## original article

# Association of interleukin-4 polymorphisms with multiple sclerosis in southeastern Iranian patients

Mohammad Kazemi Arababadi,[ab] Reza Mosavi,[c] Ali Ravari,[d] Hossein Teimori,[e] Gholamhossein Hassanshahi[b]

From the [a]Department of Microbiology, Hematology and Immunology, Faculty of Medicine, [b]Molecular Medicine Research Center, [c]Neurosurgery, Faculty of Medicine, [d]Nursing, Faculty of Nursing, Rafsanjan University of Medical Sciences, Rafsanjan, [e]Cellular and Molecular Research Center, School of Medicine, Shahrekord University of Medical Sciences, Sharekord, Iran

Correspondence: Dr. Gholamhossein Hassanshahi · Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. T: +983915234003-5, F: +983915225209, M: +989133933445 · ghassanshahi@gmail.com · Accepted: April 2011

**BACKGROUND AND OBJECTIVES:** Immune system–related factors are important in the pathogenesis of multiple sclerosis (MS). Interleukin 4 (IL-4) as a helper T cell (2TH) cytokine is involved in the regulation of immune responses. Hence, this study was designed to explore the association between MS and polymorphisms in the -590 region of IL-4.

**DESIGN AND SETTING:** A descriptive study at Rafsanjan University of Medical Sciences, Rafsnajan from September 2009 to August 2010.

**PATIENTS AND METHODS:** Blood samples were collected from 100 MS patients and 150 healthy controls on EDTA precoated tubes. DNA was extracted and analyzed for IL-4 polymorphisms using restricted fragment length polymorphism in patients and controls. Demographic data were also collected by a questionnaire that was designed specifically for this study.

**RESULTS:** We observed a significant difference in the C/C, T/C, and T/T genotypes of the -590 region of IL-4 between patients with MS and healthy controls ($P<.001$).

**CONCLUSIONS:** We conclude that functional polymorphisms of IL-4 possibly play a crucial role in the pathogenesis of MS.

Multiple sclerosis (MS) is a sophisticated disorder in which the importance of immune system-related parameters in its pathogenesis is partially evident.[1] Both the loss of myelin and central nervous system (CNS) inflammation are frequent symptoms of MS.[2] The proper mechanisms responsible for myelin degeneration in MS have yet to be clarified;[2] however, recent published studies state that cognate immune system parameters are closely involved in the pathogenesis and complications of MS.[3] Accordingly, immune regulatory factors might influence the pathogenesis of MS.[3]

Cytokines are key factors in an appropriate immune response in autoimmune diseases[4,5] and interleukin 4 (IL-4), a prevailing helper T cell (2TH) cytokine in regulation of 1TH responses of the CNS.[6] Therefore, genetic factors that lead to a decreased cytokine expression enable the immune system to induce a vigorous immune response against CNS antigens in patients with MS.[7] The involvement of IL-4 in immunological disorders including MS,[7] systemic lupus erythematosus,[8] nephrotic syndrome,[9] graft rejection,[10] asthma,[11] and type 1 diabetes[12] is well documented. The human IL-4 gene is located on a cluster of cytokine genes on the q arm of chromosome 5 (5q31.1). It is approximately 10 kb in size with 4 exons and 3 introns (a and b). The IL-4 promoter region that extends to about 500 bp from the "TATA"-like sequence contains 5 polymorphic sites. The -590C/T single-nucleotide polymorphism is the most common IL-4 promoter olymorphic site (c). The paramount role of IL-4 as an inhibitory cytokine in autoimmunity and inflammation[13] explain the challenges of this cytokine on the pathogenesis of some diseases including MS. Previous studies revealed that IL-4 ex-

pression could be regulated by its polymorphisms in the -590 region.[6,14] Therefore, we aimed to investigate the polymorphisms in the -590 region of this cytokine in relapse-remitting multiple sclerosis (RRMS).

## PATIENTS AND METHODS

### Collection of samples
Specimens were collected from 100 relapsing-remitting patients with MS and 150 healthy controls during 2008–2009 in Rafsanjan University of Medical Sciences. The occurrence of MS was diagnosed by a neurologist according to clinical and paraclinical findings (magnetic resonance imaging study, oligoclonal bands in cerebrospinal fluid, and evoked potentials) based on the McDonald criteria.[15] Healthy control cases were also selected within the Rafsanjan population and matched by sex, age, and socioeconomic status following approval by Rafsanjan University of Medical Sciences Ethical Committee. Written consent forms were filled out by both patients and controls prior to specimen collection. The socioeconomic

status was based on monthly income and education levels.

