LETTER TO THE EDITORS

IL2RA/CD25 polymorphisms contribute to multiple sclerosis susceptibility

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Without Abstract

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Sirs: Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system (CNS) whose primary mechanism of injury is by inflammatory/autoimmune demyelination and, to a variable degree, axonal damage. Epidemiological studies, genome screenings and case-control studies suggest that multiple genetic factors influence susceptibility to multiple sclerosis (MS) [1–5].

Genome-wide screens in multiplex families with MS [6], insulin-dependent diabetes mellitus (IDDM) [7] and adult-onset primary open angle glaucoma [8], have shown linkage to locus 10p15. A partial list of candidate genes located in this region includes *NET1, PRKCT, ITIH2, IL2RA, IL15RA, IT1H2, hGATA3*, the mRNA for open reading frame KIAA0019, and the gene for D123 protein. Amongst these, the interleukin 2 receptor alpha chain (*IL2RA/CD25*) gene has recently been associated with type 1 diabetes (T1D) in a study using a tag-SNP

approach [9]. Furthermore, a polymorphism in the *IL2* promoter region (−330) not only affects its level of expression but is also associated with MS diseases progression (secondary progressive MS) [10]. The protein encoded by *IL2RA* plays a key role in the activity of the high affinity IL-2R/CD25 and in the differentiation and functioning of the CD4 + CD25+ regulatory T cells [11], involved in immunological tolerance. In fact, in one patient report [12], mutations affecting its expression resulted in autoimmunity, similar to what has been observed in IL-2 and IL-2R-deficient mice [11]. Therefore, considering all of these data, and the provisional evidence of the implication of IL-2 in MS, *IL2RA* is an interesting candidate MS susceptibility gene.

We used PCR-restriction fragment length polymorphism (PCR-RFLP) analysis to genotype 4 SNPs located in regulatory regions of the *IL2AR* gene (Figure 1) in a DNA collection of 346 MS patients, clinically defined according to Poser's criteria [13], and 413 ethnically matched (Caucasian) controls. Within the cases, 247 subjects had the relapsing-remitting form of MS (RR-MS) and 99 had the secondary progressive form (SP-MS). The study was carried out after obtaining written informed consent from all participants under protocols approved by the Institutional Review Board of the Hospital Carlos Haya, Málaga, and the Hospital Clínico and the Regional Centre of Blood Transfusions, both in Granada, Spain.
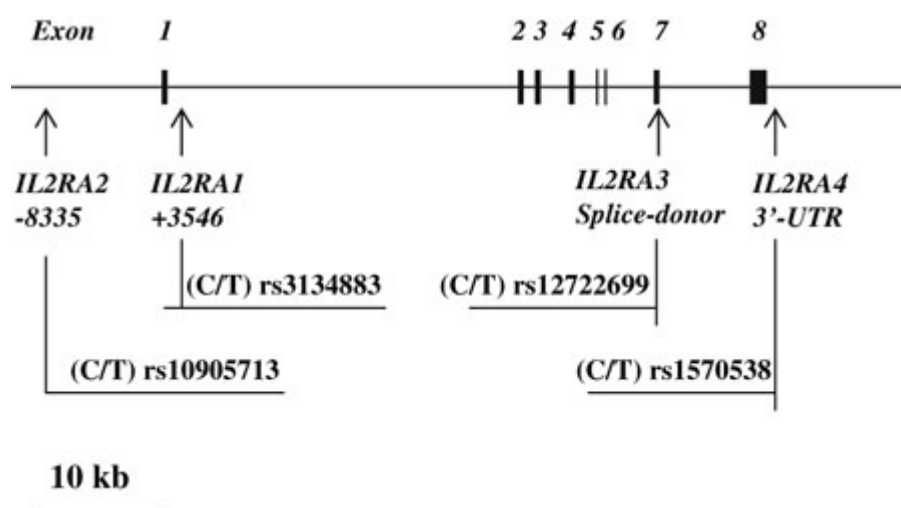

Fig. 1 Scheme of the *IL2RA* locus at semi quantitative scale, representing approximately 60 kb, indicating the position in the gene and the rs # of the four SNPs analysed according to "NCBI SNP Human Genome". Methodology details as primers and enzymes used are available from the authors. The (C/T) *IL2RA*1 polymorphism is located at the first intron, at +3546 bp from the transcription initiation site, at the Positive Regulatory Region IV (PRRIV) next to NF-AT-GAS motifs [16]. The (C/T) *IL2RA*2 is at −8335 bp of the transcription initiation start site, at the PRR CD28-responsive enhancer [17]. The (G/A) *ILR2RA*3 is located at splicing site donor consensus sequence of the exon 7. The *IL2RA4* polymorphism is located at the 3′ untranslated region +50704 bp from the initiation start site

