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Association of *HLA-DRA* and *IL2RA* Polymorphisms with the Severity and Relapses Rate of Multiple Sclerosis in an Iranian Population

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Abstract

Background: Multiple sclerosis (MS) is a multifactorial condition in which many genetic and environmental factors interfere. The association between genes involved in the immune system and MS was previously reported. The aims of this study were to evaluate 14 SNPs of *HLA-DRA*, 14 SNPs of *IL2RA* with severity of MS through Expanded Disability Status Scale (EDSS) and Annualized Relapse Rate (ARR).

Methods: 102 patients with MS referred to Sina hospital in Tehran, Iran, were diagnosed and studied based on McDonald's guideline, clinical signs, and brain imaging procedures. All patients were included in the study following informed consent. Genotyping study of 14 variants in the *HLA-DRA*, and 14 variants in *IL2RA* was conducted by Sanger sequencing. Disease outcomes including EDSS and ARR were registered. Outcome measures between different genotypes of each SNPs were compared separately.

Results: Among 14 SNPs in IL 2RA the genotypes of rs12722489 showed a significant association with ARR in two consecutive years. Mean ARR1 was 1.06±1.12, 0.20±0.34 and 0.31±.50 for AA, GA, and GG genotypes, respectively (*p* value= 0.008). Mean ARR2 was 1.5±1.08, 0.28±0.40, and 0.42±0.55 for AA, GA, and GG, respectively (*p* value= 0.001). Regression analysis showed a significant association between rs12722489 with ARR1 and ARR2, removing the potential confounding mediators. No significant association was found between SNPs in *HLA-DRA* with the attack rate and severity of MS.

Conclusions: The rs12722489 of IL-2RA has an association with ARR, but not with EDSS.

Keywords: Annualized Relapse Rate, Expanded Disability Status Scale, *HLA*, Multiple Sclerosis, SNP.

Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease that can lead to demyelination of central nervous system (CNS) neurons and axon damage (1, 2). The symptoms generally begin at a young age and cause disability throughout life (3). Although MS is considered a progressive neurological condition, its clinical manifestations may vary from mild to disabling (4). The epidemiology of this condition also varies based on geographical distribution, with greater prevalence in the northern than in the southern hemisphere (5). The prevalence of MS has increased significantly

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in recent decades, particularly in Asians and African Americans (6). A nationwide population-based study in Iran reported an overall incidence rate of 6.7/100,000, with a mean age of 31.6 ±0.9, and female-to-male ratio of 3.11. Therefore, Iran might be considered a high-risk area for MS development (7).

This disease is a multifactorial condition affected by many genetic and environmental factors (3, 8). The interaction of genetic with environmental factors also plays a critical role in this context. The studies on the concordance rate in monozygotic twins and the disease incidence rate in MS patient relatives have provided strong evidence in for the role of genetics in disease etiology. However, these patterns do not follow Mendelian traits (4, 9-11).

The association between human leukocyte antigen (*HLA*) and MS has been reported previously (12, 13). Allelic variation at *HLA*, particularly the *HLA* class II region, associates with MS (14). A strong association was also reported between *HLA* and phenotypic traits in MS (3). Many studies have shown that the class II *HLA* (*HLA-DRB1*) is a strong locus for the development of MS. For example, the consistent relationship of *DRB1*1501* with MS has been shown (15). *HLA-DRB1**15:01 not only is the main genetic driver of MS development, but also takes part in disease progression (3).

A relationship between some single nucleotide polymorphisms (SNPs) in the interleukin-2 receptor A (*IL2RA*) subunit with MS susceptibility has been reported in some populations (16-20).

Studies also showed a common susceptibility region in *IL2RA* in patients with type 1 diabetes, MS, systemic lupus erythematous, and rheumatoid arthritis (15, 21-24). In a previous genome-wide association study (GWAS), it was shown that two SNPs in *IL2RA*, including rs12722489 and rs2104286, can cause susceptibility to MS development (14). A coding SNP (rs6897932) in exon 6 of *IL7RA* also showed high association with MS (13).

It is worth noting that although many studies evaluated the roles of SNPs in *HLA-DRA* and *IL2RA*, few studies to date have reported relationships between these SNPs and MS severity, particularly in Middle Eastern countries. The present study evaluated 14 *HLA-DRA* and 14 *Il2RA* SNPs and their potential association with MS severity by measuring Expanded Disability Status Scale (EDSS) and annualized relapse rate (ARR).

