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The Association Between C424c/A Polymorphism Within the IL-25 Gene and Multiple Sclerosis

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Abstract

Background: Multiple Sclerosis (MS) is a common autoimmune system disease which affects the central nervous system. It has been documented that interleukin-25 (IL-25) plays key roles in suppressing Th1 responses, which is increased during MS.
Objectives: The aim of this study was to investigate the c424C/A polymorphism within the IL-25 gene in MS patients in comparison to healthy controls.
Patients and Methods: In this case-control study, 74 patients with MS and 75 healthy controls were selected. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used in order to determine c424C/A polymorphism within the IL-25 gene.
Results: The results showed that there was no statistical significant difference in distribution of genotype (AA, AC and CC) and allele (A and C) frequencies between MS patients and healthy controls (P = 0.901 and P = 0.728, respectively).
Conclusions: In conclusion, it appears that the c424C/A polymorphism within the IL-25 gene has no significant relationship with MS, and this polymorphism is probably not associated with MS complications, its onset and gender distribution.

Keywords: Multiple Sclerosis, Polymorphism, Genetic, CCL25

1. Background

Multiple Sclerosis (MS) is a common autoimmune system disease which affects the central nervous system with network vascular inflammation, demyelination and axonal damage symptoms (1, 2). It is a chronic disease of the central nervous system and causes severe cognitive and physical impairments (3, 4). Accordingly, it has been documented that white matter lesion may affect the cognition of MS patients (5). Among the various clinical forms, relapsing-remitting is the most common type, found in approximately 85% to 90% of cases (6). Although, it is difficult to differentiate Primary Progressive Multiple Sclerosis (PPMS) from Relapsing Remitting Multiple Sclerosis (RRMS) in the early phases, yet previous studies have demonstrated that MRS can be used as a potential way to diagnose PPMS and RRMS (2). In fact, after traumatic and rheumatic diseases, MS is the third factor for asthenic diseases, and has been the subject of extensive studies worldwide (7). Currently, more than 1.3 million people are affected by MS worldwide (8). Multiple Sclerosis is a disease of young adults and most of patients are diagnosed between the ages of 20 and 40 (9). Although the exact reason for this disease is unknown, it has been reported that several factors such as genetic and environmental factors (especially viruses) are involved in the pathogenesis of MS (1, 10, 11). Accordingly, several investigators are trying to improve symptoms of MS by alteration in the behavior of MS patients including exercise (4). In spite of such efforts, it appears that genetic factors play key roles in the pathogenesis of MS. Interestingly, researchers do not limit this disease just to a single gene and candidate several immune and non-immune genes in the pathogenesis of MS.

Recently, scientists have investigated Interleukin-25 (IL-25) including its effects on the immune system. This cytokine has a similar structure to IL-17 and plays a key role in stimulating and development of T helper 2 (Th2) responses (12). Furthermore, IL-25 is involved in the control function of endothelial cell, Th1 and Th17, which are the main cells involved in the pathogenic activities of the immune system (12). Therefore, any factor, which can regulate the expression of the cytokine, can be considered as a candidate for investigation in the MS dis-

ease process. It has been reported that there is a known polymorphism (c424C/A polymorphism) within exon 2 of the IL-25 gene (13), which may be associated with immune system-related disease. Therefore, according to this explanation, it seems that the polymorphism may be associated with MS.

2. Objectives

Thus, the main aim of this study was to evaluate the association between c424C/A polymorphism within the IL-25 gene with MS in an Iranian population.

3. Materials and Methods

3.1. Subjects

In this case-control study, according to the study of Arababadi et al. (1) and using sample-size estimation we compared two binomial independent proportions with the following equation:

$$n_1 = \left[\frac{Z_{1-\frac{\alpha}{2}} \times \sqrt{\bar{p}\bar{q}\left(1+\frac{1}{k}\right)} + Z_{1-\beta} \times \sqrt{p_1 q_1 + \frac{p_2 q_2}{k}}}{\Delta} \right]^2 \quad (1)$$

