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Interleukin 18 gene polymorphism is a risk factor for multiple sclerosis

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Abstract Proinflammatory cytokines with immunosuppressive properties play an important role in the pathogenesis of multiple sclerosis (MS). Interleukin 18 (IL-18) is one of the most important innate cytokines produced from macrophages in the early stages of the inflammatory immune response. The purpose of this study was to determine whether there was any relationship between *IL18* gene polymorphisms and MS. *IL18* genotyping were performed in 101 MS patients and 164 control subjects by using the PCR–restriction fragment length polymorphism (PCR–RFLP) method. The frequency of MS patients with the CC genotype of the *IL18* gene at position −137 was significantly higher than with the GG genotype [$p = 0.01$, odds ratio (OR) 3.17]. In haplotype analysis of two SNPs in the *IL18* gene, frequency of the CC haplotype was significantly higher in MS patients ($p = 0.002$, OR 3.0). However, the genotype distribution of the *IL18* −607 C/A polymorphism in the MS patient group was not significantly different from that of the control group. These data suggest that *IL18* gene polymorphisms at position −137 might be a genetic risk factor for MS in the Turkish population.

Keywords Interleukin 18 · Polymorphism · Multiple sclerosis · Cytokine

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Introduction

Multiple sclerosis (MS) is the major inflammatory condition affecting the central nervous system, and it is characterized by disseminated focal immune-mediated demyelination [1, 2]. Susceptibility to MS is thought to be conferred by the combination of genetic and environmental factors. Polymorphisms of many cytokine genes affect the transcriptional activities, resulting in individual variation in cytokine production was shown to be associated with MS [3, 4]. Cytokines, chemokines, and their receptors have an important role in the evolution of demyelinating lesions in the central nervous system, and the levels of proinflammatory and anti-inflammatory molecules have been found to correlate with changes in MS disease activity [5].

Interleukin-18 (*IL-18*) is a pleiotropic, proinflammatory cytokine that functions in the inflammatory cascade. *IL-18* is able to induce interferon-g (IFN-g), initially described as an interferon (IFN)-γ inducing factor [4]. IFN-γ, *IL-12*, and *IL-18* have been found in the brain, cerebrospinal fluid, and peripheral blood of MS patients, especially during acute exacerbations [6–11]. Experimental allergic encephalomyelitis (EAE) provides a key model for exploring disease mechanisms with potential relevance for MS, and increased production of *IL-18* was observed in the acute phase of EAE [9]. Moreover, anti-*IL-18* mAbs administered to rats during EAE prevented the development of lesions [12].

The human *IL18* gene is located on chromosome 11q22.2–q22.3. 11 different polymorphisms within the *IL18* gene have recently been identified [13]. Two of these polymorphisms, −607C/A (rs1946518) and −137G/C (rs187238), are known to influence expression from the *IL18* gene [14, 15]. At position −607, the change from cytosine to adenine disrupts a binding site for cyclic AMP responsive element-binding protein (CREB), and at position −137, the change from guanine to cytosine affects the human histone H4 gene-specific transcription factor-1 (H4TF-1) binding site. Upon stimulation, low promoter activity was observed in the A and C alleles at positions −607 and −137, respectively. Specifically, the haplotype that bears C at position −607 and G at position −137 was shown to be associated with a significantly higher expression of the *IL-18* protein [15].

Accordingly, functional polymorphisms in *IL18* gene promoters are attractive candidates, due to their impact on cytokine level production, and subsequently, on inter-individual disease susceptibility. The role of the other cytokine gene polymorphisms in MS, as a chronic immune-mediated neurodegenerative disease, has been previously reported in various populations [16]. However, few investigations have been undertaken on the association between *IL18* gene polymorphisms and MS. As such, in this study, we evaluated the possible associations of *IL18* rs187238 and rs187238 promoter polymorphisms with MS in the Turkish population.

