Association of TNF-related apoptosis inducing ligand receptor (TRAIL-R) gene polymorphisms in Iranian Azeri patients with multiple sclerosis

Bita Amir Taghavi[1], Mehrdad Hashemi[1], Gholamreza Niaei[2], Seyed Ali Rahmani*[,3]

[1]Department of Genetics, Islamic Azad University, Tehran Medical Sciences Branch, Tehran, Iran
[2]Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
[3]Department of Biology, Ahar Branch, Islamic Azad University, Ahar, Iran

[*]**Corresponding Author:** email address: rahmaniseyedali@yahoo.com (A. Rahmani)

ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system with various degrees of axonal damage. The TNF-related apoptosis inducing ligand receptor (TRAIL-R) might be playing an important role in the pathogenesis of MS. The objective of our study was to evaluate the association of two common polymorphisms is located in the TRAIL-R1 and TRAIL-R2 gene, in the pathogenesis of MS.DNA was extracted from whole blood using the salting-out procedure. We genotyped two single nucleotide polymorphisms in particular regions with single strand conformation polymorphism (SSCP) and Results obtained from the sequence of some samples, were analyzed using DNAMAN software. The distribution of genotype frequencies was analyzed using Pearson's x^2 test. Statistical significance was defined as $p < 0.05$.No Significant differences in SNP rs4872077 were found between the PRMS and PPMS groups and No association was found between the genotype status of the rs1001793 and rs4872077 polymorphisms and the age at onset, disease duration, EDSS.
Our study suggests no association between TRAILR polymorphisms and MS Disease. Nevertheless, this polymorphisms does not appear to be a severity marker of the disease, neither modifying the clinical progression of MS nor its therapeutic response.

Keywords: Multiple sclerosis; TRAIL; TRAIL receptor; SSCP

INTRODUCTION

Multiple sclerosis (MS, OMIM 126200) is one of the most common causes of neurological disability in young adults which is caused by interaction between environmental and inherited factors, where the transmission of signals in the central nervous system (CNS) vary from each other by lesion size and number, pathology, and clinical outcome. The most frequent forms of MS are relapsing remitting MS (RRMS), in which acute attacks are followed by complete or partial recovery, and primary progressive MS (PPMS), characterized by disease progression from onset [1-4].To date, it is supposed that MS is a chronic inflammatory condition, probably of autoimmune nature in which myelin-specific T cells seem to be responsible for the orchestration and prolongation of the autoimmune reaction that leads to oligodendrocyte damage and demyelination, in addition to axonal and neuronal damage [1-4]. A remarkably low number of susceptibility genes for MS have been identified so far. The disease shows familial clustering, and twin studies have revealed that a large portion of this clustering can be attributed to shared genes. But none of the candidate genes suggested to date has been shown to be essential or sufficient for disease development; the most association has obviously been proven with alleles of the major histocompatibility complex (MHC) and in particular with the haplotypes HLA-DQB1*0602, -DQA*0102, -DRB1*1501, -DRB5*0101 [3,4,11]. However, MS is a heterogeneous condition in which the involved genes may be

different in different subsets of patients [3-6].Genome wide linkage studies have identified MS susceptibility genes on at least eight human chromosomes, including chromosome 6, which carries the genes for TNF. TNF-related apoptosis inducing ligand (TRAIL) is a newly identified member of the TNF/nerve growth factor super family, mapped on chromosome 3q26 [7, 13, 15]. Both TRAIL and TRAIL receptor genes are potential candidates for involvement in the development of multiple sclerosis [13-15]. Carlos Lopez-Gomez et.al has previously reported the effects of SNPs in TRAIL and TRAIL Receptors genes on MS susceptibility [13]. Several studies have searched for allelic variants associated with the response to IFN beta treatment in MS genetic and alterations in TRAIL/TRAIL receptor system might compromise apoptotic cell signaling [13-15]. TRAILR-1 and TRAILR-2 genes which are the two common polymorphisms rs4872077 (T>C) and rs1001793 (G>A) transition polymorphisms are potential candidates for involvement in the development of multiple sclerosis [13]. The objective of this study is to investigate the association between TRAILR-1 and TRAILR-2 gene polymorphisms (rs4872077 and rs1001793) and susceptibility to MS and clinical course of the disease.

