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Research article

Association of *OX40L* gene polymorphism with multiple sclerosis in Iranians

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ABSTRACT

Introduction: The exact etiology of multiple sclerosis is unknown but recent studies indicated a link between *tumor necrosis factor superfamily member 4* and the disease. Polymorphisms located in the regulatory region of the gene may affect its phenotype. Hence, we aimed to investigate the association of promoter polymorphisms of the gene with multiple sclerosis and also to estimate the frequency of haplotypes in the patients and healthy subjects.

Methods: Two hundred age- and sex-matched subjects including 100 patients and 100 healthy subjects were investigated in the study. Genotype and allele distributions of rs3850641, rs1234313, and rs10912580 polymorphisms in the promoter region of the gene were investigated by polymerase chain reaction-restriction fragment length polymorphism. In addition, haplotype frequencies estimation and linkage disequilibrium analysis were performed by SNPStats web tool.

Results: The distribution of *AA, AG* and *GG* genotypes of rs3850641 was significantly different between the patient and healthy groups ($P = 0.009$). In addition, frequencies of *A* and *G* alleles of rs3850641 were different between the groups ($P < 0.001$). Also the distribution of rs3850641 genotypes was different between the women of the both groups ($P = 0.007$). Our analysis revealed that rs3850641 AG (Odds ratio = 0.393, 95 % confidence interval = 0.170–0.907, $P = 0.029$) and *GG* (Odds ratio = 0.373, 95 % confidence interval = 0.168–0.830, $P = 0.016$) genotypes were associated with decreased risk of the disease. However, rs1234313 genotype and allele distributions were not different between the groups. The distribution of rs10912580 polymorphism. AA, *AG*, and *GG* genotypes was significantly different between the groups ($P = 0.007$). rs10912580 AG genotype was associated with low risk of the disease (Odds ratio = 0.252, 95 % confidence interval = 0.102–0.623, $P = 0.003$). The distribution of haplotypes was statistically different between the patient and healthy groups ($P < 0.001$). *A-G-A* was the most frequent haplotype among the patients and the estimated frequency was higher than that of the control group (0.5527 versus 0.3739).

Conclusion: The distribution of rs3850641 and rs10912580 genotypes was different between the patients and healthy subjects. Moreover, rs3850641 AG and *GG* genotypes and also rs10912580 AG genotype were associated with low risk of the disease in Iranians. Further studies with large groups are recommended to show whether genotype variation in the patients could alter the response to treatment or not.

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1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) involving different pathogenic pathways that usually causes persistent disability at advanced stages. The breakdown of the myelin sheath causes MS which is one of the most prevalent inflammatory disorders of CNS. It is more common among young individuals (particularly women) between the ages of 20–50 years [1]. The exact etiology of the disease is unknown but according to clinical and animal research [2] T lymphocyte interactions with neural antigens could contribute to the development of MS [3]. In healthy individuals, inflammatory T cytokines (TH-1) and anti-inflammatory cytokines (TH-2) are in balance, whereas in MS patients, the balance is disrupted by increasing the ratio of TH-1-like cytokines (Interleukine (IL) −2, Interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), etc.) to TH-2-like cytokines (IL-4 and IL-10) that consequently lead to demyelination and neuronal damage [4].

Multiple signaling pathways may induce MS through proinflammatory cytokines such as IL-17, IL-22, TNF-α, IL-1, IL-12, and IFN-γ. However, anti-inflammatory cytokines like IL-4 and IL-10 are reduced in the blood and may have a direct protective effect under these circumstances [5]. Immune system inefficiency, in regulating T cells activity, increases the responsiveness of cluster of differentiation (CD) 4+ T cells and leads to overactivity of TH-1 and TH-17 T cells [6]; leading to myelin destruction and neuroinflammation [7].

Based on stem cell studies on dendritic cell lines, regulatory T cell provocation might alter the expression of many cytokines including TNF-α. TNF superfamily has a member known as the *OX40 (CD134) ligand* (*OX40L*; Gene ID: 7292) that is expressed in dendritic and activated B cells, and appears to be vital for immune reactions mediated by T cells [8,9]. *OX40L* is one of the most recently evaluated genetic factors thought to be linked with regulatory T-cell function in MS [3,10,11].

