Stromal Cell Derived Factor-1 Genetic Variation at Locus 801 in Patients with Myasthenia Gravis

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ABSTRACT

Background: Myasthenia gravis (MG) is the most common disorder of neuromuscular junction in which autoantibodies develop against nicotinic acetylcholine receptor for unknown reasons. The association of immunomodulator genes with different autoimmune disease has been studied in recent years. **Objective:** The aim of this study was to investigate correlation between a genetic variation in Stromal Cell Derived Factor-1 (SDF1) and susceptibility to MG in an Iranian population. **Methods:** Genotyping of SDF1 at position 801 G/A was performed by Polymerase Chain Reaction-Restriction Length Polymorphism (PCR-RFLP) in 87 patients with confirmed myasthenia gravis and 261 normal control subjects. **Results:** No statistically significant differences were observed in the frequencies of genotypes and alleles between patients and controls ($p>0.05$). Furthermore, no significant differences in the genotype distribution were found between the cases with different stages ($p>0.05$). **Conclusion:** Our data suggest that the SDF1 gene polymorphism at position 801 G/A is not associated with myasthenia gravis.

Keywords: Polymorphism, Myasthenia Gravis, SDF1

INTRODUCTION

Myasthenia gravis (MG), the autoimmune disease characterized by muscle weakness or dysfunction, is generally believed to be triggered by binding auto-antibodies to the nicotinic acetylcholine receptor at the neuromuscular junction. Although autoimmune initiating factors in myasthenia gravis are still under debate, it is generally accepted that autoreactive lymphocytes are the main initiators of the adverse complications (1). As a small chemokine, Stromal Cell Derived Factor-1 (SDF-1) (also known as CXCL12) controls trafficking, homing and maturation of B and T lymphocytes (2) and has been suggested, in animal model, to have an essential role in the development of immune

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related disorders including autoimmune diseases (3). Up-regulation of SDF-1 and its selective receptor (CXCR-4) has been observed in rheumatoid arthritis (4) and inflammatory component of allergic airway disease (5). SDF-1 is also one of the effective elements in the development of autoreactive B cells in murine model of lupus (6) as well as non obese diabetic (NOD) mice (3,7).

Human SDF1 gene is located on chromosome 10q11 with a nucleotide transition from G to A at position 801 in 3-untranslated region (8,9). This single nucleotide polymorphism (SNP) has been indicated to be associated with early onset of type1 diabetes (IDDM) as well as other immune related diseases including AIDS and cancer (9-13).

The aim of present study was to investigate the probable association of this genetic variation with myasthenia gravis in an Iranian population.

MATERIALS AND METHODS

Subjects. A total of 87 Iranian patients with myasthenia gravis, 34 male and 53 female, with mean age of 35.3 ± 12.2 years consisted our study group. 261 healthy individuals (52 ± 15 years, 81 male and 181 female) with no family history of autoimmune diseases or malignancy from the same area in the south of Iran were also recruited as control group. Table 1 illustrates the clinicopathological characteristics of the patients with myasthenia gravis (MG).

Table 1. Clinicopathological characteristics of the patients with Myasthenia Gravis.

Characteristics		Value	Percent
Number of patients		87	-
Age, Years (Mean ± SD)		35.3 ± 12.2	-
Male/Female		34/53	-
Stages	I	8	9.2
	II	60	69.0
	III	7	8.0
	IV	12	13.8
Duration of the disease, Years (Mean ± SD)		6.2 ± 3.1	-
Edrophonium test result	Positive	76	87.4
	Negative	1	1.1
	Missing	10	11.5
MG Crisis	No MG Crisis	66	75.9
	One MG Crisis	12	13.8
	Two MG Crisis	9	10.3
Thymectomy	Positive	75	86.2
	Negative	2	2.3
	Missing	10	11.5

Sixty patients (69%) were in stage II of the disease. Edrophonium test was performed for 77 patients; out of them 76 showed a positive result. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences and blood samples (approximately 7 ml) were collected from individuals after informed consent was obtained.

