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Combination of interleukin-10 gene promoter polymorphisms with HLA-DRB1*15 allele is associated with multiple sclerosis

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***Background & objectives*: Multiple sclerosis (MS) is common in some ethnic groups. Interleukin-10 (IL-10) is a potent anti-inflammatory and immunosuppressive cytokine that may be an important regulator in MS disease pathogenesis. IL-10 promoter includes several single nucleotide polymorphisms and the level of IL-10 expression is related to these polymorphisms. Furthermore, loci within the histocompatibility regions are responsible for susceptibility to MS. The aim of this study was to investigate the association of IL-10 gene promoter polymorphisms and HLA-DRB1*15 allele frequencies with MS susceptibility in an Iranian population.**

***Methods*: In this study 336 MS patients and 454 healthy controls were included. Genomic DNA was purified from peripheral blood samples by a standard protocol. Genotyping was performed by the sequence-specific primer polymerase chain reaction method.**

***Results*: IL-10 −1082 G/G and IL-10 −819 C/C genotypes were more frequent in MS patients than healthy individuals. DRB1*15 allele showed a higher frequency among MS patients compared to controls.**

***Interpretation & conclusions*: The IL-10 and HLA-DRB1*15 polymorphisms were associated with the susceptibility to MS in Iranian patients. Our results suggest that gene-gene interaction of IL-10 polymorphisms and HLA-DRB1*15 alleles may be important factors in the development of MS.**

Key words Cytokine - HLA-DRB1*15 allele - interleukin-10 - multiple sclerosis - single nucleotide polymorphism

Multiple sclerosis (MS) is the most common demyelinating disorder, mostly affecting young women. According to the previous studies concerning the incidence rate of MS[1,2], Iran was categorized as a low-risk region[3]. However, a later study showed that the incidence of MS was more than 35/100,000 in Isfahan, Central Province of Iran[4]. The autoimmune process results in the destruction of myelin sheaths surrounding axons of the central nervous system leading to neurodegeneration and permanent neurologic deficits[4]. Though the initiating mechanism is not clear, it seems that the activation of pro-inflammatory cytokine cascade, besides dysregulation of anti-inflammatory processes, has an important role in the pathogenesis of the disease[5].

Since the incidence of MS is found to be significantly higher in monozygotic twins in comparison to dizygotic twins[6], a genetic basis is suggested and the role of human leukocyte antigens (HLAs) is now widely accepted, especially in the case of HLA-DRB1*15[7]. Further, it has been shown that polymorphisms in the genes encoding cytokines may play a role in the susceptibility to MS[8] or disease course[9]. Ramagopalan *et al*[10], in a large cohort study, have shown that HLA-DRB1*15 allele is overtransferred to MS patients, especially from their mothers. In a collaborative genome-wide association study involving 9772 cases of European descent, the identity of the HLA-DRB1 risk alleles was refined in MS patients. It has been confirmed that variation in the HLA-A gene underlies the independent protective effect attributable to the Class I region[11]. Tuwir *et al*[12] found that Irish individuals who were positive for the HLA-DRB1*15 allele were more susceptible to develop optic neuritis. They also found that people with optic neuritis and HLA-DRB1*15 allele were at higher risk for developing MS.

Interleukin 10 (IL-10) is an anti-inflammatory cytokine mainly produced by macrophages and T-lymphocytes during autoimmune and infectious diseases[13,14]. The progression or recovery of MS seems to have a direct association with the amount of IL-10 production[15]. The production of IL-10 is regulated by its gene located on chromosome 1q31-32[16]. The three single nucleotide polymorphisms (SNPs) in the promoter region of IL-10 gene, 1082 G/A, 819 T/C and 592 A/C, have been shown to be in association with high or low production of this cytokine[16]. Myhr *et al*[17] have reported that −819 and −592 alleles have complete linkage where TA/CC, TT/CC and AA/CC haplotype combinations are formed. It is proposed that these haplotypes determine IL-10 mRNA levels and regulate its production. Mihailova *et al*[18] found significant differences in the frequency of genotypes between MS patients and controls in Bulgarian population. Luomala *et al*[19] demonstrated that these polymorphisms were in correlation with disease severity through the regulation of production of IL-10 but not with susceptibility to disease.

