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High frequency of the IL-2 −330 T/HLA-DRB1*1501 haplotype in patients with multiple sclerosis☆

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Abstract We have evaluated the role of the HLA-DRB1*1501 allele and the IL-2 −330 T/G polymorphism and their interaction in susceptibility to multiple sclerosis on 360 patients and 426 matched healthy individuals. We used the SSP-PCR method to determine the alleles. Fisher's exact test was used to analyses. We observed a significant increase in the T allele at IL-2 −330 position in patients (OR=1.34, $P<0.05$), and the T/T and T/G genotypes were more frequent among patients than controls. The HLA-DRB1*1501 allele was overrepresented in patients as compared to the control group (OR=1.7, $P=0.0006$). The two-locus analysis of the interaction between the IL-2 promoter polymorphism and the HLA-DRB1 allele showed that the HLA-DRB1*1501/T haplotype was more frequent in patients than controls (OR=16, $P<0.0001$). Our findings support previous findings about the role of the HLA-DRB1*1501 allele in susceptibility to MS. This work also provides new findings about the importance of gene–gene interactions in the development of MS.

☆ Predisposing factor of multiple sclerosis.

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Introduction

Multiple sclerosis (MS) is a challenging disorder for neuroscience researchers because of the complexity of its pathophysiology. Although the triggering event is not yet understood, however, it is known that activation of the immune system against self myelin antigens is a common process that occurs in the course of this disease. There is also evidence supporting the presence of self-reacting immune components, i.e., the complement system, antibodies and lymphocytes, in MS patients [1,2].

There is no doubt that genetic polymorphisms are involved in susceptibility to MS [3–5]. HLA alleles and haplotypes might be the most relevant genetic predisposing factors for multiple sclerosis [6,7]. Fernández et al. [8] found a significant association between the HLA-DRB1*1501 allele, as well as some haplotypes that included this allele, and MS. A recent publication by the ANZ consortium revealed similar results in a large population [9].

The cytokine network is a cornerstone of the human immune system. Activation of the cytokine network, whether primary or secondary, results in complex connections that determine the course of the disease. Some believe that multiple sclerosis is a result of dysregulation of the immune response. There is evidence that the immune system shifts from Th2 to Th1 in MS patients [10].

Interleukin 2 (IL-2) is an important cytokine that is produced by activated T cells and promotes the proliferation of lymphocytes, macrophages and NK cells [11,12]. It has both pro- and anti-inflammatory activities. It promotes an inflammatory response through the generation of Th1 and Th2 effector cells. It also blocks the differentiation of T cells into Th17 effectors and promotes the development and maintenance of T regulatory cells (reviewed in [13]).

The relevance of IL-2 in multiple sclerosis had been elucidated by studies of MS patients and experimental autoimmune encephalomyelitis (EAE) [14,15]. Levels of IL-2 mRNA were increased in CNS lesions of EAE models during the acute phase [15].

A single nucleotide polymorphism (SNP) at the −330 position of the IL-2 gene was identified by John and colleagues [16]. According to a study by Hoffmann et al. [17], stimulation with anti-CD3/CD28 induced higher levels of IL-2 in individuals who were homozygous for the G allele relative to those who had the G/T or T/T genotypes. However, there is disagreement about the impact of this SNP on the production of IL-2 [18].

Matesanz et al. [19] reported that the G/T and T/T genotypes at the −330 position of the human IL-2 promoter are associated with susceptibility to MS. However, Shokrgozar and colleagues [20] did not observe a significant association between the IL-2 −330 SNP and susceptibility to MS.

The aim of this study was to assess the frequencies of the IL-2 −330 T and G alleles among Iranian MS patients and healthy individuals as well as the interaction between these genotypes and the HLA-DRB1*1501 allele.

Materials and methods

Subjects

We studied the distribution of IL-2 −330 allele and genotypes and the HLA-DRB1*1501 allele in 360 unrelated MS patients from a single center and 426 healthy controls to evaluate the impact of these variations and their interaction on susceptibility to MS. Expert neurologists confirmed the occurrence of MS according to clinical and paraclinical findings (MRI, oligoclonal bands in CSF and evoked potentials) based on McDonald's criteria [21]. Age-, sex- and ethnicity-matched control subjects with no history of autoimmune or inflammatory disorders were selected from the northeast of Iran in order to preclude environmental factors. A demographic questionnaire that included sex and age for both groups and the type of MS, age at onset and the Expanded Disability Status Scale (EDSS) for MS patients was prepared. The study was performed with the approval of the local ethics committee, and informed consent was obtained from all recruited individuals. None of the approached subjects refused to participate.

