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Association of interleukin-1 gene cluster polymorphisms and haplotypes with multiple sclerosis in an Iranian population

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ABSTRACT

Multiple sclerosis (MS) is a multi-factorial autoimmune disease of the central nervous system. The exact etiology of MS is still unknown. Due to the important roles that cytokines play as mediators in immune and inflammatory responses, we have evaluated the association of IL-1 gene cluster polymorphisms and haplotypes with MS susceptibility in 306 unrelated MS patients and 312 healthy matched controls. A significant association was found for the IL-1β +3953 T allele [OR = 1.43, 95% CI (1.14–1.79), *P* value = 0.002, *Pc* = 0.01] and for IL-1β +3953 T/T genotype and MS risk [OR = 1.92, 95% CI (1.25–2.96), *P* value = 0.005, *Pc* = 0.01]. Interestingly, the genotypes of the polymorphisms remained significant under recessive, co-recessive and dominant models. However, no significant differences were found between MS patients and controls in the genotype and allele frequencies of the IL-1β −511, −31 and IL-1Ra polymorphisms. Haplotype analysis for IL-1β −31 and IL-1β −511, with moderate linkage disequilibrium (LD), using the EM algorithm revealed a significant global association of haplotype differences between the two groups. Lower presence of two haplotypes (H3: C-T and H4: T-C) was observed in the MS patients than healthy controls. However, after applying Bonferroni's correction the differences were not significant. To our knowledge, this is the first study reporting the association of the IL-1β +3953 gene polymorphism and MS susceptibility.

1. Introduction

Multiple sclerosis (MS) is a multi-factorial autoimmune disease of the central nervous system (CNS) in which genetic, environmental and geographical factors are involved in the etiology of the disease (Handel et al., 2010, Kallaur et al., 2011). Environmental factors (e.g. vitamin D exposure, EBV infection and smoking) interact with genetic factors to produce an overall disease risk to MS (Handel et al., 2010, Pravica et al., 2012). Cytokines are important mediators in immune and inflammatory responses. Therefore, genes encoding cytokines can be suitable candidates for investigation into immunoinflammatory conditions, such as multiple sclerosis (Schrijver et al., 2003). Interleukin-1 (IL-1) is a multifunctional cytokine that is involved in several processes including immunoregulation, inflammation, neurodegeneration and neuroregeneration (Dinarello, 1996, Dinarello and Wolff, 1993).

Several reports have implicated the role of the IL-1 family in the MS pathophysiology (Schrijver et al., 2003). On the other hand, the presence of specific human gene single nucleotide polymorphisms (SNPs) in the non-coding regions of genes has been associated with susceptibility to several diseases (Knight, 2003). The IL-1 gene cluster is considered logical candidate for the loci determining susceptibility to the MS disease (Feakes et al., 2000). The IL-1 gene family (IL-1α, IL-1β and IL-1RA) located in a cluster on the long arm of human chromosome 2 (2q13–14), have several recognized polymorphisms (Dinarello, 1996). IL-1α and IL-1β are agonists and IL-1Ra is a specific receptor antagonist (Dinarello, 1996). Two biallelic bases-exchange polymorphism in the IL-1α gene have been described at position −889 C/T (rs1800587) and +4845 (rs17561), both are C to T single nucleotide polymorphism (SNP) (Erbek et al., 2007, Mcdonald et al., 2001). The IL-1β gene has three described biallelic polymorphisms at positions −511 (rs16944), −31 (rs1143627), and +3953 (rs1143634) from the transcriptional start site, all representing a C to T substitution (El-Omar et al., 2000, Di Giovine et al., 1992, Pociot et al., 1992). The IL-1Ra gene (IL-1RN) contains a variable number of tandem repeat (VNTR) polymorphism of 86 base pairs in intron 2 (Tarlow et al., 1993). The association of IL-1 gene polymorphisms with MS susceptibility has been investigated in several populations (Aggelakis et al., 2010, Borzani et al., 2010, Crusius et al., 1995, De La Concha et al., 1997, Dominici et al., 2002, Ferri et al., 2000, Hooper-Van Veen et al., 2003, Huang et al., 1996, Kantarci et al., 2000, Luomala et al., 2001, Mann et al., 2002, Mirowska-Guzel et al., 2011, Niino et al., 2001, Sarial et al., 2008,

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Schrijver et al., 1999, Sciacca et al., 1999); however, the findings are controversial. This may be partly due to small sample sizes and different populations (Huang et al., 2013). The purpose of the present study was to address the association between polymorphisms and haplotypes of the IL1 gene cluster and MS susceptibility in an Iranian population.

