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ORIGINAL ARTICLE

High-resolution melting curve analysis of polymorphisms within CD58, CD226, HLA-G genes and association with multiple sclerosis susceptibility in a subset of Iranian population: a case–control study

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system with unknown etiology, which typically is manifested in early to middle adulthood. Recently, genome-wide association studies have identified susceptibility of immune-related genes to be involved in MS predisposition. The goal of the current study was to investigate the association of single nucleotide polymorphisms (SNP) with the immunologically related genes responsible for the disease, composed of CD58 (rs2300747 A>G), CD226 (rs763361 C>T), and HLA-G (rs1611715 A>C), with MS susceptibility. In this case–control study, a total of 200 patients suffering from relapsing-remitting multiple sclerosis and 200 healthy individuals were recruited. DNA was extracted from blood and then all subjects were genotyped for the polymorphism within mentioned genes by high-resolution melting (HRM) real-time PCR method. Statistical analyses were performed using SPSS software (version 20; SPSS, Chicago, IL, USA). Our finding showed that there are significant differences in genotype and allele frequencies between two groups regarding rs763361 ($P=0.035$, OR 0.64, CI 95% for C allele) and rs1611715 ($P=0.038$, OR 1.57, CI 95% for AA genotype) polymorphisms within CD226 and HLA-G genes, respectively. Concerning rs2300747 polymorphism on CD58 gene, no significant differences were found between cases and controls. In general, results from the current study indicate that CD226 and HLA-G, but not CD58 genetic polymorphisms are associated with increased risk of MS in Isfahan population similar to European populations. However, to elucidate how these SNPs contribute to MS pathogenesis, functional studies are needed.

Keywords Multiple sclerosis · CD58 · CD226 · HLA-G · Single nucleotide polymorphisms

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Introduction

Multiple sclerosis (MS) is an autoimmune T cell mediated disease affecting the central nervous system (CNS) and it is commonly found among young adults. The etiology of MS is poorly understood, but it has been suggested that it is affected by both genetic and environmental factors [1]. Genome-wide association studies (GWAS) throughout the human genome using single nucleotide polymorphism (SNP) markers enable scientists to detect common genetic variants that affect the susceptibility to complex diseases like MS. A number of genetic susceptibility factors have been recently identified in MS through GWAS association studies. These risk-conferring genes, which are associated with MS susceptibility, are classified into two main groups: human leukocyte antigen (HLA) and non-HLA genes that are mostly involved in the immune response [2, 3]. SNPs within the susceptibility genes are appropriate variants that provide insight into common gene function and pathways and shed light into the genetic predisposition for complex diseases. Among the HLA susceptibility genes, HLA-DRB1 is the most remarkable locus contributing to MS genetic susceptibility. Other HLA haplotypes involved in MS predisposition are DRB1*1501-DQA1*0102-DQB1*0602. In this regard, HLA-G has been hypothesized to be the primary HLA genetic susceptibility factor for MS [4, 5]. Recent investigations have identified a broad spectrum of non-HLA genes prominently associated with MS including CD58, RPL5, DBC1, ALK, FAM69A, ANKRD15, EVI5, KLRB1, CBLB CLEC16A, IL7RA, IL2RA, CLEC16A, and CD226 that have been found to be associated with susceptibility to MS [3, 6, 7]. Of these genes, we classified SNPs into three candidate genes that are involved in disease susceptibility (CD58 rs2300747, CD226 rs763361, and HLA-G rs1611715) as identified by GWAS [8–10]. The CD58 (LFA-3) gene on chromosome one (1p13.1) that is critically involved in proliferation, differentiation, and activation of T cells [11]. The rs2300747 polymorphism of CD58 gene is found within the first intron and does not have a known considerable consequence. The protective effect within the CD58 locus has been suggested to be captured by the rs2300747 G allele that may exert its effect on disease risk by a specifically dose-dependent increase in CD58 mRNA expression both in vitro and ex vivo. This protective role is supported by finding that mRNA expression of CD58 is higher in MS patients during clinical remission which is about 1.7-fold greater than the baseline expression in healthy control subjects [12, 13]. Moreover, the CD58 risk allele leads to lower CD58 expression and consequently downregulation of FoxP3, an important transcription factor for regulatory T cells resulting in dysfunction of regulatory T cells in MS patients. Functional studies suggest a potential mechanism by which, the protective allele-mediated increase in CD58 expression up-regulates the expression of FoxP3 through engagement of the CD58 receptor, CD2, leading to the enhanced function of $CD4^+$ regulatory T cells that are defective in subjects with MS [13, 14]. Due to the important role of CD58 in stimulation and enhancing T cell receptor signaling by engaging CD2 [15], SNPs within CD58 locus provide an attractive target for perceiving the effect of genetic variation in immune system dysfunction associated with MS.