The official full name of *OX40L* is *TNF superfamily member 4* (*TNFSF4*) which also known as *Glycoprotein34 (GP34); CD252; transcriptionally-activated glycoprotein 1 (TXGP1); CD134L; OX-40L* and *Tumor Necrosis Factor Ligand 2B (TNLG2B*) (https://www.ncbi.nlm.nih.gov/gene/7292). *OX40L* gene located on 1q25.1 encodes a cytokine of the TNF ligand family. The encoded protein functions in T cell and antigen-presenting cell (APC) interactions, and mediates the adhesion of activated T cells to endothelial cells [12,13]. It has been discovered that the *OX40L* interaction with *OX40* on activated T cells plays a crucial role in immune system function [9]. T-cell stimulants such as *OX40L/OX40* interaction can enhance the inflammatory response, which might be linked to an immunostimulatory role in this pathway [14].

OX40L single nucleotide polymorphisms (SNPs) have been studied in some diseases including breast cancer [15], atherosclerotic disorders [16], behcet´s disease [17], asthma [18], hepatitis C virus (HCV) infection [19], systemic lupus erythematosus [20] and autoimmune thyroid disease [21]. However, there is no study regarding the association of rs3850641, rs1234313, and rs10912580 polymorphisms with MS susceptibility in Iranian patients. These polymorphisms are located on the regulatory regions of OX40L on chromosome 1, positions 173206693, 173197108 and 173287411, respectively (https://www.ncbi.nlm.nih.gov/snp/). The polymorphisms are reported in the forward orientation. Haplotype-specific chromatin immunoprecipitation of activated polymerase II indicated that the haplotype including rs3850641G-allele being associated with lower transcriptional activity in cells [22]. Moreover, some studies identified a haplotype block containing rs10912580 in the upstream region of OX40L which was correlated with expression of OX40L [23]. Polymorphisms located in the regulatory region of the gene may affect its phenotype. Therefore, we aimed to evaluate the relationship between these polymorphisms of *OX40L* gene with MS and also to estimate the frequency of haplotypes in MS and healthy subjects.

2. Materials and methods

2.1. Subjects

Two hundred age- and sex-matched subjects including 100 healthy individuals and 100 MS patients referred to Peymanieh Hospital (Jahrom city, Iran) were recruited in the study (from November 2018 to February 2020). Patients whose diseases were approved by a neurologist were divided into secondary-progressive (SP), primary-progressive (PP), and relapsing-remitting (RR) subtypes. Patients and healthy subjects with a history of any inflammatory diseases, autoimmune diseases, cancer, and neurological diseases were excluded from the study. Also, healthy people who had autoimmune disease in their first degree relatives were excluded from the study. Methylprednisolone, plasmapheresis and intravenous immunoglobulin (IV-IG) were used to treat patients in the acute and symptomatic stage of the disease. After treatment, IFNβ-1b, IFNβ-1a, Glatiramer acetate, Fingolimod and dimethylformamid drugs were used in order of priority for maintenance and prevention of relapsing multiple sclerosis. For the treatment of relapsing-remitting multiple sclerosis (RRMS) Rituximab (anti-CD20), Ocrelizumab (anti-CD20) and Natalizumab were used.

2.2. Extraction of genomic DNA and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Five ml of venous blood was taken and collected in EDTA-containing tubes, and stored immediately at −80 °C. The salting-out method was used to extract the genomic DNA [24]. The purity and quality of the extracted DNA were checked by Gene Quant spectrophotometer and electrophoresis on agarose gel (Supplementary Fig. 1). The absorption ratio (260nm/280 nm) of the extracted DNA was about 1.8. In this study rs3850641 with minor allele frequency (MAF) G = 0.167, rs1234313 with MAF A = 0.312, and rs10912580 with MAF G = 0.213 in the promoter region of *OX40L* gene were investigated by PCR-RFLP. The sequences of forward and reverse primers are summarized in Table 1 [25].

