Original Article

Genetic association of rs1520333 G/A polymorphism in the *IL7* gene with multiple sclerosis susceptibility in Isfahan population

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Abstract

Background: Multiple sclerosis (MS) is an inflammatory neurodegenerative disease in which the insulating membrane of central nervous system is damaged. The etiology of MS includes both genetic and environmental causes. A Genome — Wide Association Study (GWAS) recognized genetic single nucleotide polymorphisms (SNP) linked with MS predisposition among which immunologically related genes are considerably over signified.

The purpose of the present study is to explore the association of rs1520333 C/T polymorphism in the *IL7* gene variants with the risk of MS in a subset of Iranian population.

Materials and Methods: In this case — control study, 110 cases with MS and 110 controls were contributed. DNA was extracted from blood samples and to amplify the fragment of interest contain rs1520333 SNP, polymerase chain reaction — restriction fragment length polymorphism method was implemented for genotyping of the DNA samples with a specific restriction enzyme (*MwoI*).

SPSS for Windows software (version 18.0; SPSS, Chicago, IL, USA) was used for statistical analysis.

Result: We demonstrated the important association between G allele [odds ratio (OR) =1.6614, confidence interval (CI) =1.12-2.47, $P = 0.0124$] and GG genotype (OR = 7.45, 95% CI = 2.13-25.97, P 0.0016) of the rs1520333 SNP for susceptibility to MS after adjustment for age, and gender. OR adjusted for age, gender, and body mass index has displayed similar outcomes.

Conclusion: These results indicate that the rs1520333 SNP is a significant susceptibility gene variant for development of MS in the Iranian population. Nevertheless, functional studies are required to completely elucidate how this SNP contributed to MS pathogenesis.

Key Words: GWAS, *IL7* gene, multiple sclerosis, polymorphism

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INTRODUCTION

Multiple sclerosis (MS) is considered a common inflammatory neurodegenerative disease of the central nervous system that mostly affects young adults and leads to important disability, including physical, mental, and occasionally psychiatric complications.[1,2] The quick growing in prevalence

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of MS has been a leading public health problem worldwide, including Iran.[3] The etiology of MS remains mysterious, but both intricate genetic components with numerous predisposing-conferring genes as well as environmental components have been identified as important causes.[4] Genetic predisposition to MS is likely to be under a polygenic manner, but the majority of the genes involved is yet to be recognized.[5,6] Over the past decade, weighty efforts have been invested in the exploration for MS susceptibility genes.[7,8] Recently, a remarkable progress was made in the detection of susceptible genes in which single nucleotide polymorphisms (SNPs) complicated in MS through genome-wide association studies (GWAS).[9-13] From these, various association studies of polymorphic immune-associated genes described for MS.[8,10,14] Most of these genes are investigated because they are presumed to be relevant to the pathogenesis of MS based on the function of the gene.[8,9] However, additional genes complicated in MS predisposition and in the change of its clinical course stay to be identified.[15,16] One of the new susceptibility genetic variants that have been introduced by a new GWAS is rs1520333 SNP in the IL-7 gene related to MS.[17] The protein encoded by this gene is a cytokine that is secreted by stromal cells in the bone marrow and thymus.[18,19] IL-7 cytokine plays an important role in immune system such as T-cell development, peripheral T-cell homeostasis, pre-B-cell growth factor and immune tolerance.[18,20,21] MS usually has an autoimmune pathology in which TH1 and TH17 lymphocytes have a key contribution.[22,23] In this pathology pathway, IL-7 cytokine directly elevated effector TH17 cells in human TH17 cells from subjects with MS; however, it is not necessary for TH17 differentiation.[22,24] According to the results of this GWAS study, the rs1520333 SNP of the IL-7 gene is a new candidate variant known to be associated with MS. Given complex genetic effects and multifaceted gene — environment interaction nature of MS, frequencies of genetic SNP polymorphisms are diverse among ethnic populations.[8] It is especially important to investigate its association in Isfahan population, where the number of cases with MS is rising quickly. Thus, the aim of the present study is to determine whether the rs1520333 SNP in the IL-7 gene identified by the genome-wide association studies in European populations is associated with the susceptibility to MS in Isfahan population.

MATERIALS AND METHODS

A case-control study was conducted to assess the association between rs1520333 SNP and MS. The patient population consists of 110 subjects (27 men and 83 women) with MS diagnosed, according to the McDonald criteria. The control group, including the 110 healthy subjects (27 men and 83 women) matched healthy controls were qualified to the study randomly selected from the general population. All of the patients and controls were Iranians. None of the participants were related, and no cases of familial MS were included in the study.