Recently, a non-synonymous polymorphism (Gly307Ser/rs763361 C > T) in the CD226 gene has been identified as a genetic risk factor for autoimmunity [16]. rs763361 risk variant was first discovered to confer the risk of rheumatoid arthritis in populations with European and Colombian ancestry [17]. CD226 (also known as DNAM-1) is a transmembrane 67-kDa glycoprotein and a member of the immunoglobulin superfamily encoded by a gene on human chromosome 18q22.3 that mainly participated in the activation and differentiation of T cells, cytotoxicity of T cells and NK cells, and apoptosis [18–20]. This intercellular adhesion molecule is constitutively expressed on the majority of $CD4^+$ and $CD8^+$ T cells, natural killer cells, monocytes, platelets, megakaryocytes, mast cells and can act as a co-stimulator that contributes to multiple innate and adaptive immune responses [20].

The exon-7 variant (rs763361) in the C-terminal domain can affect the signaling function in T cells by alteration of the exon-splicing silencer sequence, which may affect the CD226 molecule expression. Moreover, the underlying cellular mechanisms may increase activation of T and NK cells that subsequently leads to different phenotypes in inflammatory autoimmune diseases [21, 22]. Another hypothesis in this regard is that this variant could interfere in the phosphorylation of CD226 at 329Ser and 322Tyr residues, leading to the modified downstream signal transduction may be these posttranslational modifications [23]. It was, therefore, of interest to investigate whether rs763361 SNP was the susceptibility variant in the region and a shared risk locus for MS disease.

The last SNP evaluated is HLA-G gene rs1611715 A > C that is located on chromosome 6 (p21.3). A recent publication on the effect of GWAS in MS, reports this HLA variant as a risk locus that could influence MS susceptibility [24]. The nonclassical MHC class Ib molecule HLA-G, has well-recognized immunoregulatory activities, including regulation of both innate and adaptive immune responses and induction of tolerance. In physiological conditions, HLA-G is expressed restrictively in different tissues and also on different immune cell subsets in the peripheral blood of healthy adults. In contrast, upregulation of HLA-G can be observed in pathological conditions such as malignant transformation,

grafted organs, viral infections, and inflammatory and autoimmune disorders [25–27]. Polymorphisms within HLA-G gene may potentially affect the biological properties of the protein. $CD4^+$ HLA-G^+ regulatory T cells have an important role to play in MS pathophysiology, by interacting with inhibitory receptors, including the killer immunoglobulin-like receptor (KIR) 2DL4, immunoglobulin-like transcript 2 (ILT2) and ILT4, and with CD8, while HLA-G directly inhibits NK cells, cytotoxic T-lymphocyte (CTLs), B cells, and dendritic cells (DCs). Therefore, $CD4^+$ HLA-G^+ regulatory T cells counteract peripheral and central immune responses in MS potentially ameliorating neuroinflammatory and neurodegenerative CNS damage [25, 28].

To the best of our knowledge, no study has yet evaluated the role of CD58 rs2300747, CD226 rs763361, and HLA-G rs1611715 polymorphisms in MS risk in an Iranian population. Therefore, the present study was conducted to evaluate the impact of these variants on the susceptibility to MS in a population sample from Isfahan City, Iran.

Materials and methods

Patients and controls

This case–control study was performed on 200 patients diagnosed with RRMS according to the McDonald criteria referring to MS clinic of Isfahan, a city in the central part of Iran with a high prevalence of MS. In the case group, assessment of disability was done using magnetic resonance imaging (MRI) and Kurtzke's Expanded Disability Status Scale (EDSS). For the control group, 200 age- and sex-matched healthy subjects were randomly selected from the general population of the city. After obtaining informed consent from all patients and healthy individuals, the blood sample was taken and collected in EDTA-containing tubes for DNA isolation. The demographic characteristics of patients and normal controls are presented in Table 1.