$$n_1 = \mathrm{kn}_1$$

Where significance level was 0.05, the expected power was considered 90%, $P_1 = 1\%$ (frequency of AA genotype in MS patients), $P_2 = 17\%$ (frequency of AA genotype in healthy controls), K = 1 (sample size was considered equal in both groups), and $\Delta = 15\%$ (the minimum difference in frequency of AA genotype in the two groups, which was considered clinically important). Based on the above parameters, sample size was determined as 75 subjects in either group. In this study, 80 patients with MS were recruited for blood sampling yet according to the inclusion and exclusion criteria, 74 patients with relapse and remitting MS (RRMS) and 75 healthy controls were finally enrolled during August 2013 to September 2014. "Blood samples were collected from consecutively admitted patients to the Sina hospital, the main referral hospital for MS patients from all provinces of Iran. Age and gender matched control samples were also collected from the Tehran Blood Transfusion Services.

The occurrence of MS was confirmed based on McDonald's criteria (Diplopia, blurry vision, finger pruritus and disability) (14) and brain Magnetic Resonance Imaging (MRI) results by an expert neurologist, according to clinical and preclinical findings. Having at least one Central Nervous System (CNS) demyelinating event and being at least 18 years old to give informed consent were considered as the main inclusion criteria. The two groups were matched in terms of age and sex and subjects with genetic diseases, diabetes, autoimmune disease and smokers were excluded from the study.

Informed consent was filled out by all participants and this study was approved by the ethics committee of Rafsanjan University of Medical Sciences (RUMS) with number 9/1077 on the 15th of July 2013. Regarding ethical considerations, patients' data were collected by their nurses and the data and blood samples were trans-located to the laboratory without names. The patients were aware of the purpose of the research. The authors declared that they kept the patients' data private.

3.2. DNA Extraction, Polymerase Chain Reaction Amplification and Restriction Fragment Length Polymorphism

DNA was purified from peripheral blood immune cells of participants using a commercial kit (Cinnaclon, Tehran, Iran), according to the manufacturer's instructions. The PCR amplification of exon 2 of the IL-25 gene and RFLP (using BsrFI restriction enzyme) was performed according to the study of Buning and et al. (13).

3.3. Statistical Analysis

Numerical variables are presented as Mean ± SD, while categorical variables are summarized by absolute frequencies and percentages. Continuous variables were compared using independent two-sample t-test, and categorical variables were compared using the chi-square test across the two study groups. One-sample Kolmogorov-Smirnov test was applied to test for normal distribution in cases and controls. No statistically significant violation of normality was met.

The logistic regression model was established to estimate the Odds Ratio (OR) and 95% Confidence Interval (CI) of MS development in patients with the AC and AA compared to patients with the CC genotype. For the statistical analysis, the SPSS version 18.0 software for windows (SPSS Inc., Chicago, IL) was used. All P values were two-tailed with statistical significance defined by $P \leq 0.05$. The results also revealed 1 to 2% missing values in the evaluated genotypes and alleles. Thus, the statistical analysis was performed on the valid data.

4. Results

The age and gender characteristics of the participants are presented in Table 1. The results revealed that eight (11.3%) out of 74 patients with MS had the AA genotype, while 16 (22.5%) had AC and 47 (66.2%) had CC genotypes (Table 2). The results also showed that 8 (11.4%), 18 (25.7%) and 44 (62.9%) of the healthy controls had the AA, AC and CC genotypes, respectively. The chi-square test revealed that the frequency distribution of the genotypes was not statistically different between patients with MS and the controls (P = 0.901) (Table 2).

Table 1. Comparison of Age and Frequency Distribution of Gender in Patients With Multiple Sclerosis and Healthy Controls

Variables	MS Patients (n = 74)	Healthy Controls (n = 75)	P Value
Age, y[a]	36.66 ± 9.57	34.67 ± 7.78	0.267
Gender[b]			
Male	17 (23)	23 (30.7)	0.289
Female	57 (77)	52 (69.3)	

Abbreviation: MS; Multiple Sclerosis.
[a]Values are expressed as Mean ± SD.
[b]Values are expressed as No. (%).