There was no evidence of deviation from Hardy–Weinberg equilibrium in controls or cases for any of the SNPs genotyped. The distribution of genotypes by affected status is shown in Table 1. Allele and genotype frequencies were compared using Pearson´s chi-square test. The data revealed a significant over-representation of the *IL2RA4* T allele among cases compared with controls, with frequencies of 0.54 and 0.48 respectively $(P = 0.03)$, and more so for those with RR-MS (frequency = 0.55, $P = 0.01$). The estimated odds ratio (OR) for carriers of the T/T genotype of *IL2RA4* relative to homozygotes in the C allele was 1.51 (95% CI, 1.02–2.22; $P = 0.04$) and, again this was higher for RR-MS (OR = 1.69, $P = 0.02$). The

estimated OR per T allele from logistic regression analysis was of 1.23 (95% CI, 1.01–1.49, P = 0.04), which again, was stronger for RR-MS (OR = 1.30, P = 0.02). Both of these associations were observed independently of age and sex, with ORs of 1.27 (P = 0.02) and 1.32 (P = 0.02) observed respectively after adjustment for sex and age, the latter in 5-year categories. High linkage disequilibrium was observed between the *IL2RA2* and *IL2RA1* polymorphisms with an $r^2 = 0.8$. A comparison of haplotype frequencies between cases and controls using HaploView program found no evidence of an association.

Table 1 Distribution of genotypes of *IL2RA* polymorphisms in MS cases and controls*

		Controls *n* (%)	Cases *n* (%)	OR (95% CI)	P
IL2RA1	C/C	243 (58)	207 (60)	1.00	
	C/T	147 (35)	123 (36)	0.98 (0.73–1.33)	0.9
	T/T	28 (7)	16 (5)	0.67 (0.35–1.27)	0.2
			per T allele	0.90 (0.71–1.14)	0.4
IL2RA2	C/C	232 (56)	200 (58)	1.00	
	C/T	154 (37)	125 (36)	0.94 (0.70–1.27)	0.7
	T/T	32 (8)	21 (6)	0.76 (0.43–1.36)	0.4
			per T allele	0.90 (0.72–1.14)	0.4
IL2RA3	TT	418 (100)	346 (100)		
	TC				
	CC				
IL2RA4	C/C	119 (28)	79 (22)	1.00	
	C/T	193 (46)	161 (47)	1.26 (0.88–1.79)	0.2
	T/T	106 (25)	106 (31)	1.51 (1.02–2.23)	0.04
			per T allele	1.23 (1.01–1.49)	0.04

n, number of subjects; percentages shown in parenthesis.

* Statistical analysis. Comparisons of genotype and allele frequencies between healthy controls and MS patients were performed by contingency table 3 × 2 (genotypes) or 2 × 2 (alleles) chi-square (χ^2) test, using the package available from the web of the Institute of Human Genetics of the Technical University of Munich (http://www.ihg.gsf.de/cgi-bin/hw/hwa1.pl). We performed haplotype frequency estimation testing for differences between cases and controls using the HaploView (MJ Daly and JC Barrett, Whitehead Institute, MA, USA) and FAMHAP12 (T. Becker and M. Knapp, University of Bonn, Germany) softwares. No association was found (data not shown).

Because the *IL2RA4* polymorphism is at the 3′- untranslated region (3′-UTR), it could affect the level of the IL-2 receptor alpha expression and consequently the amount of CD4 + CD25+ regulatory T cells (Fig. 1). This may be very relevant for controlling the autoimmune attack against myelin and neurons in MS and therefore influence disease progression or severity. Animal models of MS have shown that CD4 + CD25+ regulatory T cells are directly involved in the natural recovery from, and protection against, experimental autoimmune encephalomyelitis (EAE model) [*14*].

The apparently weak association of the *IL2RA4* polymorphism with MS may be explained by diseases complexity that makes the potential set of genes involved in the pathology in each patient heterogeneous and by the complex inheritance involving interactions between

combinations of loci that may influence the immune response. On the basis of neurobiological and immunological markers, Luchinetti et al, [*15*] identified four fundamentally different patterns of demyelination in MS. Two of these patterns (I and II) were found to resemble T-cell-mediated or T cell plus antibody-mediated autoimmune encephalomyelitis, while patterns III and IV were characteristic of primary oligodendrocyte dystrophy rather than autoimmunity. On the other hand, we cannot discard the possibility that the association observed at the *IL2RA4* polymorphism could be due this SNP being in linkage disequilibrium with other causal variants at the *IL2RA* region.

In conclusion, this is the first study that identifies a significant association between a *IL2RA* polymorphism and MS, employing a case-control approach with samples collected from Caucasians in Spain. Replication in independent samples is the next important step in the validation of this gene as genetic factor involved in MS.

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