Interferon-Gamma Gene Polymorphism in Iranian Patients with Multiple Sclerosis

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ABSTRACT

Interferon- gamma (IFN-γ) is an important immune regulator and inflammatory cytokine which is implicated in the pathogenesis of multiple sclerosis (MS). A single nucleotide polymorphism, T to A, at position +874 in the first intron has previously been shown. This polymorphism is associated with IFN-γ production level. To study the effect of this polymorphism on susceptibility to multiple sclerosis, we screened genomic DNA samples from clinically definite MS patients and their unaffected first-degree relatives as controls, using sequence-specific primers (PCR-SSP). The results indicated that MS patients showed a lower TT (21.2% vs. 30.3%) and higher AA (21.2% vs. 12.1%) genotypes compared to controls, although there were statistically no differences in the IFN-γ genotype distribution between these two groups. Thus, our data indicate that there is no association between IFN-γ +874 polymorphism and MS susceptibility or severity of the disease.

Key words: Gene Polymorphism, Interferon-γ, Multiple Sclerosis

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) in which abnormal immune response to one or several CNS myelin antigens results in focal lesions and clinical symptoms.1 Environmental and genetic factors are thought to be involved in the pathogenesis of the disease. Many genes have been investigated but no major MS susceptibility locus has been identified, apart from the repeated reports of the association between MS and some HLA class I and II haplotypes. Genes coding for Cytokines are also candidate genes for MS predisposition.2,3

The role of IFN-γ in the pathogenesis of the disease has been extensively studied. Elevated levels of IFN-γ are observed in the blood, CSF and CNS lesions of MS patients in active phase.4,8 In contrast, peripheral blood mononuclear cells (PBMC) derived from patients in remission or inactive phase produce a lower amount of IFN-γ compared to controls.8-10 Therefore alteration in IFN-γ production may influence the susceptibility to MS and this alteration could be due to gene polymorphism. A biallelic polymorphism, T to A, has been determined at position +874 in the first intron. The homozygous T/T, A/A and heterozygous A/T alleles are associated with high, low and intermediate production of IFN-γ, respectively.11

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INF-γ Gene Polymorphism in MS

The aim of present study was to analyze whether there was an association between IFN-γ gene polymorphism at this locus and susceptibility to MS in the Iranian population.

MATERIALS AND METHODS

Patients

Thirty three MS patients (21 female, 12 male; aged: 29.8 ± 8.6) with clinically definite MS, according to the criteria of Poser were studied. Patients were divided into 2 groups according to clinical patterns at onset, namely: 1) relapsing remitting MS (RRMS) including patients with secondary progressive MS (n=27) and 2) primary progressive MS (PPMS; n=6). The mean age of the disease onset was 21.1 ± 6.4 and the average disease duration was 8.75 ± 6.5 years. The median of expanded disability status scale (EDSS) score was 5.75 (range 0-9.5).

The severity of disease was assessed for each patient using progression index (PI= EDSS/duration (yr). Forty-nine percent of patients showed visual disorders as initial symptoms while 51% had other symptoms at the onset of disease. The mean number of relapses in RRMS was 5.4 ± 2.9.

For each patient only one member of his/her first degree relatives with no history of MS, mostly their father or mother, (n= 33; mean age: 55.1 ± 9.4; 21 female, 12 male) was assessed as a healthy subject. All patients and controls were of Iranian Caucasian origin.

DNA Preparation

Five ml of whole blood from patients and controls were used for DNA extraction by a modified proteinase k/salting-out method. Briefly, red blood cells were lysed using cold lysis-buffer I (0.3 M sucrose, 10mM Tris-HCl (pH: 7.5), 5mM MgCl2, 1% Triton x-100). The pellet was washed with phosphate buffer saline (PBS) once. To the pellet 3 ml lysis-buffer II (10mM Tris-HCl, 400mM NaCl, 2mM Na2-EDTA), 200µl of 10% SDS and 40 µl proteinase K were added and incubated in 37°C overnight.

To remove proteins, 1 ml of 6M NaCl was added and centrifuged for 5 min at 1500 g. For extraction of DNA, 2 volumes of absolute ethanol were added to the supernatant. The extracted DNA was washed twice in 70% ethanol and dried at 37°C and recovered in sterile water. Extracted DNA was stored at -20°c until utilization.

