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Promoter Sequence of IL7RA Gene Reveals an Association with Multiple Sclerosis in Iranian Patients

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ABSTRACT

Multiple Sclerosis (MS) is the most inflammatory, demyelinating, neurodegenerative and disabling disorder of central nervous system with a strong genetic component. In this investigation we examined the variations of IL7Rα gene promoter among Iranian MS patients. In this case-control study, one hundred Iranian RR-MS patients and eighty ethnically, sex and age matched healthy controls with no personal or family history of autoimmune diseases were investigated for variations in the IL7Rα gene promoter based on PCR and sequencing strategy , in this Chi square was applied to analyze. The value of p <0.05 was considered significantly. A significant positive association between T allele of rs11567685 (P: 0.04; OR: 2.426; 95%CI: 1.581-3.722) and T/C genotype (P: 0.0001; OR: 0.407; 95%CI: 0.217-0.766) were demonstrated. C/C genotype of rs11567685 (P: 0.00003; OR: 0.273; 95%CI: 0.145-0.513) have negative association to MS. Also A allele rs11567686 (P: 0.01; OR: 0.545; 95%CI: 0.327-0.91) and G allele rs7718919 (P: 0.02; OR: 0.417; 95%CI: 0.119-0.921) show a negative association to MS. DNA sequencing of IL7Rα promoter demonstrated an association between three SNPs (rs11567686, rs11567685 and rs7718919) and MS disease. Further studies on large sample size are required to bring about more authentic results. In addition, more studies are required to define the effects of these variations on the IL7R protein in multiple sclerosis. Finally the functional effects of these SNPs need to further investigations.

Key words: MS, IL7Rα, Promoter, Polymorphism.

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1. INTRODUCTION

Multiple sclerosis (MS) is an ambiguous autoimmune disorder of central nervous system among young adults that due to inflammatory responses leads to demyelinating of neurons. This autoimmune disorder is characterized by inflammation, demyelination, primary or secondary axonal degeneration, diplopia, ataxia and finally relapses (1). The classified clinically courses can be divided into four different subtypes: Relapsing-Remitting MS (RR-MS) that initially characterized by relapses with full recovery. 80% of MS patients experience a Relapsing-Remitting course with clinical exacerbations of neurologic symptoms and followed complete or incomplete remission. Although the pathogenesis of the disease has not yet been fully elucidated, but complex interplay of environmental and genetic factors are likely causes for disease development and increasing evidences that indicate MS in genetically occurs in predisposed persons (2). In fact like most other autoimmune-mediated diseases, MS belongs to the large group of multifactorial disorders which interact with both environmental and genetic factors and contribute to MS susceptibility. In migration studies when people migrate from low-risk to high-risk areas, strong environmental

component is particularly evident and changes the risk of MS (3). Several considerable environmental factors that influence the risk of MS are virus infections, geographical distribution, diet and exposure to sunlight. Although the link of each of these factors with the pathogenesis of MS still is under controversy (4); In Twin studies, monozygotic twins in comparision with dizygotic twins have 30% higher chance and more concordance rates also suggest a strong genetic component. On the other hand, siblings and children of probands have 1–2% chance of the prevalence compare normal population (3, 5). The first Genetics Etiology of MS has described with the human leukocyte antigen (HLA) class II genes, located on chromosome 6 and represents the strongest risk for MS (6). Researchers showed that candidate gene, HLADRB1* 1501, is not the unique susceptible allele but maybe is a part of a susceptible haplotype which this haplotype includes at least HLA-DQA1*01:02 and HLADQB1*06:02, that through epistatic mechanisms interact with HLA-DRB1*15:01 (7). The inflammatory damages display the autoimmune trait of MS, that we studied some genes, especially HLA and cytokine genes, in Iranian patients with MS previously (7-9). Recently, showed that the interleukin7 receptor α chain (IL7Rα) is one of the non HLA genes have been associated with MS. IL7Rα complex receptor consist of IL7R and IL7 to shapes an IL-7Rα . IL7Rα is a member of the type I cytokine receptor family that combined with common gamma chain of IL2Rα cytokine receptor, forms a complete figure of receptor for IL7 ligand. The rs6897932 of the interleukin 7 receptor α, is one of the first non HLA genetic polymorphisms that found to be associated with an increased risk of developing MS (10-12). Many studies reported a significantly higher association of IL7Rα in promoter single nucleotide polymorphism (SNP) like rs11567685 to affect the CD4 T cells in MS patients (10). According to the records a trios, −504 C, on promoter of IL7Rα observed in MS carriers (11). In this investigation we examined the variations of IL7Rα gene promoter among Iranian MS patients compared to healthy controls for detecting novel or related SNPs by DNA sequencing technique.