For detecting the genetic role in susceptibility to MS disease, previously we showed the correlation between polymorphisms in the CCR5 (CC chemokine receptor 5), IL-6 (Interleukin 6), IL-2 (Interleukin 2), TNF-α (Tumor necrosis factor alpha) and CD-24 genes with MS[20-23]. In this study, the frequency of HLA-DRB1*15 allele and SNPs in the IL-10 promoter region were studied among Iranian MS patients and healthy controls to evaluate the possible role of IL-10 gene polymorphism in MS.

Material & Methods

A total of 336 patients and 454 healthy controls were screened for genetic variations in the promoter region of the IL-10 gene and HLA-DRB1*1501 allele in the Medical Cellular and Molecular Research Center, Gorgan, Iran. Sampling was carried out from volunteer patients during 2009-2011. MS patients were diagnosed by expert neurologists based on clinical and paraclinical findings (magnetic resonance imaging, oligoclonal bands in the cerebrospinal fluid and evoked potentials) according to the McDonald's criteria[24]. The sample size was calculated to have the minimum effect size with at least 80 per cent power and significance of 95 per cent under the dominance model by the Quanto software V-1.2 (University of Southern California, USA) based on frequency of disease. Moreover, the minor allele frequency was chosen to be 10 per cent and a type 1 error level of 0.05.

Control individuals were people referred to Gorgan blood donation centres in Golestan province who were matched by sex, ethnicity and age, and there were no autoimmune or inflammatory disorders in their history. A pretested demographic questionnaire was used and which included sex and age for both groups, age at onset and expanded disability status scale for MS patients. Demographic information of MS patients is shown in Table I. The mean age for MS patients was

Table I. Demographic data of multiple sclerosis (MS) patients (n=336)

Parameter	MS patients (n=336)
Mean age (yr), mean±SD	36.8±8.6
Female/male	239/97
EDSS, mean±SD	3.4±1.9
Age at onset (yr), mean±SD	27±8
Duration of the disease (yr)	5.8±3.9
Clinical subtype, n (%)	
RR	283 (84.2)
PP	44 (13.1)
SP	5 (1.5)
PR	4 (1.2)

EDSS, expanded disability status scale; RR, relapsing-remitting; PP, primary-progressive; SP, secondary-progressive; PR, progressive-relapsing

Table II. Primer sequences used for the sequence-specific primer genotyping method

Primer position	Primer sequence
hGH	
Sense	5'-GCCTTCCCAACCATTCCCTTA-3'
Antisense	5'-TCACGGATTTCTGTTGTGTTTC-3'
IL-10 (−1082)	
G	5'-CTA CTA AGG CTT CTT TGG GAG-3'
A	5'-ACT ACT AAG GCT TCT TTG GGA A-3'
GEN	5'-CAG TGC CAA CTG AGA ATT TGG-3'
IL-10 (−819)	
C	5'-CCC TTG TAC AGG TGA TGT AAC-3'
A	5'-ACC CTT GTA CAG GTG ATG TAA T-3'
GEN	5'-AGG ATG TGT TCC AGG CTC CT-3'

IL, interleukin; HGH, human growth hormone

Table III. Frequencies of interleukin-10 −1082 alleles and genotypes in patients and controls

Allele and genotypes	Control n (%)	MS n (%)	OR	95% CI	*P*
G	382 (42)	337 (50)	1.00	-	-
A	526 (58)	335 (50)	1.38	1.13-1.69	0.001
G/G	47 (10)	53 (16)	1.00	-	-
G/A	288 (63)	231 (69)	1.98	1.36-2.89	<0.001
A/A	119 (26)	52 (15)	2.59	1.54-4.35	

MS, multiple sclerosis; OR, odds ratio; CI, confidence interval

Table IV. Frequencies of interleukin-10 −819 alleles and genotypes in patients and controls

Allele and genotypes	Control n (%)	MS n (%)	OR	95% CI	*P*
C	560 (62)	442 (66)	1.00	-	-
T	348 (38)	230 (34)	0.83	0.68-1.03	0.09
CC	192 (42)	144 (43)	1.00	-	-
CT	176 (39)	154 (46)	1.21	0.88-1.65	0.005
TT	86 (19)	38 (11)	0.59	0.38-0.91	

MS, multiple sclerosis; OR, odds ratio; CI, confidence interval

36.8±8.6 yr (ranging from 18-62 yr) and for controls was 35.45±6.9 yr (ranging from 21-62 yr).

