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Interleukin 7 Receptor Alpha Gene Variants Are Correlated with Gene Expression in Patients with Relapsing-remitting Multiple Sclerosis

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ABSTRACT

The association of single nucleotide polymorphisms (SNPs) of the IL-7Rα gene with multiple sclerosis (MS) have been documented in various populations. This study aimed to evaluate the genotype distributions of two SNPs, rs6897932 and rs201084372, and the functional association of rs6897932 in relation to IL-7Rα gene expression in a group of Iranian relapsing-remitting MS (RRMS) patients.

Genotyping for both SNPs in the IL7Rα gene and relative quantification of mRNA expression for both isoforms of IL-7Rα were performed in 100 RRMS patients and 100 ethnic-matched healthy controls.

Higher significant frequencies of the T allele and TT genotype for rs6897932 (C/T) were observed in patients comparing to controls (p=0.006). Higher frequencies of the T allele and the TT and TG genotypes and lower frequencies of the G allele and GG genotypes for rs201084372 (G/A) were found in patients comparing to controls (p<0.0001). A decreased level of mRNA expression for the membrane-bound IL-7Rα (mbIL-7Rα) and an increased level of mRNA for the soluble IL-7Rα (sIL-7Rα) were observed in patients versus controls (p=0.005 and p=0.002 respectively). A significant decreased level of mRNA expression for mbIL-7Rα (p=0.01) and an increased level of mRNA for sIL-7Rα (p=0.008) were observed in RRMS patients compared to healthy controls carrying the TT+CT genotypes.

The higher levels of mRNA expression for the sIL-7Rα isoform in MS patients carrying the IL7R*TT genotype is a new finding not previously reported in studies on the genotype-induced effects of IL-7Rα expression in multiple sclerosis.

Keywords: Gene expression; IL7Rα gene; Multiple sclerosis; Polymorphism

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INTRODUCTION

Multiple sclerosis (MS) is the most common cause of acquired neurological disability, which occurs mainly in young adults with an incidence of 0.1%.[1,2] The onset and progression of disease may vary markedly in individuals affected by MS.[3] For example, some individuals with MS show minor disability for several decades after onset, whereas others can become wheelchair dependent shortly after disease diagnosis. Thus, this condition has a complex inheritance with many different genetic and environmental factors contributing to its pathogenesis and more than 100 gene variants possibly involved in its development and progression.[4,5] Among them, HLA genes are known to be one of the most relevant genetic factors which could be able to influence not only the susceptibility to MS disease but also the clinical outcome of immuno-therapy for the disease.[6]

In addition the activation of myelin reactive T cells are known to have a role in the onset of MS.[7] These auto-reactive T-cells in MS patients appear to be skewed towards a pro-inflammatory Th1 phenotype with interleukin 7 (IL-7), a possible key factor for the stimulation and expansion of these cells in response to antigens.[8] The function of IL-7 is mediated by binding to an alpha chain (IL-7Rα or CD127) of the IL-7 receptor, a membrane glycoprotein that consists of two different protein chains.[9] The *IL-7Rα* gene is located on chromosome 5p13.3 and various studies have suggested that this region is linked with MS disease.[10] The association of numerous single nucleotide polymorphisms (SNPs) of the IL-7Rα gene with MS have been documented in different populations.[11] Accordingly, association studies of the IL-7Rα gene variants showed that rs6897932 in the exon 6 could be a risk factor for MS developing in several European populations and in Japanese.[9,12-14] This codon-changing polymorphism leads to alternative splicing of IL-7R exon 6, which influences the amount of membrane-bound (exon 6 included) and soluble (exon 6 skipped) IL7R isoforms.[12] Another SNP (rs201084372) with RNA splicing has been reported to be relevant to MS.

Many cytokines have been associated with the pathology of MS such as IL2, which is known to be one of the crucial immunoregulatory cytokines. Previously, we investigated various polymorphisms of the IL2 gene[16,17] and other genes,[18,19] which are involved in the immune system dysfunction of MS patients. In this study, our aim was to explore the role of the *IL7Rα* gene polymorphisms, rs6897932 and rs201084372, and examine their effects on IL-7Rα gene expression in relation to the pathogenesis of MS.