### DNA extraction and detection of polymorphisms
The peripheral blood was collected on EDTA precoated tubes and then genomic DNA was extracted by a commercial kit (Bioneer, South Korea). The extracted DNA samples were then stored at −20°C for further use.

IL-4 (-590C/T) gene polymorphism was analyzed by the polymerase chain reaction (PCR)-restricted fragment length polymorphism method. The sequences of primers were as follows: sense - 5'-TAAACTTGGGAGAACATGGT-3' and antisense - 5'-TGGGGAAAGATAGAGTAAT-3'. The PCR mixture was made up by addition of the following reagents to a 0.2 mL microcentrifuge tube on ice: 2.5 µL of Taq DNA polymerase buffer (10×), 0.5 µL of MgCl2 (stock concentration 1.5 mM), 0.5 µL of each dNTP [(dATP, dCTP, dGTP, and dTTP) stock concentration of 10 mM], 1 µL of each primer [(forward and reverse), stock concentration of 25 ng/µL], 1 µL of prepared DNA, and sterile double distilled water to a final volume of 25 µL. The PCR condition was an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of melting at 95°C for 50 seconds, annealing at 53°C for 50 seconds, and extension at 72°C for 40 seconds, with a final extension step of 5 minutes at 72°C using thermal cycler (C1000; Bio-Rad, USA). The PCR product of IL-4 (-590C/T) was a 195-bp fragment and was digested with AvaII into 175- and 20-bp fragments. The digested products were run on a 2.5% agarose gel (Cinnagen, Iran) and studied on Chemi-Doc model XRS (Bio-Rad) after staining with ethidium bromide. The assay yielded a single 195-bp band related to heterozygote C/T genotype; 2 bands of 20 and 175 bp related to the hemozygote CC genotype; and 3 bands of 20, 175, and 195 bp corresponded to homozygote TT genotype.

### Statistical analysis
The Hardy-Weinberg equilibrium was assessed using genotype data. Allele and genotype frequencies were calculated in patients and healthy controls by direct gene counting. The statistical analysis of the differences between groups was determined by the chi-square test using EPI 2000 and SPSS software version 13 (IBM Corp., Armonk, NY USA). A $P$ value less than .05 was considered as significant.

## RESULTS
The evaluation of the polymorphisms in -590 of IL-4

**Table 1.** Frequency of polymorphisms of IL-4 gene in multiple sclerosis patients and controls.

| Condition Genotype (n, %) | Patients | Control | P value |
|---|---|---|---|
| C/C | 76 (76%) | 108 (72%) | <.001 |
| T/C | 23 (23%) | 38 (25.3%) | |
| T/T | 1 (1%) | 4 (2.7%) | |
| **Alleles** | | | |
| C | 175 (87.5%) | 254 (84.6%) | <.001 |
| T | 25 (12.5%) | 46 (15.4%) | |

**Table 2.** Demographic and socioeconomic conditions of multiple sclerosis patient and controls.

| Variant | Healthy control | MS patient | P value |
|---|---|---|---|
| Age | 31 (8) | 31 (6) | .85 |
| **Sex** | | | |
| Female | 69 (46%) | 45 (45%) | .90 |
| Male | 81 (54%) | 55 (55%) | |
| **Socioeconomic status** | | | |
| Weak | 31 (21%) | 22 (22%) | |
| Medium | 72 (48%) | 47 (47%) | .90 |
| High | 47 (31%) | 31 (31%) | |

by AvaII restriction enzyme showed that the prevalence of C/C genotype was 76 (76%) in patients with RRMS and 108 (72%) in controls. We found that the frequency of T/C genotype was 23 (23%) and 38 (25.3%) in patients with RRMS and controls, respectively. The frequency of T/T genotype in patients with RRMS was 1 (1%), whereas the frequency of this genotype in controls was 4 (2.7%) (**Table 1**). The statistical analysis of our data confirmed a significant difference between two groups in genotypes ($P<.001$). The frequency of the C allele was 175 (87.5%) and 254 (84.6%) in patients with RRMS and controls, respectively. Twenty-five (12.5%) cases of T allele were observed in patients, whereas the frequency of this allele was 46 (15.4%) in controls. To test for Hardy-Weinberg equilibrium, the chi-square in the patients group ($\chi^2=0.058$, $P=86.5$) and in the control ($\chi^2=0.036$, $P=88.5$) strongly indicated that both groups were in Hardy-Weinberg equilibrium. The statistical analysis of alleles indicated no significant difference between patients with RRMS and controls ($P= .374$) (**Table 1**). The calculated odds ratio of alleles was 0.79 for patients with RRMS and controls. There was no statistically significant difference between groups in mean age ($P=.85$), gender ($P=.90$), and or socioeconomic status of the participants ($P=.90$) (**Table 2**).

