

# Polymorphism in the third intron of the interferon- $\gamma$ gene is associated with susceptibility to multiple sclerosis

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**ABSTRACT.** The present study aims to examine the relationship between polymorphisms in the third intron of the IFN- $\gamma$  gene and their influence on susceptibility to multiple sclerosis. A population-based case-control study was used for this purpose. Multiple sclerosis patients and healthy controls were interviewed. Genetic polymorphisms of IFN- $\gamma$  intron III at the +2118 A/G and +3586 G/ACT sites were detected using polymerase chain reaction-restriction fragment length polymorphism. Genotypes and allele frequencies of IFN- $\gamma$  intron III at the +2118 position were significantly different between multiple sclerosis patients and controls ( $P \geq 0.05$ ). However, no difference in allele frequencies was observed at the +3586 position between the two groups ( $P \leq 0.05$ ). Thus, polymorphisms at the +2118 A/G site in the IFN- $\gamma$  intron III gene may be associated with susceptibility to multiple sclerosis.

**Key words:** Polymorphism; Interferon- $\gamma$ ; Multiple sclerosis

## INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination in the central nervous system, and has been shown to be associated with a number of genetic variations. Interferon-gamma (IFN- $\gamma$ ), which is a pro-inflammatory cytokine secreted by Th1 cells, can regulate the activity of immune cells and induce general inflammation. Studies have shown that level of IFN- $\gamma$  increases in the acute phase of MS. IFN- $\gamma$  may therefore act as a direct inducer of demyelination in the central nervous system (Horwitz et al., 1997).

The IFN- $\gamma$  genomic DNA contains 4 exons and 3 introns. Several single nucleotide polymorphisms (SNPs) exist in the IFN- $\gamma$  gene, and play critical roles in the pathophysiology of MS. In addition, several studies have found that SNPs also appear in the third intron of the IFN- $\gamma$  gene (Bream et al., 2000; Henri et al., 2002). In our previous study, we found three SNP sites in IFN- $\gamma$  intron III (+2118 site A/G, +2707 site G/A and +3586 site G/ACT). However, the +2707 G/A SNP was found to be unassociated with MS susceptibility. In this study, we used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to explore the relationship between MS susceptibility and IFN- $\gamma$  intron III SNPs at the +2118 A/G and +3586 G/ACT sites. We found that MS patients and healthy controls differ significantly in their genotype and allele frequencies at the IFN- $\gamma$  intron III +2118 A/G site. The IFN- $\gamma$  intron III +2118 A/G polymorphism may be associated with MS genetic susceptibility.

## MATERIAL AND METHODS

### Patient recruitment

The MS group consisted of 58 patients (21 males and 37 females) from our hospital that were recruited between 2006 and 2007, and ranged between 17 and 70 years of age (average 37.2 years). All patients were diagnosed by two or more specialists in the Department of Internal Medicine, and disease state was confirmed by the 1983 MS diagnostic criteria (Gellein K et al., 2003). In the control group, 40 healthy individuals (14 males, 26 females) between the ages of 14 and 65 were recruited (average 34.7 years). These individuals were relatives or neighbors of the patients, and had no consanguinity between each other. In addition, interviews and examinations were carried out to ensure that the control individuals had no history of recent infections, and did not suffer from diseases of the nervous system and other autoimmune diseases. Age and gender were matched between MS and control groups.

### Methods

#### *Reagent and instrument*

Endonuclease XMN I was purchased from NiuYingLun Biotechnology (Beijing, China); PCR instrument was purchased from MJ Research Inc. (Waltham, USA), and 2X Taq PCR Master Mix was purchased from Tiangen Biotech (Beijing, China).

#### *DNA extraction*

DNA was extracted from 2 mL venous blood.

### ***Primer design and synthesis***

Gene sequences were obtained from the GenBank (NM\_000619.2). Primers were designed using the DNA Primer 5 software, and were synthesized by Shanghai Biological Engineering Technology Co., Ltd. The sequences of the primers are as follows: +2118 Forward, 5'-TCCATTCGTGTTTGGGTGAC-3'; +2118 Reverse, 5'-TGGGCAGTACAATCTGATAGGT-3'; +3586 Forward, 5'-GGGCAATCTTGAGTGAGC-3'; +3586 Reverse, 5'-CAGGTGCATGAAGTGGAA-3'.

### ***Gene amplification***

Reaction mixtures were assembled as follows: 10  $\mu$ L 2X PCR Taq Mix Master, 2  $\mu$ L forward and reverse primers, 2  $\mu$ L DNA template (200-400 ng), and distilled water. PCR tubes containing the reaction mixtures were placed in the thermocycler, and the cycling parameters were as follows: denaturation at 94°C min for 5 min; 30 cycles of 94°C for 30 to 45 s, 58°C for 45 s, 72°C for 45 s, followed by a final extension at 72°C for 5 min.

### ***Restriction enzyme digestion of the amplified products***

Restriction enzyme digestion reactions (20  $\mu$ L) were set up as follows: 5  $\mu$ L *ScaI* or *Tsp45I*, 0.5 U *XmnI* endonuclease, 2  $\mu$ L 10X NEB Buffer, 0.2  $\mu$ L 10X BSA, and sterilized H<sub>2</sub>O. Enzyme digestion products were separated on 8% agarose gel electrophoresis, and the results were observed by ethidium bromide staining.

