Original Article

STUDY OF ASSOCIATION BETWEEN SUSCEPTIBLE VARIANTS IN GENES WITH VITAMIN D RESPONSE ELEMENTS AND THE RISK OF RELAPSING-REMITTING MULTIPLE-SCLEROSIS IN IRANIAN AZERI POPULATION

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Abstract

Background & Aims: Multiple sclerosis (MS) (MIM #126200) is a chronic, inflammatory, demyelinating disorder of the central nervous system (CNS) that is the most common reason for non-traumatic neurological defects among young people. The aim of this study was to evaluate the association between single-nucleotide polymorphisms (SNPs) in genes with vitamin D response elements (VDREs) in their regulatory regions and the risk of relapsing-remitting multiple sclerosis (RR-MS) in the Iranian Azeri population.

***Materials & Methods*:** A total of 129RR-MS cases and 200 normal controls from West Azerbaijan were recruited in this study. We genotyped the fourteen MS susceptible variants in genes with vitamin D response elements that emerged from previous genome-wide association studies (GWAS), using Tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) assay in every participant. Chi-square, Fisher's exact test, and allelic and genotypic regression analysis were used to investigate the association of these polymorphisms with RRMS.

***Results*:** Three polymorphisms showed significant association (p-value < 0.05) with RR-MS. In particular, rs4410871 of PVT1 (p-value= 0.035, TT vs CC OR[95%CI]= 2.775 (1.253-6.146)), rs212405 of TAGAP (p-value=0.016, AA vs TT OR[95%CI]= 2.463 (1.255-4.835)), and rs7090512 of IL2RA (p-value= 0.008, CC vs TT OR[95%CI]= 2.865 (1.433-5.731)) were identified as susceptible risk factors in our group.

***Conclusion*:** The current study replicated fourteen susceptible variants in genes with vitamin D response elements and the risk of RR-MS in the Iranian Azeri population, which implies the existence of some similarities between the MS genetic structure of the GWAS populations and the studied Iranian Azeri population.

Keywords: Single Nucleotide Polymorphism, Relapsing-Remitting Multiple Sclerosis, Vitamin D Response Elements, PVT1, TAGAP, IL2RA

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Introduction

Multiple sclerosis (MS) is an autoimmune disease and the leading cause of non-traumatic disability in young adults, formed by attacking the immune system to the myelinated axons in the neurons of the central nervous system (CNS), destroying the myelin in these neurons (1). Epidemiologic data reveals that up to 2.8 million people live with MS worldwide, with a frequency of 36 per 100,000, whereas the prevalence of MS is being continuously increased from 2013 (2). The etiology of MS is not exactly unraveled, however, it appears that a complicated interaction of genetic background and non-genetic factors, including viruses and other environmental factors are involved. These factors additively result in the action of the immune system against healthy cells in one's body (3). Taken together, the disease is formed by autoreactive lymphocytes that trans-migrate across the blood-brain barrier (BBB) and enter the brain parenchyma, where they can cause local inflammation and lead to demyelination and subsequent axonal loss (4).

According to the disease course, MS patients can be categorized into four main groups: relapsing-remitting (RR-MS), primary progressive (PP-MS), secondary progressive (SP-MS), and progressive relapsing (PR-MS) (5). RR-MS is the most common form, contributing to 85% of the patients, which is marked by relapses or exacerbations of the symptoms followed by subsequent remission periods when the symptoms almost disappear (6).