2. Materials and methods

2.1. Subjects

In this population-based association study, 618 Iranian subjects including 306 unrelated MS patients and 312 healthy controls were investigated for genetic variations in the IL-1 gene cluster. The study was approved by the Ethics Committee of Golestan University of Medical Sciences (No: 26279010188) and written informed consent was obtained from all patients and healthy control subjects. MS patients were diagnosed by expert neurologists based on clinical and paraclinical findings (MRI, oligoclonal bands in CSF, and evoked potentials) according to McDonald's criteria (Mcdonald et al., 2001). The control group consisted of 312 healthy subjects of the same ethnic origin as MS patients also matched for age and sex. They were recruited from the Blood Transfusion Organization of Gorgan city, northeast of Iran. None of them had a history of autoimmune or inflammatory diseases. A demographic questionnaire that included sex, age, age at onset, expanded disability status scale (EDSS) and the type of MS was prepared for MS patients. The demographic data for MS patients is summarized in Table 1.

2.2. Nucleic acid extraction and genotyping

DNA was extracted from peripheral blood cells as previously described (Shahbazi et al., 2002). Polymorphisms of the IL-1 gene cluster including IL-1α −889, IL-1β −511, IL-1β −31, and IL-1β +3953 were genotyped by sequence-specific primer-polymerase chain reaction (SSP-PCR) method as described previously (Bioque et al., 1995, Hayashi et al., 2009, Hutyrova et al., 2002, Tarlow et al., 1993). An internal positive control primer pair, which amplifies a conserved region of the Human Growth Hormone gene, is included in every PCR reaction mix. After PCR and agarose gel electrophoresis, the genotyping results were evaluated by the presence or absence of an allele-specific PCR product (graphical abstract). A subset of samples was subject to DNA sequencing to confirm the genotyping results (data not shown). Each allele of IL-1RA VNTR was identified according to its size, as previously described by others (Hutyrova et al., 2002).

2.3. Statistical analysis

Genotype frequencies were tested for Hardy–Weinberg equilibrium by $\chi 2$ analysis. The possible differences in genotype and allele frequencies were assessed by the Pearson's Chi-square test, and the risk associated with genotypes/alleles was calculated as the odds ratio (OR) with 95% confidence intervals (CIs). A multiple logistic and linear regression analysis was performed to evaluate possible associations between the variables. All statistical analysis was performed using the SPSS software, version 16. Differences were considered significant when the *P* value was <0.05. Bonferroni correction for multiple testing was used for significant *P* values of the genotypes. Corrected (*Pc*) values <0.05 were considered significant.

Table 1
demographic data of MS patients (n = 306).

Mean age	32.2 ± 8.6 years
Female/male	183/123
EDSS	3.4 ± 1.9
Age at onset	26 ± 7
Duration (years)	
Clinical subtype	
Relapsing-remitting (RR)	254 (83%)
Primary-progressive (PP)	44 (14.4%)
Secondary-progressive (SP)	4 (1.3%)
Progressive-relapsing (PR)	4(1.3%)

Abbreviations: EDSS expanded disability status scale.

2.4. Linkage disequilibrium (LD) and haplotype analysis

The estimation of haplotype frequency and the analysis of associations between different haplotypes and MS risk were implemented with SNPStats online software [http://bioinfo.iconcologia.net/snpstats/start.htm] using the expectation–maximization (EM) algorithm (Sole et al., 2006). Also, LD analysis was performed using this program based on the genotype data of the IL-1β −31 (rs1143627), and IL-1β −511 (rs16944) polymorphisms.

3. Results

To examine the association of IL-1 cluster gene polymorphisms with MS, five polymorphic regions (four SNPs and one VNTR) were investigated as follows. The distribution of genotypes and alleles of the IL-1α −889, IL-1β −511, IL-1β −31, IL-1β +3953 and IL-1Ra VNTR in healthy controls and MS patients is shown in Table 2.

Table 2
Genotype and allele frequencies of IL-1 gene polymorphisms in MS patients and healthy controls.