The study was approved by the Ethics Committee of Golestan University of Medical Sciences (No: 100590041313), and written informed consent was obtained from all patients and healthy controls.

DNA extraction and genotyping: Genomic DNA was purified from 10 ml peripheral blood samples by a standard protocol with some modifications[25]. Genotyping was done by sequence-specific primer polymerase chain reaction (SSP-PCR) method using a Thermal Cycler (Techne, UK) as described previously[21]. An internal positive control primer pair, which amplifies a conserved region of the human growth hormone gene was sourced from " TIB MOLBIOL" company (Berlin, Germany) and was included in every PCR reaction mix. The sequence of the primers is shown in Table II.

After PCR, the electrophoresis was performed in 2 per cent agarose gel (Merck, Germany) and PCR product bands were visualized by a gel documentation system (UVITEC, UK). The HLA-DRB1*1501 genotyping was carried out as previously described[26]. The genotyping results were evaluated by the presence or absence of an allele-specific PCR product.

Statistical analysis: Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). 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 per cent confidence intervals (CIs). A multiple logistic and linear regression analysis was performed to evaluate possible associations between the variables. 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[27].

Results

IL-10 −1082 and −819 SNPs and their combinations with DRB1*15 allele were analysed in MS patients and controls to find any association between these genotypes and susceptibility to MS. At the −1082 position, G allele (P=0.001) and G/G genotype (P<0.001) were more frequent in MS patients than healthy controls (Table III). At the other polymorphic site, IL-10 −819, C allele had a higher frequency in MS patients compared with controls (P=0.09). The C/C genotype was significantly more frequent among patients (P=0.005) compared to controls (Table IV).

The association of the two SNPs with MS was estimated by calculating pooled OR and 95% CI under co-dominant, dominant and recessive genetic models using the SNPStats online software. In the IL-10 −1082 position, these genetic models compared A/A genotypes to G/A+G/G genotypes in dominant model and A/

A+G/A to G/G genotypes in recessive model. The data are shown in Table V. DRB1*15 allele showed a higher frequency among MS patients as compared with control subjects (OR=1.6, CI=1.2-2.1, *P*=0.001).

Analysis of IL-10 polymorphisms and HLA-DRB alleles showed that some combinations were significantly more frequent among MS patients (Tables VI and VII). At the −1082 position, individuals having both G and DR15 alleles (determined as risk alleles) were at the highest risk for developing MS as compared with those who did not have these alleles (OR=3.7, CI=2.1-6.6, *P*<0.001). In addition, having one of these alleles was significantly associated with the risk of developing MS, compared with lacking both alleles.

Table V. Association of interleukin-10 −1082 and interleukin-10 −819 genotypes with multiple sclerosis (MS) under different inheritance models (n=790, adjusted by age + sex)

Model	Genotype	Control n (%)	MS n (%)	OR (95% CI)	*P*
IL-10 −1082					
Co-dominant	A/A	119 (26.2)	52 (15.5)	1.00	<0.001
	G/A	288 (63.4)	231 (68.8)	1.98 (1.36-2.89)	
	G/G	47 (10.3)	53 (15.8)	2.59 (1.54-4.35)	
Dominant	A/A	119 (26.2)	52 (15.5)	1.00	<0.001
	G/A-G/G	335 (73.8)	284 (84.5)	2.07 (1.43-3.00)	
Recessive	A/A-G/A	407 (89.7)	283 (84.2)	1.00	0.047
	G/G	47 (10.3)	53 (15.8)	1.54 (1.01-2.36)	
Overdominant	A/A-G/G	166 (36.6)	105 (31.2)	1.00	0.044
	G/A	288 (63.4)	231 (68.8)	1.37 (1.01-1.86)	
IL-10 −819					
Co-dominant	C/C	192 (42.3)	144 (42.9)	1.00	0.005
	T/C	176 (38.8)	154 (45.8)	1.21 (0.88-1.65)	
	T/T	86 (18.9)	38 (11.3)	0.59 (0.38-0.91)	
Dominant	C/C	192 (42.3)	144 (42.9)	1.00	0.99
	T/C-T/T	262 (57.7)	192 (57.1)	1.00 (0.75-1.33)	
Recessive	C/C-T/C	368 (81.1)	298 (88.7)	1.00	0.002
	T/T	86 (18.9)	38 (11.3)	0.53 (0.35-0.81)	
Overdominant	C/C-T/T	278 (61.2)	182 (54.2)	1.00	0.028
	T/C	176 (38.8)	154 (45.8)	1.38 (1.04-1.85)	