The contribution of genetic factors in the occurrence of MS was established early on. The first studies witness the role of genetic background in the susceptibility to MS. These findings came from the studies on twins, which showed a higher concordance in monozygotic twins, compared to dizygotic ones (7). Thereafter, it became apparent that up to 20% of MS patients had a positive familial history, which was significantly higher than its rate in the general population (8). These studies instigated the researchers to investigate the role of genetic factors in the pathogenesis of MS with the hope to find candidate genes underlying the susceptibility to MS. During the first decade of the 21st century, various linkage studies on association of candidate gene in MS were performed on large cohorts of MS patients. According to these studies, the human leukocyte antigen (HLA) class II-DRB1 locus on chromosomal location 6p21 has been consistently identified as the strongest genetic locus for MS (9). However, during the past few years, the genetics of MS has been revolutionized by genome-wide association studies (GWAS), which have led to introduction of more than 200 non-HLA loci as the MS predisposing genetic factors. In 2011, two GWAS conducted on more than 9000 MS patients found 52 non-HLA loci to be significantly associated with MS. Interestingly, these GWAS analyses demonstrated the significance of vitamin D-processing enzymes (including CYP27B1 and CYP24A1) in MS (10). Another study showed that in lymphoblastoid cells, HLA II-DRB1 is regulated by vitamin D (11). It is now obvious that vitamin D deficiency is a major environmental factor for MS, and the mechanisms underlying the interaction between the genetic factors and vitamin D is being increasingly considered as a major topic in the etiology of MS (12). In this regard, it has been suggested that polymorphisms in genes which have vitamin D receptor (VDR) binding site may affect vitamin D metabolism and therefore, be predisposing factors for MS (13). However, these results alone do not pinpoint who will develop MS in his life, but they contribute to identify the risk factors that may ultimately predict a person's risk of developing MS and point to ways of its prevention. Since none of the previous studies included the Iranian population, we conducted this study to evaluate the association between fourteen single nucleotide polymorphisms (SNPs) in genes with

vitamin D response elements (VDREs) in their regulatory regions and the risk of RR-MS in the Iranian Azeri population.

Materials & Methods

A total of 129 RR-MS patients and 200 healthy controls from West Azerbaijan Province, Iran, matched by age, gender, and ethnicity were recruited in a case-control study.

Patients were included in the study if they had a clinically definite diagnosis of relapsing-remitting multiple sclerosis (RR-MS) according to the criteria of McDonald (20) or Poser (21). The control group consisted of healthy subjects without a history of neurological disease and was matched with cases for age, gender, and ethnicity. The demographic data of the samples are presented in Table 1. The study was approved by the Ethics Committee of the Urmia University of Medical Sciences, Urmia, Iran (Approval ID: IR.UMSU.REC.1397.131). Written informed consent was taken from all patients and controls included in the study. The sample size was calculated through Cochran's formulas:

$N = (Z1 - \alpha/2)2\ p\ (1-p)/\ d2$

$Z1 - \alpha/2 = 1.96$, $p = 0.5$, $d = 0.1$, $\alpha = 0.05$, $N=96$.

According to Cochran's formulas, the minimum sample size in this study was 96 patients. However, more people were included to better results.

Table 1. Demographic data of the studied groups.

Characteristics	Controls	Total patients
Female, n (%)	134 (67%)	82 (63.56%)
Male, n (%)	66 (33%)	47 (36.44%)
Total, n	200	129
Female: male ratio	2.03	1.74
Age at time of analysis, mean ± SD (range)	37.54±8.55 (20-60)	37.24 ±7.95 (22-57)

Isolation of DNA, SNP Selection, and Genotyping Process:

Genomic DNA was isolated from peripheral white blood cells using the salting-out standard method. The concentration and purity of the extracted DNA were assayed by Eppendorf Bio Photometer. The 260/280 ratio was between 1.76 to 1.9. DNA samples were diluted to working solutions of 50-100 ng/µl.

Fourteen susceptible SNPs in genes with vitamin D response elements which had suggestive evidence of association with multiple sclerosis were selected. Reference alleles, wild-type genotypes, and minor alleles were determined based on the comparison between case and control populations.

The SNP genotyping was performed using Tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) assay. The primer design was done using the Primer1 database site (http://Primer1.Soton.ac.uk/primer1.html) and validated through the Primer-BLAST-NCBI database (Table 2).