Materials and methods

Study Subjects

Included in this study were 101 MS patients and 164 healthy subjects. The MS patients were followed up on a regular basis, every 3–6 months, at the neurology outpatient clinic of Bulent Ecevit University, Faculty of Medicine. Informed consent was obtained from all the patients who participated in this study, and the local ethics committee of Bulent Ecevit University, Faculty of Medicine approved the study.

The Kurtzke Expanded Disability Status Scale (EDSS) scores of the patients in the followup examinations and all the clinical and demographic data of the patients were retrospectively obtained from the hospital's computer database. The progression index [EDSS/duration of disease (years)] was determined in each patient included in the study.

Genomic DNA isolation and genotype analysis

Genomic DNA was extracted from 200 µl of peripheral blood, using a Macherey–Nagel DNA Isolation Kit (Cat No: 740.951.250) according to the manufacturer's instructions. A polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method was used to genotype *IL18* −607 C/A and −137 G/C polymorphisms. For each polymorphism, the PCR was performed in a 25-µl volume containing 10× PCR buffer, 3.0 mM $MgCl_2$, 0.25 mM dNTPs, 1.5 units of Taq polymerase (Promega, Madison, WI), and 0.3 µM each primers (F; 5′-GCC CTC TTA CCT GAA TTT TGG TAG CCC TC and R; 5′-AGA TTT ACT TTT CAG TGG AAC AGG AGT CC 3′) for −607 C/A polymorphism and (F; 5-ATG CTT CTA ATG GAC TAA GGA R; 5′-GTA ATA TCA CTA TTT TCA TGA ATT) for −137 G/C polymorphism.

−607 C/A polymorphism

The amplification conditions for −607 CA polymorphism were an initial denaturation at 95 °C for 5 min; then 35 amplification cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s; and a 7-min extension step at 72 °C. The PCR products were checked on a 1.5 % agarose gel for the assay completion, and then the PCR products of 171 base pair (bp) were digested with restriction enzyme *Tru9I* by 90 min incubation at 65 °C. The −607A allele was cut into two fragments of 101 and 70 bp, while the −607C allele remained uncut (171 bp).

−137 G/C polymorphism

The conditions for −137 GC polymorphism were 5 min of initial denaturation at 95 °C; followed by 35 cycles of 45 s at 95 °C, 45 min at 50 °C, and 1 min at 72 °C; and 7 min at 72 °C for the final extension. The PCR products were incubated overnight with 5 U of *Eco*RI restriction enzyme at 37 °C. The DNA obtained from the C/C homozygote individuals could not be digested by *Eco*RI, thus revealing a full-length 131-bp product. On the other hand, the DNA obtained from the G/G homozygote individuals was digested, and two fragments of 107 and 24 bp were produced. When all three bands were visualized, the individuals were genotyped as being G/C heterozygotes.

All digestion products were electrophoresed on 3 % agarose gel, visualized by staining with ethidium bromide, and evaluated using a gel documentation system (Syngene GeneGenius BioImaging System).

Statistical analysis

A case–control study was performed, and allelic and genotypic frequency of the polymorphism was calculated in both the patients and controls. The Hardy–Weinberg equilibrium was verified using the χ^2 test and by estimating the expected genotypic frequencies on the basis of the development of the square of the binomial for these polymorphisms. The χ^2 test was used to compare genotype and allele frequency of the *IL18* polymorphisms between MS

