Genetic Susceptibility to Multiple Sclerosis: The Role of FOXP3 Gene Polymorphism

Nihal İŞIK1, Nüket YILDIZ MANUKYAN2, İlknur AYDIN CANTÜRK1, Fatma CANDAN1, Ayşen ÜNSAL ÇAKMAK1, Güher SARU HAN DİRESKENELİ3

1Clinic of Neurology, Medeniyet University Göztepe Training and Research Hospital, İstanbul, Turkey
2Clinic of Neurology, Ministry Health Fatih Sultan Mehmet Training and Research Hospital, İstanbul, Turkey
3Department of Neurology, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey

ABSTRACT

Introduction: It is well recognized that both genetic and environmental factors play an important role in the pathogenesis of multiple sclerosis (MS). Immune pathogenesis of MS focuses on pathogenic CD4+ T lymphocytes. CD4+CD25+ regulatory T cells have suppressive function in this cell group. FOXP3 (forkhead boxP3) transcription factor is a key structure in the development and function of regulatory cells. Functional alterations in FOXP3 gene expression have been observed in various autoimmune diseases.

Methods: We screened a non-synonymous coding single nucleotide polymorphism (exon +2710 C/T) (rs2232369) of human FOXP3 gene in 148 MS patients (118 with Relapsing Remitting MS, 30 with Secondary Progressive MS) and 102 age- and sex-matched healthy controls. The association of polymorphisms with susceptibility, and course of the disease was evaluated.

Results: We could not detect any single nucleotide polymorphism in MS patients, however, polymorphic allele was detected in 3% of the control group. Consequently, a genetic association between the FOXP3 gene polymorphism and MS was not revealed.

Conclusion: The distribution of this polymorphism has not been screened in any other MS populations before. Although we could not succeed to find any association between susceptibility to MS and screened FOXP3 gene polymorphisms, we suggest that this particular polymorphism is not appropriate for these kind of studies in the future.

Key words: Multiple Sclerosis, FOXP3, polymorphism, genotype

Correspondence Address
Dr. Nihal Işık, Medeniyet Üniversitesi Göztepe Eğitim ve Araştırma Hastanesi, Noroloji Kliniği, İstanbul, Türkiye
Gsm: +90 532 225 79 07 E-mail: nihal-isik@hotmail.com
Received: 18.02.2013 Accepted: 12.03.2013
©Copyright 2015 by Turkish Association of Neuropsychiatry / ©Telef Hakki 2014 Türk Nöropsikiyatri Derneği
autoimmune diseases occurred in mice whose CD4+CD25+ Treg cells were abolished (8). In studies conducted in recent years, it has been shown that disruption in the tolerance mechanisms controlling growth of pathogenic T cells directed to myelin or other “self” tissue antigens might lead to occurrence of MS and other autoimmune diseases (9,10). Controversial results have been obtained in the few number of human studies (11,12,13).

Recent studies have shown that FoxP3 (forkhead box P3-scurfin) transcription factor is important in realizing the regulatory function of Treg cells (6,14,15,16).

Single nucleotide polymorphisms (SNP) are the most simple DNA differences observed between individuals. SNP may change the amino acid encoded by the gene, may stay silent or may be present in the regions where encoding does not occur. Therefore, it may play a significant role in development of autoimmune diseases by affecting gene production, mRNA formation or protein production (17).

In this study, FOXP3 gene polymorphisms defined in MS patients and healthy controls were investigated and it was investigated if these mutations were related with predisposition to MS and other phenotypic properties in our community.

Methods

Patients and Control Groups

Our patient group was composed of 148 patients who were being followed up in the Multiple Sclerosis Outpatient Clinic and had a definite diagnosis of MS according to MacDonald criteria (18). 118 of the patients had relapsing remitting (RR) MS and 30 had secondary progressive (SP) MS. 102 age and gender matched healthy controls (68 women and 34 men) who had no history of MS, no signs of MS and no familial history of MS constituted the control group.

Before blood samples were obtained the participants were given information about the study and informed consent form was filled in.