DNA Extraction and Genotyping. DNA was extracted from anticoagulant-treated peripheral blood by using salting-out method (14). For genetic analyses, Polymerase Chain Reaction (PCR) was performed in order to amplify a fragment of 302 bp around position 801 in SDF1 gene using the primer set: F (5′-CAG TCA ACC TGG GCA AAG CC-3′) and R (5′-AGC TTT GGT CCT GAG AGT CC-3′) according to previously published protocol (9). The amplified products were then digested overnight with MSPI (HpaII) restriction Enzyme (Fermatas, Lithuania). The enzyme had the ability to cut 302 bp bands when G allele was present. After electrophoresis on 3% agarose gel, genotypes were determined by observing only one 302 bp fragment (representing the A allele) or two fragments of 202 and 100 bp (representing the G allele) (Figure 1).

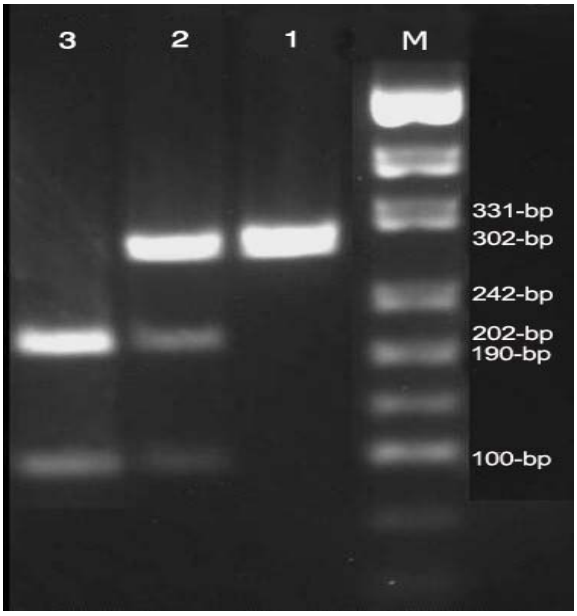

Figure 1. Results of Restriction Fragment Length Polymorphism (RFLP) for detection of SDF1 gene variation at locus 801. *MSP*I (*Hpa*II) restriction reaction left 302 bp fragment uncut when A allele was present (lane 1), but digested into 202 bp and 100 bp when G allele was preset (lane 3). A heterozygote sample with three bands has been run in lane 2. M: molecular size marker.

The result of RFLP tests was verified by automated DNA sequencing using Big Dye Terminator v3.1 sequencing chemistry/protocol and ABI 310 sequencing analyzer (Both from ABI, United states) (Figure 2).

Figure 2) ABI 310 cycle sequence analyzing of SDF1 PCR product using reverse (R) primer. Heterozygosity at locus 801 in SDF1 has been verified for a case who has already been detected to be heterozygous by *MSP*I (*Hpa*II) Restriction Fragment Length Polymorphism (RFLP) reaction.

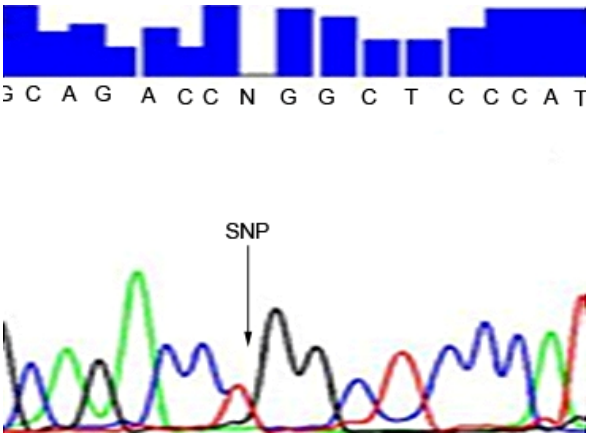

Figure 2. ABI 310 cycle sequence analyzing of SDF1 PCR product using *reverse (R)* primer. Heterozygosity at locus 801 in SDF1 has been verified for a case who has already been detected to be heterozygous by *MSP*I (*Hpa*II) Restriction Fragment Length Polymorphism (RFLP) reaction.

