Investigation of FcγRIIA and FcγRIIIA Polymorphism in Multiple Sclerosis: A Case Control Study

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ABSTRACT

Background: Multiple Sclerosis (MS), the most common demyelinating disease of the CNS, is immunologically mediated in genetically susceptible individuals. Receptors for the Fc fragment of IgG (FcγR) might induce inflammatory responses through linking the humoral and cellular immune responses by targeting immune complexes to effector cells. Polymorphisms in some FcγR genes are associated with various infectious and autoimmune diseases, probably due to their effects on different binding capacities of encoded receptors for IgG containing immune complexes. **Objective:** To investigate the importance of FcγR polymorphisms in susceptibility to MS. **Method:** One hundred and fifty MS patients and 136 age and sex matched controls were genotyped for FcγRIIA and FcγRIIIA gene polymorphisms using PCR-RFLP method. **Result:** The allelic and genotypic frequencies of the FcγRIIA and FcγRIIIA did not differ significantly between the MS patients and controls. There was no association between allelic polymorphism of FcγRIIIA and severity of disease based on Expanded Disability Status Scale (EDSS) score. However, significant association between inherited FcγRIIA genotype and disease activity (p=0.001) or progression index was revealed (p=0.014). EDSS values showed that FcγRIIA (H/H) and (H/R) genotypes were associated with a lower EDSS score in relapsing-remitting MS and in the total MS population (P=0.001) but not (R/R) genotype. **Conclusion:** Considering the detrimental role of autoantibodies in the pathogenesis of MS, our results suggest that the inherited FcγRIIA alleles could affect the severity of MS by influencing the clearance rate of immune complexes and autoantibodies. The results of the present study add the FcγRIIA gene to the gene networks which determine the severity of MS in southern Iran.

Keywords: Multiple Sclerosis, FcγRIIA, FcγRIIIA, Polymorphism

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INTRODUCTION

Multiple Sclerosis (MS) is the most common demyelinating disease in human beings, predominately affecting young adults. Environmental elements and genetic factors have both been associated with the disease. T-lymphocytes have an important role in the initiation of MS (1). On the other hand, humoral immune response has been known to contribute largely to the development of demyelination and axonal damage (2,3). Abnormal humoral response has been observed in the majority of MS patients. In this regard, increased concentration of IgG in the cerebrospinal fluid (CSF) has been associated with disease relapse (4) and a high B-cell/monocyte ratio in the CSF was associated with accelerated disease progression in a retrospective study (5). Moreover, elevated anti-myelin IgG antibody titers have been reported in patients with active disease (6,7).

The biological functions of immunoglobulins are exerted through the Fc portion. Fc portion of IgG is recognized as Fcγ-receptors (FcγRs). FcγRs are expressed by a variety of cells including macrophages and dendritic cells, linking humoral and cellular immunity. Binding of autoantibodies to myelin particles may target the inflammatory process by cross-linking FcγRs on microglia and macrophages in the CNS (8,9,10). The human genome encodes three classes of leukocyte FcγRs: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) and their subclasses (FcγRIA, FcγRIB, FcγRIC; FcγRIIA, FcγRIIB, FcγRIIC; FcγRIIIA, FcγRIIIB), indicating a characteristic expression pattern on leukocytes and binding capacity for IgG isotypes. FcγR genes have all been mapped on chromosome 1q at q21-23 (11). Genes encoding these receptors have been identified to elicit functional polymorphisms. FcγRIIA is expressed on monocytes, macrophages, and neutrophils but not on NK cells or lymphocytes and expresses a functional polymorphism in the ligand-binding domain of the receptor at amino acid position 131. Expression of histidine (H) at this position greatly enhances IgG binding capacity of receptors (12,13). In fact, FcγRIIA-H^{131} interacts more efficiently with complexed IgG2 and IgG3 than FcγRIIA-R^{131} (14). FcγRIIA shows another polymorphism at amino acid position 27, where a substitution of glutamine for tryptophan takes place that is non-functional (13). FcγRIIIA is constitutively expressed on NK cells, cultured monocytes, and macrophages (15) and exhibits a change of valine (V) to phenylalanin (F) substitution at amino acid 158. The FcγRIIIA-V^{158} allotype binds IgG1- and IgG3-containing immune complexes more efficiently than FcγRIIIA-F^{158} (16).

It seems that FcγRIIA-$H^{131}$and FcγRIIIA-V^{158} are more efficient in inducing leukocyte effector functions. Therefore, considering the importance of antibodies in MS pathogenesis, inter-individual differences in the efficacy of FcγR induced inflammation may be associated with differences in susceptibility to MS.

The aim of the present study was to determine whether there is a relation between Fcγ receptor genotypes and MS susceptibility or disease course. We investigated the distribution of FcγRIIA and FcγRIIIA genotypes in 150 MS patients and 136 ethnically matched healthy controls.

