http://pmjournal.ir Original Article
Summer 2019, Volume 4, Issue 14 (18-20)

IL7 receptor polymorphisms and Multiple sclerosis in Western Provinces of Iran

Mohammad Ali Saremi [1,*], Vahid Reza Esfahani [1]

[1] Personalized Medicine Research Center of AmitisGen, Tehran, Iran

** Corresponding author: Mohammad Ali Saremi, Personalized Medicine Research Center of AmitisGen, Tehran, Iran. E-mail:masaremi@yahoo.com*

DOI: 10.29252/pmj01026

Submitted:2019/04/18
Accepted: 2019/05/10

Keywords:
Multiple Sclerosis
Polymorphism
RFLP
Personalized Medicine

Abstract

Multiple sclerosis (MS) is an autoimmune neurodegenerative disorder. The etiology of MS is not clear but genetic and epigenetic factors are involved in MS development. Studies have shown that IL7R gene polymorphisms is capable of changing MS susceptibility. We investigated the association of MS with rs11567658, rs11567686 promoter polymorphisms of IL7R gene in western provinces of Iran. In the present study, 187 MS patients and 190 healthy control were evaluated. Polymorphic regions of IL7R promoter were amplified by appropriated primers and polymorphisms were then evaluated by RFLP method followed by validation via Sanger sequencing. Results shown rs11567685 and rs11567686 are significantly associationed with MS (P = 0.017 P = 0.046), significant association of these polymorphism with age was also found (P = 0.002). This study showed that IL7 receptor gene polymorphism has a key role in MS development and may be important opportunity for development of therapeutic and diagnostic strategies in context of personalized medicine.

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune inflammatory demyelinating disease of the central nervous system. MS attacks the myelinated axons in the CNS, leading to destruction of the myelin and the axons in different degrees [1]. MS is the most frequent CNS disorder among young adults [2]. According to the World Health Organization (WHO) in 2008, around 1.3 million people had MS worldwide, while the incidence of MS was estimated in 2013 to be 73/100,000 individuals in the world and 60/100,000 in Iran. Current studies showed that the prevalence and incidence of MS in Iran is high and is rising over time [3]. The clinical course and onset of the disease is highly variable and generally unpredictable. About 50% of the patients require a walking aid after 15 years of disease [4]. Although the initiating events are still under debate, it is generally accepted that autoreactive T lymphocytes are important mediators of the immunopathological process [5]. MS is divided into four subtypes clinically including relapsing-remitting MS (RRMS), primary progressive MS (PPMS), progressive relapsing MS (PRMS), and secondary progressive MS. The most frequent subtype has been described as RRMS with 80%, followed by PPMS (10–20%), PRMS and SPMS (rare involvement). Approximately 50% of RRMS patients turn into progressive MS [6]. The etiology of MS is not clear, but MS carries out an autoimmune attack against self-myelin or oligodendrocytic antigens by macrophages, T cells, Lymphokines, and antibodies according to a hypothesis when they enter the brain [7, 8]. Recent studies suggest that environmental, epigenetic, and genetic factors are implicated in the development of MS. Genome-wide association studies (GWAS) uncovered potential candidate genes which may lead to MS susceptibility a 25 kDa glycoprotein. IL7R works as a receptor for IL7 cytokine and regulates lymphopoiesis. The ligand-receptor complex is defined to be essential for T cell development [9]. One study further showed that both IL-7Ra and IL-7 mRNA are up-regulated in the cerebrospinal fluid of individuals suffered from MS [10]. A single polymorphism in exon 6 of IL-7Ra conferred enhanced susceptibility to the MS. It was found that this polymorphism resulted in altered splicing by excluding exon 6 from the protein, leading to increased expression of a soluble form of the receptor [11]. It has also been reported that promoter polymorphisms in IL7R gene can increase gene transcription. In this study, the IL7R gene was screened for promoter SNPs (rs11567686) and (rs11567685) and frequency of the polymorphic alleles and distribution of the genotypes were determined, where could be used to examine MS susceptibility in Iranian patients.