MATERIALS AND METHODS

This case-control study was conducted to investigate 100 Iranian relapsing-remitting multiple sclerosis (RRMS) patients including 60 females and 40 males from the west of Iran (Beesat hospital, Hamadan). All patients recruited in this study had clinically definite MS and specifically RRMS according to Poser's criteria[15] and they were under standard treatment protocol using IFN-β-1a (Cinnovex) for at least 6 months.[36,37] We also included 100 ethnic-matched healthy subjects (59 females and 41 males) with no family history for MS or any other autoimmune diseases as a control group to compare the distributions of *IL7Rα* genotypes and haplotypes as well as the gene expression level between the patient group and healthy controls. The institutional ethics committee approved (No. REC.1393.136) this study and informed consents were obtained from all patients and healthy controls.

DNA samples were extracted from 2.0 mL of peripheral blood of the study subjects using a Gene All commercial DNA extraction kit (GeneAll biotechnology Co., Ltd., Seoul, Korea, Cat. No: 106-152) based on manufacturer's instructions. After evaluation of quality and quantity of DNA samples, genotyping for two SNPs (rs6897932 and rs201084372) in the *IL-7Rα* gene was performed by the polymerase chain reaction (PCR) with capillary sequencing (Macrogen Company, Korea). The sequence of primers used for PCR and sequencing are shown in Table 1.

Total RNA was isolated from 0.5 mL of fresh peripheral blood sample using a GeneAll Hybrid-R blood RNA extraction Kit (GeneAll biotechnology, Seoul, Korea Co., Ltd., cat No.305-101). Thereafter, cDNA synthesis was performed with a Revert Aid first strand cDNA synthesis kit (Thermo Scientific, Waltham, Massachusetts, USA) according to the manufacturer's instructions. Relative quantification of mRNA for IL-7RA (both membrane-bound and soluble form) was performed by real time PCR.[20] The specific primers for IL7Rα and HPRT1 were designed using Allele ID software (Version 7, Premier Biosoft, Palo Alto, USA). The sequences of the primers are shown in Table 1.

Table1 Nucleotide sequence of probes and specific primers and the length of PCR products used for Real-time PCR of HPRT1 and IL7Ra genes

Gene name	Primer and probe sequence	Primer and probe length	Product length
HPRT1	F: AGCCTAAGATGAGAGTTC	18	88
	R: CACAGAACTAGAACATTGATA	21	
IL7Ra(F)	F: AGCACAAAGCTGACACTCCT	20	168
	R: AGGATCCATCTCCCCTGAGC	20	
IL7Ra(S)	F: CTGGAACATCTTTGTAAGAAACCAAG	26	103
	R: TAGCTTGAATGTCATC**CACCCT**	**22**	

Real-time Quantitative PCR was performed with the Corbett Rotor Gene 6000 machine (Corbett Life Science, Mortlake, Australia) and theSybr Green, Universal PCR Master Mix (Applied Biosystems, Foster City, California, USA, PN: 4304449). The levels of mRNA expression were quantified in relation to mRNA levels of HPRT1 as the housekeeping gene.

The statistical significance of the comparisons of the genotypes, alleles and haplotypes between both groups in the study were determined by the Chi-square test and Fishers' exact test when appropriate. The quantitative data were analysed using t test and one-way ANOVA when needed. p values<0.05 were considered to be statistically significant. All data were analyzed with SPSS version 18.0 for Windows (Chicago, IL, USA).

RESULTS

Demographics and some clinical characteristics of the study subjects are presented in Table 2. The mean age of RRMS patients and healthy controls were 31.7±4.2 and 30.9±8.9 years, respectively. Also, the mean age-at-onset of disease was 27.6±3.1 years in the patients and the average duration of disease was 5.2±4.3 years. All patients received IFN-β-1a (Cinnovex) for at least 6 months and the mean score of expanded disability status scale (EDSS) was 3.9±2.2 during the first year of follow up.

Genotyping for both SNPs in the *IL7Rα* gene and relative quantification of mRNA expression for both isoforms of IL-7Rα were performed for all RRMS patients and healthy controls. The frequencies of genotypes and alleles for both SNPs are shown in Table 3. We observed higher significant frequencies of the T allele and TT genotype for rs6897932 (C/T) in patients group than controls (p=0.015). Similarly, higher significant frequencies of the T allele TT and TG genotypes as well as lower frequencies of the G allele and GG genotypes for rs201084372 (G/A) were found in patients versus healthy controls (Table 3).

