

# IL-7R $\alpha$ polymorphisms in 60 Iranian multiple sclerosis patients

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Mojgan Ahmadzadeh Raji<sup>1</sup>, Alireza Khosravi<sup>2</sup>, Mohammad Hossein Sanati<sup>3</sup>,  
Seyed Massood Nabavi<sup>4</sup>, Reza Hajihoseini<sup>5</sup>, Ahmad Ebrahimi<sup>6</sup>,  
Mohammad Hossein Sabour<sup>7</sup>

<sup>1</sup> Department of Nano-Bio-Technology, School of New Sciences and Technologies, University of Tehran, Tehran, Iran

<sup>2</sup> Department of Mycology, School of Veterinary Medicine, University of Tehran, Tehran, Iran

<sup>3</sup> National Institutes of Genetic Engineering and Biotechnology, University of Tehran, Tehran, Iran

<sup>4</sup> Department of Neurology, Shahed University, Tehran, Iran

<sup>5</sup> Departments of Biochemistry, Payame Noor University, Tehran, Iran

<sup>6</sup> National Institutes of Genetic Engineering and Biotechnology, Tehran, Iran

<sup>7</sup> School of New Sciences and Technologies, University of Tehran, Tehran, Iran

## Keywords

IL-7R  $\alpha$ , Polymorphism, SNPs, SSCP, Sequencing, Multiple Sclerosis

## Abstract

**Background:** Multiple sclerosis (MS), a chronic inflammatory demyelinating disorder with neurodegenerative aspects, is more common among young adults, particularly women.

**Methods:** This molecular study was designed to investigate the IL-7R  $\alpha$  chain gene in Iranian MS patients. We studied 60 MS patients, diagnosed based on 2005 R-McDonald criteria and 60 apparently healthy individuals as the control group. DNA was extracted from whole blood cells using MBST/IRAN Extraction kit and all samples were screened for possible sequence variation in three regions including promoter, exon 2 and exon 4 with single strand conformation polymorphism (SSCP).

**Results:** The alterations were confirmed with direct sequencing by ABI 3730XL. Although no mutation was detected in the studied regions, eight single nucleotide polymorphisms (SNPs) consisting of rs71617734 in

promoter; rs35967524, rs11567704, rs1494558, rs11567705 and rs969128 in exon 2 as well as rs1494555 and rs2228141 in exon 4 were observed. The rs1494558 in exon 2 and rs1494555 in exon 4 were missense variations. Our results also showed the substitution of isoleucine with threonine in rs1494558 (P.I66T) with this accession number, FR863587 submitted in EMBL bank. The study of exon4 areas revealed two SNPs and two sequence variations, where [p.V138I] Valine substituted with isoleucine (FR863588), as well as a silent nucleotide substitution [P. H165H] in the absence of amino acid alteration. The analysis of the SNP genotype in the controls and the patients, using  $\chi^2$  showed no significant association with multiple sclerosis in this group.

**Conclusion:** Our study demonstrated the effects of some SNPs on the IL-7R  $\alpha$  protein in MS. Further studies are required to reveal the effects of these SNPs on the IL-7R  $\alpha$  protein in multiple sclerosis.

## Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder with neurodegenerative vision loss, paralysis, numbness and walking

difficulties.<sup>1</sup> Its prevalence is more in young adults and occurs 2 to 3 times more often in women. The inflammatory lesions indicate the autoimmune feature of the disease. IL-7 receptor  $\alpha$  chain, IL-2R  $\alpha$  and HLA locus has frequently been reported as the heritable risk factors associated with MS.<sup>2-4</sup> There is some evidence that the interaction of genetic and environmental factors is another cause of the disease.<sup>5</sup> Cytokine IL-7 is a 25-kDa glycoprotein,<sup>6</sup> secreted by stromal cells in the bone marrow and thymus.<sup>7</sup> IL-7, identified and characterized as a Pre-B-cell growth factor, has an important role in peripheral T-cell homeostasis. It also promotes the survival of lymphocytes and therefore is essential for naive T-cells.<sup>8-10</sup> Moreover, IL-7 is also involved in T-cell proliferation, an effect mediated by IL-7R  $\alpha$  chain (CD 127), an IL-7 receptor,<sup>11-13</sup> and common Interleukin-2 receptor  $\gamma$  chain.<sup>14</sup> This receptor is a candidate gene on the chromosome 5p14-p12,<sup>3</sup> known to be associated with multiple sclerosis.

