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Variation in the Interleukin-7Receptor Alpha Gene RS6897932 in Fars Province of Iran Multiple Sclerosis Patients

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Abstract:

Background: Multiple sclerosis (MS) is the most common chronic (inflammatory) disease of the central nervous system affecting young adults.interleukin-7 receptor is a candidate gene on the chromosome 5p14-p12, known to be associated with multiple sclerosis.

Objectives: The aim of this study was to investigate association between IL7Ra polymorphisms in MS patients from Fars state.

Materials and Methods: In this case-control study, blood samples of patients and healthy volunteers were collected (n = 60) from Neurology department of Peymanieh hospital Jahrom. The IL7Ra genotypes were determined using DNA extracted from the samples by polymerase chain reaction (PCR) techniques followed by sequencing method.

Results: Our results have revealed that allele C is present in a higher frequency in MS patients (77%) as compared to the control group(56%). allele T seems to be protective against MS development (OR =0.398, 95% CI= 0.181–0.874, p = 0.022).

Conclusions: We found significant relationship between rs6897932 genotypes and MS.

Key words: *Multiple sclerosis, Polymorphism, IL7Ra gene, PCR, SNP*

1. BACKGROUND:

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system affecting young adults. The pathogenesis of MS to be elucidated, there is increasing evidence that MS is the result of involvement of both genetic and environment factors (1). Genome-wide association studies (GWAS) have led to the identification of IL7Ra validated non-HLA risk loci for MS (2). Interleukin-7 receptor is a candidate gene on the chromosome 5p14-p12, known to be associated with multiple sclerosis (3). ***Interleukin-7receptor*** is an essential pleiotropic receptor in immunology. IL7R functions as a receptor for two signaling cascades: thymic stromal lymphopoietin and IL7 [5]. In the IL7 pathway, IL7 interacts with the gamma chain (cc) (CD132) and IL7R, forming the signaling complex. The IL7/IL7R interaction is important for the maintenance of memory T-cells and the development, survival, and proliferation of T- and B-cells, especially CD4 T-cells, which are present in inflammatory lesions of MS patients [6–8]. IL7Ra is known to play a critical role in the V (D) J recombination during lymphocyte development (4). Further, the receptor fulfills important functions in the signaling cascade for T- and B-lymphopoiesis by promoting proliferation, differentiation and survival of cells (9) as well as in the control of the T-lymphocyte receptor γ locus accessibility by STAT5 and histone acetylation (10). T-cells with altered functional characteristics are the key immuno pathological mechanism of MS (11). Single nucleotide polymorphisms in IL7Ra gene are involved in the dysregulation of immune homeostasis and susceptibility to MS (12).Alternative splicing of exon 6 of IL7RA leads to the production of a membrane-bound or a soluble form of IL7Ra (13). A non-synonymous single nucleotide polymorphism (SNP), rs6897932, located in exon 6 of the IL7Ra gene, introduces a coding change (3).

It was suggested that it has a functional effect on gene expression of the soluble form of IL7RA (14). The SNP rs6897932 was identified as an MS susceptibility-modifying polymorphism in genome-wide and gene scan studies. Still, not all results confirm its role in MS susceptibility (15, 16, 17). Here, we focused on rs6897932 polymorphisms of the *IL7Ra* gene to investigate whether it is associated with the susceptibility and risk of MS in Iranian patients.

2. MATERIALS AND METHODS

2.1. Clinical Samples

Whole blood samples were collected from 30 normal people (control) and 30 MS patients (20 female and 10 male) from Fars province, Iran. Only patients with clinically definite or laboratory-supported definite MS, according to the diagnostic criteria for multiple sclerosis of Poser *et al*, 1983 (18) were studied.

MS patients participated in this study aged 15 to 51(32.23 ± 8.94) years. And unrelated ethnically matched healthy blood donors with no history of MS or any other diseases

related to the function of IL7Ra exon6 allele's .Disability of patients was assessed using McDonald's criteria 2010.

2.2. Genotyping

Genotyping of additional MS cases and controls was performed by initial PCR amplification with the IL-7R1 primer and a modified IL-7R2 primer (3'-CCCACACTTACTGTGCTTACATC). Three extension primers were designed for promoter SNPs: IL-7R–1085: 5'-TTTGTAGATGATACACAAATGGGT, IL-7R–504: 5'-TTTTTTTTTGGCATAGTG GCATTTGCCTG, IL-7R–449: 5'- TGCTTGAACCTGGGAGGTG (Teutsch, *et al.,* 2003). SNaPshot reactions were analyzed as described by the manufacturer (ABI PRISM SNaPshot Multiplex Kit, Applied Biosystems, Foster city, CA, USA).

Peripheral blood samples (2 ml) were collected in EDTA tubes and DNA was extracted from whole blood using boiling method. Quantity and quality controls were performed by spectrophotometer and visualized by electrophoresis on 1% agarose gel. Genotyping was performed via polymerase chain reaction (PCR) with subsequent sequencing analysis. To determine the genotype of sample, approximately 100 ng of genomic DNA was amplified using master mix (bioneer ,Korea) PCR and 10 pmol of each primer, including forward (5-**CTTCAAGTGGCAGATGCTCTG-3)** and reverse **(5-CCCACACAATCACCCTCTTTA**-3)(3).The primer amplified a fragment with 349 *bp* length from genomic DNA in which cover of rs6897932. Rs6897932 amplification was performed in a20 µl reaction volume and PCR condition was initial denaturation at 94oC/ 5 min, followed by 35 cycles at 94 °C for 30 sec, 58 °C for 1min, and 72 °C 1min.Termination of cycle sequencing was performed at 72 °C for 10 min as final extension. PCR products were purified by using the bioneer PCR product purification kit (bioneer, Korea) (**Figure 1)**. DNA sequencing was performed using the ABI 3730XL machine for genotyping. PCR products were sequenced in both forward and reverse direction using the specific primer of rs6897932.