Comparing the haplotype frequencies of rs6897932 and rs201084372 revealed that the T-T haplotype either in homozygous or in heterozygous form of each SNP was more frequent in the patient group than in the control (p=0.002 and p=0.04 respectively, Table 3).
Analysis of *IL7Rα* gene expression at mRNA level showed a decreased level of mRNA expression for membrane-bound or full-length isoform of IL-7Rα (mbIL-7Rα) in patients compared to healthy controls (Figure 1a). On the other hand, an increased level of mRNA expression for soluble isoform of IL-7Rα (sIL-7Rα) was observed in patients versus controls. Also, we found a higher significant ratio of full-length/soluble expression in controls than in RRMS patients (Figures 1b and c)

Comparison of the mRNA expression levels of both IL7Rα isoforms in terms of different genotypes revealed significant differences between both groups in the study. As shown in Figure 2, a significant decreased level of mRNA expression for mbIL-7Rα and an increased level of mRNA for sIL-7Rα were observed in the RRMS patients carrying the TT+CT genotypes compared to healthy controls with the same genotypes (Figures 2a and 2b). Also, the patients with these genotypes showed a significant lower ratio of mRNA expression for mbIL-7Rα/sIL-7Rα than healthy controls (p<0.0001, Figure 2c).

Table 2. Demographic and clinical profiles of multiple sclerosis (MS) patients and healthy controls

Variables	MS patients	Controls
Female/Male [No. (%)]	60 (60%)/40 (40%)	59 (59%)/41 (41%)
Age (mean ± SD, Y)	31.7 ± 4.2	30.9±8.9
Age range (Y)	21-42	23-49
Age at onset (mean±SD, Y)	27.6 ± 3.1	-
Relapsing-remitting course (No. %)	100 (100%)	-
Duration (mean±SD, Y)	5.2±4.31	-
EDSS[a] (mean±SD)	3.9±2.2	-

a; Expanded disability status scale of Kurtzke.

Table 3. Allele, genotype and haplotype frequencies of rs6897932 and rs201084372 within IL7RA gene in multiple sclerosis patients and healthy controls

	Patients(%)	Controls(%)	OR*	CI (95%)#	*p*
rs6897932					
Allele	n=200	n=200			
C	140(70%)	161(80.5%)	0.565	(0.356-0.898)	0.015
T	60(30%)	39(19.5%)			
Genotype	N=100	N=100			
C/C	53(53%)	66(66%)	0.581	(0.328-1.028)	0.061
C/T	34(34%)	29(29%)	1.261	(0.693-2.294)	0.447
T/T	13(13%)	5(5%)	2.839	(0.972-8.29)	0.048
rs201084372					
Allele	n=200	n=200			
G	174(87%)	196(98%)	0.137	(0.047-0.399)	2.962×10^{-5}
T	24(12)	2(1)	13.5	(3.146-57.94)	8.119×10^{-6}
A	2(1)	2(1)	-	-	1
Genotype	N=100	N=100			
G/G	78(78%)	96(96%)	0.148	(0.049-0.447)	1.539×10^{-4}
G/T	16(16)	2(2)	9.333	(2.089-41.77)	0.001
T/T	4 (4%)	0			0.043
A/G	2(2)	2(2)			1
rs6897932 and rs201084372 T alleles					
Haplotype	N=100	N=100			
T^+-T^+	14(7%)	2(1%)	7.977	(1.763-36.095)	0.002
T/T^+-T/T^+	4(4%)	0			0.043

