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Original paper

The Association between HLADRB1*1501, IL7Ra, and APOE Polymorphism and MS in Iranian Population

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Abstract

Background and aim: Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system. Despite many research carried out to find the pathogenesis of this disease, the MS pathogenesis is still unclear. The association of HLA-DRB1*1501 with MS risk has been investigated in many populations. This study aimed to investigate the association of HLADRB1*1501, IL7Ra, and APOE polymorphism genes with MS in patients in Tehran.

Materials and methods: The participants in this study were selected from the neurology department of Shariati Hospital in Tehran (30 patients with MS), and 30 healthy blood donors from the Tehran Blood Transfusion Center (control group). Blood samples were obtained and DNA was extracted using the PCR method. The genotype was determined using the sequencing approach.

Results: A significant association was identified between these polymorphisms in the patient and control groups in the HLADRB1*1501 gene, G allele (p = 0.000), and in the IL7Ra gene, T allele (p=0.022). However, the PCR results for the APOE gene were unfavorable for sequencing, and the genotype was not determined.

Conclusion: The findings of this study revealed that the polymorphism of the 1501*HLADRB1 and IL7Ra genes in the Tehran patient population was similar to the European population.

Keywords: *MS, Polymorphism, HLADRB1*1501, IL7Ra, APOE*

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Introduction

MS (multiple sclerosis) is a serious disease that destroys the central nervous system and causes paralysis and death in the patient. This condition has been associated with inflammatory demyelination and myelin abnormalities [1].Multiple sclerosis is an autoimmune disease of the central nervous system in which TCD4 cells respond against myelin antigens, activating macrophages surrounding neurospinal nerves and causing irregularities in neural conduction and neurological impairments by degrading myelin [2]. Each natural person's immune system has the potential to identify, respond to, and eradicate many alien antigens, whereas it does not react negatively to individual antigens and actually exhibits tolerance. This tolerance is achieved by a variety of processes, and any anomalies in the induction or maintenance of self-tolerance result in immune responses to autoantigens and the development of autoimmune illnesses [3].

MS disease disproportionately affects young people. Various research reveal that this condition is very common in Iran. There are other theories about the cause of the disease, but the most widely accepted is that MS is triggered by an immune system attack on a person's own nerve system. Regarding the analysis of APOE gene polymorphism in various communities around the world: Tamam et al. (2011) [4] conducted a study in the population of MS patients in Turkey. The E4 allele was found to be more in the male population. Although there was no statistically significant link between this gene polymorphism and MS disease in this study, patients with the E4 allele had lower serum levels of apolipoprotein E at the time of disease recovery. Losonzci et al. [5] conducted a study in the population of MS patients in Hungary using the RFLP-PCR method in 2010. It was discovered that the existence of E2 and E4 alleles has a significant effect in illness progression. Sadeghi et al. [6] conducted a study in the population of MS patients in Iran using the RFLP-PCR method in 2011. The number of participants in two groups of 20 people, control and patient, was explored in this study. There was no statistically significant link between this gene polymorphism and MS illness. Hadavi et al. [7] did a study in the population of MS patients in Iran using the RFLP-PCR method in 2004. A strong connection was found between the occurrence of the E4 allele in the patient population and the presence of the E4 allele in the control population. In the 2004 [8] study by al- Shammri et al. in the population of patients with MS in Kuwait, the RFLP-PCR method was used. The control group included 109 participants, while the patient group included 39 participants. A strong association was found between the occurrence of the E4 allele in the female patient population and the control population. Evangelo and colleagues discovered that the abundance of E4 allele is more common in the chronic type of MS disease in their 1999 study of MS patients in England using the RFLP-PCR method. The RFLP-PCR method was used in a study conducted by da Silva et al. in 2014 [9] in a population of MS patients in Portugal. There was no significant link found between the E4 allele and MS illness. Yin and colleagues conducted a meta-analysis of twenty population groups in their 2012 study. As a control group, 4080 patients and 2897 healthy people were studied. According to the results of this study, the E2 allele enhances the progression of MS disease by 1.16 times when compared to the control group. Furthermore, the E2E4 genotype accelerates disease progression 1.74 times faster than the E3E3 genotype. The E2 allele mutation is linked to the progression of MS disease, while the E3E4 genotype was found to be protective.

The HLADRB1*1501 gene is found on the short arm of chromosome 6 at P.21.3, and these molecules help detect antigens and present them to T cells. The type 1 cytokine receptor family includes the interleukin 7 receptor. This gene is located on chromosome 5's short arm. (5p13) The interleukin-7 receptor is essential for the development of immune cells, particularly lymphocytes. The APO E gene is found on the long arm of chromosome 19 and encodes a 299 amino acid

polypeptide. According to many studies, APO E suppresses the release of cytokines, which are important for the development of chronic inflammatory processes in the advancement of central nervous system illnesses like MS. The aim of this study is to look into the polymorphism association between the HLADRB1*1501, IL7Ra, and APO E genes in MS patients in Tehran.