2. MATERIALS AND METHODS

2.1. Patients and Controls

In this case-control study, one hundred patients were in concordance with McDonald criteria and every demyelination suggestive disease was excluded. All patients had signed an agreement for participating in this project. All patients had a review about their details and are clinically definite diagnosed by neurologist. Eighty healthy individuals were selected as controls. Controls were age and sex matched to MS patients. Demographic and clinical data of MS and control groups are presented in Table 1.

Table 1. Demographic and clinical data of MS patients and healthy control

Variables	MS patients	Healthy control
Female (no, %)	85 (85%)	65 (75%)
Age (mean,Y)	31	32
Age at onset (mean, Y)	25	-
Relapsing-Remitting (no, %)	100 (100%)	-
Duration (mean, Y)	5.6	-
EDSS (mean)	3.5	-

2.2. Blood sampling

Whole blood samples were obtained from 100 Iranian MS patients and 80 healthy controls. DNA samples of control subjects were obtained from healthy donors.

2.3. DNA extraction

Genomic DNA was extracted from whole blood cells by using a standard salting out technique. DNA concentration was determined by construction of a standard curve of Nano drop. Samples stored at -80˚C and aliquots samples for further analysis.

2.4. PCR amplification

Amplification was performed by using forward and reveres primers that were utilized to generate a 1484bp product size and Table 2 depicts the sequence of primers.

Table 2. The nucleotide sequences of the forward and reverse primer

primer	sequence	Tm(°C)
Forward	5'-ATACCTAGGCACTAATTTAGTTC-3'(23-mer)	60.1
Reverse	5'-CCTGAAACCATGCTACAG-3'(18-mer)	58.2

The 32 PCR cycles program was followed as: an initial step at 95˚C for 5 min and 32 cycles followed by 95˚C for 1 min, 61.8˚C for 1min and 72˚C for 1 min and a final step is 72˚C for min and finally cooling 5 min in 4˚C. PCR products separated and electrophoresed by using agarose gel 1% for verification of correct amplification.

2.5. Sequence analysis

Sequencing was performed via the Sanger chain termination technique by the ABI automated DNA sequencer (Macrogene company, Korea).

2.6. Statistical analysis

Associations between IL7Rα promoter alleles and MS were examined in case-control by using chi-square test and Fisher's exact tests for detection and analysis frequencies of alleles and genotypes between study groups. Differences in frequencies were tested by chi square test and the SPSS statistical package was used to determine p values. The value of $p < 0.05$ was considered significantly.

3. RESULTS AND DISCUSSION

Promoter region of IL7Rα gene were checked for detecting novel or related SNPs by PCR, DNA sequencing technique. Three variations were identified in promoter region of IL7Rα by DNA sequencing. All SNPs were in hardy-Weinberg equilibrium (HWE) in both patients and control groups. Allelic and genotype frequencies and their analysis of rs11567686, rs11567685 and rs7718919 are showed in

Table 3.

Table 3. Allele and genotype frequencies of IL7Ra promoter polymorphisms in patients and controls

Allele/Genotype	Patients (%)	Controls (%)	P value	OR (95%CI)
rs11567686				
Alleles	n=200(%)	n=160(%)		
A	144(72)	132(82.5)	0.019	0.545(0.327-0.91)
G	56(28)	28(17.5)		
Genotypes	N=100(%)	N=80(%)		
A/A	72(72)	66(82.5)	0.098	0.545(0.265-1.124)
A/G	0	0		
G/G	28(28)	14(17.5)		
rs11567685				
Alleles	n=200(%)	n=160(%)		
T	133(66.5)	72(45)	0.042	2.426(1.581-3.722)
C	67(33.5)	88(55)		
Genotypes	N=100(%)	N=80(%)		
T/T	58(58)	36(45)	0.08	1.688(0.933-3.054)

T/C	17(17)	0	0.0001	0.407(0.217-0.766)
C/C	25(25)	44(55)	0.00003	0.273(0.145-0.513)
rs7718919				
Alleles	n=200(%)	n=160(%)		
G	175(87.5)	151(93.2)	0.027	0.417(0.189-0.921)
T	25(12.5)	9(6.8)		
Genotypes	N=100(%)	N=80(%)		
G/G	80(80)	71(88.8)	0.1	0.507(0.217-0.897)
G/T	15(15)	9(11.2)	0.4	1.392(0.575-3.371)
T/T	5(5)	0	0.06	-

A significant positive association between T allele of rs11567685 with P: 0.04 (OR: 2.426; 95%CI: 1.581-3.722) and T/C genotype with P: 0.0001 (OR: 0.407; 95%CI: 0.217-0.766) were demonstrated. C/C genotype of rs11567685 with P: 0.00003 (OR: 0.273; 95%CI: 0.145-0.513) have negative association to MS.