MATERIALS AND METHODS

DNA Isolation

From October 2014 to June 2015, all participants involved in this comparative case (Group 1) and control (Group 2; Absence of Clinical symptoms and Family history) study were informed about the study and written informed consent was obtained before blood collection all individuals involved in this comparative case (Group 1) and control (Group 2; Absence of Clinical symptoms and Family history) study provided us with written informed consents for the genetic analysis according to Iran Medical Committee. The study population consisted of 80 MS patients and 80 healthy individuals as controls, all being unrelated Iranian Azari from East Azerbaijan of Iran. We therefore studied age and sex-matched attendants and relatives of MS patients and healthy staff at the clinic. All patients had clinical or laboratory supported definite MS according to the Poser criteria [8] or fulfilled the criteria of McDonald for MS [19] and the patients were classified as relapsing–remitting or primary progressive according to the initial course of the disease. Blood samples were collected from volunteers and placed in tubes that contained an EDTA anticoagulant. Genomic DNA was extracted from the white blood cells using the method of Miller et al. [9]. DNA concentrations were determined by a UV spectrophotometer at 260 nm.

Genotyping

The Single-strand conformation polymorphism (SSCP) technique makes possible the detection of both known and unknown single point mutations and polymorphisms in products of the PCR. Optimization of SSCP analysis to detect the maximum number of mutations requires electrophoresis under carefully controlled conditions at different temperatures, using different gels. We have analyzed the specific region of TRAIL-R1 and TRAIL-R2 gene. SSCP analysis identified aberrant bands. A 100-bp DNA Ladder (Fermentas Vilnius, Lithuania) was used as a size standard for each gel lane. PCR was carried out at 95 ℃ denature for 5 min, followed by 35 cycles of 95 ℃ denature for 20 sec, 58 ℃ annealing for 20 sec and 72 ℃ extension 30 min. Sequences of Primers used in Polymerase Chain Reactions in 'Table 1'. To validate the obtained results, samples of PCR products were sent to Bioneer Corporation (Daejeon, Republic of Korea) for sequencing. Direct sequence analysis of these aberrantly migrating bands led to the identification of TRAIL-R1 and TRAIL-R2 mutations.

Table 1. Sequences of Primers Used in Polymerase Chain Reactions

Name	Primers sequence (5' → 3')		PCR Product (*bp*)
rs4872077	F^1	TGTACGCCCTGGAGTGACATC	246
	R^2	TTGCCCTCAGCCAGCACCTA	
rs1001793	*F*	TTCCTTGCCCACATTTTCTGGA	126
	R	TGCAGCTCTCACCTCTCAAACA	

1.F: Forward primer
2.R: Reverse primer

STATISTICAL ANALYSIS

The student t-test was used to test the difference between the groups with regards to mean age, and χ^2 statistics. The distribution of genotype frequencies was analyzed using Pearson's χ^2 test. P value less than 0.05 was considered statistically significant. In order to check if any individual SNP was associated with MS susceptibility, genotype frequencies were compared, using a likelihood ratio test.

RESULTS

Our study was performed on a case and control design consisting of 160 people. The clinical data for the two examined groups are displayed in Table 2. The median age of onset of disease in PPMS group was 38.2±9.3 years and for PRMS group was 36.5±9.4. As the groups were age and sex matched, there were no differences by gender or mean age (p=0.898); the age at onset in the PPMS patients was higher and the disease duration was shorter than in the PRMS patients but not significantly so (p=0.510 and p=0.345 respectively). No substantial differences in SNP rs4872077 were found between the PRMS and PPMS groups. (χ^2=3.040; *df* =1; *p*=0.082 and χ^2=0.720; *df*=1; *p*=0.142). According to our data, there was no significant difference between patients with MS and the healthy subjects. No association was found between the genotype status of the rs1001793 and rs4872077 polymorphisms and the age at onset, disease duration, and Expanded Disability Status Scale (EDSS). Analysis of genotype and allele frequencies of the rs1001793 polymorphism is shown in Tables 3, 4 and rs4872077 polymorphism in Table 5, 6.

The genotypes and allele frequencies were not different in patient and control groups (the frequencies of SNP rs4872077 (χ^2=0.42, P=0.514) and rs1001793 (χ^2=2.3, P=0.127)). Future studies are required to further examine the findings as these results do not support a role of TRAIL-R gene polymorphisms in susceptibility to MS.