Genetic evidence indicates that certain mutations in the  $\gamma$  c subunit of this receptor may cause X-linked severe combined immunodeficiency (X-SCID).<sup>15,16</sup> Moreover, Lundmark et al. reported altered expression of the genes encoding IL-7R  $\alpha$  and its ligand, IL7, in the cerebrospinal fluid of MS patients.<sup>17</sup> Microarray studies have also showed an increase in cDNA expression in IL-7R in peripheral blood mononuclear cells of relapsing-remitting MS patients in comparison with normal controls.<sup>18</sup> This gene has a potential functional role in MS pathogenesis.<sup>3</sup> The aim of this study was to identify single nucleotide polymorphisms in IL-7R  $\alpha$  chain gene in some Iranian MS patients. We used two techniques, single-strand conformation polymorphism (SSCP) and sequencing, for the determination of polymorphisms in our samples. The SSCP analyses were used<sup>19-21</sup> due to the fact that the technique is based on the principle that single-stranded DNA molecules form specific sequence-based secondary structures under non-denaturing conditions.<sup>22</sup>

## Materials and Methods

### (i) Patients and Controls

The patient group consisted of 60 clinically definite MS cases, with expanded disability status scale (EDSS) lower than six. The affected individuals were diagnosed based on the revised 2005 McDonald criteria.<sup>23</sup> The study was approved by the Ethical Committee of the hospital. Sixty individuals with no symptoms of MS or other neurological disease were selected as the control samples and used in SSCP gels. After sequencing, their sequences compared with sequences of selected patients.<sup>23,24</sup> Blood samples were collected using EDTA-containing Venoject tubes (Grainer/UK Company).

### (ii) DNA Extraction

DNA samples were extracted from whole blood cells

using MBST/Iran Extraction kit (Investigating Unit Molecular Biological System Transfer, Tehran, Iran), based on the manufacturer's protocol. The concentrations were measured with Eppendorf Biophotometer.

### (iii) PCR amplification

Three sets of primers for three regions of the IL-7R  $\alpha$  chain gene, including promoter, exon 2 and exon 4, were used as described by Teutsch et al.<sup>3</sup> The length of the fragments was checked with IL-7R  $\alpha$  gene using the UCSC site.<sup>25</sup> We amplified these fragments using the premix supplied by CinaGene Company (0.2 Units/ml Taq DNA polymerase in PCR buffer With 3 mM MgCl<sub>2</sub>, 0.4 mM dNTP) in a final volume of 25  $\mu$ l. PCR amplification was performed under standard reaction conditions, consisting of initial denaturation for 2 min at 94° C followed by 30 cycles of 94° C for 20 s, annealing temperatures (53° C for promoter, 55° C for exon2, 58.4° C for exon 4) for 10 s, 72° C for 30 s, followed by a 5 min extension at 72° C. PCR products were detected by Ethidium bromide on 1% agarose gel electrophoresis with TBE 1 x buffer (Lab FAQs Roche, Germany).

### (iv) SSCP analysis

SSCP was carried out using a maxi vertical gel (according to Bio-Rad's protocol).<sup>26</sup> Equal volume of PCR products and a solution containing 0.1 g bromophenol blue, 0.1 g xylene cyanol, 0.16 g NaOH, and 38 ml formamide were mixed together. The mixture was then denatured at 94° C for 4 min, chilled on ice, and loaded on a non-denaturing 8% acrylamide gel (acrylamide/bis-acrylamide gel: 39/1, Bio-Rad protocol). Electrophoresis was performed in TBE 1 x buffer at 125 volts/cm electrode for 18-20 hours at room temperature. DNA was detected using silver staining.

### (v) Sequence analysis

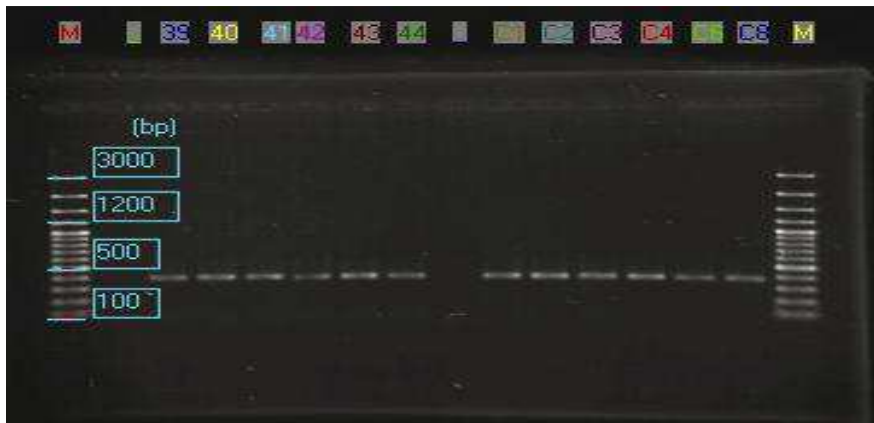
DNA fragments displaying different electrophoretic patterns were selected for sequencing. PCR products were purified (using the ABI Company's cleanup method). DNA sequencing was performed with ABI 3730XL machine (Applied Biosystems, Carlsbad, CA, USA).