PCR reactions were performed in a final volume of 20 μl containing 10 μl Taq DNA Polymerase Master Mix Red (AMPLIQON, Cat

no: A180301), 2.5 μl genomic DNA (0.2 μg), 1 μl forward primer (1 μM), 1 μl reverse primer (1 μM) and 5.5 μl PCR grade water.

PCR conditions for genotyping using either set of primers included an initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and a final extension at 72 °C for 10 min.

PCR products were 274, 305 and 372 bp for rs3850641, rs1234313 and rs10912580, respectively. PCR products were applied to RFLP to detect the tested polymorphisms genotypes (Supplementary Fig. 2). The rs3850641 PCR products were digested by *Hpy*CH4III (Fermentase Company, 10 U, 16 h overnight). *GG* genotype was digested by *Hpy*CH4III to yield two fragments of 210 and 64 bp. However, *AA* genotype had no restriction site for this enzyme. *AG* genotype produced 3 fragments of 210, 64 and 274 bp. In addition, the rs1234313 PCR products were digested by *Bsr*DI (Fermentase Company, 10 U, 16 h overnight) to yield two fragments of 165 and 140 bp for *AA* genotype. *GG* genotype had no restriction site for this enzyme. Three fragments of 165,140 and 305 bp were produced for *AG* genotype. Moreover, the rs10912580 PCR products were digested by *Acc*I (Fermentase Company, 10 U, 16 h overnight). *GG* genotype was digested by *Acc*I to yield two fragments of 199 and 173 bp. *AA* genotype had no restriction site for this enzyme. *AG* genotype produced three fragments of 199,173 and 372 bp. Digested products were run on 3% agarose gel and visualized by the green viewer on a UV transilluminator.

2.3. In silico analysis

A web based application, SNPStats, was used for prediction of haplotypes blocks frequency. We supposed rs3850641-rs1234313-rs10912580 order for haplotype block. The data was entered into the software as a text file. A regression model was used to investigate the relationship between the frequency of haplotypes and the disease. For this purpose, disease status was introduced as a response element. Since the response was binary, an unmatched case-control design and logistic regression models were used. Then, OR and 95% CI were calculated. The most frequent haplotype was selected as the reference category by the software. The two-by-two association of rs3850641-rs1234313, rs3850641-rs10912580 and rs1234313-rs10912580 polymorphism was investigated. The possibility of LD between the polymorphisms was performed and the coefficient of LD (D), normalized D (D′), Pearson's correlation coefficient (r) and P values were calculated.

2.4. Statistical analysis

Statistical analysis was performed by SPSS v.18 (Chicago). The Kolmogorov-Smirnov test was used to investigate the normality of the age in the study population. Binary logistic regression was performed to calculate odds ratio (OR) and 95% confidence interval (CI). The numeric data were reported as mean ± Standard Error (SE). Student *t*-test was performed to compare quantitative data between the groups. Moreover, Chi-square test was applied to investigate the differences of genotype, allele and haplotype frequencies between the groups. A p value less than 0.05 was considered to be significant. Haplotype frequencies estimation, linkage disequilibrium (LD), and Hardy-Weinberg equilibrium (HWE) analysis were performed by SNPStats web tool [26]. Frequency threshold for rare haplotypes was 0.01.

3. Results

3.1. Characteristics of the study population

The dataset used in our study for analysis of genotype, allele and haplotype frequencies, and LD is supplemented as Table 1. One hundred MS patients and one hundred healthy individuals were investigated in the study (Table 2). Subjects were in the range of 15–60 years. The mean age of healthy subjects and MS patients was 33.77 ± 1.15 and 32.65 ± 0.96, respectively. There was no significant difference in age between MS and healthy groups (P = 0.459). There were 37 men and 63 women in the healthy group and 32 men and 68 women in the patient group. The distribution of sex was not statistically different between the groups (P = 0.552). There were 28% SP, 3% PP, and 69% RR typing patients. The duration of MS was less than one year in 23.6%, between one and five years in 26.4%, between 6 and 10 years in 34.7%, and more than ten years in 15.3% of the patients. In addition, 23.6% of the study participants had a family history of MS.