Genotype and allele	Healthy controls (n = 312)	MS patients (n = 306)	OR (95% CI)	*P* value	P_c value
IL-1α (−889) (C/T)					
C	323 (51.8%)	351 (57.4%)	Reference	–	
T	301 (48.2%)	261 (42.6%)	0.798 (0.638–0.999)	0.048	1
CC	85 (27.2%)	107 (35%)	Reference	–	
CT	153 (49.1%)	137 (44.8%)	0.711 (0.493–1.026)	0.068	
TT	74 (23.7%)	62 (20.2%)	0.666 (0.428–1.035)	0.071	
IL-1β (−511) (C/T)					
C	323 (51.8%)	310 (50.7%)	Reference	–	
T	301 (48.2%)	302 (49.3%)	1.045 (0.836–1.307)	0.696	
CC	82 (26.3%)	74 (24.2%)	Reference	–	
CT	159 (51%)	162 (52.9%)	1.129 (0.770–1.656)	0.535	
TT	71 (22.7%)	70 (22.9%)	1.093 (0.693–1.723)	0.704	
IL-1β (−31) (C/T)					
C	347 (55.6%)	334 (54.6%)	Reference	–	
T	277 (44.4%)	278 (45.4%)	1.043 (0.833–1.305)	0.715	
CC	102 (32.7%)	96 (31.4%)	Reference	–	
CT	143 (45.8%)	142 (46.4%)	1.055(0.734–1.516)	0.772	
TT	67 (21.5%)	68 (22.2%)	1.078 (0.696–1.670)	0.735	
IL-1β (+3953) (C/T)					
C	347 (56%)	286 (47%)	Reference	–	
T	277 (44%)	326 (53%)	1.428 (1.141–1.786)	**0.002**	**0.012**
CC	104 (33.3%)	73 (23.9%)	Reference	–	
CT	139 (44.5%)	140 (45.8%)	1.43 (0.98–2.10)	**0.0001**	**0.002**
TT	69 (22.1%)	93 (30.4%)	1.92 (1.25–2.96)	**0.005**	0.01
IL-1Ra VNTR					
A_1	414(66.3%)	395 (64.5%)	Reference	–	
A_2	198(31.7%)	200 (32.7%)	0.673(0.318–1.428)	0.303	
A_3	12(2%)	17 (2.8%)	0.713(0.332–1.532)	0.386	
A_1A_1	162(52%)	148 (48.4%)	Reference	–	
A_2A_2	60(19.2%)	59 (19.3%)	1.076(0.705–1.643)	0.733	
A_1A_2	78(25%)	82 (26.8%)	1.151(0.786–1.686)	0.471	
A_1A_3	12(3.8%)	17 (5.5%)	1.551(0.717–3.355)	0.265	

Abbreviations: MS Multiple sclerosis, IL Interleukin, A allele, VNTR variable number of tandem repeats, OR odds ratio, CI confidence interval, P_c value = corrected *P* value after including Bonferroni's multiple testing correction [*P* value multiplied by the number of comparisons].

3.1. IL-1α polymorphism

There was no association between IL-1α-889 genotypes with MS (Table 2). Comparison of the IL-1α-889 genotypes revealed that the C/C genotype was overrepresented in MS patients compared with healthy controls (35% versus 27.2%), whereas the frequency of C/T and T/T genotypes in healthy controls were more than MS patients (44.8% versus 49.1% and 20.2% versus 23.7%, respectively). However, comparison of IL-1α −889 T risk allele frequencies in the subject and control groups revealed a negative, borderline- significant association with MS [OR = 0.798, 95% CI (0.638–0.999), *P* value = 0.048]. However, it failed to remain significant after applying Bonferroni correction.

3.2. IL-1 β polymorphisms

Three polymorphic regions of IL-1 β gene, including IL-1β −511, −31 and +3953 were investigated. The distribution of the IL-1β −511 genotypes and alleles did not significantly differ between the MS patients and healthy controls (Table 2). No significant differences were observed between MS patients and healthy controls in the genotype and allele frequencies of the IL-1β −31 polymorphism (Table 2).

For IL-1β +3953 gene polymorphism, distribution of the C/T genotype was significantly different between the MS patients and healthy controls when compared to the C/C reference genotype [OR = 1.43, 95% CI (0.98–2.10), *P* = 0.0001, P_c = 0.0002] (Table 2). The T/T genotype frequency of IL-1β +3953 in the MS patients and healthy controls was also significantly different when compared to the C/C reference genotype [OR = 1.92, 95% CI (1.25–2.96), *P* = 0.005, P_c = 0.001] (Table 2). The T allele frequency, compared to the C reference allele, was significantly higher in MS patients than the controls [OR = 1.428, 95% CI (1.141–1.786), *P* value = 0.002, P_c = 0.012] (Table 2).