### **Statistical analysis**

Data are reported as means, and statistical significance was determined using  $\chi^2$  tests.

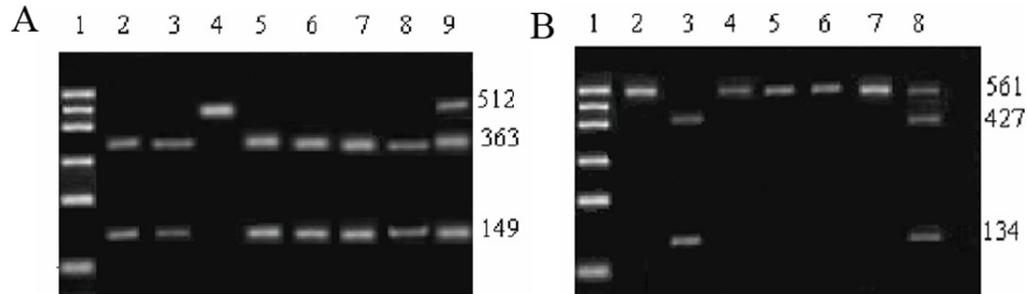
## **RESULTS**

### **Enzyme-digested product of IFN-g**

Following digestion by *XmnI* or *Tsp45I* restriction enzymes, IFN- $\gamma$  genes containing G/G homozygotes at the +2118 and +3586 sites were visualized as two fragments. At the +2118 site, A/A homozygotes were not digested, and as result, only a single band at 512 bp was observed (Figure 1A). In A/G heterozygotes, the gene product was not fully digested, and was therefore composed of three bands (512, 363 and 149 bp) (Figure 1A). In G/ACT SNP at the +3586 site, the digestion was also included one band (561bp) and three bands (561, 427 and 14 bp) (Figure 1B).

### **Hardy-Weinberg equilibrium test**

The expected and observed genotypes of IFN- $\gamma$  +2118 and +3586 sites in the MS group were not statistically different from each other ( $P \geq 0.05$ ). This result indicated that genotype frequencies of IFN- $\gamma$  at +2118 were at Hardy-Weinberg equilibrium (Table 1). In the control group, the expected and observed genotypes of IFN- $\gamma$  +3586 were also not statistically significant ( $P \geq 0.05$ ; Table 2).



**Figure 1.** DNA fragments at the (A) +2118 and (B) +3586 sites in the third intron of the IFN- $\gamma$  gene obtained following DNA digestion. In A, lanes 2, 3, 4, 5, 6, 7, and 8 are the homozygotes (A/A); lane 9 is the heterozygote (A/G); lane 4 is the homozygote (G/G). In B, lane 3 is the homozygote (G/G); lane 8 is the heterozygote (X/G); lanes 2, 4, 5, 6, and 7 are the homozygotes (X/X). Lane 1 is the molecular marker in both A and B.

**Table 1.** Expected and observed genotype distribution in the MS group.

Group	INF- $\gamma$ +2118 site genotype			INF- $\gamma$ +3586 site genotype		
	AA	AG	GG	GG	GX	XX
Expected genotype	38	18	2	0	4	54
Observed genotype	41	12	5	1	2	55
Test value	$\chi^2 = 2.600$ P = 0.273			$\chi^2 = 1.676$ P = 0.433		

Compared with the expected genotype distribution, P > 0.05.

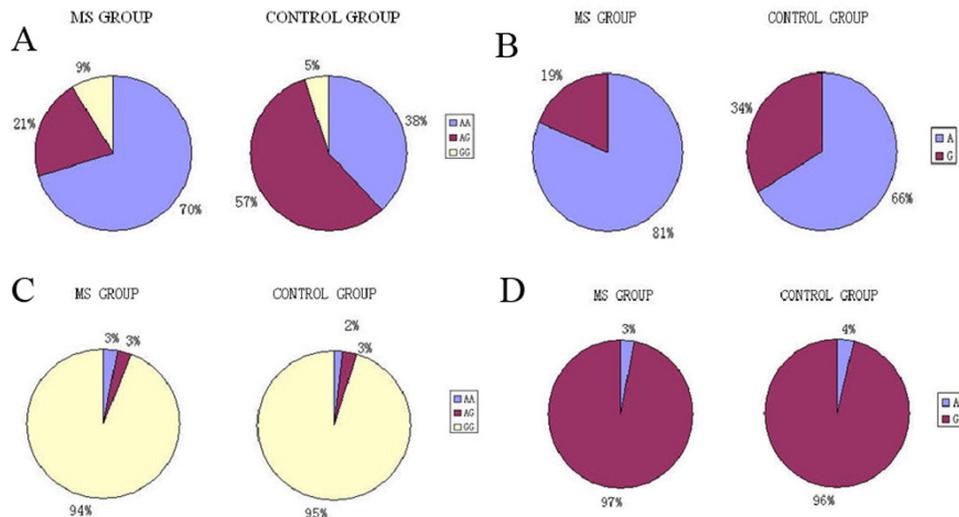
**Table 2.** Expected and observed genotype distribution in the control group.