2.3. Statistical Analysis

The frequency of alleles and genotypes for case and control groups were identified and the association analysis of rs6897932 with MS was performed using Chi-square test and regression logistic test. Hardy-Weinberg equilibrium (HWE) was used to check allelic equilibrium between samples. Odd`s ratio (OR) and 95% interval confidence (CI) were applied to estimate the contribution of the risk factors. All statistical analyses were performed using SPSS version 22 the conventional *P*-value of ≤ 0.05 was considered as overall significant level.

3 RESULTS

In our study, we observed significant differences in the *IL7Ra* allele frequencies between MS patients and control group that are shown in Table 2. Our results have revealed that allele C is present in a higher frequency in MS patients (77%) as compared to the control group (56%), which indicates for the increased risk of MS development. Interestingly, allele T was manifested in MS patients in a significantly less frequency, representing only 23% as compared to 44% in controls. This suggests that allele T seems to be protective against MS development (OR =0.398, 95% CI= 0.181–0.874, p = 0.022).The additive model fitted the best to assess association between genotypes and the MS risk.

The genotype analysis showed that the frequency of genotype CT is the highest in controls to be 67% and the lowest in MS patients 40%. The frequency of genotype CC was the highest in MS patients (56%) and the lowest in controls (23%). Conclusively, the data suggest that individuals carrying genotype TT are protected against affecting of MS.

Table 1. Demographic and clinical profiles of multiple sclerosis patient and control

variable	sex	Age, means	Age range
Ms patient	20 Female (64%) 10 Male (33%)	33.23+/-8.947	15-51
Control	20 Female (64%) 10 Male (33%)	34.2+/-9.679	18-58

Table 2: **The frequency of rs6897932 allele and genotype in Fars multiple sclerosis patients and controls**

Genotype	control subjects (n =30)	MS Subjects (n =30)	OR	P-value	CI
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

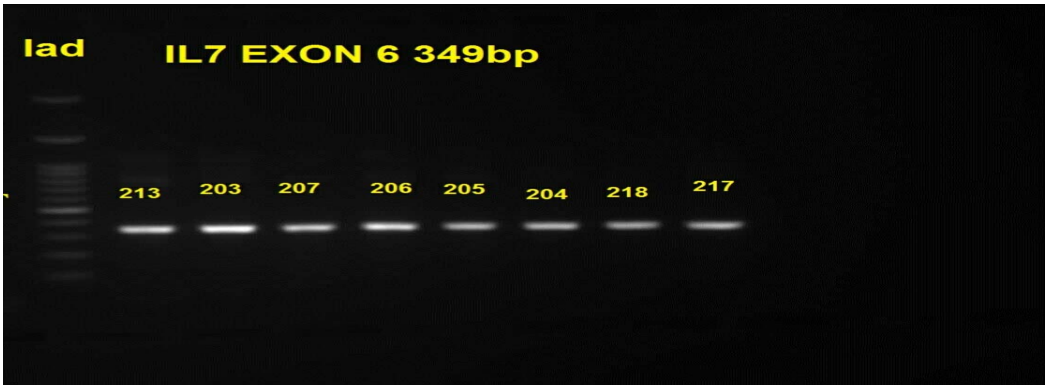

Figure 1. The amplified fragments of the il7 (rs6897932) gene (349 *bp)*, using specific primer.

4 DISCUSSION

MS is an inflammatory neurodegenerative disorder. Although the etiology of MS is not clear, MS pathology is suggested to affect genetically predisposed individuals (19). IL-7 is a critical factor for regulating T-lymphocyte development and homeostasis (20).SNP rs6897932 located on exon 6 has very important role in transmembrane and soluble form of IL-7Ra protein and represents the most consistently replicated susceptibility gene to MS after MHC region (21). Studies have investigated the correlation between MS and IL-7Ra polymorphism (10, 18-22) or a number of studies have illustrated an association between MS and the high risk allele of rs6987932. These studies covered different ethnic groups (23, 24, 25, 26, 27, and 28). Akkad *et al* confirmed the association of SNP rs6897932 with susceptibility to MS, although he suggested that the SNP rs6897932 may not be the disease causing variation in this gene (7).

Our results demonstrated that association between rs6897932 in IL7Ra gene with the risk of MS in our cohort of Fars province. We found allele C to be associated with an increased susceptibility to MS ($p = 0.034$). The frequency of allele C in the MS patients was significantly higher when compared to controls. Concordantly with our findings, the protective roll of allele T has already been reported by several other studies (27, 28). Cierny study has reported the protective effect of allele T in the control group in compared MS patient .Our finding also show approximately same results by Cierny study (28).in our study genotype TT is more frequent in control group. Our results are against with some other study in European populations which the rs6897932 C allele was also not recognized as a risk factor for MS susceptibility (15,29,30, 31, 32, 33).we can suggest ethnicity variation ,geographical location and interaction of population genetic background with the environmental, are main reason of this difference.

5. CONCLUSION

We found significant relationship between rs6897932 genotypes and MS in Fars population. We have also showed for the first time in Fars population, that allele C of rs6897932 is associated with the risk of MS and allele T has a protective additive effect against MS susceptibility. One limitation of our study was the small number of study samples. However, further studies with larger sample sizes are needed to fully confirm these findings.

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