*OR: odds-ratio; #CI: confidence-interval

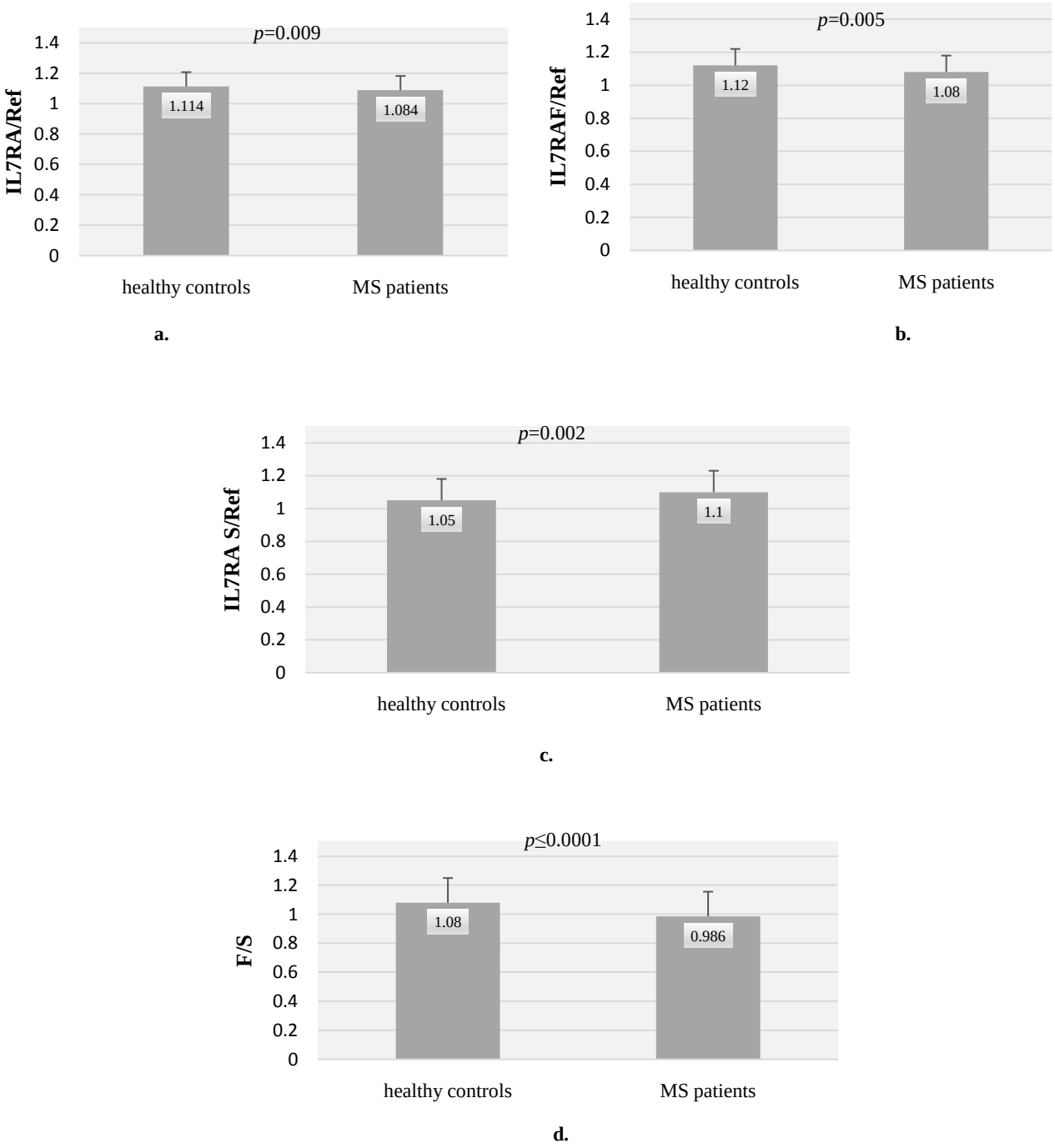

Figure 1. IL7RA isoforms expression in whole blood of multiple sclerosis (MS) patients and healthy controls. a) IL7RA expression in whole blood of MS patients and healthy controls, b) IL7RA full length (membrane bound) isoform expression in whole blood of MS patients and healthy controls, c) IL7RA soluble isoform expression in whole blood of MS patients and healthy controls, d) IL7RA full length/soluble isoform expression in whole blood of MS patients and healthy controls.