3.3. IL-1Ra polymorphism

No significant differences were observed between MS patients and healthy controls in the genotype and allele frequencies of the IL-1Ra polymorphism. A2/A3 and A3/A3 genotypes were not observed in our study (Table 2).

3.4. Association of IL-1 gene polymorphisms with MS under different inheritance models

The association of the four SNPs in the IL-1 gene with MS was estimated by calculating pooled odds ratio (OR) and 95% confidence intervals (CI) under co-dominant, dominant and recessive genetic models. Based on the assumption that T, the more frequent allele, is a risk allele, these genetic models compares C/C genotypes to C/T + T/T genotypes in dominant model and C/C + C/T to T/T genotypes in recessive model. In the co-dominant model, the genotype frequencies were compared between the MS patients and healthy controls and a significant association with MS was observed in IL-1β, +3953 (OR = 1.43, 95% CI (0.98–2.10) *p* = 0.011, P_c = 0.022) (Table 3). Also, significant association was observed in IL-1β, +3953 (OR = 1.60, 95% CI (1.12–2.27) *P* = 0.009, P_c = 0.0018) when the effect of the polymorphism was considered under a dominant genetic model (Table 4). In addition, a significant association was found in IL-1β, +3953 (OR = 1.54, 95% CI (1.07–2.21) *p* = 0.019, P_c = 0.038) under a recessive genetic model (C/C + C/T vs. T/T) (Table 5).

3.5. Association of IL-1 gene haplotypes with MS

Since only rs1143627 (IL-1β −31), and rs16944 (IL-1β −511) positions showed moderate LD (D′ = 0.1546, r = 0.143 and *P* value = 0.00001), haplotype analysis was only performed for these SNPs (Table 6). Therefore, the SNP order which defined the haplotype structure was rs1143627 (IL-1β −31), and rs16944 (IL-1β −511), respectively. Haplotype analysis with the EM algorithm revealed a significantly higher presence of two haplotypes H3 (CT) and H4 (TC) in healthy controls than the MS patients (Global haplotype association *p*-value = 0.025) (Table 6), thereby suggesting protective roles for them against MS. However, neither of them remained significant after applying Bonferroni's multiple testing corrections.

4. Discussion

The imbalance between pro- and anti-inflammatory cytokines is one of the landmarks in MS development and progression (Link, 1998, Mihailova et al., 2005). Thus, specific gene polymorphisms in IL-1 gene, as a pro-inflammatory cytokine, may contribute to the pathogenesis of MS (Dinarello, 1996, Huang et al., 2013, Reboul et al., 2000, Schrijver et al., 2003, Trevilatto et al., 2011) through dysregulation of IL-1 expression. Several studies have been carried out to test the hypothesis that genetic polymorphisms in the IL-1 gene family might be associated with MS risk; however data have yielded conflicting results. In this regard, more replication studies on different populations may be help establish a valid genotype–phenotype association (Chanock et al., 2007). In the present study, we assessed the association of IL-1 gene cluster polymorphisms with susceptibility to MS in 618 Iranian subjects including 306 MS patients and 312 healthy-matched controls. The functional significance of the promoter region in the regulation of IL-1α gene expression has been shown in several studies (Dominici et al., 2002, Hulkkonen et al., 2000, Mora et al., 1990). Dominique R, et al. showed that the IL-1α (−889) C/C genotype was associated with a significantly lower gene transcription and lower level of IL-1α in plasma compared with the T/T genotype (Dominici et al., 2002). Also, Shirodaria S, et al. showed that the presence of the T allele in the IL-1α −889 C/T promoter is associated with a fourfold increase in IL-1α expression (Shirodaria et al., 2000). Contradictory findings have reported no evidence for the association between the IL-1α −889 C/T polymorphism and MS risk (Huang et al., 2013). No association was

Table 3
polymorphisms in the IL-1 cluster gene polymorphisms and MS risk under Co-dominant model.