Materials and methods

Sample collection

Study participants included 102 MS patients who were randomly selected from individuals referring to Sina teaching hospital in Tehran, Iran. The MS diagnosis was based on McDonald criteria and clinical signs and symptoms; results were confirmed using imaging procedures including brain magnetic resonance imaging (MRI). All diagnostic procedures were performed by neurologists. Informed consent was obtained from all study subjects. The study was approved by the Ethics Committee of Pasteur Institute of Iran. Five ml of peripheral blood were obtained from each participant.

DNA extraction

Patient DNA was extracted from 200 ul serum samples according to the manufacturer's manual (South Korea, Exgene Cell SV- mini), and stored at -20 °C for nested PCR.

DNA extraction and PCR amplification

DNA was extracted and purified from whole blood lymphocytes by Mini QIAamp DNA Mini Kits (Cat. 51104; Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The DNA integrity and fragmentation were analyzed using 1% agarose gel electrophoresis.

Polymerase chain reaction (PCR) was performed using a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, USA) to detect the 28 polymorphisms. These SNPs were located in *HLA-DRA* and *IL2RA*. The specific primer sequences for each gene were designed using Primer3 online software (http://frodo.wi.mit.edu/primer3). Primer specificities were determined using Primer-BLAST and SNPCheck V3 tools. The primer sequences are shown in Table 1.

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Table 1. Primer sequences and amplicon sizes.

Gene	Forward	Reverse	Length (bp)
IL2RA	ATGCTCTGCCTCTGGAAGACAC	TATCTCAATGGGTTTCCACACTGT	1472
HLA-DRA*	TGCCTGCTTTTGCTTCTTTAGTCTC	AGGTGGTTTCAAGAATCAGTCAGAC	1173

Sanger sequencing

After gel purification, the amplicons were sent to Macrogen Company (Seoul, South Korea) for Sanger sequencing. The results were trimmed and analyzed by BioEdit and Chromas software to verify the sequencing quality and accuracy. Then, the extracted sequences were blasted against the NR database to validate the annotated regions with the gene sequences of interest.

Outcome measures

The outcome measures of our study were EDSS and ARR. EDSS was proposed by Kurtzke JF in 1983 (25). This scale assesses eight functional systems, including pyramidal (muscle weakness or difficulty moving limbs), cerebellar (ataxia, loss of balance, coordination or tremor), brain stem (problems with speech, swallowing, and nystagmus), sensory (numbness or loss of sensations), bladder-bowel, visual (problems with sight), cerebral (problems with thinking and memory), and mental systems in each patient.

We defined ARR based on the annual relapse number for each patient divided by the number of years from the time of diagnosis. The prognosis of each patient was adjusted based on the disease duration.

Statistical analysis

Means and SDs were calculated for continuous variables in a descriptive manner. The nominal and qualitative data were reported as percent and frequencies. In analytical statistics, if the data had a normal distribution, we applied parametric tests, such as the independent t-test, to compare the normal data between the two groups, and ANOVA tests to compare the normal data among three groups or more. But if the related data did not have a normal distribution, non-parametric tests, such as Mann-Whitney and Kruskal Wallis tests, were performed to compare the non-normal data between two and among three groups or more, respectively. To remove the confounding effects of confounding variables such as age and sex, multiple regression analyses were performed. Before conducting the multiple regression analyses, we checked the normal distribution of related variables. Because the ARR data did not have a normal distribution, to perform the multiple regression analyses, we converted the ARR data using the natural logarithmic function. This transformation allowed us to obtain a normal distribution of ARR data. Thus, the regression analyses were performed on the logarithmic (natural) transformation of the ARR data. We also analyzed the potential multicollinearity of independent variables using the multicollinearity test, the results of which were not problematic. *p* values less than 0.05 were considered significant. All data were analyzed using SPSS software version 21.

Results

The mean age was 31.96±8.25. Of 102 participants, 84 (82.4%) were female and 18 (17.6%) were male. Mean ARR in the first evaluation (ARR1), second evaluation (ARR2), and EDSS was 0.34±0.56, 0.47±0.62, and 2.79±1.34, respectively.

Table 2 shows the EDSS, ARR1, and ARR2 means and standard deviations for the *HLA-DRA* SNP genotypes. No significant differences were detected between the genotypes.

Table 3 shows EDSS, ARR1, and ARR2 means and standard deviations for the *IL2RA* SNP genotypes. Of the 14 *IL2RA* SNPs, only the rs12722489 variant showed significant association with ARR in two consecutive years. Mean ARRs were 1.06±1.12, 0.20±0.34, and 0.31±.50 for genotypes AA, GA, and GG, respectively (p= 0.008), in the first evaluation, and 1.5±1.08, 0.28±0.40, and 0.42±0.55 for genotypes AA, GA, and GG, respectively (p= 0.001), in the second evaluation.