MATERIALS AND METHODS

Subjects. One hundred and fifty patients (35 males and 115 females) who have been referred to Neurology outpatient clinic and Neurology wards at Namazi and Chamran hospitals at Shiraz from August 2002 to August 2004 were studied. Inclusion criteria of Poser et al. (17) were considered. Probable and possible MS patients and those received interferon-β were excluded from the study. Current and past medical histories including neurological exam and para clinical data (laboratory and imaging) of all patients were reviewed. Expanded Disability Status Scale (EDSS) was determined. Patients' neurological symptoms were recorded by a neurologist using a questionnaire.

DNA Extraction. EDTA blood was obtained from patients and 136 healthy controls. Genomic DNA was extracted from peripheral blood leukocytes by salting out method.

Determination of FcγRIIIA -158 G→A Polymorphism. Polymorphism at codon 158 of FcγRIIIA gene consists of a GTT to TTT mutation coding for valine and phenylalanine, respectively. Genotyping was performed by a method developed by Nieto et al. (18), consisting of PCR and restriction fragment length polymorphism analysis using amplification-created restriction sites. For FcγRIIIA genotyping 20 µl of PCR reaction mixture was prepared containing genomic DNA sample (125 ng), 200 µmol/L dNTPs, 2 mM $MgCl_2$, 1X Taq DNA polymerase buffer, two units of Taq DNA polymerase (Bohringer Manheim, Germany), and 10 pmol of each test primer (5'-ATA AGG TCA CAT ATT TAC AGA ATG GCC AAG-3' as sense primer and 5'-CAG TCT CTG AAG ACA CAT TTT TAC TCC GTA-3'as anti-sense primer). Cycling conditions were as follows: one cycle of initial denaturation step at 95ºC for 5 minutes followed by 31 cycles at 94ºC for 40 seconds, 52ºC for 60 seconds, and 72ºC for 60 seconds, and finally one cycle at 72ºC for 10 minutes. Subsequently two digestion reactions per sample were performed: 10 µl aliquots of PCR product were treated either with 10 unites of *Rsa* I or with 10 unites of *Rsa* I plus 15 units of *Sty* I in a 20 µl final volume of 1X reaction buffer for 16 hours at 37 ◦C. Reaction products were separated on a 3% agarose gel and stained with ethidium bromide.

Determination of FcγRIIA-H/R^{131}Polymorphism. PCR amplification was performed using the method described by Jiang et al. (19). The 5'-GGA AAA TCC CAG AAA TTC TC**G** C-3' and 5'-CAA CAG CCT GAC TAC CTA TTA CGC **G**GG-3' oligonucleotides were selected as sense and anti-sense primers, respectively. The sense primer contains a one-nucleotide substitution (in bold), which introduces a *BstU* I site into the PCR product when the next nucleotide is G, but not A. The anti-sense primer contains two nucleotide substitutions which introduce an obligate *BstU* I site into all PCR products which use this primer. PCR amplification was performed in a 100 µl reaction mixture containing 100 ng genomic DNA, 200 pM of each primer, 150 mM $MgCl_2$, 100 µM each dNTP, 10 mM Tris-HCl (pH=9), and 2 units of Taq DNA polymerase. 30 cycles (94 ºC for 15 seconds, 55 ºC for 30 seconds, and 72 ºC for 40 seconds) were performed in ependroff thermal cycler and samples were analysed on 3% agarose gel stained with ethidium bromide after restriction enzyme digestion.

Statistical Tests. Chi-square test was used for comparison of FcγR genotype distribution between groups. Fisher's exact test and Yates correction were used for small number of patients. Association of continuous data (such as age at disease onset, EDSS, and progression index) with different genotypes was determined by Kruskal-Wallis test using SPSS 11.5 software. P-values less than 0.05 were considered significant.

RESULTS

Demographic Data. One hundred fifty patients with MS were studied, 115 cases were female and 35 were male (F/M=3.28), with mean age of 30.34 years (range 14 to 46). The mean age at the onset of disease was 25.95 years (range 13 to 43) and the mean disease duration was 4.33 years (range three months to 20 years). The mean EDSS score was 3.84 (range 1.5 to 9). The initial presentation of disease was relapsing-remitting (RRMS) in 141 (94%) and primary progressive (PPMS) in 9 cases (6%).136 age and sex matched healthy controls including 104 women and 32 men were selected from the same area and ethnic groups, with mean age of 31.2 years (range 15 to 45).