Position	genotype	Healthy controls (n = 312)	MS patients (n = 306)	OR (95%CI)	*P* value	$P_c$value
IL-1α (−889) (C/T)	C/C	85 (27.2%)	107 (35%)	1.00	–	
	C/T	153 (49%)	137 (44.8%)	0.71 (0.49–1.03)	0.11	
	T/T	74 (23.7%)	62 (20.3%)	0.67 (0.43–1.04)		
IL-1β (−511) (C/T)	C/C	82 (26.3%)	74 (24.2%)	1.00	–	
	C/T	159 (51%)	162 (52.9%)	1.13 (0.77–1.66)	0.82	
	T/T	71 (22.8%)	70 (22.9%)	1.09 (0.69–1.72)		
IL-1β (−31) (C/T)	C/C	102 (32.7%)	96 (31.4%)	1.00	–	
	C/T	143 (45.8%)	142 (46.4%)	1.06 (0.73–1.52)	0.94	
	T/T	67 (21.5%)	68 (22.2%)	1.08 (0.70–1.67)		
IL-1β (+3953) (C/T)	C/C	104 (33.3%)	73 (23.9%)	1.00	–	
	C/T	29 (9.3%)	55 (18%)	1.43 (0.98–2.10)	**0.011**	**0.022**
	T/T	69 (22.1%)	93 (30.4%)	1.92 (1.25–2.96)		

$P_c$value = corrected *P* value after including Bonferroni's multiple testing correction (*P* value multiplied by the number of *comparisons*).

Table 4
polymorphisms in the IL-1 cluster gene polymorphisms and MS risk under Dominant model.

Position	genotype	Healthy controls (n = 312)	MS patients (n = 306)	OR (95%CI)	*P* value	P_c value
IL-1α (−889)	C/C	85 (27.2%)	107 (35%)	1.00	–	
	C/T–T/T	227 (72.8%)	199 (65%)	0.70 (0.49–0.98)	**0.038**	0.078
IL-1β (−511)	C/C	82 (26.3%)	74 (24.2%)	1.00	–	
	C/T–T/T	230 (73.7%)	232 (75.8%)	1.12 (0.78–1.61)	0.55	
IL-1β (−31)	C/C	102 (32.7%)	96 (31.4%)	1.00	–	
	C/T–T/T	210 (67.3%)	210 (68.6%)	1.06 (0.76–1.49)	0.73	
IL-1β (+3953)	C/C1	04 (33.3%)	73 (23.9%)	1.00	v	
	C/T–T/T	208 (66.7%)	233 (76.1%)	1.60 (1.12–2.27)	**0.009**	**0.018**

$P_c$value = Corrected *P* value after including Bonferroni's multiple testing correction (*P* value multiplied by the number of *comparisons*).

observed between IL-1α-889 genotypes with MS in our population in line with previous studies (Huang et al., 2013).

However, a mild statistically significant difference was observed between the frequency of the T allele in healthy controls and MS patients (48.2% versus 42.6%, $P = 0.048$) (Table 2) which did not remain significant after correction by Bonferroni's method. This finding supports previous reports (Huang et al., 2013). Also, a significant association found between MS and the IL-1α −889 C/T polymorphism under a dominant genetic model failed to persist after Bonferroni's correction (OR = 0.70 (95% CI) 0.49–0.98, $P = 0.038$, $Pc = 076$) (Table 4). The result of Sarial S et al. showed that the IL-1α (−889) T/C and T/T genotypes were associated with MS disease in an Iranian population (Sarial et al., 2008). Here, differences were also observed between MS and control groups in TC and TT genotype frequencies, but they were not statistically significant. Elevated levels of IL-1 β cytokine within the MS lesions and sera of MS patients have been described previously (Cannella and Raine, 1995, Dujmovic et al., 2009, Heesen et al., 2000). To date, several studies with debatable conclusions have been to perform to explain the role of genetic polymorphisms in IL-1β expression. Association of IL-1β C-511T polymorphisms with the susceptibility to several diseases has been reported (Nemetz et al., 2001, Wang et al., 2003, Wang et al., 2002), however, no association has been found between IL-1β C-511T polymorphisms and MS risk in previous studies (Aggelakis et al., 2010, Ferri et al., 2000, Hooper-Van Veen et al., 2003, Luomala et al., 2001, Mann et al., 2002, Mirowska-Guzel et al., 2011, Niino et al., 2001), except for a few cases in recent years. The results of Borzani I et al. showed the association between the IL-1β C-511T variant and MS in an Italian Caucasian population (Borzani et al., 2010). Also, the results of Isik N, et al. suggested a protective role for the 2/2 genotype of IL-1β −511 in the Turkish population (Isik et al., 2013). However, meta-analyses failed to find an association between the IL-1β C-511T and MS risk (Huang et al., 2013, Nikolopoulos et al., 2011).