Table 2. Clinical characteristics of the two examined groups

Groups	Patient number Male/Female	Age (years)	Age at onset (Years)	Disease duration (Years)	EDSS
PPMS	42/38	49.3±10.2	38.2±9.3	10.9±9.1	5.8±1.8
PRMS	42/38	49.4±9.6	36.5±9.4	12.8±7.9	2.9±1.8
P	-	0.898	0.510	0.345	<0.001

Table 3.Genotypic and Allele frequencies of the rs1001793 polymorphism

group	Genotype	Frequency (%)	PPMS (n=40)	PRMS (n=40)
1.00	AA	3 (3.8)	1	2
	GA	13 (16.2)	6	7
	GG	64 (80.0)	33	31
	Total	80 (100)	40	40
2.00	AA	1 (1.2)	-	-
	GA	9 (11.1)	-	-
	GG	70 (87.7)	-	-
	Total	80 (100)	-	-

Table 4.The distribution of genotype frequency of the rs1001793 polymorphism

Genotype	Value	*df*	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.323[a]	1	0.127	-	-
Continuity Correction[b]	1.707	1	0.191	-	-
Likelihood Ratio	2.350	1	0.125	-	-
Fisher's Exact Test	-	-	-	0.191	0.095
Linear-by-Linear Association	2.308	1	0.129	-	-
N of Valid Cases	160	-	-	-	-

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 12.50.
b. Computed only for a 2x2 table

Table 5.The distribution of genotype frequency of the rs4872077 polymorphism

group	Genotype	Frequency (%)	PPMS (n=40)	PRMS (n=40)
1.00	CC	3 (3.8)	1	2
	TC	11 (13.8)	3	8
	TT	66 (82.5)	36	30
	Total	80 (100.0)	40	40
2.00	CC	3 (3.7)	-	-
	TC	8 (10)	-	-
	TT	69 (86.3)	-	-
	Total	80 (100)	-	-

Table 6.The distribution of genotype frequency of the rs4872077 polymorphism

Genotype	Value	*df*	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.427[a]	1	0.514	-	-
Continuity Correction[b]	0.190	1	0.663	-	-
Likelihood Ratio	0.428	1	0.513	-	-
Fisher's Exact Test	-	-	-	0.664	0.332
Linear-by-Linear Association	0.424	1	0.515	-	-
N of Valid Cases	160	-	-	-	-

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 12.50.
b. Computed only for a 2x2 table

DISCUSSION

MS is a common disease which is most likely caused by interaction between multiple common allelic variations of genes. The possible association between multiple sclerosis (MS) and TRAIL Receptor polymorphisms has already been analyzed in several studies, with conflicting results [3, 13].

In general, association studies are very powerful

in dissecting the genetic contribution to complex diseases; however, there are many examples of association with a candidate gene followed by studies which could not confirm the earlier reports. It seems that other polymorphisms in different positions of TNF-α, other cytokines, and also their interaction should be taken into account in the study of MS susceptibility [4-6, 20]. Recently, some genome-wide association studies (GWAS) have been performed through analyzing a large number of SNPs, simultaneously, based on chip technology. They demonstrated no significant relationship between TRAIL gene polymorphism and MS, which is consistent with our findings [1, 10, 12]. To date, 55% of the Iranian population belongs to the 15-30 years age group. MS typically occurs in young patients and the predominance of young population in Iran may justify the high prevalence of MS in this country [16-17]. This paper did not reveal significant differences between the polymorphisms and the Expanded Disability Status Scale (EDSS). We have not found any publication in which the relationship between genotypes and EDSS was examined. A study of TRAILR-1 and TRAILR-2 genotypes in Spanish patients has shown rs4872077, in TRAILR-1 gene, $p = 0.005$, OR = 1.72; and rs1001793 in TRAILR-2 gene, $p = 0.012$, OR = 0.84. The combination of the alleles G/T/A in these SNPs appears to be associated with a reduced risk of developing MS ($p = 2.12\times10^{-5}$, OR = 0.59). The results obtained in previous studies suggest that genes of the TRAIL/TRAIL receptor system exert a genetic influence on MS [13]. It is notable that in our study, the SNP rs4872077 located in the TRAIL-R1 gene does not show any association with MS. The protective role of the A allele in rs1001793 polymorphism does not show any association with MS in our study. The findings of the present study might suggest that the rs4872077 T to C and rs1001793 G to A transition polymorphisms in the TRAILR-1 and TRAILR-2 is less common in MS patients in the Iranian Azeri Turkish population. This is not in accord with the proposed role of TRAILR in the pathophysiology of clinical characteristics in MS. This genetic complexity may also be partially responsible for the disagreement among results reported by various studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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