Also A allele rs11567686 with P: 0.01 (OR: 0.545; 95%CI: 0.327-0.91) and G allele rs7718919 with P: 0.02 (OR: 0.417; 95%CI: 0.119-0.921) show a negative association to MS.

Hence, several studies like present study Specially checked the putative promoter region of IL7R by individual DNA sequencing and identified several common polymorphisms that related with cytokine and other immunological genes in MS (12). Mazzucchelli in 2007 underlines that higher expression of IL7Rα influence the CD4+ T cells and leads to thymic emigrants of regulatory and conventional T cells in MS patients , it can alo influence T cell development and homeostasis and finally contribute to the altered immune regulation which is associated with disease development in MS patients (13). Because of dysregulation in gene expression, genetic differences in gene promoter regions maybe cause of MS which would point to inherited factors causing the gene dysregulation, and thus supporting a role for the gene in disease susceptibility and/or progression. Genotyping IL7Rα polymorphisms in MS patients and healthy controls clarify Three tagging SNPs in the promoter region of the IL7Rα gene (rs11567686, rs11567685 and rs7718919) and influence the expression of this gene and were analyzed using a sequencing procedure to determine common SNP_S genotype in MS patients and healthy controls. Broux et al. in 2010 reported that SNP rs11567686 and SNP rs11567685 which are located respectively in 449 bp and 504 bp distances from start codon on the promoter of IL7Ra gene have shown positive association with MS (14). According to the Sadeghi haj et al in 2015, a significant association was observed on genotype level : relapsing-remitting (RR-MS) in SNP rs7718919 (*P*: 0.03) and also secondary-progressive multiple sclerosis (SP-MS) is related with SNP rs11567685 (*P*: 0.009) in western populations (15). Booth in 2005 also reported that, (-504) T allele, of SNP rs11567685 was more frequent in PP- MS group (p: 0.01), and almost the same result with p: 0.042 was repeated in our data (16). Two subsequent studies mark this special SNP and its allele to PP-MS. The latter study emphasis on association between −504 C trios and PP-MS in carriers of one interleukin 7 receptor (IL7R) promoter SNP, in Victorian/Tasmanian MS DNA bank (P: 0.05), in addition in −504 trios, the T allele was more common on SPMS (17, 18). although two studies of Booth and Heidari reported high risk T allele of rs11567685 with PP-MS and Heidari in 2011 submitted an association between SNP rs11567686 and SP MS in Iranian populations (19). Overexpression of IL-7Rα is a major cause of increasing rate of T-cells and does not alter the ration of soluble to membrane-associated isoforms of IL7Rα in MS patients. We know that rs7718919 located in the promoter region of IL-7Ra gene with (*P*: 0.031) had an inconsiderable association with MS patients and till now never reported in Iranian and Australian populations. However they showed that the presence of T allele in rare allele rs7718919 SNP's facilitate the binding of different transcription factors, including nuclear factor to gene promoter in T cells (19). Also detection of potential transcription factor binding sites of the promoter region SNPs showed a binding site for nuclear factor 1 on the rarer SNP -1085 T allele, but not the G allele, and a site for nuclear factor for activated T cells in the rarer SNP -449 G allele , but not the A allele (19). In present study we observed p: 0.06 for T/T genotype and P: 0.02 for G allele in rs7718919 SNP as well as rs11567686 with p: 0.01 for high risk A allele and finally we found the association of high risk T allele with P: 0.042 and p: 0.0001 and p: 0.00003 for T/C and C/C genotype in rs11567685, respectively. Unfortunately we could not determine the

linkage between T/T genotype with rs11567685 but allele frequency of the T allele was found to be comparable with previous studies in Iran, Jordan and other Asian countries. Observed differences in our results and others finding might depend on differences in genetic and other risk factors which involved in MS between populations. This investigation was performed on Iranian MS patients, it would be so interesting to do this analaysis on large sample size of populations with different ethnics in Iran and other countries are located in Middle East to demonestrae the role of IL7Ra polymorphisms in MS disease in this erea. Further studies on large sample size are required to bring about more authentic results. In addition, more studies are required to define the effects of these variations on the IL7R protein in multiple sclerosis. Finally the functional effects of these SNPs need further investigations.

4. CONCLUSION

In this investigation, DNA sequencing of IL7Rα promoter demonstrated an association between some alleles and/or genotypes of three positions (rs11567686, rs11567685 and rs7718919) and MS disease. We know Iran is an extended country with many different ethnical backgrounds and differences in latitudes. As a result , Ethnic background play a major role in accompany with disease even specific environment triggers might interfere to the MS susceptibility, so according to the small sample sizes and limited power of this study , more investigations on IL7Rα polymorphism in Iranian population and environmental triggers like psychological stresses are still needed.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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