Table 1 Demographic features in cases and normal controls

Variables	Case ($n=200$)	Control ($n=200$)	P value
Age (mean ± SD) year	31.32 ± 8.53	32.31 ± 7.55	0.185
Gender [n (%)]			
Male	36 (18%)	50 (25%)	0.113
Female	164 (82%)	150 (75%)	
Female/male	4.55%	3%	
Education [n (%)]			
Preliminary	50 (26.6%)	51 (25.8%)	0.363
Secondary	75 (39.9%)	92 (46.5%)	
University	63 (33.5%)	55 (27.8%)	
Family status [n (%)]			
Single	12 (30.8%)	49 (24.6%)	0.427
Married	27 (69.2%)	150 (75.4%)	
EDSS [n (%)]			
≤ 1	96 (88.1%)	–	–
1–2	8 (7.3%)	–	
> 2	5 (4.6%)	–	
Treatment [n (%)]			
Treated	131 (65.5%)	–	–
Untreated	69 (34.5%)	–	

This table shows characteristics of age, sex, education, marital status in two studied groups and also degree of disability (EDSS), divided groups of patients

Extraction of genomic DNA and genotyping by HRM real-time PCR

Genomic DNA was extracted from leukocytes in peripheral blood using (Amersham Pharmacia Biotech, Buckinghamshire, UK) DNA extraction kit according to the manufacturer's protocols. Concentration and purity of the isolated DNA were checked using UV spectrophotometry and electrophoresis on 1% agarose gel, respectively, and stored at − 20 °C until further use. Polymorphisms within three genes, including CD58 (rs2300747), CD226 (rs763361), and HLA-G (rs1611715), were evaluated via real-time PCR assay. The assay was performed using the Rotor-Gene 6000-Real-Time PCR System (Corebett research) and Feldan Real-Time PCR kit (Bio Basic, Canada). PCR reactions were performed in duplicate in 10 µL of final volume using the Type-It HRM Kit (Feldan), HRM PCR buffer, nucleotides and Eva Green dye, Hot Star Taq Plus DNA Polymerase, and 30 ng DNA. Program of PCR was an initial denaturation activation step at 95 °C for 5 min, followed by a 40-cycle program (denaturation at 95 °C for 10 s, annealing conditions 60 °C for 30 s, and 72 °C for 15 s; and HRM step from 65 to 95 °C rising with a rising rate of 0.1 °C s^{-1}). Genotyping for mentioned polymorphisms was performed by High-Resolution Melting (HRM) software (1.7 version). Finally, 20% of the samples were randomly included as duplicates for determining the genotyping error rate. All primer sequences, amplicon size, and other characteristics of polymorphisms are shown in Table 2.

Statistical analysis

All statistical analyses were performed by applying the statistical SPSS software for Microsoft Windows (version 20; SPSS, Chicago, IL, USA). The association among genotypes and MS was calculated by computing the odds ratios (ORs) and 95% confidence intervals (CIs) from logistic regression analyses. All continuous variables were expressed as the mean ± standard deviation (SD). Student's t test was used to compare the continuous

Table 2 Primers used for detection of rs2300747, rs763361, rs1611715 polymorphisms in CD58, CD226 and HLA-G genes, respectively

Gene	RS number	Location	Allele	Product size (bp)	Primer sequence
CD58	rs2300747	Intron 1 1 P13	A/G	173	F: 5′GCCAAATATTACTGATACCATGAAGTTC3′ R: 5′ ATGCACAAGTT-AGTGTGGGAGATG 3′
CD226 (DNAM-1)	rs763361	Exon 7 18q22.3	C/T	193	F: 5′ACGCGTCGACAACCAGCCTTTCAAACAG3′ R: 5′CGGGATCCTGGTTATCGGTTTTACCC3′
HLA-G	rs1611715	Centromere 6 p21.3	A/C	116	F: 5′GCTTGGCTCCTCCAAGGAAT3′ R: 5′CAGGAACCAGGAAGAGGCAG3′

Forward and reverse primers specification and the product size of interested genes are explained