OR, odds ratio; CI, confidence interval; IL, interleukin

Table VI. Frequencies of risk alleles (interleukin-10 −1082 G and HLA-DRB1*1501) in multiple sclerosis (MS) patients and controls

Comparison	Control n (%)	MS n (%)	OR	95% CI	*P*
G^+ versus G^-	339 (74.7) versus 115 (25.3)	286 (85.1) versus 50 (14.9)	0.49	1.3-2.9	0.001
$DR15^+$ versus $DR15^-$	154 (33.9) versus 300 (66.1)	151 (44.9) versus 185 (55.1)	1.6	1.2-2.1	0.001
$G^+/DR15^+$ versus $G^+/DR15^-$	113 (24.9) versus 226 (49.8)	123 (36.6) versus 163 (48.5)	1.51	0.8-1.5	NS
$G^+/DR15^+$ versus $G^-/DR15^+$	113 (24.9) versus 41 (9)	123 (36.6) versus 28 (8.3)	1.6	0.9-2.9	NS
$G^+/DR15^+$ versus $G^-/DR15^-$	113 (24.9) versus 74 (16.3)	123 (36.6) versus 22 (6.6)	3.7	2.1-6.6	<0.001
$G^+/DR15^-$ versus $G^-/DR15^+$	226 (49.8) versus 41 (9)	163 (48.5) versus 28 (8.3)	1.1	0.6-1.9	NS
$G^+/DR15^-$ versus $G^-/DR15^-$	226 (49.8) versus 74 (16.3)	163 (48.5) versus 22 (6.6)	2.4	1.4-4.3	0.001
$G^-/DR15^+$ versus $G^-/DR15^-$	41 (9) versus 74 (16.3)	28 (8.3) versus 22 (6.6)	2.3	1.1-4.8	0.02

OR, odds ratio; CI, confidence interval; NS, not significant

Table VII. Frequencies of risk alleles (interleukin-10 −819 C and HLA-DRB1*1501) in multiple sclerosis (MS) patients and controls

Comparison	Control n (%)	MS n (%)	OR	95% CI	*P*
C^+ versus C^-	322 (82.4) versus 69 (17.6)	302 (89.9) versus 34 (10.1)	1.9	1.2-3.0	0.001
$DR15^+$ versus $DR15^-$	133 (34) versus 258 (66)	151 (44.9) versus 185 (55.1)	1.6	1.2-2.2	0.001
$C^+/DR15^+$ versus $C^+/DR15^-$	99 (25.3) versus 223 (57.1)	135 (40.2) versus 167 (49.7)	1.8	1.3-2.6	0.001
$C^+/DR15^+$ versus $C^-/DR15^+$	99 (25.3) versus 34 (8.7)	135 (40.2) versus 16 (4.7)	2.9	1.5-5.9	0.001
$C^+/DR15^+$ versus $C^-/DR15^-$	99 (25.3) versus 35 (8.9)	135 (40.2) versus 18 (5.4)	2.7	1.4-5.3	0.001
$C^+/DR15^-$ versus $C^-/DR15^+$	223 (57.1) versus 34 (8.7)	167 (49.7) versus 16 (4.7)	1.6	0.8-3.2	NS
$C^+/DR15^-$ versus $C^-/DR15^-$	223 (57.1) versus 35 (8.9)	167 (49.7) versus 18 (5.4)	1.5	0.8-2.8	NS
$C^-/DR15^+$ versus $C^-/DR15^-$	34 (8.7) versus 35 (8.9)	16 (4.7) versus 18 (5.4)	0.9	0.4-2.3	NS