DNA isolation

10 cc venous blood were obtained from the patients and controls and placed in EDTA tubes. After the necessary procedures required for separation of DNA (separation of leukocytes, cell explosion, nucleus explosion, abolishment of proteins) were performed, DNA strands which became visible with addition of ethanol were placed in a sterile DNA tube. The obtained DNA was homogenized with 200 μl sterile distilled water and rediluted at a ratio of 1:50. Their optic densities were measured by spectrophotometer (at 260 nm) and their amounts were specified. According to the values obtained, the DNAs were diluted as 30 μg/ml and kept at +4°C until they were studied.

Polymerase chain reaction (PCR)

+2710 C/T (rs2232369, 220.aa A/V) SNP which is found in the encoding region on the FOXP3 gene (exon) and has been reported to cause to amino acid alternation was screened by way of amplification of the targeted genomic region by PCR and specification of the pattern of cutting with restriction enzyme (polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)). The method was based on loss of the property of the FOXP3 gene of being cut with the appropriate enzyme selected according to the nucleotide differences at the point of polymorphism investigated. Primer3 (Whitehead Institute for Biomedical Research) program was used for this study. Amplification was performed with 5’-CTGAGGGCGATGCATTAGG and 5’-TTTGCAGACTGTTATCC primers and presence of C or T allele was identified by cutting the product with a length of 360 base pairs with EcI enzyme. The C allele constituted a cutting point for the restriction enzyme.

Statistical analysis

The allele and genotype frequencies found in the patient and control groups were compared using χ² test. Probable haplotype frequencies were calculated using Arlequin program.

Results

The demographic properties of the study group is summarized in Table 1. There was no difference between the patient and control groups in terms of age and gender. The distribution obtained for FOXP3 gene +2710 (C/T) polymorphism in MS and healthy controls is shown in Table 2.

When this polymorphism distribution which was identified but not screened in any population before was examined, polymorphic allele was shown with a rate of 3% only in the control group. When the allele frequencies found in MS patients and healthy controls were compared, no significant difference of distribution was observed between the two groups (Table 2).

Discussion

Although the etiology of MS which is an autoimmune disease is not known exactly, it is thought that it probably has a heterogeneous structure and both genes and environmental factors are important in development of the disease. However, a clear and homogeneous type of inheritance could not be found. Therefore, classical genetic epidemiological techniques can not be applied in studies. In all diseases with complex inheritance, the most appropriate approach for gene studies is primarily detecting the chromosomal region of the genomic effect by linkage analyses. The secondary step is to demonstrate the degree of variability in certain parts of the candidate region (exon, promoter region or intron) in terms of direct relation with the disease (19,20).

Single nucleotide polymorphisms are the most common DNA sequence variations in the human genome. SNPs are thought to be old and stable mutations found in the whole of genome. These properties make them good targets for genetic studies. Although these mutations are mostly neutral, some may play a role in predisposition to morbidity or resistance to morbidity (21,22).

CD4+CD25+Treg cells which are thought to be important in the immune etiopathogenesis of autoimmune diseases realize
been shown that mutation in the FOXP3 gene causes to IPEX syndrome which is a rare autoimmune genetic diseases in humans. IPEX syndrome (immune disregulation, polyendocrinopathy, enteropathy, X-linked inheritance) is an immune system disease characterized with hyperreactivity of T cells (23).

The number of studies investigating the role of FOXP3 in the etiopathogenesis of MS has increased in recent years. In 2003, Putheti et al. reported that CD4+ CD25+ T cells increased (11). In 2004, they reported that these cells did not change (12). However, in 2004, Viglietta showed that the suppressive functions of CD4+ CD25+ T cells decreased, but did not give information if this decrease was related with the level of expression of the FOXP3 gene (13). In 2005, Huan et al. showed that the reduction in the functionally suppressive ability of peripheral CD4+ CD25+ T cells was related with the reduction in the level of FOXP3 expression (17). Similarly, Venken reported that both the suppressive functions of CD4+ CD25+ T cells and the amount of FOXP3 protein decreased in RRMS patients in a study published in 2008 (24).