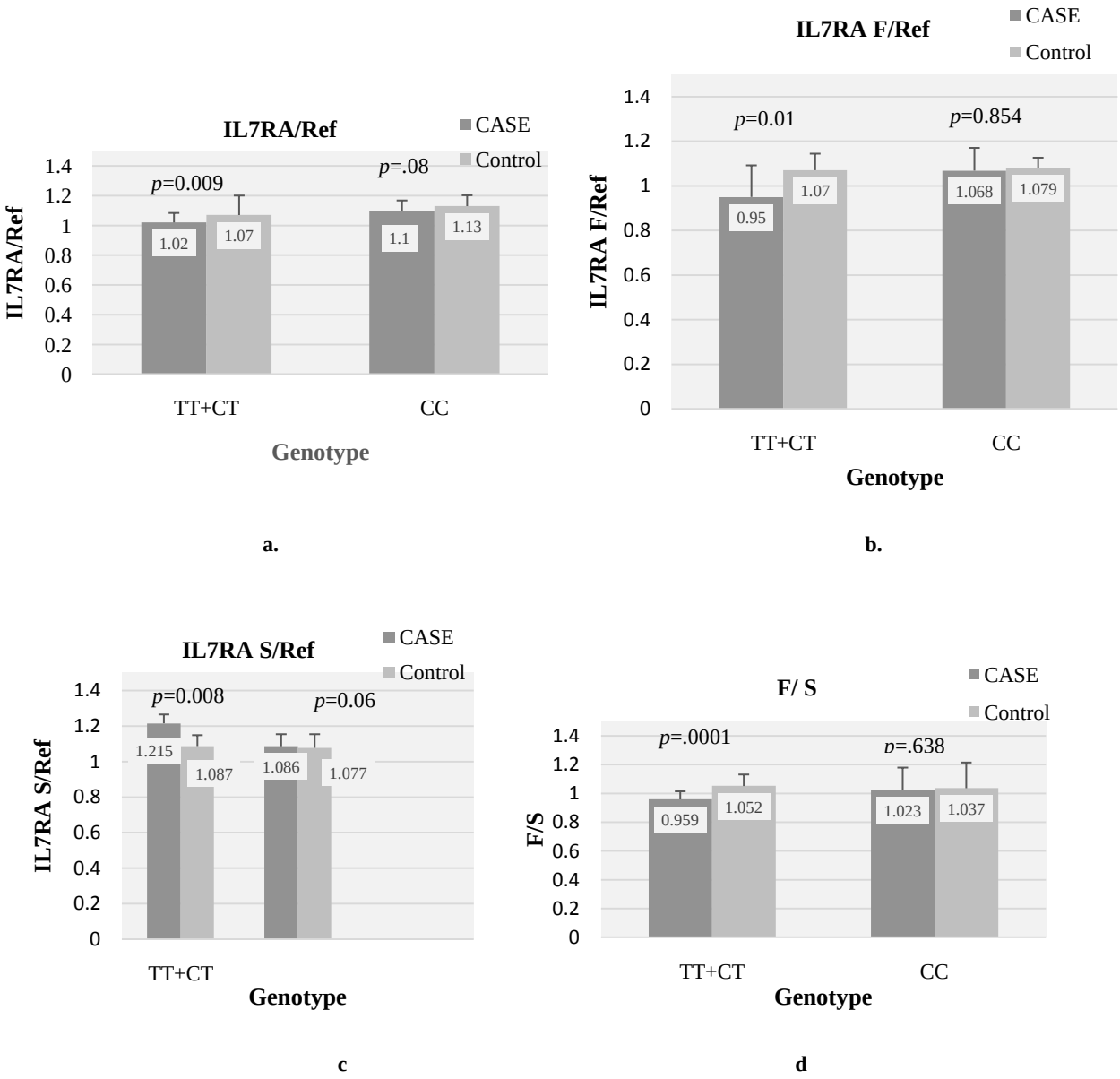

Figure 2. Correlation of IL7RA genotypes and expression in multiple sclerosis patients. a) Correlation between IL7RA expression and rs 6897932, b) Correlation between IL7RA full length expression and rs 6897932, c) Correlation between IL7RA soluble expression and rs 6897932, d) Correlation between IL7RA full length/soluble expression and rs 6897932.

DISCUSSION

The association of *IL7Rα* gene variants with MS has been investigated in different populations, but the results are still controversial.[13,21] It is suggested that the role of *IL7RA* gene variants in immunopathogenesis of MS could be related with the origin of population. For this reason, we investigated the functional association of two SNPs in *IL7Rα* gene, rs6897932 and rs201084372, with MS in a sample of the Iranian population.

We observed higher significant frequencies of the T allele and TT genotype for rs6897932 (C/T) and the T allele as well as TT and TG genotypes for rs201084372 (G/A) in RRMS patients compared to healthy controls. Several previous studies demonstrated that the allele C

of rs6897932 in exon 6 of *IL7Rα* gene is associated with increased susceptibility to MS.[13,22-27] This finding is in contrast with our results for genotyping the rs687932 in the *IL7Rα* gene of RRMS patients, which are in agreement with reports from some European countries such as Germany, Northern Ireland and Western Balkan region.[20,25] Elucidation of the probable susceptibility role of the T allele and the possible protective role of the C allele of rs687932 in RRMS that are in line with some studies. On the contrary other studies indicate the effect of ethnicity on these genotypes in MS patients. Single nucleotide variations could hypothetically influence gene expression and/or protein structure by altering cis-acting elements, RNA transcript stability, or affecting RNA splicing. To predict how these missense variants may affect protein function and stability, PolyPhen-version 2,[28] SIFT version 1.03,[29] and SNPS3D[30] programs were used. In silico protein analysis showed that both rs6897932 (p.Thr244Ile) and rs201084372 (p.Gly236Glu) polymorphisms are benign variations that do not affect the stability of the protein structure. However, it has been suggested that rs6897932 results in alternative splicing of IL-7R exon 6, which leads to membrane-bound (exon 6 included) and soluble (exon 6 skipped) IL7R isoforms.[12] Using HaploReg v4, [31] it was suggested that the mentioned polymorphisms alter enhancer histone marks and binding motifs for several transcription factors including Pax-5, CDP, Maf, and Pou2f2. Such alterations may affect gene expression level.