Statistical analysis

SPSS for Windows software (version 18.0; SPSS, Chicago, IL, USA) was used for statistical analysis. The allele and genotype frequencies were tested for Hardy–Weinberg equilibrium using the Chi-square test. Logistic regression analysis was accomplished to calculate distributions and risk allele/genotype-specific odds ratios (ORs), 95% confidence intervals (CIs), and analogous *P* values after adjustment for gender, age, as covariates between cases. All continuous variables were expressed as the mean ± standard deviation (SD). Student's *t* test was used to compare the continuous variables between the MS and control groups. Pearson's v2 test was used to evaluate the difference in the prevalence of MS among different genotypes.

DNA extraction and SNP genotyping

Peripheral blood samples of patient and control groups were collected in tubes containing ethylenediaminetetraacetic acid as anticoagulant, and then DNA was extracted from whole-blood samples using the DNG plus DNA extraction Kit (Cinnagen, Iran), according to the manufacturer's instructions. DNA integrity was checked by ultraviolet absorption at 260 and 280 nm and by agarose gel electrophoresis. Genotyping was performed by polymerase chain reaction (PCR) — restriction fragment length polymorphism. The following primers were used for PCR amplification:
Forward: 5'-AACTGCCATACCTCCTAGTACTGTTTC-3',
Reverse: 5'-TCAACTATTAAATTGTGGCTTCATTC-3'.

These specific PCR primers amplified a 482-bp fragment in which there is a specific restriction site to determine the different alleles of the rs1520333 SNP. PCR was carried out on an Eppendorf thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 45 s, and a final extension of 72°C for 5 min. PCR products were then digested with 1 U of *MwoI* restriction enzyme (Reaction volume 15 mL) (Fermentas, Lithuania). After 3-h incubation at 37°C, the enzyme cut the 482 bp PCR product into two fragments 295 and 187 bp in length [Figures 1 and 2]. The resulting products were visualized by 1.5% agarose gel electrophoresis.

Fragments size of 295 and 187 bp indicated the presence of a wild-type homozygous GG genotype; a single 482 bp fragment displayed the presence of homozygous AA genotype; and three fragments of 295, 187, and 482 bp indicated the presence of heterozygous GA genotype. To determine genotyping error rate, we performed both random duplications in 20% of the samples [Figures 3-5].

RESULTS

Demographic and clinical features of the participants selected demographic and clinical features of cases and controls in the studied population and the association with MS are demonstrated in Table 1. No major differences were revealed between the two groups concerning gender (P = 1), age (31.2±6.4 years for controls, and 30.2 ±7.5 years for controls (P = 0.258). Blood group status also was not statistically different (P = 0.065) between the two groups [Table 1]. Genotypes were effectively typed in all subjects and did not deviate from the distribution expected by the Hardy–Weinberg equilibrium. Frequencies of the AA, GA, and GG genotypes of rs1520333 SNP were 45.5%, 51.8%, and 2.8% in controls, and 37.3%, 45.5%, and 17.3% in cases, respectively, the frequency of the C allele in cases (39.5%) was more than that in the healthy control group (28.6%) [Table 2]. A notable association of the allele G (OR = 1.6614, 95% CI = 1.12-2.47, P value = 0.0124) and the GG genotype (OR = 7.45, 95% CI= 2.13-25.97, P value = 0.0016) of rs1520333 SNP was detected with higher MS risk [Table 2]. For other genotypes of the rs1520333 polymorphism, results were not significantly associated with the risk for MS in the population under study [Table 2]. In addition, when we compared the AA and GA genotypes against GG genotype as reference, the association between rs1520333 SNP genotypes and MS risk was examined in subgroups of both subjects stratified by gender, age (under and over 30 years), and blood groups. The adjusted OR for the GG and GA + AA genotypes was 1.35 (95% CI: 0.46-3.96, P = 0.7842) in males, and 0.574 (95% CI: 0.307-1.075, P = 0.1137) in females, which is indicative of no significant association. Also, we did not find significant relationship for under and over 30 years groups (OR = 0.548, 95% CI: 0.239-1.260, P = 0.2442 and OR = 0.909, 95% CI: 0.443-1.865, P = 0.0773, respectively), and the genotype distribution. In stratification analysis for blood groups, adjusted OR for the GG and GA + AA genotypes was 0.568 (95% CI: 0.199-1.621, P = 0.4251) in individuals with A blood group, 0.663 (95% CI: 0.205-2.145, P = 0.6949) in individuals with B blood group, 0.889 (95% CI: 0.206-3.831, P = 0.8279) in individuals with AB blood group, and 0.895 (95% CI: 0.355-2.256, P = 1) in individuals with O blood group [Table 3].