METHODS

One hundred eighty-seven MS cases and 190 healthy person with same age were selected as patient and control groups. DNA samples were obtained from peripheral white blood cells of 377 participants by Salting-out method. One polymerase chain reaction (PCR) was performed for both polymorphism, rs11567685 and rs11567686 in the promoter region of IL7R gene. The forward primer 5'-TGC CTA CTG TGG TGT ATG AGA TAT GAG-3' and the reverse primer 5'TGT TCC TCC TGG ATA TTC CCT GC-3' were selected (Hasan Simsek et al. 2018). The PCR cycle program was similarly performed for two polymorphism sites as follows: an initial step at 95 °C for 15 min, followed by 40 cycles of 95 °C for 30 s, 65 °C for 30 s, 72 °C for 45 s, and a final step of 72 °C for 10 min. PCR products were visualized by electrophoresis on 0.8% agarose gel.

Digestion was performed using PstI and HphI to detect the rs11567685 and the rs11567686 polymorphism regions, respectively. Then, electrophoresis was performed on 2% agarose gel. For the polymorphism of rs11567685, PstI restriction enzyme was capable not only of cleaving the position of the major allele (T), resulting in two fragments, 630 and 20 bp, but also of cleaving the fragment with the minor allele (C), generating 399, 231, and 20 bp fragments. While the 20 bp fragment was not distinguishable by DNA primer; the presence or absence of the 630 and/or the 399 and 231 bp fragments was considered for identification.

For the polymorphism of rs11567686, HphI restriction enzyme cleaved the position of the major allele (A), resulting in generation of two 461 and 189 bp fragments, but it was not capable of cleaving the fragment containing minor allele (G), leading to generation of a 650 bp fragment. The frequency of alleles and distribution of genotypes for patient and control groups were also identified. Analysis of association of two SNPs with MS was performed using Pearson's chi-square test. Odds ratio (OR) and 95% confidence interval (CI) were applied to estimate the contribution of the risk factors. All of the statistical analyses were performed using SPSS version 19.0 program. The conventional p value of = 0.05 was considered as overall significant level.

RESULTS

The statistical population of this study included 187 MS patients and 190 normal control. Female to male ratio was determined to be about 2 to 1.3 and mean age for onset of MS was 27.63 years. Additionally, the mean age of onset for female patients was determined as 26.72 years and the mean age of onset for male patients was calculated 28.46 years. The genotype distribution of IL7R rs11567686 for AA, GA, and GG in control group were 32.7, 49.4, and 17.9%, respectively. The genotype distribution of IL7R rs11567686 for AA, GA, and GG in MS patients were recorded as 38.5, 41.9, and 19.6%, respectively. This genotype distribution was not significantly different between control and MS groups (P = 0.142). The genotype distribution of IL7R rs11567685 polymorphism for TT, TC, and CC among control were determined to be 58.2, 36.3, and 7.5%, respectively. In MS patients, TC was the most frequent genotypes (52.1%), followed by TT (36.4%), and CC (11.5%), therefore, a significant difference was found between MS and control group (P = 0.017). Regarding to the age of onset, the distributions of rs11567685 (T/C) genotypes and rs11567686 (A/G) polymorphisms were found to be significantly different (P = 0.002, P = 0.148) (Table 1).

Table 1. Distribution of IL7R Polymorphism

IL7-R polymorphisms	Control group	MS group	P value
Rs11567686			0.142
AA	32.7	38.5	
AG	49.4	41.9	
GG	17.9	19.6	
Rs11567685			0.017
TT	58.2	36.4	
TC	36.3	52.1	
CC	7.5	11.5	

DISCUSSION

Multiple sclerosis (MS) is an autoimmune inflammatory demyelinating disease of the central nervous system [1]. Genetic studies have shown a significant relation between SNPs and MS. several GWASs have been applied for predicting the genetic background of this autoimmune disease [8]. IL7 and its receptor IL7R emerge as candidate genes for MS susceptibility. Previous studies have shown a relationship between IL7R SNPs and MS. Promoter and exon polymorphisms of this gene is capable of altering expression of this gene by affecting binding of transcription factors [12]. Two polymorphisms have been described for IL7R in promoter region including rs11567685 and rs11567686. In our study, we demonstrated a significant association of rs11567685 with MS disease. Transcription binding sites for RAR gamma, FoxP3, and Pax-6 were also found in the presence of minor allele A of the IL-7R – 449 (rs11567686) region, but not at the major allele G. Based on our results presented herein, we propose that these polymorphisms may be associated with increased disease susceptibility by increasing transcription of IL-7R gene expression; however, future studies are required for more clarification [13]. A recent study from Iran showed that A allele and AG genotype of - 449 polymorphism could led to MS susceptibility in female patients [14]. The association of the high risk T allele of rs11567685 with MS has been reported by two previous studies, but such linkage failed to be confirmed by a third one; however, the functional effects of these SNPs need to be further investigated. Our study had some positive

