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# IL-2, IFN-γ, and IL-12 Gene Polymorphisms and Susceptibility to Multiple Sclerosis

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**Abstract**
*Background* Multiple sclerosis (MS) is a multifactorial disease. Positive genetic background could predispose individuals to this chronic disabling disease. In order to investigate the role of some proinflammatory cytokines (interleukin (IL)-2, IL-12, and interferon-gamma (IFN-γ)) as a risk factor for MS, this study was performed.
*Methods* Two hundred and eleven patients with relapsing-remitting form of MS were enrolled in this study and compared with 359 healthy individuals. Using polymerase chain reaction based on sequence-specific primer method, the cytokine genes were amplified, and alleles and genotypes were detected on gel electrophoresis.
*Results* Significant increases for IFN-γ AT (+874) genotype (54.5% vs. 37.8%, $p=0.0002$) and IL-12 AA (−1188) genotype (60.8% vs. 49.7%, $p=0.014$) were found in MS patients in comparison with healthy controls. A significant decrease in IFN-γ TT (+874) genotype (17.7% vs. 27.5%, $p=0.01$) and IL-12 CA (–1188) genotype (30.9% *vs.* 45%, $p=0.001$) in MS patients was also detected. No significant differences of IL-2 G/T (−330) and IL-2 G/T (+166) in alleles and genotypes were observed between MS patients and normal subjects.
*Conclusions* It could be suggested that the genetic variation in IL-12 A/C (−1188) and IFN-γ A/T (+874) cytokine genes could be risk factors for MS patients.

**Keywords** Cytokines · IL-2 · IFN-γ · IL-12 · multiple sclerosis · single nucleotide polymorphism

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## Introduction

Multiple sclerosis (MS) is a complex autoimmune disease in which inflammation leads to demyelization of the neurons in the central nervous system (CNS). Although the mechanisms causing tissue injury in CNS are unknown, it seems that the cause of the disease is related to the dysregulation of the immune system in CNS [1].

Differences in cytokine production levels are related to allelic variants in cytokine genes. In the other words, high and low cytokine productions are controlled by allelic variants of cytokine genes, which could make different susceptibilities to autoimmune diseases. So, genetic polymorphisms of cytokines are considered as susceptible

factors for autoimmune diseases such as multiple sclerosis [2–5]. The role of cytokine gene polymorphisms in individual susceptibility to several diseases has been documented [6–9].

We have previously shown that there are some relations between human leukocyte antigen (HLA) and MS [10,11], whereas HLA-DRB1*15, −DRB1*04, −DQA1*0102, and −DQB1*0602 were increased in MS patients [11]. We have also recently performed genetic polymorphisms of some cytokines [12, 13], including proinflammatory cytokines, which showed that interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α) may contribute to susceptibility to MS [13]. Moreover, involvement of the genes IL2RA and IL7RA in disease susceptibility was confirmed by genome-wide association studies [14, 15]. Polymorphisms of other cytokines, such as IL-2, interferon-gamma (IFN-γ), and IL-12 genes may also contribute to susceptibility and pathogenesis of MS by altering cytokine production and inducing inflammation. In this study, some inflammatory cytokines, including IL-2, IFN-γ, and IL-12 were genotyped in MS patients in order to clarify susceptible genes related to MS in the Iranian population.

## Patients and Methods

### Subjects

Two hundred eleven (74% women, 26% men) unrelated relapsing-remitting multiple sclerosis (RRMS) patients with clinically defined disease according to the McDonald criteria [16] were recruited from the Iranian MS Society. Mean age of patients group was 31.17±7.00 years. The mean age at disease onset was 27.0 years and mean duration of disease was 6.9 years. All patients had expanded disability status scale between 2.0 and 4.0. The control group consists of 359 normal persons that have been selected randomly from an Iranian blood transfusion organization in Tehran. These subjects were randomly selected from three cities (Tehran, Yazd, and Sistan), as previously reported [17].

### DNA Genotyping

Sampling was performed after obtaining written informed consent from all participants (patients and controls). Five milliliters of venous blood was taken. Peripheral blood mononuclear cells were isolated in sterile conditions from EDTA blood by density gradient centrifuge using Ficoll/Paque (Sigma, USA) and were frozen in liquid nitrogen. After collecting all 211 specimens, genomic DNA was extracted by using Proteinase K according to the Miller method [18].