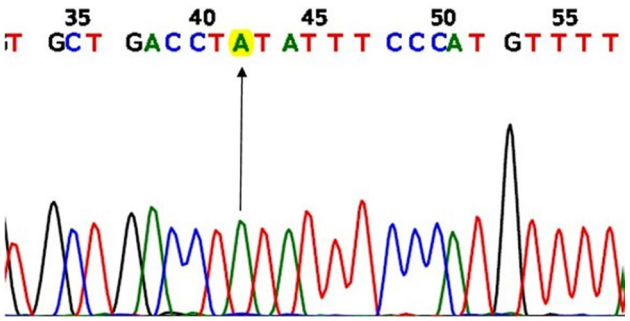

Fig. 1 A sequencing chromatogram showing AA genotype of rs2300747 in CD58 gene

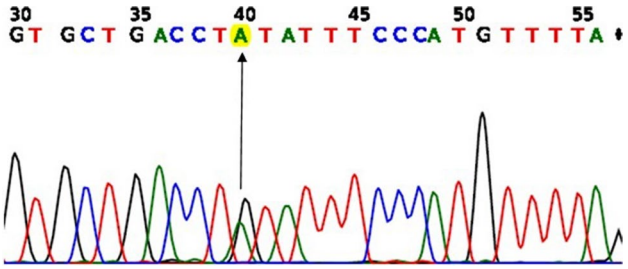

Fig. 2 A sequencing chromatogram showing AG genotype of rs2300747 in CD58 gene

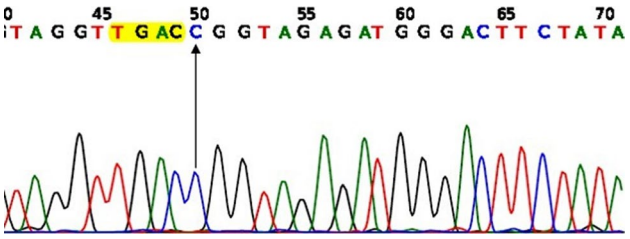

Fig. 3 A sequencing chromatogram showing CC genotype of rs763361 in CD226 gene

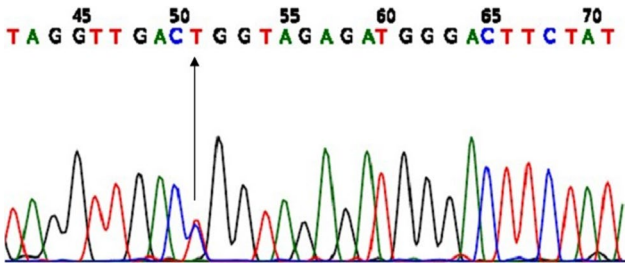

Fig. 4 A sequencing chromatogram showing CT genotype of rs763361 in CD226 gene

variables between both case and control groups. Pearson's v^2 test was used to evaluate the difference in the prevalence of MS among genotypes. *P* values were determined and those with < 0.05 were considered to be statistically significant.

Results

In the present study, the relationship of rs2300747, rs763361, and rs1611715 SNPs with MS were investigated. The summary of SNPs description is shown in Table 2. Demographic and clinical features of cases and controls in the studied population and the association with MS are demonstrated in Table 1. No major differences were observed between the two groups concerning gender ($P = 0.113$), age (31.32 ± 8.53 years for controls and 32.31 ± 7.55 years for the cases, $P = 0.185$), education status ($P = 0.363$), and family status ($P = 0.427$) between the two groups (Table 1). Genotypes of all SNPs were effectively typed in all 400 subjects and found that they did not deviate from the distribution expected by the Hardy–Weinberg equilibrium. After genotyping by HRM real-time PCR system, the results were confirmed by samples randomly selected for sequencing (Figs. 1, 2, 3, 4, 5, 6).