Table 1
Sequences of primers used for detection of rs3850641, rs1234313 and 10912580 in the OX40L gene. F: forward primer, R: reverse primer.

Primers	Sequence
rs3850641.F	5'- GAACTGGTCTCTTTCCTATTTC -3'
rs3850641.R	5'- CCCACAGCAATCGTAAAG-3'
rs1234313.F	5'- CTCCTACCATGTCTCAAAC-3'
rs1234313.R	5'- CTGTCTTCCACAGTCCTC-3'
rs10912580.F	5'- CAGGAGGATCATTTGAACC-3'
rs10912580.R	5'- CTTCGATGGAGACCATAAAC-3'

Table 2
Characteristics of the study population. SP: secondary-progressive, PP: primary-progressive, RR: relapsing-remitting, NS: not significant, Control: healthy subjects, MS: multiple sclerosis, N: number of subjects. The difference in age between the healthy and patient groups was analyzed using the Student's *t*-test. The difference in the distribution of men and women between the healthy and patient groups was calculated by the chi-square test.

Parameters	Control N = 100	MS N = 100	P value
Age(year)	33.77 ± 1.15	32.65 ± 0.96	NS
Sex(male/female)	37/63	32/68	NS
SP	–	28(28%)	
PP	–	3(3%)	
RR	–	69(69%)	

3.2. Genotype and allele distributions

Genotype and allele distributions of rs3850641, rs1234313, and 10912580 are summarized in Table 3. The tested polymorphisms were not ($P < 0.001$) in HWE (Supplementary Table 2).

3.3. rs3850641 genotype and allele frequencies

The PCR products of rs3850641 are depicted in supplementary Fig. 3 274 bp fragments were successfully amplified. The distribution of *AA, AG*, and *GG* genotypes of rs3850641 was significantly different between the MS and healthy groups ($P = 0.009$). In addition, frequencies of *A* and *G* alleles were different between the groups ($P < 0.001$). Moreover, *AG* + *GG* versus *AA* frequency was significantly different between the groups ($P = 0.003$). Since studies indicated that the prevalence of MS among the women is higher than the men so we compared the frequency of genotypes and alleles between multiple sclerotic women (MSW) and healthy women (HW). The distribution of rs3850641 genotypes in the woman of the both groups was significantly different ($P = 0.007$, data not shown). Our analysis revealed that *AG* (OR = 0.393, 95 % CI = 0.170–0.907, $P = 0.029$) and *GG* (OR = 0.373, 95 % CI = 0.168–0.830, $P = 0.016$) genotypes were associated with decreased risk of MS. There was no statistically different of genotypes and allele frequencies between MS subtypes ($P > 0.05$).

3.4. rs1234313 genotype and allele frequencies

Supplementary Fig.4 depicted the PCR products of rs1234313. 305 bp fragments were successfully amplified. Frequencies of *A* and *G* alleles of rs1234313 were not statistically different between the MS and healthy groups ($P > 0.05$). In addition, the *AA, AG* and *GG* genotypes distributions showed no significant difference between the groups ($P > 0.05$). Furthermore, the *AA* + *AG* versus *GG*

Table 3
Genotype and allele distribution of rs3850641, 1234313 and 10912580 polymorphisms of the OX40L gene in the study population. Control: healthy subjects, NS: not significant, MS: multiple sclerosis, Ref: reference allele/genotype, N: number of subjects, OR: odds ratio. The difference in the distribution of alleles and genotypes between the healthy and patient groups was calculated by the chi-square test. Odds ratio was calculated by binary logistic regression.