The functional gene polymorphisms of the cytokines were typed by using polymerase chain reaction, based on sequence-specific primer. Sequence-specific oligonucleotide primers for alleles of each selected cytokine were used. Some single nucleotide polymorphisms (SNPs) within the following positions were investigated: IL-2 (−330 G/T and +166G/T), IFN-γ (+874 A/T), and IL-12 (−1188 A/C).

### Statistical Analysis

Each allele and genotype frequency of IL-2, IFN-γ, and IL-12 genes in the MS patient group was detected and compared with the control group. Chi-square with Yates correction and Fisher's exact test and odds ratio were performed in this case control (Epi Info 6 program, version 6.2, World Health Organization, Geneva, Switzerland). $p<0.05$ was considered statistically significant.

## Results

A significant increase in genotype frequencies of AT (+874) IFN-γ genotype (54.5% vs.37.8%, OR=0.67, 95% CI 0.45–1.00, $p=0.0002$) was detected in MS patients compared with healthy subjects, while the frequency of TT genotype was significantly decreased in the patient group (17.7% vs. 27.5%, OR=0.57, 95% CI 0.36–0.89, $p=0.01$; Table I).

The frequency of IL-12 (−1188) AA genotype (60.8% vs. 49.7%, OR=1.54, 95% CI 0.58–4.16, $p=0.014$) was significantly increased in our studied MS patients in comparison with healthy control subjects, while the CA genotype (30.9% vs. 45%, OR=0.55, 95% CI 0.37–0.8, $p=0.001$) was significantly decreased in the patient group (Table I). No statistical differences were found in allele/genotype frequencies of IL-2 (−330) G/T and (+166) G/T in patients with MS (Table I).

## Discussion

Some genes, involved in the immune response, are defined as susceptible genes for MS. Among all these genes, cytokine genes are more important for polymorphism studies in autoimmune diseases like MS [19].

In our first pilot study on MS, we studied cytokine profile in 44 Iranian MS patients, but the conclusions were restricted due to limited sample size [12]. TNF-α and IL-1 genes and their association to the Iranian MS population have recently been reported [13]. In this study, we have genotyped some inflammatory cytokines including IL-2, IFN-γ, and IL-12 in a large number of MS patients in order to clarify susceptible genes related to MS in the Iranian population.

In the present study, we could not find any significant association for alleles and genotypes of IL-2 SNPs between

**Table I** Allele and Genotype Frequencies in MS and Healthy Subjects

| Allele/genotype | Patients *N* (%) | Control *N* (%) | OR (95% CI) | *p* value[a] |
|---|---|---|---|---|
| IL-2 −330 | | | | |
| G | 175 (41.5)[b] | 292 (40.8)[c] | 1.03 (0.8–1.32) | 0.87 |
| T | 247 (58.5)[b] | 424 (59.2)[c] | 0.97 (0.76–1.25) | 0.87 |
| GG | 11 (5.2)[b] | 28 (7.8)[c] | 0.65 (0.30–1.39) | 0.31 |
| GT | 153 (72.5)[b] | 237 (66.2)[c] | 1.35 (0.91–1.99) | 0.14 |
| TT | 47 (22.3)[b] | 93 (26.0)[c] | 0.82 (0.54–1.24) | 0.37 |
| IL-2 +166 | | | | |
| G | 330 (78.2)[b] | 560 (78.2)[c] | 1.00 (0.74–1.35) | 0.94 |
| T | 92 (21.8)[b] | 156 (21.3)[c] | 1.00 (0.74–1.35) | 0.94 |
| GG | 128 (60.6)[b] | 218 (60.9)[c] | 0.99 (0.69–1.42) | 0.97 |
| GT | 74 (35.1)[b] | 124 (34.6)[c] | 1.02 (0.70–1.48) | 0.98 |
| TT | 9 (4.3)[b] | 16 (4.5)[c] | 0.95 (0.38–2.33) | 0.92 |
| IFN-γ +874 | | | | |
| A | 224 (53.5)[d] | 381 (53.7)[e] | 1.0 (0.78–1.28) | 0.97 |
| T | 194 (46.5)[d] | 329 (46.3)[e] | 1.0 (0.78–1.29) | 0.97 |
| AA | 58 (27.8)[d] | 123 (34.7)[e] | 0.72 (0.49–1.07) | 0.10 |
| AT | 114 (54.5)[d] | 134 (37.8)[e] | 0.67 (0.45–1.00) | **0.0002** |
| TT | 37 (17.7)[d] | 97 (27.5)[e] | 0.57 (0.36–0.89) | **0.01** |
| IL-12 −1188 | | | | |
| A | 311 (76.2)[f] | 517 (72.2)[g] | 1.23 (0.92–1.65) | 0.16 |
| C | 97 (23.8)[f] | 199 (27.8)[g] | 0.81 (0.61–1.08) | 0.16 |
| AA | 124 (60.8)[f] | 178 (49.7)[g] | 1.54 (0.58–4.16) | **0.014** |
| CA | 63 (30.9)[f] | 161 (45.0)[g] | 0.55 (0.37–0.80) | **0.001** |
| CC | 17 (8.3)[f] | 19 (5.3)[g] | 1.62 (0.78–3.36) | 0.22 |