Table 2. The primer sequences and primer products of T-ARMS-PCR.

SNP (Gene)	Primer sequence	Primer annealing temperature (°c)	PCR product length (bp)
rs11154801 ***(AHI1)***	**Forward inner primer (C allele):** GATTCTGGGAATACAGACACTGGC**T**C**C** **Reverse inner primer (A allele):** CTCCTTCAGAAGGTCGAAACCTCAA**A**T**T** **Forward outer primer:** CTCACTCCCATCTTTTCCTCACTTTTTG **Reverse outer primer:** TAAGCAAATGTAGTTGCCATCTCTGTGC	59	C(Wild):139 A(Mutant):111 C+:195
rs140522 ***(SCO2)***	**Forward inner primer (T allele):** AGCACACACTCCATTTAATTATG**G**G**T** **Reverse inner primer (C allele):** TGGACTTAAATAGATAAGTCCTCTCT**A**T**G** **Forward outer primer:** TCACCCTTACCCAAGTTTCAGTATAC **Reverse outer primer:** CTTCTTCTTTTTTTTACATTTTGTTTGC	55.2	T (mutant): 186 C(wild):147 C+ : 278
rs10466829 ***(CLECL1)***	**Forward inner primer (A allele):** ACATGACCTGAAGCCATTGAAGA**T**T**A** **Reverse inner primer (G allele):** CCTCTAGAATCTGTCTGGCTTTG**G**T**C** **Forward outer primer:** ACAAGCAAAATTTGTGCTATAGGCAGT **Reverse outer primer:** TCTGTTCTCTGGTTATTCAGGTGAGAAA	57.5	A(wild): 201 G(mutant): 297 C+: 446
rs4410871 ***(PVT1)***	**Forward inner primer (T allele):** ATGAGAAACCGTTGCCATCTTCGGGA**C**G**T** **Reverse inner primer (C allele):** AGTGTGCCTCCCTCCCACACTGG**C**A**G** **Forward outer primer:** TTTCGCTGGATGGAACAATGCCTTGGAC **Reverse outer primer:** ACCCAGACTGAGACCCAACACCCCAGTG	60.1	T(mutant): 193 C(wild): 138 C+: 276
rs1800693 ***(TNFRSF1A)***	**Forward inner primer (T allele):** CAGGTGAGCATGGGCACCAGGTC**C**C**T** **Reverse inner primer (C allele):** AAGGGCACTGAGGACTCAGGTGAGGA**T**A**G** **Forward outer primer:** GAAAATGACCAGGGGCAACAGCACTGTG **Reverse outer primer:** CCTCCAATGGTAGGGCCTCTGTTCACCA	61	T(wild): 157 C(mutant): 192 C+: 294
rs756699 ***(TCF7)***	**Forward inner primer (T allele):** AGGAAAGGAAATGTATATTTTCAG**A**T**T** **Reverse inner primer (C allele):** GCCTGAGCCCACTTAATCATGGC**G**A**G**	60.5	T(Wild): 204 C(Mutant): 175 C+: 326