### (vi) Statistical analysis

SNP genotyping in exon 2 for the patients and the controls were analyzed by  $\chi^2$ .

## Results

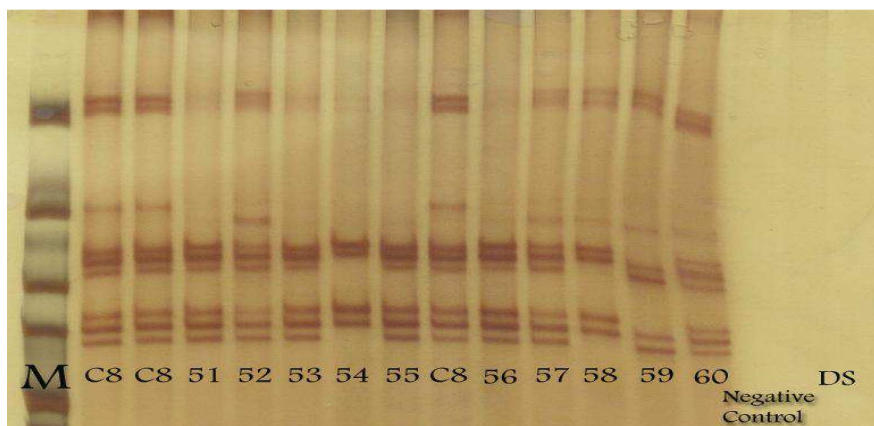
Patients were 46 (77%) females and 14 (23%) males. About 83.3% of the patients were classified to be suffering from the relapsing-remitting (RR) subtype of MS. All cases were Iranian. The promoter, exon 2 and exon 4 regions were amplified with Teutsch's primers. We expected 420 base pair (BP) fragment for promoter, 372 bp for exon 2 and 283 bp for exon 4 (Fig. 1 and 2).



**Figure 1.** 1% Agarose gel electrophoresis was used for fragment detection of exon 2



**Figure 2.** Fragments in exon 4, amplified with PCR



**Figure 3.** SSCP pattern is shown in Acryl amide gel for exon 2  
M: Molecular weight marker, C8: control sample, DS: Double-strand DNA

### Description of polymorphism

Two different patterns were obtained in exon 2 (Fig. 3), whereas the study of other regions revealed different patterns. The schematic representation of SSCP patterns in exon 2 and exon 4 showed similarity between the patients and the controls. About 63% of the patients and controls presented a similar pattern in exon 2; as for the exon 4 region, the rate was about 51.3%. Regarding the exon 2, 20% showed pattern B, 17% pattern C, and in exon 4, 1.7%, 20% and 27% represented patterns B, C and D, respectively (Table 1). The Odds Ratio and P-value of the differences in the SNPs of the exon 2 region is shown in table 2. After sequencing, different patterns were

noted in the rs1494558 of the exon 2 region of case 41 and 43. Case 41 was homozygous with a TT genotype, whereas 43 had a CC genotype. The difference in the patterns was clearly in the SSCP. In comparison with other samples in the exon 4 region, the rs1494555 of case 34 was heterozygous with AG genotype and of case 35 was homozygous with GG genotype (Fig. 4).

In the promoter, a single SNP with no variation was found in rs71617734. After sequencing the homozygous TT genotype was observed in our patients' population. It seems that this SNP is not suitable for studying the population diversity and further studies are necessary in this regard. In exon 2,

**Table 1.** Schematic representation of SSCP patterns for Exon2 and Exon4 of IL-7R  $\alpha$  gene

	Pattern A(Control)	Pattern B	Pattern C	Pattern D
Exon4				
Exon2	 51.3%	 1.7%	 20%	 27%
	63%	20%	17%	

SSCP: single strand conformation polymorphism

**Table 2.** The amounts of odds ratio and P-value in SNPs of Exon2

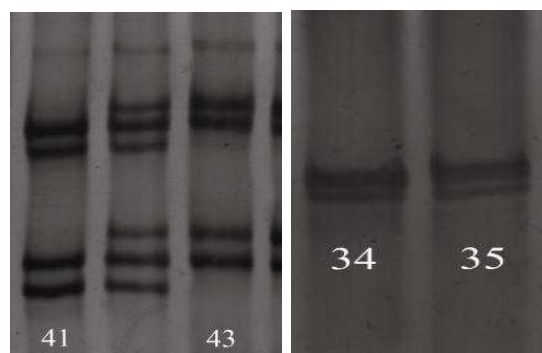
SNP	Genotype	Cases	Controls	Odds Ratio	P-value*
rs35967524	AA	0	0		
	AT	0	0		
	TT	60	60		
rs11567704	CC	60	60		
	CT	0	0		
	TT	0	0		
rs1494558	CC	22	20	1	
	CT	32	30	1.03	0.94
	TT	6	10	1.83	0.311
rs11567705	CC	28	33	1	
	CG	28	20	0.61	0.19
	GG	4	7	1.48	0.56
rs969128	AA	42	54		
	AG	0	4		0.14
	GG	0	2		0.50

