signal transduction, whereas the IL-10R2 subunit is thought to be required for signaling only. IL-10R1 is specifically expressed on immune cells (macrophages) and some few epithelia (such as colon), whereas IL-10R2 is present on all tissues. Interaction of IL-10 with the IL-10R complex stabilizes dimerization of both IL-10R subunits, activates phosphorylation of the receptor-associated Janus tyrosine kinases, and induces STAT3-mediated signal transduction (Moore et al., 2001). There is evidence of IL-10 and IL-10R gene expression in astrocytes and microglia within the central nervous system (Kronfol and Remick, 2000).

By screening for mutations in the IL-10R1 gene, two variants causing a substitution of glycine 330 to arginine (G330R; rs2229113) and of serine 138 to glycine (S138G; rs3135932) were identified (Gasche et al., 2003). Both single nucleotide polymorphisms (SNPs) are frequently present in Caucasians, 17% for the S138G and 32% for the G330R, respectively. S138G is in strong linkage disequilibrium with G330R. In a European Caucasian cohort, approximately one-half of G330R carriers also carried S138G. Structural analysis

of the S138G variant revealed that the substitution of S138G may interfere with binding of IL-10 to IL-10R1. Monocytes from individuals carrying the IL-10R1 variants are less sensitive to IL-10-mediated inhibition of TNF- α production in vitro than are monocytes from individuals who do not carry the IL-10R1 variants. The SNPs render cells IL-10 insensitive (Gasche et al., 2003) specifically when expressed homozygously.

The current study aims to investigate a possible genetic association between IL-10R1 variants and TS. Beside existing immune hypotheses of TS, the gene for IL-10R1 is located in a chromosomal candidate region for TS on 11q23.

2. Methods

2.1. Subjects

A total of 77 (23 females, 54 males; mean age 34 years) unrelated Austrian Caucasians with a consensus diagnosis of Tourette's syndrome according to DSM-IV (American Psychiatric Association, 1994) criteria were investigated. All individuals were recruited at the Department of General Psychiatry, Medical University of Vienna (Stamenkovic et al., 2000). In addition, 250 unrelated healthy controls (no history of psychiatric disorders, no first degree relatives with psychiatric disorders) were screened by two independent psychiatrists (no DSM-IV diagnosis of any psychiatric disorder) in order to apply the association strategy. All individuals gave written informed consent. The protocols were approved by ethical committee at the institution.

2.2. Diagnostics

All patients with TS showed motor and vocal tics of at least moderate severity. To assess the severity of tics and illness, the Yale Global Tic Severity Scale (Leckman et al., 1989) and the Clinical Global Impression Scale (Guy and Bonato, 1970) were used.

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-IV blind consensus diagnosis was conducted by two independent psychiatrists.

2.3. Genotyping

Two allele-specific multiplex PCRs (polymerase chain reaction) were used for detection of S138G and G330R

Table 1
Genetic association between Tourette's syndrome and IL-10R1 S138G and G330R variants

	S138G alleles		S138G genotypes			G330R alleles		G330R genotypes		
	1	2	1/1	1/2	2/2	1	2	1/1	1/2	2/2
Patients	130 (0.844)	24 (0.156)	53 (0.688)	24 (0.312)	0 (0.000)	109 (0.717)	43 (0.283)	36 (0.474)	37 (0.487)	3 (0.039)
Controls	417 (0.834)	83 (0.166)	174 (0.696)	69 (0.276)	7 (0.028)	350 (0.700)	150 (0.300)	119 (0.476)	112 (0.448)	19 (0.076)
Fisher test <i>P</i>	0.80463		0.36573			0.76098		0.56224		

G330 is allele 1 (wild type) and S138 is allele 1 (wild type), respectively. Data are given in absolute and relative numbers.

in genomic DNA. The primer sequences and PCR conditions were previously described (Gasche et al., 2003).