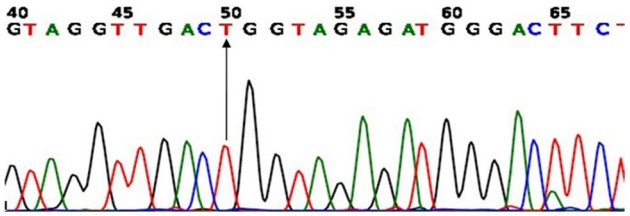

Fig. 5 A sequencing chromatogram showing TT genotype of rs763361 in CD226 gene

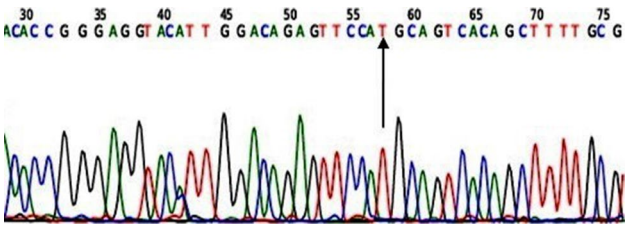

Fig. 6 A sequencing chromatogram showing TT genotype of rs1611715 in HLA-G gene

Table 3 Allele and genotype distribution of rs2300747 SNP (within CD58 gene) in cases and controls and their association with MS in this study

	Case ($n=200$) n (%)	Control ($n=200$) n (%)	OR (95% CI)	P value
Genotype frequency				
AA	149 (74.5%)	135 (67.5%)	1.40 (0.91–2.17)	0.152
AG	51 (25.5%)	65 (32.5%)		
Allele frequency				
A	174 (87%)	178 (89%)	0.82 (0.45–1.51)	0.543
G	26 (13%)	22 (11%)		

Comparison of observed distribution of heterozygosity and homozygosity of the two populations. There was no statistically significant difference in the distribution of alleles and genotypes between case and control groups

Genotype and allele frequencies of CD58 (rs2300747 A>G) polymorphism

Frequencies of the A/A, A/G genotypes of rs2300747 SNP were 67.5 and 32.5% in controls, and 74.5 and 25.5% in cases, respectively. Frequencies of the A and G alleles of rs2300747 SNP were 89 and 13% in controls and 87 and 13% in cases, respectively. Our findings revealed that there are not any significant differences in genotype ($P=0.152$, OR 1.40, CI 95%) and allele ($P=0.543$, OR 0.52, CI 95%) distribution between MS patients and controls regarding CD58 rs2236242 polymorphism. Therefore, we failed to find any evidence indicating the association of rs2300747 with MS (Table 3).

Table 4 Allele and genotype distribution of rs763361 SNP (within CD226 gene) in cases and controls and their association with MS in this study

	Case ($n=200$) n (%)	Control ($n=200$) n (%)	OR (95% CI)	P value
Genotype frequency				
CC	50 (25%)	75 (37.5%)	0.46 (0.27–0.79)	0.014
CT	86 (43%)	80 (40%)	1.61 (1.01–2.58)	
TT	64 (32%)	45 (22.5%)	0.75 (0.46–1.23)	
CC + CT	136 (68%)	155 (77.5%)	0.62 (0.39–0.96)	0.033
CC	50 (25%)	75 (37.5%)	1.80 (1.17–2.76)	0.007
CT + TT	150 (75%)	125 (62.5%)		
Allele frequency				
C	93 (46.5%)	115 (57.5%)	0.64 (0.43–0.95)	0.035
T	107 (53.5%)	85 (42.5%)		

There was a significantly different distribution of rs763361 SNP in CD226 genotype frequencies in subjects affected by MS compared with healthy controls ($P=0.014$)

Genotype and allele frequencies of CD226 (rs763361 C>T) polymorphism

As shown in Table 4, there was a significant association between C allele and T allele in rs763361 between the case and control groups. A higher frequency of T allele in the case group compared to the healthy subjects was observed. Furthermore, the frequency of CT and TT genotype in the case group was increased compared to the healthy subjects. In addition, the ratio of CT + TT genotype in MS patients compared with CC genotype was significantly higher than the control group ($P=0.007$, OR 1.80, CI 95%). The T allele was associated with the increased risk of MS compared with the C allele ($P=0.035$, OR 0.64, CI 95%).

