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 IL2RA 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 IL2RA 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 in recent decades, especially in the northern hemisphere.
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 favor of 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 erythematosus, 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-DRB1 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-DRB1 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.
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 ARRrs were 1.06±1.12, 0.20±0.34, and 0.31±0.50 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
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 rs12722498 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                 | 2.87±1.32    | 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                 | 1            | 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         |
|              | G/A                 | 3.8±1.1      | 0.070         |
|              | G/G                 | 2.70±1.3     |               |
| rs7194       | A/A                 | 2.90±1.35    |               |
|              | G/A                 | 2.46±1.29    | 0.366         |
|              | 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    |               |
|              | T/A                 | 3.45±1.31    | 0.090         |
|              | T/T                 | 2.85±1.31    |               |
| rs7197       | C/C                 | 2.87±1.32    | 0.088         |
|                  | C/T       | T/T       |
|------------------|-----------|-----------|
| rs1051336        | 3±1.23    | 0.75±1.06 |
|                  | G/A       | 2.85±1.21 |
|                  | G/G       | 2.77±1.37 |
| rs111471704      | T/A       | 2.5       |
|                  | T/T       | 2.79±1.35 |
| rs1157343109     | T/T       | 2.78±1.34 |
| rs1041885        | T/T       | 2.78±1.34 |

**ARR1**

|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|------------------|-----------|-----------|-----------|-----------|-----------|
| DelINSrs9281809  | 0.12±0.20 | 0.22±0.25 | 0.46±0.70 | 0.25±0.32 | 0.05±0.11 |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |

**rs7194**

|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|------------------|-----------|-----------|-----------|-----------|-----------|
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |

**Rs7195**

|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|------------------|-----------|-----------|-----------|-----------|-----------|
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |

**rs7197**

|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|------------------|-----------|-----------|-----------|-----------|-----------|
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |

**rs1051336**

|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|------------------|-----------|-----------|-----------|-----------|-----------|
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |

**rs111471704**

|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|------------------|-----------|-----------|-----------|-----------|-----------|
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |

**rs1157343109**

|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|------------------|-----------|-----------|-----------|-----------|-----------|
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |

**rs1051336**

|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|------------------|-----------|-----------|-----------|-----------|-----------|
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
|                  | A/A       | G/A       | G/G       | T/A       | T/T       |
| rs1157343109 | T/T | 0.34±0.55 | - |
| rs1041885 | T/T | 0.34±0.55 | - |

**ARR2**

| DelINSrs9281809 |       |     |     |     |
|-----------------|-------|-----|-----|-----|
| A/A             | 1     |     |     |     |
| 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 |     |     |     |

| rs493535 |       |     |     |     |
| A/A     | 0.48±0.64 |     |     |     |
| C/A     | 0.35±0.58 |     |     |     |
| C/C     | 0.41±0.49 |     |     |     |
| T/A     | 1     |     |     |     |
| T/T     | 0.5±0.68 |     |     |     |

| rs3135390 |       |     |     |     |
| A/A     | 1     |     |     |     |
| C/A     | 0.33±0.57 |     |     |     |
| G/G     | 0.46±0.62 |     |     |     |

| rs7194    |       |     |     |     |
| A/A     | 0.5±0.68 |     |     |     |
| A/G     | 0.36±0.51 |     |     |     |
| G/G     | 0.53±0.50 |     |     |     |

| Rs7195    |       |     |     |     |
| A/A     | 0.53±0.50 |     |     |     |
| G/A     | 0.36±0.51 |     |     |     |
| G/G     | 0.50±0.68 |     |     |     |

| rs1131541 |       |     |     |     |
| T/A     | 0.48±0.45 |     |     |     |
| T/T     | 0.45±0.63 |     |     |     |

| rs7196    |       |     |     |     |
| A/A     | 0.41±0.49 |     |     |     |
| A/T     | 0.41±0.55 |     |     |     |
| T/T     | 0.48±0.65 |     |     |     |

| rs7197    |       |     |     |     |
| C/C     | 0.49±0.63 |     |     |     |
| C/T     | 0.35±0.58 |     |     |     |
| T/T     | 0.41±0.49 |     |     |     |