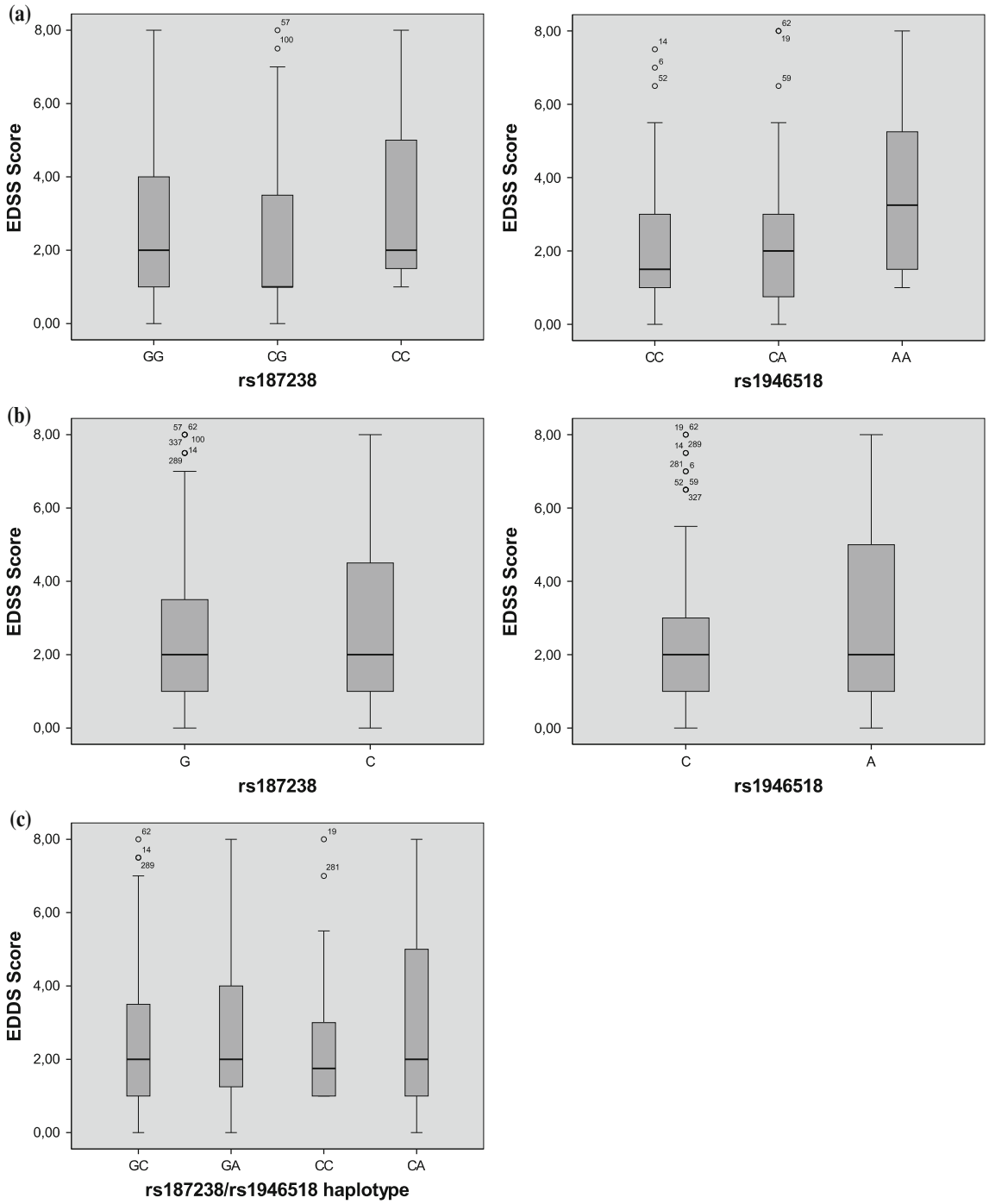

Fig. 1 EDSS score compared by **a** genotypes, **b** alleles, **c** haplotype of rs187238 and rs1946518 polymorphisms of IL-18 gene between groups

patients and controls. The association between *IL18* polymorphisms and MS was modeled through binary logistic regression analysis, and odds ratio (OR) and 95 % confidence interval (95 %CI) were calculated to compare the MS risk associated with the genotypes. Distribution of data was determined by the Shapiro–Wilks test. The Mann–Whitney U test was used to compare EDSS score with the alleles, genotypes, and haplotypes of the *IL18* gene polymorphisms. The software used for the calculations was SPSS version 18 (SPSS Inc., Chicago, IL).