	Forward outer primer: TTTACTAAATCCATCTGGGGCTCCTTT **Reverse outer primer:** TGAACCACTGTACCCTAGTGAAGAGCTT		
rs630923 ***(CXCR5)***	**Forward inner primer (A allele):** GGAGGAGAAGTGAGGGCAGCCTTTA**G**A**A** **Reverse inner primer (C allele):** AATTGTCTCAACTGGGGCCTATGAC**C**A**G** **Forward outer primer:** CCCAAGTCAAGGGACTTTTCAGTCCTTG **Reverse outer primer:** ATTTAAGCAAATGAGCTGCGCATCGTCT	61.3	A(mutant): 230 C(wild): 265 C+: 439
rs9989735 ***(SP140)***	**Forward inner primer (G allele):** GAAGAAATGTGCCTAGGAAAGGGGG**T**G**G** **Reverse inner primer (C allele):** CTGGACTCCTAATTCTCTCTCCTTCTC**G**C**G** **Forward outer primer:** CAGGAGCAGAGAATGGAGCAGAAACACA **Reverse outer primer:** TGAAGTATCTCCTAGGTGGGGCTGTCCA	62	G(Wild): 205 C(Mutant): 141 C+:288
rs2255214 ***(CD86)***	**Forward inner primer (G allele):** TCTTCATATTTCTTAGAACTTTGTG**C**T**G** **Reverse inner primer (T allele):** GTTTTGTTTTTTAATATGCAGAT**G**C**A** **Forward outer primer:** TTTAGTTCAGTTAAAATTGCATTCTTTG **Reverse outer primer:** ATCTCTCTACTTAGGAATCTACGTGCAT	53.6	G(Wild): 237 T(Mutant): 285 C+: 468
rs17066096 ***(IL22RA2)***	**Forward inner primer (A allele):** GGTGAAGGGCCTTTCTGCTTAAA**C**C**A** **Reverse inner primer (G allele):** CCAGGCAGTGCTGTGTAACTGGAAATA**A**A**C** **Forward outer primer:** AGACTTCTTTGACAAGGTGGCAAACAGG **Reverse outer primer:** CCTTCCTTCTTTCTGACTCTCAGTCGCA	59	A(Wild): 218 G(Mutant): 293 C+: 455
rs212405 ***(TAGAP)***	**Forward inner primer (T allele):** GCAGGAGAAGGGCTGCCTTCCAGG**A**A**T** **Reverse inner primer (A allele):** CCAGGGTCCCAGCAAACCTCTGAGA**G**G**T** **Forward outer primer:** TGTGGGTGGGGAAGATATGCCTGGAAGA **Reverse outer primer:** AGGGGGCAAATTTGAAATGTCTGCTTCCA	60.2	T(Wild): 165 A(Mutant): 201 C+: 311
rs7090512 ***(IL2RA)***	**Forward inner primer (C allele):** GGATTTTCTCTCCTTTATCTTGTACA**A**A**C** **Reverse inner primer (T allele):** GACCTCATGTGGACATTTCAGGT**A**C**A** **Forward outer primer:** AGAGAGCCTGCTGTTTATGTACTGGAT **Reverse outer primer:** GAATTGAATTATACTAAAGGGTGCACCC	58.5	C(Mutant): 118 T(Wild): 134 C+: 197

rs2248359 ***(CYP24A1)***	**Forward inner primer (C allele):** TGACAAACATACACAATTTCTAGAGT**G**A**C** **Reverse inner primer (T allele):** AGCGAGTTCTACTCTTGCCAGCCG**A**G**A** **Forward outer primer:** TTCTTCTACACCGACTACCTTGTGCAAA **Reverse outer primer:** GTATTGGATCAGGTTGAAAGGATTCGAG	58.3	C(Wild): 136 T(Mutant): 160 C+: 240
rs4796791 ***(STAT3)***	**Forward inner primer (T allele):** ATGCTAACTCAATGCAATATTGCAC**G**T**T** **Reverse inner primer (C allele):** TAAATTACCTCTCCCGTAAACCAA**C**C**G** **Forward outer primer:** TGATTTGTCGAGAAAGTCAGTCTGAGATG **Reverse outer primer:** TGCATGTTTTTTCACATTTTTTCTCATGT	58.8	T(Mutant): 203 C(Wild): 140 C+: 288

Bold letters indicate the mismatches at the first and third positions from the 3′ ends. C+: Positive control (the product of two non-specific outer primers).

The PCR reactions were at a final volume of 25µL, and contained amaR One PCR Master Mix (12.5µL), 0.5µL of each primer (10 pmol/µL), 2µL of DNA (50ngµL), and ddH2O (8.5µL).