## DISCUSSION

The pivotal role of the immune system in the etiology and pathogenesis of MS are well documented.[16-18] The crucial role of the cytokine network in the immune response is also documented.[4,19] It is well known that several factors—from infectious agents, hormonal conditions, to cytokine gene polymorphisms—control expression and secretion of cytokines.[19] The results of our study showed that there was a clear significant difference between control subjects and patients with RRMS in polymorphisms in the -590 region of IL-4. Previous studies have shown a close correlation between the C/C genotype and decreased expression of IL-4.[20,21] In this investigation, we observed that the C/C genotype is more frequent in patients with

RRMS than controls. Therefore, based on our results, it seems that IL-4 gene polymorphisms can influence the pathogenesis of MS in our study population (south-eastern Iranian patients). In contrast to our results, Kamali et al showed that the polymorphisms in this region of IL-4 were not associated with MS in Shiraz city of Iran (a south-central part of Iran).[22] This discrepancy between our results and the findings of Kamali et al could be explained by the fact that some parts of the Shiraz population are mixed, with both Iranian and Turk ethnic groups, which differ in race and genetic constitution from our study population. Furthermore, Kamali et al studied IL-4 polymorphisms in all different phases of MS,[22] whereas all of our patients were in relapsing-remitting phases. Our results may reveal that IL-4 polymorphisms are more related to this type of disease rather than the other phases of MS.

These data may help clinicians to predict the beginning of the relapsing-remitting course of MS. Some studies have indicated that IL-4 -590 polymorphisms are associated with MS, which is consistent with out results.[6,7,23,24] For instance, Urcelay et al showed that these polymorphisms are associated with MS in the Spanish population.[6] An investigation in the Italian population also showed a significant correlation between IL-4 polymorphisms and late onset of MS.[23] The relationship between other polymorphisms in the IL-4 gene and MS also was reported by other investigators.[7,24] Therefore, it seems that IL-4 polymorphisms can significantly affect the expression of IL-4, and hence could presumably be involved in MS progression.

Overall, further studies are needed on patients with MS in different phases of MS with a wider sample size to differentiate between various MS phases according to the polymorphisms in Iranian patients with MS.

## REFERENCES

**1.** O'Keeffe J, Gately CM, Counihan T, Hennessy M, Leahy T, Moran AP, et al. T-cells expressing natural killer (NK) receptors are altered in multiple sclerosis and responses to alpha-galactosylceramide are impaired. J Neurol Sci 2008;275:22-8.

**2.** Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. Nature 2006;441:1094-6.

**3.** Baker BJ, Akhtar LN, Benveniste EN. SOCS1 and SOCS3 in the control of CNS immunity. Trends Immunol 2009;30:392-400.

**4.** Arababadi MK, Nosratabadi R, Hassanshahi G, Yaghini N, Pooladvand V, Shamsizadeh A. Nephropatic complication of type-2 diabetes is following pattern of autoimmune diseases? Diabetes Res Clin Pr 2010;87:33-7.

**5.** Arababadi MK, Mosavi R, Khorramdelazad H, Yaghini N, Zarandi ER, Araste M, et al. Cytokine patterns after therapy with Avonex(R), Rebif(R), Betaferon(R) and CinnoVex in relapsing-remitting multiple sclerosis in Iranian patients. Biomark Med 2010;4:755-9.

**6.** Urcelay E, Santiago JL, Mas A, Martinez A, de Las Heras V, Arroyo R, et al. Role of interleukin 4 in Spanish multiple sclerosis patients. J Neuroimmunol 2005;168:164-7.

**7.** Kantarci OH, Schaefer-Klein JL, Hebrink DD, Achenbach SJ, Atkinson EJ, McMurray CT, et al. A population-based study of IL4 polymorphisms in multiple sclerosis. J Neuroimmunol 2003;137:134-9.