Group	INF- $\gamma$ +2118 site genotype			INF- $\gamma$ +3586 site genotype		
	AA	AG	GG	GG	GX	XX
Expected genotype	12	29	17	0	3	37
Observed genotype	15	23	2	1	1	38
Test value	$\chi^2 = 1.269$ P = 0.530			$\chi^2 = 2.013$ P = 0.365		

Compared with the expected genotype distribution, P > 0.05.

## Genotype distribution and allele frequency

Genotype distribution was compared at the IFN- $\gamma$  +2118 and +3586 sites between MS patients and healthy controls. In the MS group, A/A, A/G, and G/G genotypes were found at the +2118 site in 41, 12 and, 5 patients, respectively. In the control group, A/A, A/G, and G/G genotypes were found in 15, 23, and, 2 individuals, respectively. The frequency of the A allele at the IFN- $\gamma$  +2118 site was increased in the MS group (81.03%) as compared with the control group (66.25%) ( $\chi^2 = 5.520$ , P  $\leq$  0.05) (Figure 2A and B). However, no significant difference was observed between the MS and control groups in genotype distribution and allele frequency at the IFN- $\gamma$  +3586 site (P  $\geq$  0.05) (Figure 2C and D).



**Figure 2.** Genotype distribution and frequency. Gene polymorphisms of IFN- $\gamma$  intron III at (A) +2118 and (C) +3586 sites in multiple sclerosis. Distribution of IFN- $\gamma$  intron III polymorphism at (B) +2118 and (D) +3586 sites in multiple sclerosis.

## DISCUSSION

Multiple sclerosis is an autoimmune disease characterized by demyelination in the central nervous system. Recently, much research has been conducted in the field of molecular biology on the genetic predisposition to MS via examinations of gene polymorphisms. Some genes of interest include the human leukocyte antigen (HLA) gene, the T cells receptor (TCR) gene (Dyment et al., 2004), the tumor necrosis factor (TNF) (Fernandez-Arquero et al., 1999) gene, the vitamin D receptor (VDR) gene (Fukazawa et al., 1999; Niino et al., 2000), the female hormone estrogen receptor (ER) (Savettieri et al., 2002), and the low molecular weight polypeptide (LMP) gene (Liblau et al., 1993; Omran et al., 2013). However, many of the early association studies cannot be duplicated due to differences in disease etiology and clinical methodologies. Currently, researchers generally employ genome scanning and gene linkage analysis to explore gene polymorphisms (Kenealy et al., 2004; Bergman et al., 2014). Researchers also found that SNP analysis of candidate genes may be more efficient in determining genetic predispositions than linkage analysis. IFN- $\gamma$  is a pro-inflammatory cytokine secreted by Th1 cells, and has a wide range of functions such as induction of inflammation and regulation of immune cell activities. Some studies have indicated that the IFN- $\gamma$  gene is implicated in the development of a number of diseases including hepatitis B, rheumatism, and systemic lupus erythematosus. Domestic and foreign studies show that IFN- $\gamma$  is a key factor in the development of MS. However, correlation studies examining the relationship between the IFN- $\gamma$  gene and MS are scarce. This study aims to explore the pathogenesis of MS, and provide a novel treatment strategy for MS.

IFN- $\gamma$  exerts its biological function through binding to the specific receptor, IFN-gR. Dysfunction of IFN-gR can inhibit differentiation of Th0 cells into Th1 cell. IFN-gR is closely associated with MS development by regulating the balance between Th1 and Th2

cytokines. The IFN- $\gamma$ R gene is located on the sixth chromosome, and variant forms of IFN- $\gamma$ R1 and IFN- $\gamma$ R2 include Val14Met and Gln64Arg. Schrijver et al. (2004) suggested that gene polymorphisms of IFN- $\gamma$  intron I, IFN- $\gamma$ R1, and IFN- $\gamma$ R2, were not correlated with MS. However, the Gln64Arg amino acid polymorphism in IFN- $\gamma$ R2 was associated with progressive MS (Schrijver et al., 2004).

Studies have shown that intron III of IFN- $\gamma$  has three SNP sites (+2118 site A/G, +2707 site G/A, and +3586 site G/ACT). In our previous study, we found that +2707 A/G was not associated with susceptibility to MS. In the current study, we aimed to investigate the relationship between polymorphisms at the +2118 A/G and +3586 G/ACT sites and MS susceptibility. We found that IFN- $\gamma$  +2118 and +3586 sites of MS group have distinct genetic profiles. Results indicated that frequency of the A allele at the IFN- $\gamma$  +2118 site was significantly increased in the MS group as compared with the controls. However, no difference in allele frequency was detected between the two groups at the IFN- $\gamma$  +3586 sites. These results indicated that IFN- $\gamma$  +2118 SNPs might be associated with susceptibility to MS. However, MS is a complex disease that is associated with many factors, and the mechanisms of MS need to be further explored.

### Conflicts of interest

The authors declare no conflict of interest.

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