Multiple regression analyses were performed on EDSS and the natural logarithms of ARR1 (ln

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ARR1) and ARR2 (ln ARR2). Because the ARR1 and ARR2 data did not have a normal distribution, the logarithmic (natural) functions of ARR1 and ARR2 were applied. Applying this function, the related data of ARR1 and ARR2 followed a normal distribution, thus the normality condition of outcome variable to conduct the linear regression was met. In the multicollinearity test, we found no multicollinearity between the potential independent or confounding variables. In regression analyses, the various SNPs were considered as independent variables. In multiple regression analyses, to remove the confounding effects of potential mediators, we entered the sex and age into the model, in addition to the SNPs. In multiple regression analyses, a significant association was obtained only between *IL2RA* rs12722489 in with ARR1 (standardized β=0.319, t=2.884, and P=0.005) and ARR2 (standardized β=0.392, t=3.784, and p <0.001), removing the potential confounding mediators.

Table 2. EDSS, ARR1, and ARR2 means and standard deviations in the *HLA-DRA* SNP genotypes.

Polymorphism	Genotype	Mean±SD	*p* value
EDSS			
DelINSrs9281809	-/-AACTAACT	1.87±1.65	0.366
	AACTAACT/AACTAACT	2.90±1.35	
	INS/-	2.82±1.24	
rs4935356	A/A	1	0.104
	G/A	4±0.5	
	G/G	2.84±1.34	
	G/T	2.68±1.15	
	T/A	5	
	T/T	1.87±1.65	
rs3135390	A/A	2.87±1.32	0.092
	C/A	3±1.25	
	C/C	1.16±1.04	
rs4935354	C/C	1.87±1.65	0.366
	C/T	2.82±1.24	
	T/T	2.9±1.35	
rs3177928	A/A	1	0.090 0.070
	G/A	3.8±1.1	
	G/G	2.70±1.3	
rs7194	A/A	2.90±1.35	0.366
	G/A	2.46±1.29	
	G/G	1.87±1.65	
rs7195	A/A	1.87±1.65	0.366
	G/A	2.82±1.24	
	G/G	2.90±1.35	
rs1131541	T/A	2.85±1.21	.883
	T/T	2.77±1.37	
rs7196	A/A	1.16±1.04	0.090
	T/A	3.45±1.31	
	T/T	2.85±1.31	
rs7197	C/C	2.87±1.32	0.088

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	C/T	3±1.23	
	T/T	0.75±1.06	
rs1051336	G/A	2.85±1.21	0.441
	G/G	2.77±1.37	
rs111471704	T/A	2.5	0.831
	T/T	2.79±1.35	
rs1157343109	T/T	2.78±1.34	-
rs1041885	T/T	2.78±1.34	-
	ARR1		
DelINSrs9281809	-/-AACTAACT	0.12±0.20	0.224
	AACTAACT/AACTAACT	0.43±0.67	
	INS/-	0.23±0.32	
rs4935356	A/A	0	0.423
	G/A	0.22±0.25	
	G/G	0.46±0.70	
	G/T	0.25±0.32	
	T/A	0	
	T/T	0.05±0.11	
rs3135390	A/A	0.39±0.62	0.472
	C/A	0.23±0.33	
	C/C	0.06±0.12	
	T/A	0	
rs4935354	C/C	0.05±0.11	0.190
	C/T	0.24±0.32	
	T/T	0.43±0.67	
rs3177928	A/A	0	0.635
	G/A	0.18±0.24	
	G/G	0.360.58	
rs7194	A/A	0.43±0.67	0.190
	G/A	0.24±0.32	
	G/G	0.05±0.11	
Rs7195	A/A	0.05±0.11	0.190
	G/A	0.24±0.32	
	G/G	0.43±0.67	
rs1131541	T/A	0.24±0.33	0.601
	T/T	0.35±0.58	
rs7196	A/A	0.06±0.12	0.526
	T/A	0.30±0.50	
	T/T	0.37±0.59	
rs7197	C/C	0.39±0.61	0.361
	C/T	0.23±0.33	
	T/T	0.06±0.12	
rs1051336	G/A	0.25±0.32	0.612
	G/G	0.35±0.58	
rs111471704	T/A	1	0.239
	T/T	0.33±0.55	