Further investigations using larger sample sizes are needed to determine a clearer association of this SNP and its functional influences with MS in our population.

Our finding of an over expression of mRNA for sIL-7Rα and an under expression of mRNA for mbIL-7Rα in RRMS patients compared to healthy controls is consistent with previous reports by McKay et al.[33] As we did not measure the sIL-7Rα or mbIL-7Rα protein levels in serum of the patients, our results about the clinical relevance of mRNA levels in MS should be interpreted cautiously.

With regard to the functional association of rs6897932 (C/T) in *IL7Rα* gene with MS, we evaluated the mRNA expression levels of both mbIL-7Rα and sIL-7Rα isoforms and analysed the results according to different genotypes of rs6897932. A significant decreased level of mRNA for mbIL-7Rα and increased level of mRNA for sIL-7RA were observed in those RRMS patients carrying TT+CT genotypes compared to healthy controls with the same genotypes. Also, the patients with these genotypes showed a significant lower ratio of mRNA for mbIL-7RA than healthy controls. Surprisingly, our findings are in contrast to similar studies that showed a higher level of mRNA expression for sIL-7Rα in patients carrying the IL7R*CC genotype for this SNP.[33-35] This codon-changing single nucleotide polymorphism (rs6897932 C→T, The244Ile) has been reported mainly in relation to variable mRNA expression, although some studies have shown no correlation between genotypes of this SNP and mRNA expression for the IL-7Rα isoforms.[35] Analysis of the mRNA expression for both isoforms of IL-7Rα according to the IL7R*CC genotype between patients and control did not show significant differences, which is contrary to previous studies.[33-35] Because of the ethnic variation in different studies, it is reasonable to observe discrepant results for the functional association of IL-7Rα gene polymorphism, which is the second most relevant gene polymorphism associated with MS after HLA-DRB1*1501. However, further well-designed comparative studies in various populations are warranted to determine the exact role of this SNP in the immunopathogenesis of MS.

Analyses of the second SNP in the IL-7Rα gene (rs201084372 (G/A)) revealed higher and lower significant frequencies for the G allele and T allele, respectively in RRMS patients than controls. Additionally, the GG and GT genotypes were found to be more and less frequent, respectively in patients versus controls. To our knowledge, this is the first report on this SNP in the IL-7Rα gene in MS patients and the functional impacts of this SNP on IL-7Rα gene expression in MS is still undetermined.

Haplotype analysis of both SNPs in the IL-7Rα gene revealed an increased frequency of the T-T haplotype in patients versus healthy control, which is not easily interpretable because of the undetermined role of the second SNP (rs201084372 (G/A)) in the *IL-7Rα* gene in relation to MS. Our data should be interpreted with caution. Further studies are necessary to find out the exact role of this SNP in MS pathogenesis.

In summary, our results demonstrate that the *IL7Rα* gene variants, rs6897932 and rs201084372, are functionally relevant to susceptibility or protection for multiple sclerosis. Although our finding in regard to the

increased level of sIL-7Rα as a modulator of the quality of IL-7 signal in RRMS patients is in agreement with previous studies, the genotype-mediated expression of IL-7Rα isoforms in our patients is surprisingly different from those in other similar studies. We observed higher levels of mRNA expression for sIL-7Rα isoform in patients carrying the *IL7R**TT genotype, which is a remarkable genetic difference when compared to previous studies on the genotype-induced effects of IL-7Rα expression in MS. A plausible explanation for this discrepancy could be ethnic variations in the studied populations. In this regard, further investigations using larger groups of MS patients in our population either as cohorts or as case-control studies are needed to find out the exact role of these two SNPs in the *IL7Rα* gene and their functional relevance in a predisposition to MS or protection against this autoimmune disease.

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