DISCUSSION

Although etiology of the MS still remains blurred, the genetic impact on both predisposition and clinical outcomes is undeniable.[1,7] Study of potentially key polymorphisms in the some genes of the genome has developed as a robust approach in understanding of

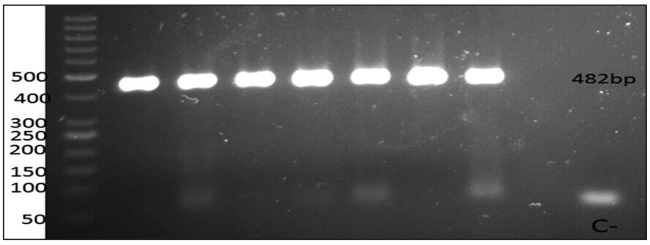

Figure 1: 482 bp PCR product of IL-7 gene polymorphism (rs1520333). Last lane is negative control

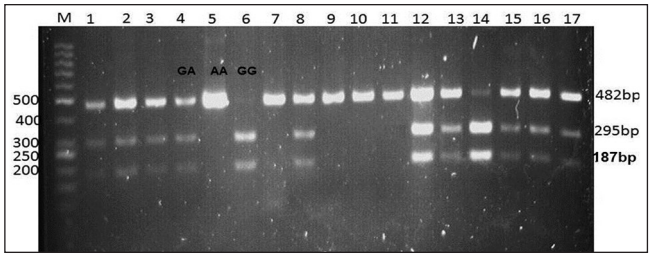

Figure 2: Enzyme digestion. M = Marker (50 bp), Lanes 5, 7, 9, 10, 11 (AA genotypes), Lane 6 (GG genotype), and other lines are (GA genotypes)

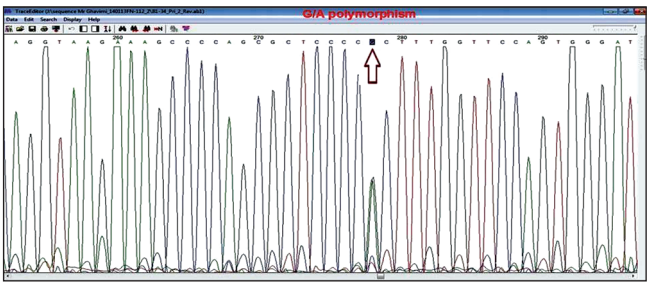

Figure 3: A sequencing chromatogram showing GA genotype of rs1520333 G > A

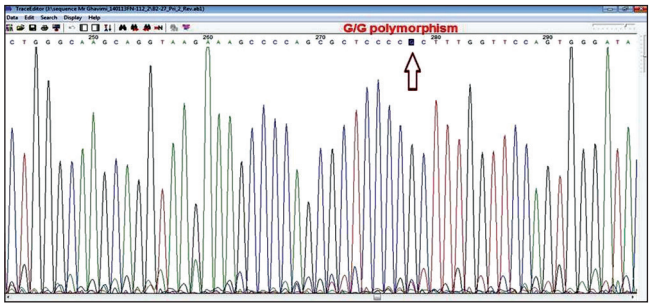

Figure 4: A sequencing chromatogram showing GG genotype of rs1520333 G > A

the intricate interaction between genotype of subjects and multifactorial diseases such as MS.[25-27] As the leading finding of our study, the rs1520333 SNP was displayed to confer susceptibility to MS risk, with the G allele and GG genotype having a risk outcome. We observed the MS association of rs1520333 SNP representative risk allele and genotype for *IL-7* gene found in Europeans, proposing effect of this variant associated with MS in Europeans.[17,22] is also related in Isfahan population. Our association study is the first study after which GWAS study has confirmed the risk association of this SNP with MS in a case — control method. The *IL7* pathway is crucial for the expansion, maturation, and survival of T cells, and it is hypothesized to be where there is failure in immune response.[20,28] This failure may possibly play a role in the hostile autoimmunity occurrence in MS cases. In our study, a significant increase of the G allele and GG genotype in MS patients was shown. The G allele carriers (GG genotype carriers) were more frequent in patients with MS (39.5%, OR = 1.6614, 95% CI = 1.12-2.47, *P* value = 0.0124) than control subjects (28.6%); the ORs for GA heterozygotes and AA homozygotes were 0.3454 and 0.2185, respectively. Moreover, in the current study, the association between rs1520333 SNP and MS risk as well as the influence of other parameters such as age, gender, and blood groups, were considered. But, we failed to find any evidence for association between genotypes of rs1520333 SNP and these parameters regarding the MS risk in population under study. These findings offer firm evidence for the notion that this genetic variant independently contributes to the risk of MS in Isfahan population. Although the mechanism by which rs1520333 SNP in the *IL7* gene influences MS pathogenesis is not distinct, the association with allele G carriage supports the hypothesis that an increased overall risk of the disease may be linked to alterations in immune function and might relate to allele G-linked differences in the regulation of autoreactive immune cells, which precipitate inflammatory disease activity. It is reasonable that immunologic impacts of MS pathogenesis cause heightened immune stimulation that reveals these genotype-induced effects, and this hypothesis could be tested by assessing rs1520333 SNP function in other studies with MS according to genotype. Therefore, it is hard to interpret the results from association studies due to the lack of understanding of the cellular and molecular process influenced