Allele/Genotype		Conrol (N = 100)	MS (N = 100)	OR	P value
rs3850641					
Allele	A	137(68.5%)	168(84%)	Ref	–
	G	63(31.5%)	32(16%)	0.414(0.256–0.670)	<0.001
Genotype	AA	59(59%)	79(79%)	Ref	–
	AG	19(19%)	10(10%)	0.393(0.170–0.907)	0.029
	GG	22(22%)	11(11%)	0.373(0.168–0.830)	0.016
	AG + GG/AA	41	21	0.383(0.205–0.714)	0.003
rs1234313					
Allele	A	49(25.5%)	47(23.5%)	1.056(0.668–1.672)	NS
	G	151(75.5%)	153(76.5%)	Ref	–
Genotype	AA	20(20%)	17(17%)	1.160(0.561–2.397)	NS
	AG	9(9%)	13(13%)	1.699(0.584–4.944)	NS
	GG	71(71%)	70(70%)	Ref	–
	AA + AG/GG	29	30	1.049(0.571–1.927)	NS
rs10912580					
Allele	A	157(78.5%)	169(84.5%)	Ref	–
	G	43(21.5%)	31(15.5%)	0.670(0.402–1.116)	NS
Genotype	AA	67(67%)	81(81%)	Ref	–
	AG	23(23%	7(7%)	0.252(0.102–0.623)	0.003
	GG	10(10%)	12(12%)	0.993(0.404–2.440)	NS
	AG + GG/AA	33	19	0.476(0.248–0.913)	0.025

frequencies showed no significant difference between the groups ($P > 0.05$). Our analysis showed that there is no association between rs1234313 genotypes and the risk of MS. There was no statistically difference in genotype and allele frequencies between MS subtypes ($P > 0.05$). In addition, the distribution of genotypes and alleles were not statistically different between the MSW and HW ($P > 0.05$, data not shown).

3.5. rs10912580 genotype and allele frequencies

The PCR products of rs10912580 are depicted in supplementary Fig. 5 372 bp fragments were successfully amplified. The distribution of the *AA, AG*, and *GG* Genotypes of rs10912580 was significantly different between the MS and healthy groups ($P = 0.007$). However, frequencies of *A* and *G* alleles were not statistically different between the groups ($P > 0.05$). *AG* + *GG* versus *AA* frequency was significantly different between the groups ($P = 0.003$). In addition, the distribution of rs10912580 genotypes between the MEW and HW was significantly different ($P = 0.001$, data not shown). Our analysis revealed that the *AG* genotype was associated with reduced risk of MS (OR = 0.252, 95 % CI = 0.102–0.623, $P = 0.003$). There was no statistically difference in genotype and allele frequencies between MS subtypes ($P > 0.05$).

3.6. Haplotype frequency and linkage disequilibrium

Haplotype frequency estimation (Table 4) revealed that the distribution of haplotypes was statistically different between the MS and healthy groups ($P < 0.001$). A-G-A (The alleles were for rs3850641, rs1234313, and 10912580, respectively) was the most frequent haplotype among the MS patients and the estimated frequency was higher than that of the control group (0.5527 versus 0.3739). However, G-G-G was the rare haplotype in the patient group (0.0058). In addition, A-A-G haplotype was not seen among the control subjects. Moreover, the predicted haplotypes were associated with low risk of MS ($P < 0.05$). Our analyses showed that there was no linkage between the tested polymorphisms (Table 5).

4. Discussion

The main finding of our study was that *OX40L* gene polymorphism is associated with low risk of MS in Iranians. MS is a prevalent demyelinating disease with the highest rate in North America and Europe (>100/100,000 inhabitants), and the lowest rate in Eastern Asia and sub-Saharan Africa (2/100,000 population) [27]. The main cause of the disease is unknown but studies indicated that both genetic and environmental factors are involved in the pathogenesis of MS [28]. Some studies indicated that gene variations could have a relation to MS [29].

The HWE explains that genetic variations remain constant from one generation to the next if no disturbing factors occur. This equation is violated by factors including mutations, natural selection, nonrandom mating, genetic drift, and gene flow [30]. Our tested polymorphisms were not in HWE. Other studies have also shown the deviation of the studied populations from HWE. Abramovs group finding [31] on a large genomic dataset indicated that the deviation from HWE is observed in the populations and the major cause of deviation,especially in South Asian (SAS) and Latino/Ad-mixed American, is due to heterozygote deficiency rather than heterozygote excess. Garnier-Géré group [32] suggested that non-random mating due to geographical location may be a common cause of deviation from HWE in large populations of different ethnicities. It should be noted that the population of Iran consists of different ethnicities; therefore, this may be one of the reasons for deviation of the tested polymorphisms from HWE. Moreover, this deviation from the balance, as suggested by Graffelman et al. [30], can also be due to mutation. Our study revealed that the polymorphic variants were associated with low risk of MS. Some studies have indicated that in cases where polymorphic changes are advantageous, these changes may be due to natural selection [31].