Statistical Analysis. SPSS software version 11.5 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis and statistical significance was defined at p-value less than 0.05. The test for Hardy-Weinberg Equilibration was performed by using Arlequin software package version 3.1 (15).

RESULTS

We investigated allele and genotype frequencies at position 801 G/A in SDF1 gene among MG patients and a healthy control group. The distribution of genotypes were not deviated from Hardy-Weinberg Equilibration as investigated by Arlequin 3.1 (15) in both patients and control group ($p>0.05$).

The distribution of genotypes and alleles are illustrated in Table 2. The mutant allele A was observed with nearly the same frequency among the patients and control subjects (49/174 (28.2%) and 137/522 (26.2%), respectively; $p=0.67$). Wild type genotype GG was the most frequent genotype in both patients and control group (45/87 (51.7%) and 144/261 (55.2%) respectively) and no significant differences were found in the distribution of different genotypes between study populations ($p=0.84$, Table 2).

The distribution of investigated genotypes in different stages of the disease was then investigated. Statistical analysis also revealed no significant association between genotype frequencies of SDF1 gene and different stages of the disease ($p>0.05$)

DISSCUSION

By recruiting the leukocytes to the site of inflammation, SDF-1 has been hypothesized to play a crucial role in the pathogenesis of autoimmune diseases (2). SDF-1 is believed to be able to recruit CXCR-4^+ T and B lymphocytes to inflammatory sites and lymphoid organs, and consequently lead to the production of autoantibodies against nicotinic acetylcholine receptor at the neuromuscular junction (1,2). Observing the critical role of

the pair SDF-1/CXCR-4 in autoimmune disorders has led to in-vivo experiments indicating that blocking this interaction is a suitable approach to handle autoimmune diseases (16).

Table 2. The frequencies of genotypes and alleles at position 801 in SDF-1 gene in patients with myasthenia gravis and control group.

Loci			Myasthenia Gravis patients	Control group	P value
SDF1 801 G/A	Genotypes	GG	45 (51.7%)	144 (55.2%)	0.84
		GA	35 (40.2%)	97 (37.2%)	
		AA	7 (8%)	20 (7.6%)	
		Total (N)	87 (100%)	261 (100%)	-
	Alleles	G	125 (71.8%)	385 (73.8%)	0.67
		A	49 (28.2%)	137 (26.2%)	
		Total (2N)	174 (100%)	522 (100%)	-

In this study which, to our knowledge, is the first to investigate SDF1 gene in MG, alleles and genotypes of SDF1 at position 801 G/A were investigated. The data indicated that G and A alleles were not differentially distributed between MG patients and control group. There was also no association between SDF1 801 genotypes and the stages of the disease. In consistent with these findings, other investigations indicated that SDF1 gene polymorphism is not associated with autoimmune thyroid disease in Japanese (17) and with systemic lupus erythematous in Mexican individuals (18). Although a study in French population suggested that SDF1 801 A allele is associated with the early onset of type1 diabetes (10), another study among Japanese was not able to find such association (19).

Despite such negative results in autoimmune diseases, previously published data from our group revealed that SDF1 801 gene polymorphism may be considered as a factor increasing the susceptibility to several types of cancer including lung, breast and head and neck carcinomas (12,20,21). Similar studies by other groups on prostate and oral cancers were also in consistence with these findings (11,22). Investigations also provided evidences that this genetic variation may delay the onset of AIDS by affecting the SDF-1 role as an innate barrier against propagation of HIV-1 virus (9,23).

Collectively, it seems that SDF1 gene polymorphism at position 801 is not as important in autoimmune diseases as in cancers as well as HIV infection. However, it should be noted that single SNP studies might be miss-leading when no association is observed. Further investigations, specially, multi-SNP analysis are accordingly suggested to verify the observed data in the field of autoimmune diseases.

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