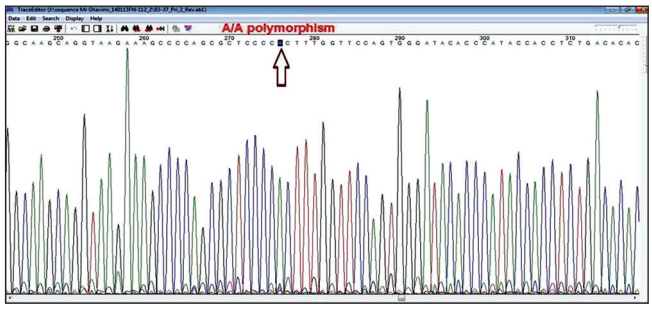

Figure 5: A sequencing chromatogram showing AA genotype of rs1520333 G > A

Table 1: Demographic features in cases and normal controls

Variables	Controls (*n* = 110) (%)	Cases (*n* = 110) (%)	*P* value
Age (mean±SD)	31.2±6.4	30.2 ±7.5	0.258
Gender			
Male	27 (24.5)	27 (24.5)	1
Female	83 (75.5)	83 (75.5)	
Blood group			
A	33 (30)	28 (25.5)	0.065
B	29 (26.4)	20 (18.2)	
AB	12 (10.9)	26 (23.6)	
O	36 (32.7)	36 (32.7)	
EDSS			
1		74 (67.3)	
1.5		19 (17.3)	
2		13 (11.8)	
3-5		4 (3.6)	
Symptoms at the onset of MS			
Dizziness		41 (37.3)	
Numbness in the limbs		35 (31.8)	
Blurred or double vision		34 (30.9)	

EDSS: Expanded disability status scale, MS: Multiple sclerosis

Table 2: Allele and genotype distribution of rs1520333 SNP in cases and controls and their association with MS in this study

Group	Controls		Cases		Risk or protective allele/genotype	OR (95% CI)*	*P* value**
	N	%	*N*	%			
Allele frequency (rs1520333)							
G	63	28.6	88	39.5	G	1.6614 (1.12-2.47)	0.0124
A	157	71.4	132	60.5			
Genotype frequency							
AA	50	45.5	41	37.3	–	0.71 (0.42-1.22)	0.2185
GA	57	51.8	50	45.5	–	0.77 (0.46-1.32)	0.3454
GG	3	2.7	19	17.3	GG	7.45 (2.13-25.97)	0.0016

$P < 0.05$, *The OR with 95% CI shown is for the risk allele/genotype, **P allele is the P value for comparison of the allele distribution between the cases and controls, P genotype is the P value for comparison of genotype distribution between the cases and controls

Table 3: Stratification analysis of rs1520333 genotype frequency in cases and controls

Group	Genotype		OR (95% CI)	*P* value*
	AA (%)	GA/GG (%)		
Male				
Control	11 (45.8)	16 (53.36)	1.35 (0.46-3.96)	0.7842
Case	13 (53.2)	14 (46.7)		
Female				
Control	39 (58.2)	44 (44.4)	0.574 (0.307-1.075)	0.1137
Case	28 (41.8)	55 (55.6)		
Age <30 years				
Control	19 (51.4)	22 (36.7)	0.548 (0.239-1.260)	0.2442
Case	18 (48.6)	38 (63.3)		
Age >30 years				
Control	31(57.4)	38(55.1)	0.909 (0.443-1.865)	0.0773
Case	23 (42.6)	31 (44.9)		
Blood group				
A				
Control	15 (62.5)	18 (48.6)	0.568 (0.199-1.621)	0.4251
Case	9 (37.5)	19 (51.4)		
B				
Control	13 (65)	16 (55.2)	0.663 (0.205-2.145)	0.6949
Case	7 (35)	13 (44.8)		
AB				
Control	4 (33.3)	8 (30.8)	0.889 (0.206-3.831)	0.8279
Case	8 (66.7)	18 (69.2)		
O				
Control	18 (51.4)	18 (48.6)	0.895 (0.355-2.256)	1
Case	17 (48.6)	19 (51.4)		

by this polymorphism. Taken together with our present results indicate that the distribution of the rs1520333 SNP investigated is a strong risk factor for MS susceptibility. Nevertheless, because the Europeans, Iranians, and other populations are dissimilar in their environmental risk profiles, body composition, and genetic backgrounds, additional studies in other MS association studies will be needed to further replicate rs1520333 SNP and to better define the risk roles that *IL-7* gene variant may play in the disease pathogenesis.

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