Stephen's group [33] indicated that gene variations are involved in immunological processes and most of the variations are located in non-coding and regulatory regions rather than coding regions. To the best of our knowledge, our study is the first one that investigated the tested polymorphisms in the regulatory region of OX40L gene in Iranian MS patients. Song group [21] investigated the relation of rs1234313 and rs3850641 to susceptibility to autoimmune thyroid diseases (AITDs) in the Han Chinese population. They

Table 4
Haplotype frequency estimation (n = 200) in the study population. * The frequency of A-A-G haplotype in the control group was zero, so A-A-G was excluded from Chi-square analysis. MS: multiple sclerosis, Control: healthy subjects, SNP1: rs3850641, SNP2: rs1234313, SNP3: rs10912580, Ref: reference haplotype, n: number of subjects, OR: odds ratio. The difference in the distribution of haplotypes between the healthy and patient groups was calculated by the chi-square test. Odds ratio was calculated by binary logistic regression.

	SNP1	SNP2	SNP3	Total	MS n = 100	Control n = 100	OR	P value
1	A	G	A	0.4666	0.5527	0.3739	Ref	
2	A	A	A	0.1586	0.1573	0.1683	0.627(0.486–0.809)	<0.001
3	G	G	A	0.1482	0.1019	0.1961	0.348(0.265–0.458)	<0.001
4	A	G	G	0.1203	0.1047	0.1428	0.495(0.372–0.658)	<0.001
5	G	A	A	0.0416	0.0331	0.0467	0.485(0.304–0.773)	0.002
6	G	G	G	0.0249	0.0058	0.0422	0.080(0.032–0.205)	<0.001
7	G	A	G	0.0229	0.0192	0.03	0.428(0.237–0.772)	0.005
8	A	A	G	0.017	0.0253	0*	_	

Table 5
Multiple-SNP analysis to calculate LD for the tested polymorphisms. LD for rs3850641-rs1234313, rs3850641-rs10912580 and rs1234313-rs10912580 was calculated. LD: linkage disequilibrium, D: the coefficient of LD, D′: normalized D, r: Pearson's correlation coefficient, Snp1: rs3850641; Snp2: rs1234313; Snp3: rs10912580. SNPStats web tool was used to calculate LD.

D statistic	Snp2	Snp3
Snp1	0.0072	0.0027
Snp2	.	−0.0036
D' statistic	Snp2	Snp3
Snp1	0.0397	0.0195
Snp2	.	0.0801
r statistic	Snp2	Snp3
Snp1	0.0394	0.0166
Snp2	.	0.0214-
P-values	Snp2	Snp3
Snp1	0.4309	0.7395
Snp2	.	0.6681

found that rs3850641GG genotype frequency was lower in AITDs and suggested that this genotype probably could decrease susceptibility to AITDs. In addition, their findings indicated that rs3850641G allele was in strong association with hypothyroidism in Hashimoto's thyroiditis (HT). They observed that the distribution of rs1234313 genotypes and alleles in AITDs patients was not different compared to the control group. In line with Song's findings, our analysis showed that the frequency of rs3850641GG genotype and G allele was lower in MS patients and this genotype and allele may be associated with decreased risk of the disease. Also, the distribution of rs1234313 genotypes and alleles were the same between the groups. However, contrary to Song's findings, the results of our study showed that the rs1234313 G allele has a higher frequency than the A allele (albeit not significant), and the G allele may be the dominant allele in the Iranian population.

Coustet group [34] investigated the association of OX40L polymorphisms including rs10912580 with systemic sclerosis (SSc) in a meta-analysis study. They have concluded that OX40L polymorphisms were associated with SSc. Our analysis revealed that the frequency of rs10912580 genotypes was different between MS patient and healthy subjects. In addition, rs10912580 AG genotype was associated with low risk of MS in Iranian.