**8.** Wu MC, Huang CM, Tsai JJ, Chen HY, Tsai FJ. Polymorphisms of the interleukin-4 gene in chinese patients with systemic lupus erythematosus in Taiwan. Lupus 2003;12:21-5.

**9.** Liu HM, Shen Q, Xu H, Yang Y. [Significance of polymorphisms in variable number of tandem repeat region of interleukin-4 gene in recurrence of childhood steroid sensitive nephrotic syndrome]. Zhonghua Er Ke Za Zhi 2005;43:431-3.

**10.** Amirzargar M, Yavangi M, Basiri A, Moghadam SH, Khosravi F, Solgi G, et al. Genetic association of interleukin-4, interleukin-10, and transforming growth factor-beta gene polymorphism with allograft function in renal transplant patients. Transplant Proc 2007;39:954-7.

**11.** Wang XH, Zhao W, Liu SG, Feng XP. [Correlation of IL-4 and IL-13 gene polymorphisms with asthma and total serum IgE levels]. Zhonghua Jie He He Hu Xi Za Zhi 2009;32:161-4.

**12.** Bugawan TL, Mirel DB, Valdes AM, Panelo A, Pozzilli P, Erlich HA. Association and interaction of the IL4R, IL4, and IL13 loci with type 1 diabetes among Filipinos. Am J Hum Genet 2003;72:1505-14.

**13.** Arababadi MK, Pourfathollah AA, Jafarzadeh A, Hassanshahi G, Daneshmandi S, Zarandi ER, et al. Evaluation of Relation between Polymorphisms in -590 Region of IL-4 and Occult HBV Infection. J Guil Univ Med Sci 2009;18:1-8.

**14.** Arababadi MK, Pourfathollah AA, Daneshmandi S, Hassanshahi G, Zarandi ER, Shamsizadeh A, et al. Evaluation of relation between IL-4 and IFN-g polymorphisms and type 2 diabetes. IJBMS 2009;12:100-4.

**15.** McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: Guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121-7.

**16.** Arababadi MK, Hassanshahi G, Azin H, Salehabad VA, Araste M, Pourali R, et al. No association between CCR5 ? 32 mutation and multiple sclerosis in patients of south-eastern of Iran. Lab Medicine 2010;41:31-3.

**17.** Pulkkinen K, Luomala M, Kuusisto H, Lehtimaki T, Saarela M, Jalonen TO, et al. Increase in CCR5 Delta32/Delta32 genotype in multiple sclerosis. Acta Neurol Scand 2004;109:342-7.

**18.** Welsh CJ, Steelman AJ, Mi W, Young CR, Storts R, Welsh TH Jr, et al. Neuroimmune interactions in a model of multiple sclerosis. Ann N Y Acad Sci 2009;1153:209-19.

**19.** Arababadi MK, Naghavi N, Hassanshahi G, Mahmoodi M. Is CCR5-Delta32 mutation associated with diabetic nephropathy in type 2 diabetes? Ann Saudi Med 2009;29:413.

**20.** Rosenwasser LJ, Klemm DJ, Dresback JK, Inamura H, Mascali JJ, Klinnert M, et al. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 1995;25 Suppl 2:74-8; discussion 95-6.

**21.** Gonzales JR, Groger S, Haley G, Bodeker RH, Meyle J. The interleukin-4 -34TT and -590TT genotype is correlated with increased expression and protein production in aggressive periodontitis. Mol Immunol 2009;1:1.

**22.** Kamali-Sarvestani E, Nikseresht A, Aflaki E, Sarvari J, Gharesi-Fard B. TNF-alpha, TNF-beta and IL-4 gene polymorphisms in Iranian patients with multiple sclerosis. Acta Neurol Scand 2007;115:161-6.

**23.** Vandenbroeck K, Martino G, Marrosu M, Consiglio A, Zaffaroni M, Vaccargiu S, et al. Occurrence and clinical relevance of an interleukin-4 gene polymorphism in patients with multiple sclerosis. J Neuroimmunol 1997;76:189-92.

**24.** Suppiah V, Goris A, Alloza I, Heggarty S, Dubois B, Carton H, et al. Polymorphisms in the interleukin-4 and IL-4 receptor genes and multiple sclerosis: A study in Spanish-Basque, Northern Irish and Belgian populations. Int J Immunogenet 2005;32:383-8.