| rs1051336 |       |     |     |     |
| G/A     | 0.42±0.45 |     |     |     |
| 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 |     |     |     |
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 |       |
|              | GA       | 2.56±1.27 | 0.558 |
|              | GG       | 2.65±1.17 |       |
| rs78556477   | CC       | 2.78±1.34 | -      |
| rs41294925   | TC       | 2.80±1.35 | 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 | -      |
| SNP             | Allele 1 | Allele 2 | p-value |
|-----------------|----------|----------|---------|
| rs992067421     | GG       | -        | -       |
| rs959264277     | TT       | 0.45±0.61 | -       |
| rs11597542      | AA       | 0.45±0.61 | -       |
| rs140806467     | AA       | 0.45±0.61 | -       |
| rs17149458      | AA       | 0.45±0.61 | -       |
| rs12722489      | GA       | 0.26±0.37 | -       |
|                 | GG       | 0.47±0.63 | -       |
| rs3118470       | AA       | 0.52±0.76 | 0.595   |
|                 | GA       | 0.45±0.5  |         |
|                 | GG       | 0.30±0.37 |         |
| rs78556477      | CC       | 0.45±0.61 | -       |
| rs142994925     | 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* rs12722489 is 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 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-*
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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References
1. Alcina A, Abad-Grau Mdel M, Fedetz M, Izquierdo G, Lucas M, Fernandez O, et al. Multiple sclerosis risk variant HLA-DRB1*1501 associates with high expression of DRB1 gene in different human populations. PLoS One. 2012;7(1): e29819.
2. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron. 2006;52(1):61-76.
3. Isobe N, Keshavan A, Gourraud P-A, Zhu AH, Datta E, Schlaefer R, et al. Association of HLA genetic risk burden with disease phenotypes in multiple sclerosis. JAMA Neurol. 2016;73(7):795-802.
4. Hollenbach JA, Oksenberg JR. The immunogenetics of multiple sclerosis: a comprehensive review. J Autoimmun. 2015;64:13-25.
5. Simpson S, Blizzard L, Otahal P, Van der Mei I, Taylor B. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. J Neurol Neurosurg Psychiatry. 2011;82(10):1132-41.
6. Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol. 2010;9(5):520-32.
7. Hosseinizadeh A, Baneshi MR, Sedighi B, Kermanchi J, Haghdoot AA. Incidence of multiple sclerosis in Iran: a nationwide, population-based study. Public Health. 2019;175:138-144.
8. Stadelmann C, Wegner C, Brück W. Inflammation, demyelination, and degeneration—recent insights from MS pathology. Biochim Biophys Acta. 2011;1812(2):275-82.
9. Ebers G, Sadovnick A, Risch N. A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. Nature. 1995;377(6545):150-1.
10. Ebers GC, Yee IM, Sadovnick A, Duquette P. Conjugal multiple sclerosis: Population-based prevalence and recurrence risks in offspring. Canadian Collaborative Study Group. Ann Neurol. 2000;48(6):927-31.
11. Willer C, Dyment D, Risch N, Sadovnick A, Ebers G, Group CCS. Twin concordance and sibling recurrence rates in multiple sclerosis. Proc Natl Acad Sci U S A. 2003;100(22):12877-82.
12. Bertrams J, Kuwert E, Liedtke U. HL‐A susceptibility loci. Proc Natl Acad Sci U S A. 2003;100(22):12877-82.
13. Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, De Jager PL, et al. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med. 2007;357(6545):150-1.
14. Alcina A, Fedetz M, Ndagire D, Fernández-Arquero M, Bartolomé M, et al. Polymorphisms in the IL2, IL2RA and IL7RA genes in multiple sclerosis risk. PLoS Genet. 2009;5(1):e1000342.
15. Bartsch H, Stolte G, Hartung HP. Conjugal multiple sclerosis: Population-based study. Public Health. 2019;175:138-2010;18(7):794-9.
16. I. Multiple sclerosis: association with HL...
southern Japanese. J Neurol Sci. 2014;337(1-2):147-50.
28. Dai Y, Li J, Zhong X, Wang Y, Qiu W, Lu Z, et al. IL2RA allele increases risk of neuromyelitis Optica in Southern Han Chinese. Can J Neurol Sci. 2013;40(6):832-5.
29. Matiello M, Weinshenker BG, Atkinson EJ, Schaefer-Klein J, Kantarci OH. Association of IL2RA polymorphisms with susceptibility to multiple sclerosis is not explained by missense mutations in IL2RA. Mult Scler J. 2011;17(5):634-6.
30. Wang X-X, Chen T. Meta-analysis of the association of IL2RA polymorphisms rs2104286 and rs12722489 with multiple sclerosis risk. Immunol Invest. 2018;47(5):431-442.
31. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. Nat Rev Immunol. 2012;12(3):180-90.
32. Chistiakov DA, Voronova NV, Chistiakov PA. The crucial role of IL-2/IL-2RA-mediated immune regulation in the pathogenesis of type 1 diabetes, an evidence coming from genetic and animal model studies. Immunol Lett. 2008;118(1):1-5.
33. Rose JW, Giovannoni G, Wiendl H, Gold R, Havrdova E, Kappos L, et al. Consistent efficacy of daclizumab beta across patient demographic and disease activity subgroups in patients with relapsing-remitting multiple sclerosis. Mult Scler Relat Disord. 2017;17:32-40.
34. Baecher-Allan C, Hafler DA. Human regulatory T cells and their role in autoimmune disease. Immunol Rev. 2006;212:203-16.
35. Bennetts BH, Teutsch SM, Buhler MM, Heard RN, Stewart GJ. HLA-DMB gene and HLA-DRA promoter region polymorphisms in Australian multiple sclerosis patients. Hum Immunol. 1999;60(9):886-93.
36. Morrison BA, Ucisik-Akkaya E, Flores H, Alaez C, Gorodezky C, Dorak MT. Multiple sclerosis risk markers in HLA-DRA, HLA-C, and IFNG genes are associated with sex-specific childhood leukemia risk. Autoimmunity. 2010;43(8):690-7.