PCR-SSP

IFN-γ polymorphism at position +874 in the first intron (T versus A) was determined by allele-specific PCR (PCR-SSP) according to manufacturer’s recommendations (One Lambda, Inc, Canoga Park, CA). Briefly, the PCR was performed with 100-200 ng of isolated genomic DNA as template in reaction mixture containing appropriate primer pairs, dNTP-buffer mix (D-mix) and Taq polymerase, in a final volume of 10 ul. 30 cycles of amplification were carried out as follows: one cycle at 96°C for 130 seconds and 63°C for 60 seconds, 9 cycles at 96°C for 10 seconds and at 63°C for 60 seconds and the final 20 cycles including 96°C for 10 seconds, 59°C for 50 seconds and 72°C for 30 seconds. PCR products were then loaded into a 2.5% agarose gel and photographed using an ultraviolet transilluminator.

Statistical Analysis

Statistical tests were performed using SPSS version 11.5 software. Comparison of alleles and genotypes frequencies between patients and controls were performed by Chi-square and Fisher exact tests. The progression index in patients with different genotypes was compared by Mann-Whitney U test. A p value lower than 0.05 was considered significant.

RESULTS

The IFN-γ gene frequencies of all patients and controls are shown in table 1. There were no differences in allele frequencies in the polymorphisms in IFN-γ between patients and controls. Most of the MS patients and controls showed TA genotype (57.6% and 57.6% respectively) which is associated with intermediate IFN-γ production. Although the differences in the genotype distribution were not statistically significant (p=0.6, df=2), however MS patients showed a decreased frequency of TT (21.2% vs. 30.3%) and increased frequency of AA (21.2% vs. 12.1%) genotype in comparison with
controls. There were also no statistically differences in IFN-γ genotypes between patients with different initial symptoms (visual and non-visual) and clinical patterns. There was no difference in progression index between patients with different genotypes.

**DISCUSSION**

A dysregulated production of IFN-γ has been shown in MS. Increased levels of IFN-γ and IFN-γ mRNA have been detected in peripheral blood of relapsing-remitting MS (RRMS) patients at the time of relapses.\(^4,9\) Similarly, CD4+ and CD8+ T cells isolated from patients with primary progressive and secondary progressive MS produced higher levels of IFN-γ compared to clinically inactive MS patients and healthy controls.\(^8,15\) In contrast, several studies demonstrated that inactive MS or RRMS patients treated with IFN-β produced lower level of IFN-γ compared to unaffected individuals.\(^8-9,15-16\) Other studies also found peripheral blood cells from MS patients with different clinical patterns produced lower level of IFN-γ than controls.\(^10,17\)

According to these observations, we speculated that this variation in IFN-γ production rate could be involved in the pathogenesis of MS and probably this variation is under genetic control. A single nucleotide polymorphism (SNP), T to A, located at position +874 in the first intron could influence IFN-γ production levels. The association of different genotypes at this position, with a low (AA), medium (AT) and high (TT) cytokine production has been shown in vitro.\(^11\) To investigate whether polymorphisms at this locus influenced susceptibility to MS, we analyzed genotype frequencies in the MS patients compared to their first degree healthy relatives as controls. This is the first study investigating the genetic association of polymorphisms in the +874 IFN-γ gene with MS compared to their relatives using PCR-SSP. In the present study we found no significant differences in genotype frequency between patients and controls. Our results are in the line with studies on the IFN-γ intron 1 CA-repeat polymorphisms in European MS patients (18-20). Only in the Swedish MS patients, a weak disease association of the IFN-γ allele I1 has been reported.\(^19\)

Our study also showed that despite the non-significant differences, MS patients showed a higher rate of the genotype related to low IFN-γ production (AA) than the controls (21.2% vs. 12.1%). This finding suggests that MS patients may produce a lower IFN-γ.

The dual role of IFN-γ as a pro-inflammatory and immunosuppressive cytokine has been described using models of autoimmune disease.\(^21\) It is postulated that the time of production and concentration of pro-inflammatory cytokines during the inflammation process may be critical to dampening T cell responses.\(^22-23\) Chu et.al found that IFN-γ deficiency increased the severity of experimental autoimmune encephalomyelitis (EAE) and decreased the apoptosis of activated T cells in the CNS (24). Thus, IFN-γ may play its anti-inflammatory role via induction of T cell apoptosis. On the other hand, several regulatory T cell populations seem to require IFN-γ secretion to exert their suppression effect on the autoreactive T cells.\(^25\)

In conclusion, although our study showed no statistically significant differences in the genotypes of IFN-γ, but production level of this cytokine could affect susceptibility to MS. Because patients’ families are at higher risk for MS than the general population, in order to obtain significant results, we suggest that population-based case-control studies of candidate genes instead of family-based studies should be considered. Since MS is a polygenic disease in which, each gene probably contributes a moderate effect to the overall risk, it is...
likely that patients and their families are similar in a number of genes.

REFERENCES