OR, odds ratio; CI, confidence interval; NS, not significant

Table VIII. Haplotype association with multiple sclerosis (MS) (n=790, adjusted by age + sex)

Haplotype	IL-10		Controls (%)	MS (%)	OR (95% CI)	*P*
	−819	−1082				
H1	C	G	28	39	1.00	-
H2	C	A	34	26	0.46 (0.33-0.65)	<0.001
H3	T	A	24	23	0.58 (0.43-0.79)	<0.001
H4	T	G	14	10	0.55 (0.35-0.86)	0.01

Global haplotype association $P<0.001$. OR, odds ratio; CI, confidence interval; IL, interleukin

At the IL-10 −819 position, a significant difference was observed between patients and controls when both C and DR15 alleles were present simultaneously as compared with other combinations. Haplotype analysis with the EM (Expectation–Maximization) algorithm[27] revealed a significantly higher occurrence of two haplotypes H2 (CA) and H4 (TG) in healthy controls than the MS patients (global haplotype association, $P<0.001$) (Table VIII), thereby suggesting protective roles for them against MS.

Discussion

This study evaluated IL-10 promoter polymorphisms and their combinations with HLA-DRB1*15 allele in Iranian MS patients and healthy controls. The results showed that −1082 G/G genotype and −819 CC genotype were associated with higher risk of developing MS. Together with HLA-DRB1*15 allele, IL-10 promoter SNPs and haplotypes were associated with susceptibility to MS.

Studies have shown that CD4+ T-cells are involved in the development of autoimmune diseases such as MS[28,29]. Among CD4+ T-helper (Th) cell subsets, Th1 and Th17 CD4+ cells play a critical role in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), the most commonly used experimental model for human MS[30]. In contrast, regulatory T-cells and IL-10 have been shown to play a major role in protecting against and recovery from EAE[31]. Tullius *et al*[29] showed that IL-10−/− mice were more susceptible to EAE when compared with their wild-type counterparts. These findings, in line with the previous studies, have shown that IL-10 production by interferon-γ-producing Th1 cells can prevent tissue damage and autoimmune diseases[29].

Studies on human and animal models have shown that the amount of IL-10 production is closely correlated with disease course and also it differs in remission phase from relapse periods[15]. It is well known that the high production of IL-10 is associated with the suppression of inflammation in many autoimmune disorders including MS[15]. It has been shown that three SNPs in IL-10 promoter region, including −1082 (G/A), −819 (T/C) and −592 (A/C), regulate the expression of this cytokine[17].

In the present study it was found that IL-10 −1082 G/G genotype was associated with higher risk of MS in Iranian population, while G/A and A/A genotypes reduced the risk of the disease. Luomala *et al*[19] showed that −1082 SNP was not associated

with the occurrence of MS, but it was associated with disease severity. They found that patients with G/A genotype suffered less severe disorder than other genotypes[19]. Mihailova *et al*[18] studied IL-10 promoter polymorphisms in Bulgarian population and found that distribution of C/C genotype in −819 position was increased significantly in MS patients. They did not observe any significant difference in other genotype or allele frequencies between patients and controls[18]. In the present study, IL-10 polymorphisms were seen to be associated with susceptibility to MS against previous studies.

Several HLA allele groups have been a candidate as predisposing factor for MS. Among these, HLA-A, HLA-DRB and HLA-DQB1 alleles seem to be associated with susceptibility to MS[11].

Among HLA-DRB alleles, HLA-DRB1*15 allele group seems to have the strongest association with MS[12]. Our study also confirms this fact. Hence, HLA alleles may influence role of other genes in the pathogenesis of MS[32]; lack of data about the effect of combination of IL-10 SNPs and HLA-DRB allele on MS indicates the need for such a study. The association between genetic polymorphisms and MS susceptibility may also vary with ethnicity. Racial and ethnic differences may affect not only susceptibility but also the phenotypic expression of MS, including clinical manifestations, site of lesions, disease course and prognosis[33].