The thermal cycling condition of all SNPs includes 1) Pre-denaturation: 95°C, 5 min, 1 cycle, 2) Denaturation: 95°C, 40s, 30 cycles, 3) Annealing: 30s, 30 cycles, 4) Extension: 72°C, 30s, 30 cycles, and 5) Final extension: 72°C, 5 min, 1 cycle.

Statistical Analysis:

The Chi-square test was used to evaluate differences in genotype frequency between MS patients and healthy controls. *P-values*< 0.05 are considered significant.

The Hardy-Weinberg exact equilibrium test and the analysis of SNP association with MS (Odds ratio (OR) and 95% confidential interval (95% CI)) were performed using the SPSS Statistics 23.

Bonferroni correction was applied for fourteen independent tests, and *p-values* < 0.0035 were considered significant.

Results

The distribution of SNPs' genotypes, using the Chi-square test and Fisher's exact test showed significant differences between the RR-MS cases and controls at rs4410871 *(PVT1)*, rs212405 *(TAGAP)*, and rs7090512 *(IL2RA)*. The remaining eleven SNPs showed no association with RR-MS, therefore excluded from further analysis (Table 3).

Table 3. Characteristics of the studied SNPs

	SNP	Gene	Chr: Position	Risk allele	MAF (1000Genomes)	AF (Case)	AF (Control)	*P-value*
1	**rs11154801**	AHI1	6:135418217	A	0.29	A:0.23 C:0.77	A:0.28 C:0.72	0.166
2	**rs140522**	SCO2	22:50532837	T	0.38	T:0.32 C:0.68	T:0.37 C:0.63	0.884

3	**rs10466829**	CLECL1	12:9723495	G	0.47	G:0.43 A:0.57	G:0.42 A0.58	0.341
4	**rs4410871**	PVT1	8:127802783	T	0.28	T:0.34 C:0.66	T:0.25 C:0.75	**0.035**
5	**rs1800693**	TNFRSF1A	12:6330843	C	0.299	C:0.23 T:0.77	C:0.28 T:0.72	0.754
6	**rs756699**	TCF7	5:134110884	C	0.23	C:0.25 T:0.75	C:0.27 T:0.73	0.348
7	**rs630923**	CXCR5	11:118883644	A	0.1	A:0.13 C:0.87	A:0.1 C:0.9	0.43
8	**rs9989735**	SP140	2:230250739	C	0.1	C:0.13 G:0.87	C:0.12 G:0.88	0.596
9	**rs2255214**	CD86	3:122051692	T	0.48	T:0.42 G:0.58	T:0.45 G:0.55	0.945
10	**rs17066096**	IL22RA2	6:137131771	G	0.17	G:0.22 A:0.78	G:0.18 A:0.82	1
11	**rs212405**	TAGAP	6:159049527	A	0.29	A:0.37 T:0.63	A:0.29 T:0.71	**0.016**
12	**rs7090512**	IL2RA	10:6068866	C	0.35	C:0.42 T:0.58	C:0.3 T:0.7	**0.008**
13	**rs2248359**	CYP24A1	20:54174979	T	0.45	T:0.37 C:0.63	T:0.42 C:0.58	0.894
14	**rs4796791**	STAT3	17:42378745	T	0.46	T:0.43 C:0.57	T:0.45 C:0.55	0.803

Information regarding the characteristics of the studied SNPs. The second and third column contains the dbSNP rs-number and the gene name. Column four represents the chromosome positions according to GRCh38.p2. Column five contains the minor allele. Column six reflects the minor allele frequencies in 1000 Genome. Column seven and eight comprises allele frequencies in case and control groups. Column nine shows the distribution of SNPs' genotypes between the RR-MS cases and controls (p-values), calculated with one degree of freedom (df=1).

Regression analysis for alleles under the multiplicative model was done, and statistically significant associations were found for three polymorphisms, rs4410871, rs212405, and rs7090512. rs7090512 remained significant after Bonferroni correction ($p < 0.0035$) (Table 4).