Because of the involvement of internal nerve sections, neurological symptoms in MS are various. We will see varied symptoms, especially in the initial attack of the disease, due to the peculiarities of the disease and the involvement of different sections of the CNS and how effective variables affect the development of the disease, so we can never consider the classical form of the disease. The optic nerve, brain stem, cerebellum, and spinal cord, on the other hand, are more implicated in this condition, and the clinical manifestations induced by these lesions can be the diagnostic key in dealing with these patients. In 45% of patients, the disease begins with a single symptom that can be linked to CNS injury in a specific region, and in 55% of patients, the onset is followed by a slew of clinical symptoms. In some circumstances, the onset is a significant symptom, and in others, the symptoms are so weak that the patient ignores them. The most typical symptom at the start of the disease, according to most research, is weakness and weakness in organs or sensory abnormalities [10].

MS is classified clinically into 3 types: (1) Relapsing-remitting condition (RR-MS), (2) Secondary progressive form (SP-MS), and (3) Primary progressive form (PP-MS). The relapsing- remitting type (RR-MS) is present in 80-90% of affected persons. Periods of intermittent attacks and relative recovery are noticed in this form, which, with the passage of time and the progressive growth in nervous system damage, will likewise result in severe disability, with approximately 50% of patients requiring a wheelchair after 19 years. This will result in another form of the disease, known as (SP-MS) or secondary progressive. The third type of PP-MS, or primary progressive, accounts for around 10-20% of patients who will have progressive disability without acute attacks [3].

Despite the disease's intricacy and unclear mechanism, it is thought that in genetically susceptible people, such as those with the region (HLA), the intervention of environmental and epigenetic variables can cause MS. Environmental risk factors seen in sick persons include living in temperate zones, a lack of vitamin D, stress, and viral disorders [10].

MicroRNAs (miRNAs) are short non-coding RNAs that limit protein creation by damaging or inhibiting mRNA translation. The importance of these genetic components in the regulation of many cellular processes such as growth, cell cycle, differentiation, apoptosis, metabolism, and angiogenesis is now well understood [11]. Furthermore, miRNAs have been shown to influence the regulation of gene expression networks in oncogenic or tumor suppressor pathways. MiRNAs are highly expressed in immune cells and the brain system, indicating their role in neuroimmunology. Because of the identification and stability of these components in human fluids, they can now be used as new biomarkers in clinical diagnostics. Recent research has demonstrated that single nucleotide alterations in miRNA sequences might affect their production or affinity for binding to target genes, resulting in pathogenic changes. Certain miRNAs involved in the regulation, development, and differentiation of various cell lines have altered the biological activity of T cells and B cells [11].

T cell growth and differentiation in the thymus, as well as their activity in the peripheral blood, are linked to a complex protein signaling network regulated by miRNAs. Autoimmunity is influenced by the innate immune system. According to research, the dynamic interplay of certain transcription factors and miRNAs is responsible for the formation and function of myeloid cells. These miRNAs regulate granulocytes, monocytes, macrophages, CDs, and natural killer cells [12]. Some investigations have found that MS patients have aberrant miRNA expression when

compared to healthy controls [13]. MiRNA genetic alterations and mutations influence immune system evolution and immunological response, and they play a role in the etiology of autoimmune illnesses such as MS.

Despite recent developments in laboratory and radiology in order to detect MS, there is currently no laboratory method or particular biomarker that can aid to diagnose this condition. MS is currently diagnosed using a combination of clinical and paraclinical data [14].

Single nucleotide polymorphisms are mutations caused by a single nucleotide substitution in DNA, and their prevalence in the population is 1%. These types of mutations are the most prevalent type of genetic diversity in humans, accounting for 90% of individual variances. Single nucleotide polymorphism (SNP) is strongly linked to a variety of disorders. A single nucleotide alteration in a regulatory, intronic, or exonic region can impact gene translation and expression.

A synonymous polymorphism is an SNP in which both alleles produce the identical polypeptide sequence. Insertion polymorphism occurs when a distinct polypeptide sequence is generated. An insertion polymorphism might be missense, resulting in a different amino acid, or it can be nonsense, resulting in a premature stop codon. Insertion polymorphisms cause more than 60% of mutational disorders [15].