The mean age was 31 ± 9 years for MS patients (ranging from 19 to 57 years old) and 39 ± 7 years for control subjects (ranging from 35 to 64 years old). The mean age of onset and EDSS were 26 ± 6 years (10–46 years old) and 3.5 ± 2 (ranging from 1 to 8), respectively. The female/male ratio in the patient group was 5:1. According to clinical subtypes, 306 patients (85%) had relapsing-remitting MS and 43 (12%) had primary-progressive MS. There were also eight patients with secondary-progressive MS and three with progressive-relapsing MS.

DNA extraction and genotyping

Genomic DNA was extracted from 10 ml of peripheral whole blood by a standard protocol with some modifications [22]. Briefly, red blood cells were lysed three times with a buffer containing ammonium chloride, potassium dihydrogen phosphate and disodium hydrogen phosphates. SDS (10%), EDTA and 10 μl proteinase K were then added to the pellet, which was incubated for 1 hour at 65 °C. After incubation, a phenol/chloroform/isoamyl alcohol mix was added to samples, which were then centrifuged. To visualise and precipitate the DNA, isopropanol and sodium acetate were added to the supernatant and DNA was extracted after centrifugation. DNA samples were aliquoted in graded distilled water, and DNA concentrations were determined by a UV spectrophotometer at 260 nm (Techne, UK). All samples were diluted and stored at −80 °C for future analysis.

To detect the IL-2 polymorphism at −330 position, multiplex SSP-PCR was performed according to Reynard and colleagues [23]. The HLA-DRB1*1501 Genotyping was carried out by methods and primers previously described [24] in a thermocycler (Techne, UK). The PCR products were electrophoresed on a 2% agarose gel (Merck, Germany), and bands were visualised with a gel documentation system (UVITEC, UK).

Statistical analysis

Data were recorded in the program SPSS v-16, and the means of parametric variables were calculated. Data are presented as means ± SD for parametric variables and as percentages for non-parametric variables. Allele and genotype frequencies were calculated and compared between groups by non-parametric tests followed by Fisher's exact analysis using

Table 1 Frequencies of IL-2 −330 alleles and genotypes in patients and control subjects.

	MS	Control	OR	CI	*P*-value
Allele					
G	262 (36%)	375 (44%)	1	—	—
T	458 (64%)	477 (56%)	1.34	1.1–1.7	0.0047
Genotypes					
G/G	14 (4%)	68 (16%)	1	—	—
G/T	234 (65%)	239 (56%)	4.76	2.6–9.4	<0.0001
T/T	112 (31%)	119 (28%)	4.6	2.4–9.3	<0.0001

MS = multiple sclerosis; OR = odds ratio; 95% CI = 95% confidence interval.

STATA v-8 (CA, US). *P*-values were determined, and those less than 0.05 were considered to be significant.

Results

The frequency of the T allele at the IL-2 −330 SNP position was significantly higher in MS patients than in controls (OR = 1.34, 95% CI = 1.1–1.7, *P* = 0.0047). Furthermore, the G/T and T/T genotypes were more frequent in patients than in control subjects. Complementary data are shown in Table 1. The IL-2 −330 SNP did not show any significant impact on the age at onset or the EDSS of MS patients (data not shown).

In the IL-2 −330 position, the T allele was present in 96.2% of patients and 84.4% of healthy individuals (OR = 4.7, 95% CI = 2.6–9.2, *P* < 0.0001) (Table 2).

Of 360 patients, 107 (45.1%) carried the HLA-DRB1*1501 allele, which was significantly higher than that in healthy controls (Table 2). The age at onset and EDSS were not significantly different between HLA-DRB1*1501-positive and HLA-DRB1*1501-negative patients.

The haplotype containing both the HLA-DRB1*1501 and IL-2 −330 T alleles showed a higher frequency in MS patients than in the general population. Data on the frequencies of these haplotypes are shown in Table 2.

Discussion

We have studied the IL-2 −330 T/G SNP and the HLA-DRB1*1501 allele in Iranian MS patients and healthy controls. Our results show that the IL-2 −330 T allele and the G/T and T/T genotypes were associated with a higher risk of developing MS. Furthermore, when combined with the HLA-DRB1*1501 allele, the IL-2 T allele was strongly associated with susceptibility to MS.

In our study, significantly more patients (46%) than controls (34%) were positive for the HLA-DRB1*1501 allele. The prevalence of this allele was 46% in a previous study on an Iranian MS population [24]. This rate was found to be 38.6% in Basque, Spain [8]. Chao and colleagues [25] found that some HLA-DRB1*15 haplotypes determined susceptibility to MS while others did not. They concluded that HLA-DRB1*15 is part of a susceptibility haplotype but cannot be the susceptibility allele itself as it requires the further contribution of either epistatic interactions, epigenetic modifications or nearby structural variations [25].