Results

A total of 101 patients with MS (56 women and 45 men; mean age, 33.6 ± 9.2 years) and 164 control subjects (81

Table 1 Describes the distribution of the cases, and the controls by sex, age as appropriate and clinical types of MS

	MS Cases *n* (%)	Controls *n* (%)	*p* value
Total	101 (100)	164 (100)	
Age, years mean ± SD	33.6 ± 9.2	36.3 ± 10.03	0.59
Sex			0.37
Female	56 (52.0)	81 (54.0)	
Male	45 (48.0)	83 (46.0)	
Clinical types	*n* (%)		
Relapsing-remitting MS	77 (76.2)		
Secondary-progressive MS	22 (21.8)		
Progressive MS	2 (2)		

women and 83 men; mean age, 36.3 ± 10.03 years) participated in this study (Table 1). There were no significant differences in the distribution of age and gender between the MS patients and the controls ($p > 0.05$).

The distribution of each genotype for *IL18* in MS patients and controls is shown in Table 2. The frequencies of GG, GC, and CC genotypes for the *IL18* −137 G/C polymorphism were 46.5, 34.7, and 18.8 % in the patients and 62.2, 29.9, and 7.9 % in the controls, respectively. The *IL18* −137 CC genotype indicated an increased risk of developing MS (OR 3.17, 95 % CI 1.446–6.9581). In addition, the frequency of the *IL18* −607 C/A genotype was slightly higher in the patient group (49.0 %) compared with the control group (40.1 %), but this difference did not reach statistical significance (OR 1.580, 95 % CI 0.913–2.734).

Table 3 *IL18* rs187238/rs1946518 haplotypes and the risk of developing MS

Haplotype	Controls *n* (%)	MS patients *n* (%)	OR	95 % CI
rs187238/rs1946518				
GC	188 (57.3 %)	89 (44.1 %)	Reference	
GA	65 (19.8 %)	40 (19.8 %)	1.300	0.814–2.075
CC	19 (5.8 %)	27 (13.4 %)	3.002	1.585–5.686
CA	56 (17.1 %)	46 (22.8 %)	1.735	1.091–2.761

The patients were also compared with the control group according to clinical types (Table 2). In the Relapsing-Remitting MS (RR-MS) and Secondary-Progressive MS (SP-MS) patient groups, the frequency of the CC genotype for the *IL18* −137 G/C polymorphism was higher than that of the control group; these differences were significant ($p = 0.03$ and $p = 0.033$, respectively; Table 2). No statistical comparisons were made with the Primary Progressive MS (PP-MS) group, due to the small sample size ($n = 2$).

After grouping according to allele frequencies, the *IL18* −137 C allele was found to be a significant risk factor for MS (OR 1.909, 95 % CI 1.298–2.808), but the *IL18* −607 alleles were not associated with an increase in the risk of developing MS (Table 2). When the haplotypes for the *IL18* −137 and −607 polymorphisms were determined, CC haplotype frequency was higher in the patients (13.4 %) than in the controls (5.8 %), and the difference was significant (OR 3.002, 95 % CI 1.585–5.686, Table 3).

Table 2 *IL18* genotypes and alleles and the risk of developing MS

	Controls ($n = 164$) *n* (%)	Multiple sclerosis patients ($n = 101$) All *n* (%)	RR-MS *n* (%)	SP-MS *n* (%)	PP-MS *n* (%)	OR (95 % CI) for all MS
rs187238/Genotype						
GG	102 (62.2 %)	47 (46.5 %)	36 (46.8 %)	9 (40.9 %)	2 (100.0 %)	Reference
GC	49 (29.9 %)	35 (34.7 %)	28 (36.4 %)	7 (31.8 %)	0 (0.0 %)	1.550 (0.890–2.699)
CC	13 (7.9 %)	19 (18.8 %)	13 (16.9 %)	6 (27.3 %)	0 (0.0 %)	3.172 (1.446–6.958)
rs187238/Allele						
G	253 (77.1 %)	129 (63.9 %)	90 (65.2 %)	22 (61.1 %)	4 (100.0 %)	Reference
C	75 (22.9 %)	73 (36.1 %)	48 (34.8 %)	14 (38.9 %)	0 (0.0 %)	1.909 (1.298–2.808)
rs1946518/Genotype						
CC	72 (43.1 %)	34 (33.3 %)	26 (33.3 %)	8 (36.4 %)	0 (.0 %)	Reference
CA	67 (40.1 %)	50 (49.0 %)	40 (51.3 %)	8 (36.4 %)	2 (100.0 %)	1.580 (0.913–2.734)
AA	28 (16.8 %)	18 (17.6 %)	12 (15.4 %)	6 (27.3 %)	0 (0.0 %)	1.361 (0.663–2.794)
rs1946518/Allele						
C	211 (63.2 %)	118 (57.8 %)	84 (60.0 %)	18 (50.0 %)	2 (50.0 %)	Reference
A	123 (36.8 %)	86 (42.2 %)	56 (40.0 %)	18 (50.0 %)	2 (50.0 %)	1.250 (0.876–1.785)