Changes in the DNA sequence can influence illness development and response to viruses, chemicals, medications, vaccinations, and other agents. Although their primary value in biological research is to compare sections of the genome between homologous groups, investigations connected to SNPs are also essential in cattle products and associated programs. SNPs are typically biallelic and thus quantified. SNPs typically do not act alone, but rather collaborate with other SNPs to represent the state of a disease [16].

Material and Methods

Sampling

The participants in this study were selected from the neurology department of Shariati Hospital in Tehran (patients), and healthy blood donors from the Tehran Blood Transfusion Center who served as a control group. Blood samples were sent to the laboratory and DNA was extracted. This case-control study included 60 partcipants: 30 patients and 30 healthy people (Table 1).

Table 1. Information on the population studied in the study

Gender	Healthy	Patient
Male	10	10
Female	20	20
total	30	30

For DNA extraction, 2 mL of blood was obtained from each participant and stored in tubes containing EDTA at 20°C until testing. To lyse the red blood cells, 200µL of blood was mixed with 800 µL of fresh ammonium chloride and placed on a rotator for 20 minutes. The solution was centrifuged for 2 minutes at 10,000 rpm, then washed with 400 µL of EDTA-NaCl three times. The separated sediment was washed again, and the supernatant was removed. The red blood cells were therefore totally removed. 500 µL of 50mM NaOH was added to the obtained sediment and mixed using a vortex for 10 seconds before immersing it in boiling water for 5 minutes. 100 µL of 1 M HCl-Tris was added and mixed using and vortex until the solution is neutralized. To eliminate cell debris, solution was centrifuged. The supernatant solution containing the isolated DNA was kept at 20°C.

The isolated DNA samples were diluted 1:50 and the concentration was determined using a biophotometer. A pure double-stranded DNA solution with a concentration of 50 g/mL at a wavelength of 260 nm has a concentration of 1 and an OD280/OD260 ratio of 1.8. If the sample contains protein or phenol, this ratio will be much less than 1.8, whereas if the sample contains RNA, this ratio will be greater than 1.8. Nucleic acids absorb UV light in the 260 and 280 nm range and can be observed due to the binding of these acids to ethidium bromide, which is visible at this wavelength, as measured by ethidium bromide fluorescence. As a result, the presence of DNA can be demonstrated using the electrophoresis method. To determine the sequence of the PCR product, 25 to 50 µL of the sample was sent to Tekaposist Company, Tehran-Iran for sequencing. To assess the quality of extracted DNA, two methods were utilized. It was measured using an optical absorption spectrophotometer at 260 and 280 nm in the first method. The quantitative test results showed that the OD280/OD260 ratio for the samples was in the range of 1.83-1.65, indicating that the spectrophotometry results had the optimal quality of DNA extraction. The second method employed 1% agarose gel and the existence of extracted DNA was confirmed.

Results

Polymorphism Analysis Results

The Results of the rs3135388 Polymorphism Analysis

Comparison of genotypic and allelic frequencies of rs3135388 between control and patient groups indicated that the GG genotype of the rs3135388 polymorphism of the HLADRB1*1501 gene considerably reduces the probability of having MS. Furthermore, the G allele of this polymorphism was found to be less likely to acquire MS than the A allele. The AA genotype was significaltly associated with an increased risk of MS ($p<0.001$).

Table 2. The genotype distribution of the rs3135388 polymorphism in the patient and control groups.

Genotype	control subjects (n=30)	MS Subjects (n=30)	OR	P-value	CI
HLADRB1 1501(rs3135388)					
AA	1(3%)	6(20%)	1	0.001	------
AG	4(13%)	15(50%)	0.625	0.70	0.057-6.801
GG	25(84%)	9(30%)	0.060	0.014	0.006-0.569
Frequency of A allele	10%	45%	1	-----	------
Frequency of G allele	90%	55%	0.136	0.000	0.051-0.364

Our results revealed that the genotypic frequency of the 1501*HLADRB1 gene polymorphism (rs3135388) followed the Hardy-Weinberg equilibrium.

The comparison of genotypic and allelic frequencies of rs6897932 between control and patient groups revealed that that the T allele of the IL7Ra gene's rs6897932 polymorphism significantly reduced the probability of developing MS. Furthermore, the results revealed that the CC genotype (p=0.034) and CT genotype (p=0.016) had a significant link with MS disease, which is caused by the presence of the C risk allele.

Table 3. The genotype distribution of the rs6897932 polymorphism in the patient and control groups.

Genotype	control subjects (n=30)	MS Subjects (n=30)	OR	P-value	CI
IL7Ra(rs6897932)					
CC	7(23%)	17(56%)	1	0.034	------
CT	20(67%)	12(40%)	0.247	0.016	0.079-0.768
TT	3(10%)	1(4%)	0.137	0.109	0.012-1.556
Frequency of C allele	56%	77%	1	------	------
Frequency of T allele	44%	23%	0.398	0.022	0.181-0.874

The genotypic frequency of the IL7Ra gene polymorphism (rs6897932) followed the Hardy-Weinberg equilibrium.