In the present study, we found no significant difference in the allele frequency of the IL-1β C-511T variant between healthy controls and MS patients (OR = 1.045, 95% CI (0.836–1.307) $P = 0.696$) (Table 2). (Ferri et al., 2000, Luomala et al., 2001, Niino et al., 2001, Mann et al., 2002, Hooper-Van Veen et al., 2003, Aggelakis et al., 2010, Mirowska-Guzel et al., 2011). Also, no association was observed between MS and the IL-1β C-511T polymorphism when it was considered under co-dominant, dominant and recessive genetic models (Table 2). This finding is consistent with those of some previous investigations (Huang et al., 2013). The results of Kimura et al. showed that expression of the IL-1β −31T allele is significantly higher than that of the IL-1β −31C allele in EBV-transformed lymphoblastoid cell lines (Kimura et al., 2004). This finding may correspond to the fact that -31T allele is located in a TATA box facilitating the formation of the transcription initiation complex (El-Omar et al., 2000), and the change from IL-1β −31T to IL-1β −31C (e.g. the change of motif from TATAAA to CATAAA) leads to decrease efficiency of the promoter, thereby decreasing the IL-1β expression (Hayashi et al., 2009, Lind et al., 2007, Wobbe and Struhl, 1990). In this study, the allele frequency of IL-1β −31T was not significantly higher in the MS patients (OR = 1.043, 95% CI (0.833–1.305) $P = 0.715$) (Table 2). Also, no association was observed between the MS risk and the IL-1β −31C > T polymorphism when it was considered under co-dominant, dominant and recessive genetic models (Table 2). In our population, in agreement with recent findings in the Italian population (Borzani et al., 2010), we did not find any association between the IL-1β -31C > T polymorphism and MS.

A polymorphism in the IL-1β +3953, especially the IL-1β +3953 T allele has been associated with the increased IL-1β synthesis in several immune-inflammatory diseases (Chen et al., 2006, Ferreira et al., 2008, Gore et al., 1998, Hulkkonen et al., 2000, Pociot et al., 1992). However, previous reports failed to find evidence supporting the contribution of the IL-1β +3954 gene in susceptibility to MS (Aggelakis et al., 2010, Borzani et al., 2010, Dincic et al., 2006, Hooper-Van Veen et al., 2003, Kantarci et al., 2000, Luomala et al., 2001, Mann et al., 2002, Niino et al., 2001, Schrijver et al., 1999, Wansen et al., 1997). Our results support the possible role of IL-1β +3953 polymorphism in susceptibility to MS in the studied Iranian population. A significant association was found for the IL-1β +3953 T/T genotype and MS risk [OR = 1.92, 95% CI (1.25–2.96), *P* value = 0.005, $Pc = 0.01$]. Interestingly, the risk IL-1β +3953T allele was significantly higher than the common IL-1β +3953 C allele in MS patients [OR = 1.428, 95% CI (1.141–1.786), *P* value = 0.002, $P_c = 0.01$]. This inconsistency may be due to ethnicity-related differences in the frequencies of polymorphic alleles. In addition, there was a significant association between IL-1β +3953T/C polymorphism and MS risk in our population when the effect of the polymorphism was considered under co-dominant and recessive genetic models (for more detail, see Table 2).

Table 5
polymorphisms in the IL-1 cluster gene polymorphisms and MS risk under Recessive model.

Position	genotype	Healthy controls (n = 312)	MS patients (n = 306)	OR (95% CI)	*P* value	P_c value
IL-1α (−889)	C/C–C/T	238 (76.3%)	244 (79.7%)	1.00		
	T/T	74 (23.7%)	62 (20.3%)	0.82 (0.56–1.20)	0.3	
IL-1β (−511)	C/C–C/T	241 (77.2%)	236 (77.1%)	1.00	–	
	T/T	71 (22.8%)	70 (22.9%)	1.01 (0.69–1.47)	0.97	
IL-1β (−31)	C/C–C/T	245 (78.5%)	238 (77.8%)	1.00	–	
	T/T	67 (21.5%)	68 (22.2%)	1.04 (0.71–1.53)	0.82	
IL-1β (+3953)	C/C–C/T	243 (77.9%)	213 (69.6%)	1.00	–	
	T/T	69 (22.1%)	93 (30.4%)	1.54 (1.07–2.21)	**0.019**	**0.019**

Pc value = Corrected *P* value after including Bonferroni's multiple testing correction (*P* value multiplied by the number of *comparisons*).