and negative results which were similar or opposite to earlier reports. The difference between studies may be due to ethnicity, environmental factors, other genomic and epigenetic factors, number of patients, or misdiagnosis. However, additional studies are necessary on a larger number of patients from different ethnicities to confirm the genetic association of these polymorphisms with MS, there is little doubt that IL7R polymorphisms have effects on MS development.

REFERENCE

1. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron. 2006;52(1):61-76. doi: 10.1016/j.neuron.2006.09.011 pmid: 17015227
2. Liguori M, Marrosu MG, Pugliatti M, Giuliani F, De Robertis F, Cocco E, et al. Age at onset in multiple sclerosis. Neurol Sci. 2000;21(4 Suppl 2):S825-9. doi: 10.1007/s100720070020 pmid: 11205357
3. Eskandarieh S, Heydarpour P, Elhami S-R, Sahraian MA. Prevalence and incidence of multiple sclerosis in Tehran, Iran. Iran J Pub Health. 2017;46(5):699.
4. Ghezzi A, Deplano V, Faroni J, Grasso MG, Liguori M, Marrosu G, et al. Multiple sclerosis in childhood: clinical features of 149 cases. Mult Scler. 1997;3(1):43-6. doi: 10.1177/135245859700300105 pmid: 9160345
5. International Multiple Sclerosis Genetics C, Wellcome Trust Case Control C, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011;476(7359):214-9. doi: 10.1038/nature10251 pmid: 21833088
6. Stuve O, Oksenberg J. Multiple Sclerosis Overview. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, editors. GeneReviews. Seattle: University of Washington; 1993.
7. Lundstrom W, Fewkes NM, Mackall CL. IL-7 in human health and disease. Semin Immunol. 2012;24(3):218-24. doi: 10.1016/j.smim.2012.02.005 pmid: 22410365
8. Gregory SG, Schmidt S, Seth P, Oksenberg JR, Hart J, Prokop A, et al. Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. Nat Genet. 2007;39(9):1083-91. doi: 10.1038/ng2103 pmid: 17660817
9. Zenatti PP, Ribeiro D, Li W, Zuurbier L, Silva MC, Paganin M, et al. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet. 2011;43(10):932-9. doi: 10.1038/ng.924 pmid: 21892159
10. Hoe E, McKay FC, Schibeci SD, Gandhi K, Heard RN, Stewart GJ, et al. Functionally significant differences in expression of disease-associated IL-7 receptor alpha haplotypes in CD4 T cells and dendritic cells. J Immunol. 2010;184(5):2512-7. doi: 10.4049/jimmunol.0902900 pmid: 20097866
11. Bashinskaya VV, Kulakova OG, Boyko AN, Favorov AV, Favorova OO. A review of genome-wide association studies for multiple sclerosis: classical and hypothesis-driven approaches. Hum Genet. 2015;134(11-12):1143-62. doi: 10.1007/s00439-015-1601-2 pmid: 26407970
12. Albert PR. What is a functional genetic polymorphism? Defining classes of functionality. J Psychiatry Neurosci. 2011;36(6):363-5. doi: 10.1503/jpn.110137 pmid: 22011561
13. Simsek H, Geckin H, Sensoz NP, List EO, Arman A. Association Between IL7R Promoter Polymorphisms and Multiple Sclerosis in Turkish Population. J Mol Neurosci. 2019;67(1):38-47. doi: 10.1007/s12031-018-1205-0 pmid: 30443838
14. Haj MS, Nikravesh A, Kakhki MP, Rakhshi N. Association study of four polymorphisms in the interleukin-7 receptor alpha gene with multiple sclerosis in Eastern Iran. Iran J Basic Med Sci. 2015;18(6):593.