Table 1. The demographic data and distribution of clinical properties in the control and patient groups

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS group</td>
<td>48</td>
<td>100</td>
<td>148</td>
</tr>
<tr>
<td>Age</td>
<td>40.9±9</td>
<td>37.5±8</td>
<td>38.3±9</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>11±9</td>
<td>9±5</td>
<td>9.5±6</td>
</tr>
<tr>
<td>EDSS (mean)</td>
<td>2.7±1</td>
<td>1.9±1</td>
<td>2.1±1</td>
</tr>
<tr>
<td>RR MS</td>
<td>34</td>
<td>84</td>
<td>118</td>
</tr>
<tr>
<td>SP MS</td>
<td>14</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>Control Group</td>
<td>34</td>
<td>68</td>
<td>102</td>
</tr>
<tr>
<td>Age</td>
<td>41.2±8</td>
<td>35.2±9</td>
<td>37.9±8</td>
</tr>
</tbody>
</table>

Table 2. Distribution of FOXP3 +2710 (C/T) polymorphism in the MS and healthy control group

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele frequency</td>
<td>n=148</td>
<td>n=102</td>
</tr>
<tr>
<td>C</td>
<td>296</td>
<td>198</td>
</tr>
<tr>
<td>T</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype frequency</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>148</td>
<td>99</td>
</tr>
<tr>
<td>TT</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>TC</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

It has been shown that CD25+ cell group can prevent increase in CD4+ pathogenic cells and cytokine release. Recently, the view that CD4+ CD25+ Treg cells prevent autoimmunity by way of Foxp3 has been widely accepted (6). Foxp3 is the key factor in development and function of Treg cells which express IL2 receptor α chain (CD25) and is effective in regulation of both natural and acquired immunity. FOXP3 gene is located on the X-chromosome. The FOXP3 transcription factor is basically produced by T cell sequence and is the main regulator in inhibition of T cell activation (14,15,16). Deletion in the gene coding FOXP3 results in increased proliferation of active T cells and development of both autoimmune and allergic diseases. It has been shown that mutation in the FOXP3 gene causes to IPEX syndrome which is a rare autoimmune genetic diseases in humans. IPEX syndrome (immune disregulation, polyendocrinopathy, enteropathy, X-linked inheritance) is an immune system disease characterized with hyperreactivity of T cells (23).
screened in any other population before was examined with this objective, polymorphic allele was found in 3% of the control group, while it was not found in the patients (Table 2).

In 2003, Bassuny et al. compared 199 type 1 diabetes patients and 289 healthy controls in terms of predisposition to disease in the Japanese population and screened single nucleotide polymorphisms in the protein coding region of microsatellite and FOXP3 gene in the intron and promoter areas. They found a significant relation between FOXP3 gene and predisposition to type 1 diabetes (28). On the other hand, Zavattari et al. showed that there was no relation between FOXP3 gene and type 1 diabetes in the population who lived in Sardinia island (29). In another recent study, it was reported that there was no association with FOXP3 gene polymorphism in autoimmune thyroiditis and Addison disease (30).

When examined in terms of functionality, it can be thought that Treg cells and FOXP3 molecule may have important immune functions in terms of autoimmune diseases and thus predisposition to MS. However, controversial results have been obtained in few genetic studies conducted in this area. The reason for this may be inappropriateness of the genetic site selected for screening, differences which may be created by the method which is substantially sophisticated and inconvenient and insufficient number of subjects which is a very important issue in genetic studies. All these factors were also limitations of our study.

We could find no evidence to demonstrate the relation between SNP in the exon +2710(C/T) region of the FOXP3 gene and predisposition to MS. However, we thought that it was worth to publish our results, because there was no other study where FOXP3 gene polymorphism was screened in MS and we wanted to share the information that polymorphism in this region should not be screened in future studies. We think that identification of chromosomal regions and specific target genes will be helpful in development of new and more efficient methods in terms of treatment of the disease by helping in understanding the pathogenesis of MS.

References