Regression analysis for genotypes under the additive model identified rs4410871, rs212405, and rs7090512 as significant RR-MS susceptibility risk factors in our group. rs7090512 remained significant after Bonferroni correction (p-value< 0.0035) (Table 4).

Table 4. Regression Analysis for the alleles and genotypes.

SNP	Gene	H-W P (Fisher's exact) control	Analysis Model	OR(95% CI)	*P-value*
rs4410871	*PVT1*	0.965	Multiplicative Model T vs C	1.506 (1.069-2.122)	0.019
			Additive Model TT vs CC	2.775 (1.253-6.146)	0.01
rs212405	*TAGAP*	0.75	Multiplicative Model A vs T	1.495 (1.064-2.099)	0.02
			Additive Model AA vs TT	2.463 (1.255-4.835)	0.008
rs7090512	*IL2RA*	0.982	Multiplicative Model C vs T	1.687 (1.217-2.338)	0.002
			Additive Model CC vs TT	2.865 (1.433-5.731)	0.002

Discussion

MS is an autoimmune disease of the CNS resulting from demyelination of the axons in the brain neurons. This demyelination takes place because there are autoreactive T cells and macrophages in the patients, which could transmigrate across the BBB and enter the brain, where they recognize the HLAs in the myelin sheath of neurons as foreign antigens and hurts them, resulting in the demyelination of these neurons and local inflammations in the brain (14). The inflammatory patches formed on the myelin sheath of neurons will result in the loss of function of neurons for transmitting impulses. This dysfunction usually occurs on particular nerves, e.g., the optic nerve, the pyramidal tracts, and the cerebellum, which would finally result in loss of function in these nerves (15). The first findings regarding the role of genetics in the pathogenesis of MS, which were obtained from familial clustering and twin studies, paved the way for further investigations to find candidate genes and predisposing genetic factors for MS. The first genetic factors which were shown to be involved in the pathogenesis of MS were serological alleles of HLA on locus 6p21 (16, 17). This finding and the autoimmune entity of MS led scientists to come to a conclusion that various genes encoding the immune system components may underlie the genetic background to MS, although these components have broad effects and it is not easy to exactly attribute their aberrations to MS (18, 19).

An increasing body of evidence has reported the significance of vitamin D metabolism-related genes in the pathogenesis of MS. A plethora of studies including the study of Sintzel et al. in 2018 have shown that adequate vitamin D supplementation may have a protective effect against MS and also help postpone the time at which the patients will progress from RR-MS to SP-MS form (20). Furthermore, it has been reported that the interaction between the genetic factors and vitamin D is being increasingly considered as a major topic in

the etiology of MS (12). In this regard, it has been suggested that polymorphisms in the genes which have VDREs may affect vitamin D metabolism and therefore, be predisposing factors for MS (13). In the present study, we evaluated the association between fourteen SNPs in the genes with VDREs in their regulatory sites and RR-MS. Our results demonstrated significant association of rs212405 (in *TAGAP*), rs7090512 (in *IL2RA*), and rs4410871 (in *PVT1*) polymorphisms with the risk of RR-MS.