In conclusion, our data showed that gene-gene interaction of IL-10 and HLA-DRB1*15 polymorphisms might have an important role in the susceptibility to MS in Iranian population. This association may be due to ethnic differences and differences in the genetic background of the Iranian population. Further studies on other genetic polymorphisms may reveal other candidate genes associated with pathogenesis of MS and clarify complex interactions between genes and the environment.

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***Conflicts of Interest*:** None.

References

1. Flachenecker P. Epidemiology of neuroimmunological diseases. *J Neurol* 2006; *253* (Suppl 5) : V2-8.
2. Rosati G. The prevalence of multiple sclerosis in the world: An update. *Neurol Sci* 2001; *22* : 117-39.
3. Kurtzke JF. Multiple sclerosis in time and space – Geographic clues to cause. *J Neurovirol* 2000; *6* (Suppl 2) : S134-40.
4. Etemadifar M, Janghorbani M, Shaygannejad V, Ashtari F. Prevalence of multiple sclerosis in Isfahan, Iran. *Neuroepidemiology* 2006; *27* : 39-44.
5. van Boxel-Dezaire AH, Hoff SC, van Oosten BW, Verweij CL, Dräger AM, Adèr HJ, *et al.* Decreased interleukin-10 and increased interleukin-12p40 mRNA are associated with disease activity and characterize different disease stages in multiple sclerosis. *Ann Neurol* 1999; *45* : 695-703.
6. Hansen T, Skytthe A, Stenager E, Petersen HC, Kyvik KO, Brønnum-Hansen H. Risk for multiple sclerosis in dizygotic and monozygotic twins. *Mult Scler* 2005; *11* : 500-3.
7. Dyment DA, Herrera BM, Cader MZ, Willer CJ, Lincoln MR, Sadovnick AD, *et al.* Complex interactions among MHC haplotypes in multiple sclerosis: susceptibility and resistance. *Hum Mol Genet* 2005; *14* : 2019-26.
8. Otaegui D, Sáenz A, Camaño P, Blázquez L, Goicoechea M, Ruíz-Martínez J, *et al.* CD24 V/V is an allele associated with the risk of developing multiple sclerosis in the Spanish population. *Mult Scler* 2006; *12* : 511-4.
9. Djuric T, Zivkovic M, Stankovic A, Dincic E, Raicevic R, Alavantic D. Association of the MMP-3 5A/6A gene polymorphism with multiple sclerosis in patients from Serbia. *J Neurol Sci* 2008; *267* : 62-5.
10. Ramagopalan SV, Herrera BM, Bell JT, Dyment DA, Deluca GC, Lincoln MR, *et al.* Parental transmission of HLA-DRB1*15 in multiple sclerosis. *Hum Genet* 2008; *122* : 661-3.
11. International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 2011; *476* : 214-9.
12. Tuwir I, Dunne C, Crowley J, Saddik T, Murphy R, Cassidy L. The relationship between HLA-DRB1 alleles and optic neuritis in Irish patients and the risk of developing multiple sclerosis. *Br J Ophthalmol* 2007; *91* : 1288-92.
13. Hurme M, Lahdenpohja N, Santtila S. Gene polymorphisms of interleukins 1 and 10 in infectious and autoimmune diseases. *Ann Med* 1998; *30* : 469-73.
14. Saxena R, Chawla YK, Verma I, Kaur J. Association of interleukin-10 with hepatitis B virus (HBV) mediated disease progression in Indian population. *Indian J Med Res* 2014; *139* : 737-45.
15. Hellings N, Raus J, Stinissen P. Insights into the immunopathogenesis of multiple sclerosis. *Immunol Res* 2002; *25* : 27-51.
16. Eskdale J, Kube D, Tesch H, Gallagher G. Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. *Immunogenetics* 1997; *46* : 120-8.
17. Myhr KM, Vågnes KS, Marøy TH, Aarseth JH, Nyland HI, Vedeler CA. Interleukin-10 promoter polymorphisms in patients with multiple sclerosis. *J Neurol Sci* 2002; *202* : 93-7.
18. Mihailova S, Ivanova M, Mihaylova A, Quin L, Mikova O, Naumova E. Pro-and anti-inflammatory cytokine gene polymorphism profiles in Bulgarian multiple sclerosis patients. *J Neuroimmunol* 2005; *168* : 138-43.