The implication of IL-2 in the pathogenesis of multiple sclerosis is well documented [26]. The first evidence supporting the effect of the IL-2 −330 SNP on susceptibility to MS was provided by Matesnaz et al. [19]. They concluded that the G/T and T/T genotypes at this position were associated with a higher risk of developing MS [19]. Amirzargar et al. [27] reported controversial findings in an Iranian population; they found that the IL-2 −330 G/G genotype was more frequent among MS patients. In another study by Kikuchi and colleagues [28], the frequencies of the IL-2 −330 alleles and genotypes were not significantly different between Japanese MS patients and healthy controls. More recently, Shokrgozar et al. [20] reported that the frequencies of genotypes at position −330 did not differ significantly between Iranian MS patients and controls. Most of these studies were performed on a small number of samples, and it seems that large population studies are needed to provide more reliable results.

There are controversial reports of the impact of the IL-2 −330 SNP on the production of this cytokine [17,18]. In one study, Matesnaz and colleagues [18] evaluated the expression of the IL-2 −330 G and T alleles in vivo and in vitro. This transfection study, which was performed in Jurkat cells, demonstrated differential promoter activity between the G and T alleles. The promoter with the G allele was twice as active as the one with the T allele. Conversely, quantification of allelic expression in lymphocytes showed that the −330 T allele was associated with a higher level of transcription than the −330 G allele. They also found higher levels of IL-2 mRNA expression in samples of individuals with −330 T/T and G/T genotypes compared to individuals with

Table 2 Frequencies of risk alleles (IL-2 −330 T and HLA-DRB1*1501) in patients and control subjects.

Comparison	MS		Control		OR	95% CI	*P*-value
T^+ vs. T^-	346 (96%)	14 (4%)	358 (84%)	68 (16%)	4.7	2.6–9.2	<0.0001
$DR15^+$ vs. $DR15^-$	166 (46%)	194 (54%)	144 (34%)	282 (66%)	1.7	1.2–2.3	0.0006
$T^+/DR15^+$ vs. $T^+/DR15^-$	156 (43%)	190 (53%)	129 (30%)	229 (54%)	1.5	1.1–2.0	0.0172
$T^+/DR15^+$ vs. $T^-/DR15^+$	156 (43%)	10 (3%)	129 (30%)	15 (4%)	1.8	0.7–4.7	NS
$T^+/DR15^+$ vs. $T^-/DR15^-$	156 (43%)	4 (1%)	129 (30%)	53 (12%)	16.0	5.6–62.2	<0.0001
$T^+/DR15^-$ vs. $T^-/DR15^+$	190 (53%)	10 (3%)	229 (54%)	15 (4%)	1.2	0.5–3.2	NS
$T^+/DR15^-$ vs. $T^-/DR15^-$	190 (53%)	4 (1%)	229 (54%)	53 (12%)	11.0	3.9–42.4	<0.0001
$T^-/DR15^+$ vs. $T^-/DR15^-$	10 (3%)	4 (1%)	15 (4%)	53 (12%)	8.8	2.1–42.8	0.0007

MS = multiple sclerosis; OR = odds ratio; 95% CI = 95% confidence interval.

the −330 G/G genotype [18]. They suggested that the difference between the in vivo and in vitro influence of the −330 IL-2 promoter polymorphic site suggested the existence of additional, unknown polymorphisms that affect gene regulation [18].

Marrosu et al. [29] suggested that the MHC gene(s) might be primarily responsible for genetic susceptibility to MS. They also noted that the presence of complex interactions between different HLA haplotypes, other non-HLA predisposing genes and environmental factors might explain different associations in different populations [29]. It is well documented that HLA alleles may influence other genes in the pathogenesis of MS [30]. The lack of data on the effects of combinations of the IL-2 SNPs and HLA-DRB alleles on MS elucidates the need for such a study.

This study provides the first evidence that there is an interaction between the HLA-BRD1*1501 allele and the IL-2 −330 T allele in susceptibility to MS. Individuals carrying both the IL-2 −330 T and HLA-DRB1*1501 alleles had 16 times higher susceptibility to MS relative to those lacking both. Furthermore, having only one of these alleles was associated with approximately 9 to 11 times increased susceptibility to MS relative to having neither allele.

In conclusion, our study revealed that the IL-2 −330 T allele and the G/T and T/T genotypes were associated with a higher risk of developing MS in the studied population. In accordance with many previous reports, the HLA-DRB1*1501 allele was associated with a higher risk of developing MS in the studied population. We have provided evidence of an interaction between the IL-2 −330 T and HLA-DRB1*1501 alleles in the genetic susceptibility to MS.

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