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rs1157343109	T/T	0.34±0.55	-
rs1041885	T/T	0.34±0.55	-
ARR2			
DelINSrs9281809	-/-AACTAACT	0.12±.20	0.719
	AACT/AACT	0.37±0.51	
	AACTAACT/AACTAACT	0.50±0.68	
	INS/-		
rs493535	A/A	1	0.849
	G/A	0.40±0.61	
	G/G	0.46±0.70	
	G/T	0.37±0.51	
	T/A	0	
	T/T	0.53±0.50	
rs3135390	A/A	0.48±0.64	0.733
	C/A	0.35±0.58	
	C/C	0.41±0.49	
	T/A	1	
rs4935354	C/C	0.53±0.50	0.632
	C/T	0.36±0.51	
	T/T	0.5±0.68	
rs3177928	A/A	1	0.611
	G/A	0.33±0.57	
	G/G	0.46±0.62	
rs7194	A/A	0.5±0.68	0.632
	A/G	0.36±0.51	
	G/G	0.53±0.50	
Rs7195	A/A	0.53±0.50	0.632
	G/A	0.36±0.51	
	G/G	0.50±0.68	
rs1131541	T/A	0.48±0.45	0.923
	T/T	0.45±0.63	
rs7196	A/A	0.41±0.49	0.907
	A/T	0.41±0.55	
	T/T	0.48±0.65	
rs7197	C/C	0.49±0.63	0.743
	C/T	0.35±0.58	
	T/T	0.41±0.49	
rs1051336	G/A	0.42±0.45	0.864
	G/G	0.46±0.63	
rs111471704	T/A	-	-
	T/T	0.45±0.61	
rs1157343109	T/T	0.45±0.61	-
rs1041885	T/T	0.45±0.61	-

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Table 3. EDSS, ARR1, and ARR2 means and standard deviations in the *IL2RA* SNP genotypes.

Polymorphism	Genotype	Mean±SD	*p* value
EDSS			
rs12722489	AA	2.25±1.76	0.472
	G/A	2.25±1.86	
	G/G	2.89±1.25	
rs917751277	TT	2.78±1.34	-
rs992067421	GG	2.78±1.34	-
rs959264277	TT	2.78±1.34	-
rs11597542	AA	2.78±1.34	-
rs140860467	AA	2.78±1.34	-
rs17149458	AA	2.78±1.34	-
rs12722490	GA	2.62±1.12	0.712
	GG	2.82±1.39	
rs3118470	AA	3.02±1.47	0.558
	GA	2.56±1.27	
	GG	2.65±1.17	
rs78556477	CC	2.78±1.34	-
rs41294925	TC	2	0.559
	TT	2.80±1.35	
rs12722491	CC	2.78±1.34	-
rs550805995	CC	2.78±1.34	-
rs12722621	GG	2.78±1.34	-
ARR1			
rs12722489	AA	1.06±1.12	**0.008**
	G/A	0.2±0.34	
	G/G	0.31±0.50	
rs917751277	TT	0.34±0.55	-
rs992067421	GG	0.34±0.55	-
rs959264277	TT	0.34±0.55	-
rs11597542	AA	0.34±0.55	-
rs140860467	AA	0.34±0.55	-
rs17149458	AA	0.34±0.55	-
rs12722490	GA	0.44±0.40	0.618
	GG	0.33±0.57	
rs3118470	AA	0.37±0.63	0.330
	GA	0.39±0.55	
	GG	0.13±0.29	
rs78556477	CC	0.34±0.55	-
rs41294925	TC	0.18±0.09	0.682
	TT	0.34±0.56	
rs12722491	CC	0.34±0.55	-
rs550805995	CC	0.34±0.55	-
rs12722621	GG	0.34±0.55	-
ARR2			
rs12722489	AA	1.5±1.08	**0.001**
	G/A	0.28±0.40	
	G/G	0.42±0.55	
rs917751277	TT	0.45±0.61	-

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rs992067421	GG	0.45±0.61	-
rs959264277	TT	0.45±0.61	-
rs11597542	AA	0.45±0.61	-
rs140860467	AA	0.45±0.61	-
rs17149458	AA	0.45±0.61	-
rs3118470	GA	0.26±0.37	0.595
	GG	0.47±0.63	
	AA	0.52±0.76	
	GA	0.45±0.5	
	GG	0.30±0.37	
rs78556477	CC	0.45±0.61	-
rs41294925	TC	0.22±0.14	0.588
	TT	0.46±0.62	
rs12722491	CC	0.45±0.61	-
rs550805995	CC	0.45±0.61	-
rs12722621	GG	0.45±0.61	-

Discussion

We evaluated the association between the outcome variables of MS patients and the genotypes of some polymorphisms in *HLA-DRA* and *IL2RA*. Fourteen SNPs from each locus were included and the related outcomes were compared between different genotypes for the associated SNPs.