Inheritance of FcγRIIIA and FcγRIIA Genes in MS Patients and Normal Controls. The genotype and allele frequency of FcγRIIA and FcγRIIIA are shown in table 1. No significant difference in the distribution of FcγRIIIA-V (patients=45.68%, controls= 40.47%) and FcγRIIIA-F (patients=54.31%, controls=59.52%) alleles were observed comparing patients and controls (P=0.5). There was no association between inherited alleles and disease activity (EDSS score) (P=0.75) or disease phenotype (PP-MS, RR-MS, and SP-MS) (P=0.49). Results still remained non-significant when patients were categorized based on gender (P=0.89).

Table 1. FcγRIIIA-V/F^{158} and FcγRIIA-H/R^{131} genotypes in Iranian MS patients and healthy controls

Groups	FcγRIIA-H/R^{131}			FcγRIIIA-V/F^{158}		
	HH(%)	HR(%)	RR(%)	FF(%)	VF(%)	VV(%)
RR-MS	56(39.7)	64(45.4)	21(14.9)	28(35.9)	31(39.7)	19(24.4)
PP-MS	2(22.2)	6(66.7)	1(11.1)	3(37.5)	2(25)	3(37.5)
Controls	43(31.6)	77(56.6)	16(11.8)	56(44.4)	38(30.2)	32(25.4)

RR-MS: Relapsing remitting-MS
PP-MS: Primary progressive-MS

Similar to FcγRIIIA genotype, there was no statistically significant difference in the frequency of FcγRIIA alleles in patients (H=62%; R=38%) compared with controls (H=59.92%; R=40.07%) (P=0.2). No association between FcγRIIA alleles or genotypes and disease presentation was found (P=0.086). However, the results of the present study revealed a significant association between inherited FcγRIIA genotypes and disease activity (p=0.001) or progression index (p=0.014). In fact carriage of FcγRIIA-H allele was associated with a lower EDSS score in RRMS and in the total MS population (P=0.001). Analysis of progression index (EDSS/ duration of disease) showed that FcγRIIA (H/H) genotype was associated with a lower progression index (P=0.014).

DISCUSSION

In the current study, association of FcγR genotypes with susceptibility to and severity of MS disease was investigated. No difference in the distribution of FcγRIIA and FcγRIIIA alleles was observed comparing patients with controls (P=0.5). There was no association between inherited alleles and disease phenotype (PPMS, RRMS, and SPMS). These associations remained non-significant when patients were categorized

based on gender. We found a strong association between the inheritance of FcγRIIA H allele and lower EDSS score (p=0.001).

In other studies, clinical implications of FcγR polymorphism on the course of infectious diseases have been described (20), patients homozygous for FcγRIIA-H allele had a better clinical course of meningococcal disease than those heterozygous or homozygous for FcγRIIA-R allele (20). Several lines of evidence suggest that immune complexes may influence the course of MS. Viral infections inducing formation of immune complexes, have been associated with exacerbation in MS (21). Increased levels of circulating immune complexes in MS have also been related to disease severity (22) and these patients have been shown to benefit from treatment with plasma exchange (23). The association of FcγRIIA-H allele with lower EDSS score in our study could be explained by higher ability of H allele encoded receptors in processing and clearance of immune complexes. In fact, if immune complexes are not sufficiently reduced or neutralized in circulation they may gain access to the CNS by leakage through a defective blood-brain barrier (or possibly by uptake and transport by endothelial cells) and exacerbate the disease (24).

To our knowledge, two other previous studies have focused on FcγR polymorphism in MS patients. In one, FcγRIIA, FcγRIIIA, and FcγRIIIB polymorphisms were studied in 432 Dutch Caucasian patients and 515 healthy controls (25). Similar to our study, the results of Dutch study showed no difference in the distribution of FcγRIIA and FcγRIIIA polymorphisms between patients and controls. Furthermore, FcγRIIA and FcγRIIIA genotype distributions did not differ between MS subtypes and no association with disease course and MS susceptibility was found. In another study by Myhr et al, (26) 136 Norwegian patients were compared with 96 healthy controls and distribution of genotypes and alleles of FcγRIIA or FcγRIIIB showed no significant difference between MS patients and healthy subjects. However, patients homozygous for FcγRIIIB neutrophil antigen (NA) 1 allele experienced a significantly more favourable course of disease than those heterozygous or homozygous for FcγRIIIB-NA2 allele. Patients homozygous for FcγRIIA-H allele also had a more benign course than those heterozygous or homozygous for FcγRIIA-R allele. These results and ours are similar and depict no association between FcγRIIA allele or genotype frequency and disease types.

In conclusion, the results of the present study showed that the FcγRIIA gene have disease-modifying properties in Iranian MS patients. A more favourable course of disease was observed in patients homozygous for FcγRIIA-H allele. As ethnic variations in FcγRIIA gene polymorphism has been described with an increased frequency of H/H genotype in Asian populations (Chinese, Japanese, Asian Indian) compared with Caucasians (20,27), further investigations on other ethnic populations is recommended.

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