Table 6
Analysis of haplotype frequencies and their association with MS disease, adjusted by sex and age.

Haplotype	IL-1β −31	IL-1β −511	Healthy controls	MS patients	OR (95% CI)	*P* value	$P_c$value
H1	C	C	29.08%	35.02%	1	–	
H2	T	T	21.7%	29.8%	1.07 (0.78–1.45)	0.69	
H3	C	T	26.53%	19.55%	0.66 (0.45–0.96)	**0.029**[a]	0.087
H4	T	C	22.69%	15.63%	0.65 (0.45–0.94)	**0.023**[b]	0.069
Global haplotype association *P*-value		0.025[c]					

Data are expressed as (%). Haplotype frequencies and *P* values were estimated using the implementation of the EM algorithm executed by SNPStats. The most frequent haplotype among patients served as the referent.
Pc value = Corrected *P* value after including Bonferroni's multiple testing correction (*P* value multiplied by the number of *comparisons*).
[a] These haplotypes were negatively associated with susceptibility to MS.
[b] These haplotypes were negatively associated with susceptibility to MS.
[c] Significant global haplotype association *P* value.

The interleukin-1 receptor antagonist (IL-1ra) is a naturally occurring antagonist that competes with IL-1β for the IL-1 receptor, thereby preventing activation of the target cells (Arend, 1991). Previous studies performed in the animal model for MS and experimental autoimmune encephalomyelitis (EAE) indicated that IL-1Ra might affect disease susceptibility and progression (Badovinac et al., 1998). However, the influence of IL-1ra VNTR polymorphism on MS risk is controversial (Crusius et al., 1995, Kantarci et al., 2000, Mann et al., 2002, Niino et al., 2001, Schrijver et al., 1999, Sciacca et al., 1999, Wansen et al., 1997). Previous studies have reported the association of different IL-1ra alleles with MS, including the A2 allele (Crusius et al., 1995), the A1 allele (Sciacca et al., 1999) and the rare IL-1ra allele 3 (Kantarci et al., 2000). However, we could not find any association between these polymorphisms and MS risk. Thus, our results are in line with previous studies (Huang et al., 2013). Although five alleles have, so far, been described in IL-1ra VNTR polymorphism, two alleles (A1 and A2) account for approximately 99% of all identified IL-1ra polymorphisms (Tarlow et al., 1993). In this study, approximately 97.6% of identified IL-1ra alleles were A1 and A2, and A3 comprised 2.4% (see Table 2).

In this study, a significant association was found between IL-1β + 3953 T/C polymorphism and MS risk in our population under co-dominant, dominant and recessive genetic models (for more detail, see Tables 3-5). However, no association was observed between MS and either of the IL-1β −511C > T or IL-1β −31C > T polymorphisms under all genetic models (Table 2). Statistically significant LD was identified among IL1β −31 (rs1143627) and the IL1β −511 (rs16944) alleles (D' = 0.1546, r = 0.143 and *P* value = 0.00001). Our finding was in agreement with the study of Borzani et al. in an Italian population (Borzani et al., 2010). Furthermore, Haplotype analysis initially identified two haplotypes in IL-1 gene (H3 and H4) that were negatively associated with susceptibility to MS (*p* value = 0.029 and 0.023, respectively (Table 6). Upon applying bonferroni's multiple testing correction, neither of these haplotypes remained significant. Since the latter correction is very conservative and may sometimes lead to false negative results the data should be interpreted cautiously.

5. Conclusion

Our data suggest that the IL-1β + 3953 T/C gene polymorphism may be associated with susceptibility to MS in the Iranian population, in contrast to previous studies. Given that there are no previous reports about the association of the IL-1β + 3953 T/C polymorphism and MS, this association may be due to ethnic differences. Although our results together with the others contribute to understanding the genetic susceptibility of MS risk, further comprehensive clinical and molecular studies are needed to elucidate the role of IL-1 family in MS.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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