In 2007, GWASs have identified *IL2RA* (along with *IL7RA*) as the first non-HLA gene to be associated with MS (21). Subsequent studies have confirmed the association of *IL2RA* and the susceptibility to MS in various populations, including Caucasians and Indians (22-24). However, most of these studies have focused on rs2104286 and rs12722489 polymorphisms, and there is a paucity of data regarding the association between rs7090512 and the risk of MS. In a study from a Japanese population, Ainiding et al. (2014) evaluated the associations between rs2104286, rs12722489, and rs7090512 polymorphisms and the risk of neuromyelitis optica (NMO) or MS (25). They reported that in MS patients (but not in NMO patients), rs2104286 and rs12722489 polymorphisms had significant associations with the annualized relapse rates in females. Regarding rs7090512 polymorphism, they reported a shorter disease duration in NMO patients with CC genotype, compared to non-CC (CT+TT) genotype. In line with this study and by using additive model, we observed a significant association between CC genotype of rs7090512 polymorphism and the risk of RR-MS (OR= 2.865, *P-value*= 0.008). Despite the intronic rs2104286 and rs12722489 polymorphisms, rs7090512 is located in the promoter region of *IL2RA*. Therefore, the genotype of this variant may affect the expression of *IL2RA* or alter the binding site of the VDRE, which is located in the regulatory site of *IL2RA*. There are, however, paucity of information for this phenomenon and functional studies are needed to unravel the exact role of rs7090512 polymorphism in the occurrence of RR-MS.

TAGAP is a gene that encodes a member of Rho GTPase-activator protein superfamily. This protein functions as a GTPase-activating protein in T cells, and alterations in the encoding gene has reported to be related with several autoimmune diseases, including rheumatoid arthritis and MS (26, 27). In fact, various GWAS analyses have unraveled the associations between *TAGAP* polymorphisms and the risk of MS. Berge et al. demonstrated that *TAGAP* is a vitamin D target gene in the CD4+ T cells (28). One such a polymorphism in *TAGAP* gene is rs212405 which, according to GWAS analysis by Patspoulos et al., had a highly significant correlation with MS (joint p value of the discover and replication: 8×10^{-21}) (29). In the present study, we evaluated the association between rs212405 polymorphism and the risk of RR-MS. Our results demonstrated that either using a multiplicative model or an additive model, rs212405 polymorphism had a significant correlation with the risk of RR-MS. Given the previous studies and regarding that rs212405 polymorphism is located in the promoter region of *TAGAP* and also in the vitamin D response element of this gene (28), alteration in this variant may affect the responsiveness of *TAGAP* to vitamin D in the T cells and thereby affect the T cells' function.

PVT1 is a long non-coding RNA (lncRNA), which is a candidate gene in the pathogenesis of MS. Studies have reported the role of this lncRNA in the regulation of mitochondria, whose dysfunction is a key pathway in the development of MS (30). In this regard, GWAS analyses have reported the association between *PVT1* polymorphisms and the predisposition to MS. Kiselev et al. (2020) reported that carrying G allele of rs2114358 in PVT1 is associated with the severe course of the

disease, and the significance of this association also increases the simultaneous carriage of T allele in another *PVT1* variant, rs4410871 (31). In the present study, using an additive model, we observed that TT genotype of rs4410871 in *PVT1* gene is significantly associated with the risk of MS (OR = 2.775, *P-value*= 0.01) which is, to some extent, compatible with the previous study. In another study, Kozin et al. (2020) stated that the polymorphisms in the mitochondrial genome and the interactions of mitochondrial and nuclear genes (mitonuclear interactions) might possibly be a source of "missing heritability" (32). To assess the interactions between mitochondrial and nuclear genomes in the pathogenesis of MS, they searched for biallelic combinations from 10 mitochondrial polymorphisms and 35 variants in nuclear genes. Interestingly, they identified an epistatic interaction between m.13708*A in the mitochondrial genome and T rs4410871*T in *PVT1* gene, highlighting the existence of an intricate mitonuclear epistasis, which may be a predisposing factor for MS.

Conclusion

Taken together, this research is the first study evaluating the association between fourteen polymorphisms in genes with VDREs and the risk of RR-MS in the Iranian Azeri population. Our results showed significant associations between rs212405 (*TAGAP*), rs7090512 (*IL2RA*), and rs4410871 (*PVT1*), and the risk of RR-MS. Given that most of the SNPs evaluated in this study were selected from GWAS analyses, further studies are needed to confirm our data and unravel the significance of these SNPs in the pathogenesis of RR-MS in the populations.

Conflict of interest

The authors declare that they have no conflict of interest.

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