We found that *IL2RA* rs12722489is significantly associated with the annual attack rate adjusted by the disease duration. Weber et al. showed a significant association between this SNP and MS development in unrelated French and German MS patient populations with odds ratios varying from 1.1 to 1.5 (26). In the International Multiple Sclerosis Genetics Consortium (IMSGC), based on a genome-wide study, Hafler et al. reported that some *IL2RA* intron 1 SNPS, particularly rs12722489 and rs2104286, have roles in MS (14).

In agreement with our results, Aniding et al. revealed a significant association between rs12722489 and the ARR. They reported a greater CC than TT or TC genotype frequency (27); but we found greater a greater AA than GG or GA genotype frequency in rs12722489. However, other studies found no significant association between rs12722489 and MS (20, 28, 29). Finally, a recent meta-analysis including the above studies showed that the rs12722489 C allele is associated with elevated MS risk in Caucasians but not in Asians (30).

IL2RA is an important component in lymphocyte differentiation and immune homeostasis (31). The activation of CD4+ T helper and CD8+ effector T cells is affected by IL-2 receptor signaling (32). Thus, blockade of IL-2 signaling can lead to the inhibition of T-cell effector functions (32). Some effective MS treatment drugs act through this mechanism. For example, daclizumab increases CD56 natural killer cell function, and thus leads to activated T cell killing (33). Daclizumab also inhibits the trans-presentation of IL-2 by mature dendritic cells to primed T cells (30). The therapeutic effects of this drug indicate an important role for *IL2RA* in T cell immunity and MS pathogenesis (30).

rs12722489, a polymorphism in the first intron of *IL2RA*, may increase *IL2RA* expression, likely by affecting mRNA processing and half-life (29, 30). Overall, current evidence indicates that certain *IL2RA* alleles are related to MS, and that polymorphisms involved in the immune response can affect MS pathogenesis (14, 34).

The EDSS and ARR analyses of the 14 different *HLA-DRA* SNP genotypes found no significant association between the SNPs and related outcomes.

Although the association between *HLA* antigens and MS has been known for more than four decades, the confirmed association is limited to a small number of genes and alleles, including *HLA-*

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DRB1 and the DRB1*15:01 allele (24, 25).

Based on GWASs, 10.5% of genetic variation of the underlying risk of MS may be explained by *HLA-DRB1* in the MHC class II region (4). The association between *HLA* and MS primarily involves *HLA-DRB1*; however, one study on an Australian population reported complete linkage between DRB1*1501 and the *HLA-DRA* promoter A allele. This issue shows that MS susceptibility haplotype (DRB1*1501-HLA-DQB1*0602-HLA-DQA1*0102) may be considered as a mediating factor between the *HLA-DRA* locus and MS pathogenesis (35).

In addition, the *HLA-DRA* rs3135388 variant is also associated with MS (36). This SNP is a proxy marker for DRB1*1501, and its relationship with MS was reported in a previous study (36). We did not evaluate the association between rs3135388 and MS, but a possible association between a polymorphism of a near locus with this polymorphism, rs3135390, and MS, was assessed. The results were not statistically significant.

Our study had some limitations. We did not include the MRI results as an outcome measure; however, the EDSS score assesses the functional status of patients based on anatomical involvement in brain that is clinically valuable. We included ARR as another outcome measure. This measure evaluates the annual number of attacks adjusted for the disease duration. Because MS is usually progressive, this adjustment helped us to control the bias of disease duration in our analyses. The small sample size may be considered another limitation of our study. This issue might have led to a lower power of the study to detect statistically significant differences or associations, particularly if these differences or associations were small. However, finding a significant association, particularly between the *IL2RA* rs12722489 variant and the ARR, despite the study's low power, indicates that the association may be considerable; our descriptive data also supports this issue. Multicenter studies with large sample sizes are suggested to evaluate other SNPs with MS severity and relapse rates. We did not conduct the subgroup analysis as a result of small sample size and power problem issues; however, we conducted multivariate analyses to adjust the results according to sex and age.

The *IL2RA* rs12722489 variant associated with ARR but not with EDSS. No significant association was found between *HLA-2RA* SNPs and MS attack rates or severity. Further multicenter studies with larger sample sizes are required to establish the associations between other polymorphisms with and MS severity and relapse rates.

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