19. Luomala M, Lehtimäki T, Huhtala H, Ukkonen M, Koivula T, Hurme M, *et al.* Promoter polymorphism of IL-10 and severity of multiple sclerosis. *Acta Neurol Scand* 2003; *108* : 396-400.

20. Shahbazi M, Ebadi H, Fathi D, Roshandel D, Mahamadhoseeni M, Rashidbaghan A, *et al.* CCR5-delta 32 allele is associated with the risk of developing multiple sclerosis in the Iranian population. *Cell Mol Neurobiol* 2009; *29* : 1205-9.

21. Shahbazi M, Ebadi H, Fathi D, Roshandel D, Mohamadhosseni M, Tahmasebi A, *et al.* HLA-DRB1*1501 intensifies the impact of IL-6 promoter polymorphism on the susceptibility to multiple sclerosis in an Iranian population. *Mult Scler* 2010; *16* : 1173-7.

22. Shahbazi M, Roshandel D, Ebadi H, Fathi D, Zamani M, Boghaee M, *et al.* High frequency of the IL-2 -330 T/HLA-DRB1*1501 haplotype in patients with multiple sclerosis. *Clin Immunol* 2010; *137* : 134-8.

23. Shahbazi M, Roshandel D, Omidnyia E, Rshaidbaghan A. Interaction of HLA-DRB1*1501 allele and TNF-alpha -308 G/A single nucleotide polymorphism in the susceptibility to multiple sclerosis. *Clin Immunol* 2011; *139* : 277-81.

24. McDonald WI, Compston A, Edan G, Goodkin D, Hartung H-P, Lublin FD, *et al*. Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; *50* : 121-7.

25. Shahbazi M, Pravica V, Nasreen N, Fakhoury H, Fryer AA, Strange RC, *et al.* Association between functional polymorphism in *EGF* gene and malignant melanoma. *Lancet* 2002; *359* : 397-401.

26. Ghabaee M, Bayati A, Amri Saroukolaei S, Sahraian MA, Sanaati MH, Karimi P, *et al.* Analysis of HLA DR2&DQ6 (DRB1*1501, DQA1*0102, DQB1*0602) haplotypes in Iranian patients with multiple sclerosis. *Cell Mol Neurobiol* 2009; *29* : 109-14.

27. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: A web tool for the analysis of association studies. *Bioinformatics* 2006; *22* : 1928-9.

28. Wu Y, Cai B, Feng W, Yang B, Huang Z, Zuo C, *et al.* Double positive CD4+CD8+ T cells: Key suppressive role in the production of autoantibodies in systemic lupus erythematosus. *Indian J Med Res* 2014; *140* : 513-9.

29. Tullius SG, Biefer HRC, Li S, Trachtenberg AJ, Edtinger K, Quante M, *et al.* NAD+ protects against EAE by regulating CD4+ T-cell differentiation. *Nat Commun* 2014; *5* : 5101.

30. Duhen R, Glatigny S, Arbelaez CA, Blair TC, Oukka M, Bettelli E. Cutting edge: the pathogenicity of IFN-γ-producing Th17 cells is independent of T-bet. *J Immunol* 2013; *190* : 4478-82.

31. Bettelli E, Das MP, Howard ED, Weiner HL, Sobel RA, Kuchroo VK. IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. *J Immunol* 1998; *161* : 3299-306.

32. Duvefelt K, Anderson M, Fogdell-Hahn A, Hillert J. A NOTCH4 association with multiple sclerosis is secondary to HLA-DR*1501. *Tissue Antigens* 2004; *63* : 13-20.

33. Khan O, Williams MJ, Amezcua L, Javed A, Larsen KE, Smrtka JM. Multiple sclerosis in US minority populations Clinical practice insights. *Neurol Clin Pract* 2015; *5* : 132-42.

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