*OR* odds ratio
[a] Yates-corrected
[b] $N=211$
[c] $N=358$
[d] $N=209$
[e] $N=354$
[f] $N=204$
[g] $N=358$

MS patients and controls. IL-2 has a major role in innate and specific immunity, by induction inflammation and accelerating T-cell proliferation during cell-mediated responses. Natural killer (NK) cells activated by IL-2 result to increased IFN-γ production which could promote cytotoxic effects on neural lesions of MS. IL-2 is mainly produced by Th1 cells, and the profile of Th1 secretions could be changed in MS lesions [20–22]. The distributions of genotypes at position −330 in the promoter region and position +166 in the first axon did not differ significantly between our patients and controls. This finding is similar to a Japanese study by Kikuchi et al. who found no relations between IL-2 and susceptibility to MS [21]. However, comprehensive surveys by Matesanz et al. showed some positive findings between IL-2 SNPs and susceptibility to MS [23–26].

The relation between +874 A/T IFN-γ and susceptibility to MS showed significant differences in the genotypes AT and TT in our study. IFN-γ, which is produced by T cells and NK cells, has an activating role in sustaining inflammation in MS [20]. IFN-γ has been found in CNS at the onset and relapse course of disease [27]. This cytokine may also have some regulatory functions that become apparent in its complete absence [28–30]. Recently, it has been shown that IFN-γ is associated with sex bias in MS susceptibility and with expression of IFN-γ in MS [31]. In contrast to our results, Wansen et al. found no association between IFN-γ and MS by analyzing a multiallelic microsatellite marker in the first intron of the gene [32]. Nevertheless Mihailova et al. have not found any associations between +874 IFN-γ and MS [33]. T allele of this position is correlated with allele 2 of a penta allelic CA microsatellite polymorphism, which is in association with a high level of production of IFN-γ [34]. Our results in IFN-γ (+874) showed high frequent AT genotype, which is associated with intermediate IFN-γ production. The TT genotype low-producing IFN-γ was less frequent in our studied MS patients. Our results showed the dominance of heterozygote AT vs. homozygotes TT and AA, which could suggest the intermediate production (not low, not high) of IFN-γ in lesions of MS patients.

In −1188A/C IL-12 promoter position, increased frequency of AA genotype and decreased frequency of CA genotype were found in MS patients. IL-12, which is produced principally by monocytes and dendritic cells, is one of the major mediators of immune response that is critical for the differentiation of Th1 cells [35]. Although overexpression of IL-12 causes more plaques in the CNS of

an animal [36], AA genotype seems to be associated with lower production of IL-12, which may suggest involvement of additional mechanisms other than IL-12 in the process of inflammation in MS. Alifirova et al. showed that the C allele and CC genotype of −1188 IL-12 are related to shortened duration of first remission and mean rate of relapses; thus, it could be related to susceptibility and pathogenesis of MS [37]. In accordance with our results, Veen et al. showed that IL-12 is a susceptible gene for MS [38]. Also, Seegers et al. have shown that the presence of the rarer allele was correlated with increased IL-12 p70 secretion by stimulated monocytes [39]. However, other studies by Hall et al. and Martinez et al. did not support such associations [40,41]. Although selecting the relapse-remitting MS patients in our study could be the reason for this difference, it could be due to the fact that SNPs segregate on ethnic grounds.

It could be concluded that SNPs in IFN-$\gamma$ and IL-12, not IL-2, may contribute in the susceptibility to MS in the Iranian population. However, considering higher frequencies of specific genotypes, which are not associated with high production of these cytokines, other molecules such as proinflammatory cytokines could be involved in the pathogenesis of the disease. Differences in our data with other studies could indicate that genetic polymorphisms and MS susceptibility vary with ethnicity. Further investigations on MS in different regions and also further researches on this group of patients for other genes, particularly IL2RA and IL7RA, could also be suggested.

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