2.4. Statistics

Fisher's exact test was employed to evaluate possible differences between the groups in the frequencies of alleles, genotypes and the distribution of homozygotes and heterozygotes. The significance level was set at $\alpha=0.05$. Standard chi-square tests (χ^2 , $df=1$) were used for testing Hardy–Weinberg equilibrium. A power analysis was conducted for the allele distribution of our sample. All statistical analyses were performed using the statistical computing environment R version 2.2.1 (<http://www.R-project.org>).

3. Results

The allele frequencies of the S138G and the G330R alleles in the disease cohort were 15.6% and 28.3%, respectively. No deviation from Hardy–Weinberg equilibrium was found in Tourette's syndrome (S138G: $P=0.1052$, $\chi^2=2.6244$; G330R: $P=0.08137$, $\chi^2=3.0374$) and in controls (S138G: $P=0.9596$, $\chi^2=0.0026$; G330R: $P=0.2918$, $\chi^2=1.1111$). No difference in allele frequency was seen between patients and controls (S138G: 16.6%, G330R: 30.0%) by Fisher's exact test (S138G: $P=0.80463$, G330R: $P=0.76098$). Similarly, no difference in genotype distribution was observed between patients and controls (S138G: $P=0.36573$, G330R: $P=0.56224$) (see Table 1). For the results of our power analysis, see Table 2.

Table 2
Power analysis for the sample (77 cases, 250 controls)

	OR=1.5	OR=2.0
SNP3	0.525	0.95
SNP4	0.690	0.989

Power against odds ratios of 1.5 and 2.0 for SNP3 (allele frequency approx. 16%) and SNP4 (allele frequency approx. 30%).

4. Discussion

Studies have shown increased autoantibody binding in the putamen, globus pallidus and caudate tissue of TS patients (Swedo et al., 1997; Singer et al., 1998) highlighting a role for autoimmunity in this disease. By producing various cytokines including IL-10, Th2-lymphocytes drive antibody production by B-cells and are thought to also control autoantibody formation. This is one of the mechanisms whereby IL-10 signaling might contribute to the pathogenesis of TS.

In line with these findings, Leckman et al. (2005) propose an inflammatory course in TS patients with upregulation of TNF- α . IL-10 is a potent inhibitor of TNF- α production. An increase of IL-10 during symptom exacerbation showed trend levels, whereas baseline levels were not statistically different from controls. The biological relevance of systemic cytokine levels with regard to neurological disease is incompletely understood, but might parallel their intra-cerebral activity.

Parametric multipoint linkage analyses yielded a LOD score of 3.24 for D11S1377 on chromosome 11q23 (Pauls, 2003). As the 11q23 appears to be a promising candidate region for TS (Hoekstra et al., 2001) and the functional SNPs were found within this region, it seemed reasonable to investigate a possible association between TS and the IL-10R1 SNPs, located on 11q23.

At the moment, 11 SNPs of the IL-10 R1 gene are known (National Center for Biotechnology Information SNP database — GenBank accession no. NM_001558), where only eight out of these are located within a coding region. From the latter eight SNPs, four SNPs cause an amino acid substitution (S138G, I203V, G330R, S420L). As SNPs G330R and S138G are in strong linkage disequilibrium and may act as loss of function alleles (but not I203V and S420L), we concluded that they are of greatest interest for association testing.

The diagnosis of TS according to DSM-IV is relatively straightforward. Nevertheless, a TS spectrum including chronic vocal and motor tics and tic-related obsessive compulsive disorder, as well as attention deficit hyperactivity disorder, has been proposed by some authors

(Abelson et al., 2005). The degree to which these disorders may be associated with the syndrome is not yet clear, and the results of phenomenological and neurobiological studies are contradictory (Alsobrook and Pauls, 2002). Therefore, we decided to select a sample consisting of TS patients diagnosed according to DSM-IV — thus a cohort of a clearly defined and stringent phenotype. The male/female ratio in our cohort is somewhat lower than expected (less than 2:1), but this is unlikely due to referral bias.

Our results reveal no significant association between the IL-10 receptor 1 alleles S138G and G330R with TS. This may reflect a lack of power due to a restricted sample size (Table 2). Since the TS phenotype is quite uncommon, less frequent alleles are more likely to play a role (Abelson et al., 2005).

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