Table 2. Comparison of Frequency Distribution of Genotypes and Alleles of C424c/A Polymorphism Within the IL-25 Gene in Patients with Multiple Sclerosis and Healthy Controls[a]

Variables	MS Patients (n = 74)	Healthy Controls (n = 75)	P Value
Genotypes			0.901
AA	8 (11.3)	8 (11.4)	
AC	16 (22.5)	18 (25.7)	
CC	47 (66.2)	44 (62.9)	
Alleles			0.728
A	110 (77.5)	106 (75.7)	
C	32 (22.5)	34 (24.3)	

Abbreviation: MS; Multiple Sclerosis.
[a]Values are expended as No. (%) unless otherwise indicated.

According to the logistic regression model, the OR of MS development in patients with AC versus patients with CC genotypes was 0.832 (95% IC: 0.378-1.832) (P = 0.648). The corresponding results for AA versus CC genotypes was 0.936 (95% IC: 0.323-2.710) (P = 0.903). The results also indicated that the frequencies of A and C alleles were 110 (77.5%) and 32 (22.5%), respectively, in the patients with MS and were 106 (75.7%) and 34 (24.3%), respectively, in the healthy controls. The statistical analysis found that the differences in distribution of A and C alleles between groups were not statistically significant (P = 0.728) (Table 2). The OR of MS development in patients with the A allele versus patients with the C allele was 0.907 (95% IC: 0.522-1.574), which did not reach statistical significance level (P = 0.729).

5. Discussion

Multiple Sclerosis is an autoimmune disease, with several immune-related genes involved in its pathogenesis (15). It has been confirmed that Th1 and Th17 responses are the main causes of progression of MS (16). Therefore, Th2 responses, which regulate Th1 and Th17, can improve the clinical and laboratory outcome of the disease (16). It is believed that IL-25, as a Th2 cytokine, can participate in the regulation of immune responses during inflammatory diseases including MS. Accordingly, previous studies suggested that serum levels of IL-25 were decreased in MS patients when compared with the healthy controls (17). It seems that expression of IL-25 is disrupted in MS patients. It has been proposed that polymorphisms within cytokine genes are associated with their expression (18, 19). The authors of this study hypothesized that the polymorphisms within the IL-25 gene may be associated with MS disease; hence, the c424C/A polymorphism within the IL-25 gene was evaluated in this study. The results of our study identified that neither AA, AC and CC genotypes nor A and C alleles were associated with MS in the Iranian population. To the best of our knowledge, this is a unique study, which evaluated the c424C/A polymorphism within the IL-25 gene in patients with MS, yet there is a study, which evaluated this polymorphism in Inflammatory Bowel Disease (IBD), as an inflammatory disease (13). Parallel to our results, researchers have reported that the c424C/A polymorphism within the IL-25 gene was not associated with IBD (13). Interestingly, this study documented that the expression level of IL-25 decreased in IBD patients (13). Again, as mentioned previously, it has also been revealed that expression of IL-25 decreased in MS patients (17). Based on our results and the mentioned study it may be concluded that although IL-25 may play significant roles in the pathogenesis of inflammatory diseases such as MS and IBD, c424C/A polymorphism is not important in the pathogenesis of these diseases. On the other hand, another study on Iranian patients with MS revealed that other polymorphisms, except c424C/A, within IL-25 are associated with MS (17). Thus, it may be concluded that other polymorphisms like 4076A > A, 3672T > TA, 3712G > GA, and 3463C > CA, but not the c424C/A polymorphism, may regulate IL-25 expression. According to our and previous studies it appears that evaluation of the c424C/A polymorphism, as the first report, and lack of evaluation of other polymorphisms within the IL-25 gene as well as lack of evaluation of serum levels of IL-25 are the strong and weak points, respectively, of our study. Additionally, it can be proposed that using a large sample size may be better for understanding the roles of c424C/A polymorphism in the pathogenesis of MS.

According to the earned results of our investigation it can be hypothesized that c424C/A polymorphism within the IL-25 gene is not associated with MS and cannot be considered as a risk factor for MS development.

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Footnote

Author's Contribution: Lida Zare: performed the experiments, Mahmood Sheikh Fathollahi: did the statisti-

cal analysis; Mohammad Kazemi Arababadi: helped with the project and writing of the article; Ali shamsizadeh: helped with the design of the project; Behnam Daneshpajouh: selected the patients and facilitated the sampling; Nahid Zainodini: helped with the project, Mohammad Allahtavakoli: designed the project and followed the experimental process and also the writing of the paper.

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