SNPs: single nucleotide polymorphisms

five SNPs were found, rs35967524, rs11567704, rs1494558, rs11567705 and rs969128. One with rs1494558 could change amino acid isoleucine to threonine P.I66T 27 and its population diversity is similar with CEPH population in NCBI that findings are shown in table 3. The study of IL-7R  $\alpha$ 's exon 4 regions revealed two SNPs (rs1494555, s2228141) in the patients. Two sequence variations as P.V138I resulted in valine to isoleucine substitution in rs1494555 27 but a silent nucleotide substitution as P. H 165H had no amino acid alteration in rs2228141

### Discussion

In line with many studies pointing out to the role of IL-7R  $\alpha$  chain in MS,<sup>3,17,18</sup> the present study approved that the SSCP cannot be used as a valuable technique for analyzing and detecting SNPs in single strand

**Figure 4.** Banding pattern of the samples in SSCP gel is different

The alleles of the case No. 41 and 43 were homozygous (with TT and CC genotype) in rs1494558 (in exon 2 region). Line 4 and 5 illustrates two genotypes cases in rs1494555 (in exon 4) of two cases. Sample 34 and 35 have AG and GG genotype, respectively

DNA in polyacrylamide gel. As far as our knowledge, there is no study on the population diversity in promoter region in the NCBI website. The rs71617734 can be used as a molecular marker for early detection of MS if the variations are found in the control samples. Similar to the results of the Belfast population, the present study failed to show any association between the SNPs in exon 2 and MS.<sup>28</sup> Sequence variations including P.I66T and P.V138I were found in some patients and submitted in the EMBL Bank. In rs1494558, similarities were reported between our patients and the CEPH population, the genomic DNA samples obtained for a panel of 92 unrelated individuals chosen from the Centre d'Etude du Polymorphisme Human pedigrees [The genomic DNA was extracted from individuals from the UTAH (93%), France (4%) and Venezuela (3%)]. In other SNPs of the exon 2 region, rs35967524 and rs11567704, there was no heterozygosity or allelic variation; and thus were believed to play no important role in this regard. The rs11567704 differed from that obtained from the European, Asian and African-American populations as mentioned in table 3; this comes while

the rs11567705's allelic frequency was close to that of the Asian and the European societies. Despite the sequence variations found in our patients, we could not study the control group for exon 4.

We found a similarity in the rs2228141's allelic frequency between our group and that of the HSP GENO PANEL, PGA-EUROPEAN PANEL registered in NCBI (Table 4). The results also illustrated a resemblance in the rs1494555 of our patients and that of the PAC1 and Hap Map-HCB (Hap Map project). Further studies will be required to reveal the effects of these SNPs on the IL7R- $\alpha$  protein and in multiple sclerosis.

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**Table 3.** Population diversity for Exon2

Population	Sample Ascertainment	Chrom. Sample Cnt.	Genotype Detail					Alleles	
			A/A	A/G	G/G	HWP	A	G	
Selective Iranian Population	Case	120	0.1	0.533	0.370		0.370	0.633	rs1494558
	Control	120	0.170	0.500	0.330		0.416	0.583	
CEPH		184					0.360	0.640	
Selective Iranian Population	Case	120	C/C	C/G	G/G	HWP	C	G	rs11567705
	Control	120	0.470	0.470	0.100		0.700		
Total	African American	162	0.689	0.260	0.050	0.701	0.819	0.180	
Total	European	46	0.435	0.435	0.130	1.000	0.652	0.348	
Total	Asian	174	0.609	0.263	0.254	0.500	0.741	0.258	

**Table 4.** Population diversity for Exon 4

Population	Sample Ascertainment	Chrom. Sample Cnt.	Genotype Detail				Alleles		
			C/C	C/T	T/T	HWP	C	T	
Selective Iranian Population	Case	120	0.733	0.220	0.500		0.841	0.158	rs2228141
HSP_GENO_PANEL		114	0.684	0.316		0.200	0.842	0.158	
PGA-EUROPEAN-PANEL		46	0.783	0.130	0.087	0.020	0.848	0.152	

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