Chen group [35] investigated the genotypes of rs1234313 in systemic lupus erythematosus (SLE). They showed that heterozygote and mutant homozygote are related to the disease. However, our finding revealed that this polymorphism has no relation to MS. The different results between the two studies could be due to racial difference. Meanwhile, the mutant allele they investigated is C.

Previous studies have shown that *OX40L/OX40* interaction can enhance the inflammatory response [14]. Their interactions promote an effector T cell phenotype and cause increasing production of effector cytokine. Hence, blocking OX40L may be effective in decreasing autoimmunity [11]. Our tested polymorphisms were located on regulatory region of OX40L gene. The SNPs in this region may interact with transcription factors. So, we hypothesized that the observed genotype variations may affect the expression of OX40L and consequently OX40/OX40L interaction. This change may affect T cell activity and immune response.

Chen group [35] also showed that rs1234314 and rs45454293 haplotypes were associated with SLE. Our predicted haplotypes were associated with low risk of MS in Iranians. Although the results of our study did not show linkage disequilibrium between polymorphisms, these findings are based on in silico analysis, and for the certainty of this issue, it is necessary to check the haplotypes on each pair of chromosomes experimentally using the direct haplotyping technique.

It has been found that the pathogenesis of MS is a complex issue with known and unknown factors such as genetics, sex, age, obesity, diet, family history, and other environmental factors. Some studies have indicated that the prevalence of MS among the women is higher than the men [1]. Our analysis showed that the distribution of rs3850641 and rs10912580 genotypes in MSW is different from that in HW.

The limitation of our study was that our sample size was relatively small; hence, the results should be interpreted with caution. Future experimental studies are suggested to show whether there is linkage between the tested polymorphisms or not. Further studies with large groups are recommended to show whether genotype variation in MS patients could alter the response to treatment.

5. Conclusion

Our finding showed that the distribution of rs3850641 and rs10912580 genotypes is different between healthy subjects and MS patients. Moreover, rs3850641AG, rs3850641GG and rs10912580 AG genotypes and the predicted haplotypes were associated with reduced risk of MS. In addition, according to in silico analysis, there is no linkage between rs3850641, rs10912580 and rs1234313 polymorphisms.

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Ethics declarations

The study protocol was approved by the ethics committee of Jahrom University of Medical Sciences (Research ethical code: IR.JUMS.REC.1397.063). A written informed consent form was obtained for blood sampling and publishing clinical data of the patient(s)/participants in the study.

Data availability statement

The data used in our study is supplemented as Table 1. Also the data would be available upon a reasonable request from corresponding author.

CRediT authorship contribution statement

Abdolreza Sotoodeh Jahromi: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Saiedeh Erfanian:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Abazar Roustazadeh:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None of the authors have conflict of interest.

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Abbreviations

AITDs	autoimmune thyroid diseases
APC	antigen-presenting cell
CD	cluster of differentiation
CI	confidence interval
CNS	central nervous system
GP34	Glycoprotein34
HCV	hepatitis C virus
HT	Hashimoto's thyroiditis
HW	healthy women
HWE	Hardy-Weinberg equilibrium
IFN-γ:	Interferon-gamma
IL:	Interleukine
IV-IG	intravenous immunoglobulin
LD	linkage disequilibrium
MAF	minor allele frequency
MS	Multiple sclerosis
MSW	multiple sclerotic women
OR	odds ratio
PCR-RFLP	polymerase chain reaction-restriction fragment length polymorphism
PP	primary-progressive
RR	relapsing-remitting
SAS	South Asian
SE	Standard Error
SLE	systemic lupus erythematosus
SNPs	single nucleotide polymorphisms
SP	secondary-progressive
SSc	systemic sclerosis
TNF-α:	Tumor necrosis factor-alpha
TNFSF4	TNF superfamily member 4
TNLG2B	Tumor Necrosis Factor Ligand 2B

TXGP1 transcriptionally-activated glycoprotein 1

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27304.

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