The comparison of genotypic and allelic frequencies of rs2104286 between control and patient groups indicated that there was no significant link between the genotypes of the IL2Ra gene polymorphism rs2104286 and MS disease in the examined population; nevertheless, homozygous GG lowers the chance of getting the condition. Although this rs did not have a significant link with MS disease in this population, the G allele or = 0.553 showed that this allele has a protective role in the study population.

Table 4. The frequency distribution of genotypes for the rs2104286 polymorphism in the patient and control groups.

Genotype	control subjects (n=30)	MS Subjects (n=30)	OR	P-value	CI
IL2Ra(rs2104286)					
AA	14(47%)	19(63%)	1	0.403	------
AG	12(40%)	9(30%)	0.553	0.293	0.183-1.67
GG	4(13%)	2(7%)	0.368	0.285	0.059-2.30
Frequency of A allele	66%	78%	1	------	------
Frequency of G allele	34%	22%	0.553	0.155	0.245-1.25

Our results revealed that the genotypic frequency of the IL2Ra gene polymorphism (rs2104286) in the group followed the Hardy-Weinberg equilibrium.
In the present study the PCR results for the APOE gene were unfavorable for sequencing, and the genotype was not determined.

Discussion

MS is a complex central nervous system illness that, despite an unknown cause, is likely developed by the mutual effects of inflammatory processes and neuronal loss, and it is estimated that more than two million people worldwide suffer from it. The primary feature of this disease is the breakdown of axon myelin sheaths and the creation of astrocyte lesions. It is not a hereditary disease in the traditional sense, but it appears that genetic background, as well as environmental factors (such as infection, nutrition, stress, and even location of residence), play a role in vulnerability to the condition. MS is a disease that has a strong genetic impact, according to studies on family and twins cases. The prevalence of this disease is 20 to 40 times higher among first degree relatives of patients than in the general population. The fact that identical twins have a greater concordance rate (25-34%) than non-identical twins (2-5%) demonstrates their strong heritability.
In line with our findings, the results of studies investigating the association of rs3135388 polymorphism of the HLADRB1*1501 gene with MS disease, reveal a link between this polymorphism and MS. According to the findings of this study, the genotype of rs in the patient population of Tehran province is similar to European population [17].
Data analysis revealed that the T allele of the IL7Ra gene's rs6897932 polymorphism significantly reduced the likelihood of MS. Haj et al. [6] discovered a strong association between CC and CT genotypes and MS in a study conducted in the population of Eastern Iran. According to Omraninava's [18] study, there is a link between the rs6897932 polymorphism and an elevated risk of MS in the Australian population. The study by Stankovic et al. [19] found no significant variation in genotype and allele frequency between the patient and control groups. The findings of this study indicated that further gene variants must be identified in order to evaluate the genetic propensity to MS in the study population. The interleukin 7 receptor (IL7R) has been identified as a risk gene for MS. A single nucleotide polymorphism (SNP) analysis of rs6897932, which is significantly linked to MS, revealed the impact of genotype on the relative expression of the membrane-bound IL7R gene on the total amount of this gene in mRNA. In the study by Lundmark et al. [20] three SNPs were shown to have a significant connection with higher susceptibility to MS in the examined population, one of which was rs6897932. According to the results of Yoshimura's [21] study, the CC genotype of rs6897932 IL7RA in Japanese patients with MS is clearly associated with vulnerability to the condition.
Despite evidence of an association between the IL2Ra gene polymorphism rs2104286 and MS, in some studies researchers found no significant association in the Fars province population, which is similar to the Asian population studied by Yoshimura [21]. Of course, when discussing why the findings obtained in some different societies differ or agree with the findings obtained in our study, it should be noted that, in addition to the differences in the selection conditions of patients and controls in different studies, factors such as ethnicity, race, environmental factors, and so on should be considered, and more research should be conducted to prove the role of this polymorphism as an independent risk factor in MS disease.

Conclusion

Our findings revealed the existence of a link between the IL7Ra and HLADRB1 genes, as well as the absence of a link between the IL2Ra gene and MS disease in the study population. Furthermore, the results of this study revealed that the HLADRB1 and IL7Ra genes in the study population were similar to the European population. However, our findings about the IL2Ra gene were more similar to the Asian population and the European population of the German race. To achieve more documented data, further research with large population size are required.

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Conflict of interests

The authors declare that there are no competing interests.

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