Genotype and allele frequencies of HLA-G (rs1611715 A>C) polymorphism

The distributions of genotype and allele frequencies of rs1611715 A>C SNPs are summarized in Table 5. A statistically significant difference was observed in rs1611715. Also, the frequencies of genotypes AA and AC in the case group were 42.5 and 57. 5% and in controls were 32 and 68%, respectively. There was a statistically significant difference in the genotype frequency between cases and controls ($P=0.038$, OR 1.57, CI 95%). Meanwhile, the distributions of allele frequency in cases (A = 71%, C = 29%) and control (A = 66%, G = 34%) were not significantly different ($P=0.333$). This SNP on HLA-G gene presented

Table 5 Allele and genotype distribution of rs1611715 SNP (within HLA-G gene) in cases and controls and their association with MS in this study

	Case ($n=200$) n (%)	Control ($n=200$) n (%)	OR (95% CI)	P value
Genotype frequency				
AA	85 (42.5%)	64 (32%)	1.57 (1.04–2.36)	0.038
AC	115 (57.5%)	136 (68%)		
Allele frequency				
A	142 (71%)	132 (66%)	1.26 (0.82–1.92)	0.333
C	58 (29%)	68 (34%)		

Comparison of genotype distribution of the rs1611715 SNP in HLA-G gene showed statistical difference in two studied groups (P value 0.038)

a significantly increased susceptibility to MS with AA genotype.

Discussion

MS is an autoimmune disease caused by the interaction of both environmental and genetic risk factors [29]. In our case–control study, using the HRM real-time PCR and DNA sequencing methods, possible association of three genetic variants (rs2300747, rs763361, and rs1611715 within CD58, CD226, and HLA-G susceptibility genes, respectively) with MS pathogenesis was evaluated. The results showed that, of these immune-related gene variants, polymorphism within CD226 rs763361 and HLA-G rs1611715, but not CD58 rs2300747, increased the risk of MS in a subset of the Iranian population.

CD58 is a molecule expressed abundantly on the surface of antigen-presenting cells and is involved in the proliferation and differentiation of T lymphocytes. According to De Jager et al., the possible role of CD58 in the MS pathogenesis may be related to alterations in immune function. They suggested that CD58 risk allele leads to a lower CD58 expression and consequently downregulation of FoxP3 leads to the dysfunction of regulatory T cells in MS patients [13, 30]. There is little known about the association of rs2300747 A>G genetic polymorphism with autoimmune disorders. Several case–control and familial studies have shown an association between other CD58 variants and MS susceptibility [7]. With respect to the CD58 rs2300747 polymorphism, we did not find any association between rs2300747 A>G variant and MS risk. The results of the recent meta-analysis study on rs2300747 support our findings. Jiahe Liu et al. [31] conducted a meta-analysis and indicated the absence of a relationship between this polymorphism and MS. CD58 rs2300747 polymorphism was found to be associated with decreasing MS risk in three genetic models (allelic: OR 0.86, 95% CI 0.78–0.94, $P<0.01$; heterozygous: OR 0.85, 95% CI 0.76–0.94, $P<0.01$, and dominant: OR 0.84, 95% CI 0.76–0.93, $P<0.01$). Our data are in agreement with previously obtained results from the North Indian population, as well. In a study by Pandit et al. [32] in the Indian MS population, 15 MS loci outside MHC region were validated with MS susceptibility. According to their result, the frequency of risk allele (A) of CD58 rs2300747 polymorphism was 65.7 and 64.7% in case and control groups, respectively, and they were not able to detect a significant association between rs2300747 SNP and MS [OR: 1.05, 95% CI (0.77–1.41), $P=0.7763$]. The importance of CD226 gene polymorphism (Gly307Ser/rs763361) in MS pathology has been previously confirmed in an experimental mouse model. In that study, the experimental autoimmune encephalomyelitis (EAE) with treatment having anti-CD226 monoclonal antibody delayed the onset and reduced the severity of EAE [33]. Hafler et al. hypothesized that CD226 SNP could alter RNA splicing by disrupting a splice site enhancer or silencer, resulting in either a non-functional CD226 isoform or a CD226 isoform with a novel function [22]. Our study provided evidence for the genetic association between Gly307Ser/rs763361 genetic variant and increased risk of MS. There are some evidences regarding the role of CD226 rs763361 polymorphism in various inflammatory autoimmune diseases, including type 1 diabetes, celiac disease, rheumatoid arthritis, MS, Grave's disease, psoriasis, Wegener's granulomatosis, and primary sicca syndrome in European Caucasian populations. Most of these studies conducted on the association between CD226 polymorphisms and MS are limited to the Caucasian population. Because Iranians are categorized as Caucasian, so our results regarding rs763361 SNP on CD226 gene are consistent with the results from mentioned studies. The results of the meta-analysis conducted by Qiu et al. revealed that the SNP reported to be associated with MS (rs763361) in previous studies was significantly associated with other inflammatory autoimmune diseases, as well. This study provided evidence that CD226 Gly307Ser (rs763361) is significantly associated with the risk of multiple autoimmune diseases [34]. Although there exist some differences in etiology between MS and other autoimmune disorders, all of them still share similar onset mechanisms in which the body's immune systems are misdirected to attack its own CNS. Therefore, by relying on the meta-analysis results, we confer that rs763361 in the CD226 gene can affect the development of inflammatory autoimmune diseases by altering the signaling cascade and expression of CD226 on human immune cells. Further functional studies, potentially using RNA interference technology, are now needed to determine how the CD226 influences responses of the immune system. It is noteworthy that in some other studies, the investigators did not find significant associations between rs763361 polymorphism with autoimmune diseases [35, 36].