EDDS scores were compared with the alleles, genotypes, and haplotypes of the *IL18* gene polymorphisms; results of this analysis indicated no association between EDDS scores and *IL18* gene polymorphisms ($p > 0.05$; Fig. 1).

Discussion

Some genes involved in the immune response are defined as susceptible genes for MS, among which cytokine genes are quite important, as MS is a cell-mediated autoimmune disease characterized by type-1 cytokine production [16–18]. IL-18 is a novel proinflammatory cytokine that plays an important role in Th-1 response through its ability to induce IFN-gamma production in T cells and NK cells. Environmental and individual genetic backgrounds might influence Th-1 response, particularly in cytokine gene polymorphisms [19]. In addition, it has been proposed that a variety of inflammatory and autoimmune diseases might share common pathogenic mechanisms and polymorphisms in the *IL18* gene and have been associated with several autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, and asthma [20–23].

The *IL18* gene is highly polymorphic and contains several polymorphisms that have been described in the promoter region of *IL18*. In particular, the *IL18* gene rs1946518 and rs187238 polymorphisms might be related to transcriptional regulation of this gene [15]. Therefore, in our study, these two SNPs at positions −607 and −137 were examined. We found that individuals carrying the CC genotype of the −137 polymorphism demonstrated increased MS risk, indicating that *IL18* polymorphisms might play a crucial role in MS development. The risk of MS was more than 3.3 times higher in individuals with the CC genotype compared to the GG genotype for the *IL18* −137 polymorphism. The reason for this difference might be a change from G to C at position −137, which disrupts the promoter H4TF-1 nuclear factor binding site [15, 24], resulting in low IL-18 production. However, no relationship was found between the −607 C/A polymorphism and MS. Haplotype frequencies were used to determine the genetic correlation between these two SNPs, and this analysis showed that −137C/−607C haplotype frequency was higher in the patients than in the controls. These data suggested that *IL18* played an important role in the immunopathogenesis of MS.

Gutcher et al. [25] investigated the role of IL-18 and its receptor in EAE, and they found that IL-18-deficient mice are susceptible to EAE. This observation supports our study, wherein the *IL18* −137C allele was associated with low IL-18 production, and low levels of IL-18 were associated with MS; therefore, the *IL18* −137C allele might carry a risk factor for MS. One paper has been published on *IL18* polymorphisms (promoter SNPs) in MS populations, but contrary to our study, no outstanding association was found between *IL18* genotypes and susceptibility to MS [15]. This difference in reports might be attributed to a number of factors including ethnic variability in *IL18* genotype distribution, which differs among ethnic populations, and to the type(s) of environmental factors involved in the pathogenesis of MS.

MS is an autoimmune disease, and *IL18* could be involved in MS through functional polymorphisms. In addition, it might contribute to enhanced innate immunity and Th-1-driven immune responses. This is the first report on MS susceptibility and *IL18* polymorphisms in the Turkish population. We speculated that genetic polymorphisms within the promoter region of the *IL18* gene might contribute to the pathogenesis of MS. Further studies in other populations are required to validate our findings.

In conclusion, we analyzed *IL18* gene polymorphisms in Turkish MS patients, using PCR–RFLP. Our findings suggest that the *IL18* −137G/C (rs187238) gene polymorphism increases susceptibility to MS.

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