The third SNP evaluated in the current study is HLA-G gene rs1611715 A>C that is located in the centromeric region on chromosome 6 (p21.3). Although association between rs1611715 SNP and risk of MS has not drawn much attention, other loci of this gene have been investigated, including − 725 C/G exchange in the HLA-G promoter region, HLAG* 0105N, a 14 bp insertion/deletion in the untranslated exon 8 [37], + 3142C>G [38], and 716T>G polymorphisms [39]. To the best of our knowledge, at present, our study is the first investigation on the possible association between rs1611715 gene polymorphisms and risk of MS. Our results revealed that although allele frequency is not significantly different between RRMA patients and healthy controls, there are considerable differences between them with respect to the HLA-G gene polymorphism, suggesting the role of rs1611715 SNP in susceptibility to MS. Previous studies presumed that HLA-G is implicated in immunoregulation of MS pathogenesis through inducing apoptosis of NK cells and cytotoxic T cells, suppressing the $CD4^+$ cell proliferation, and causing a shift from Th-1 to Th-2 profile. In this regard, our results could support the role of HLA-G gene polymorphism in risk of MS. Interestingly, a similar study was conducted by Kroner A et al. [37] on 698 MS patients from Germany in which they investigated some variations in HLA-G gene, including the HLA-G 14 bp insertion/deletion and the HLA-G-725 polymorphism; however, it was inconsistent with our results, as they found that these variations cannot prevent functional expression of HLA-G molecules and also are not significantly associated with susceptibility and severity of MS. Therefore, they suggested also investigating other SNPs within HLA-G gene in the future. It is of note that one reason for failure to finding an association between the HLA-G-725 polymorphism and MS is due to genotyping methods of this study, which could not find T allele. Another investigation in this regard examined the role of HLA-G 14 bp deletion/insertion and + 3142C>G polymorphisms in the production of sHLA-G molecules in RRMS, which was performed earlier by Rizzo et al. [38]. In this study, they reported that HLA-G polymorphisms can affect the serum and CSF sHLA-G levels of MS patients regardless of the inflammatory microenvironment. Similarly, Wisniewski et al. [39] investigated the association between different genotypes of three regions in HLA-G gene, including − 725C>G>T, − 716T>G and 14 bp indel. Their results showed significant differences in MS patients and healthy controls with respect to the genotypes of − 725C>G>T and in three-point haplotypes. However, regarding HLA-G rs1611715 A>C polymorphism, further investigations with larger sample size and also in different ethnical populations will be needed to establish this SNP as a modifier of susceptibility to MS.

Previous results regarding the association of genetic variants with MS risk are contradictory. Since gene polymorphisms are affected by various factors, including race, geographical area, and lifestyle, the differences between our results and previous research may be attributed to the effects of these factors, the administered techniques, differences in study designs, different genetic background, and also the sample size.

Conclusion

In summary, our study provided evidence that CD226 rs763361 C>T and HLA-G rs1611715 A>C variants were associated with a higher risk of MS in Iranian population, similar to European populations, but there was no association between CD58 rs2300747 A>G SNP and risk of MS. However, functional studies are needed to demonstrate how these SNPs contribute to MS pathogenesis.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This study was funded by the Deputy of Research, Isfahan University of Medical Sciences. (Grant number: 190115). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent After approving of study by the Ethical Committee on Human Research, Isfahan University of Medical Sciences, informed written consent was obtained from patient participating in this study.

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