INTEGRIN SUBUNIT ALPHA 4 (ITGA4) VARIANT IS ASSOCIATED WITH RELAPSING-REMITTING MULTIPLE SCLEROSIS IN AN IRANIAN POPULATION

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ABSTRACT

Introduction*: Multiple sclerosis (MS) is an inflammatory demyelinating disorder of central nervous system (CNS). As a multifactorial disorder, several genetics and environmental factors contribute in its pathogenesis. Among them are genetic variants in the integrin subunit alpha 4 (ITGA4) gene. The encoded protein has been shown to assist in migration of leukocytes across the blood brain barrier participating in the pathogenesis of neuroinflammatory disorders.*

Materials and methods*: The current case-control association study enrolled 410 unrelated MS patients and 477 healthy matched controls. The rs1143676 within ITGA4 gene, which leads to an arginine to glutamine transversion, was genotyped in all study participants using tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR).*

Results*: The rs1143676 polymorphism showed significant differences in allele and genotype frequencies between the MS patients and healthy subjects. In addition, genotypes were risk associated in dominant and codominant models.*

Conclusion*: The rs1143676 polymorphism is associated with MS risk in Iranian population. Future studies are needed to evaluate its significance in MS pathogenesis in other populations. These results are in agreement with the supposed biological role of ITGA4 in immune cell trafficking in the CNS.*

Keywords*: multiple sclerosis, ITGA4, integrin, polymorphism.*

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Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of central nervous system with several genetics and environmental factors contributing in its occurrence[1]. The usual clinical course of MS consists of several years' duration of relapses and remissions of neurological signs (relapsing–remitting MS [RRMS]), which results in a state of progressive disability (secondary progressive MS [SPMS]). Yet, in a subset of patients a stable worsening situation (primary progressive MS [PPMS]) occurs[2]. T helper-1 (Th1)-type skewing of the immune response towards proinflammatory cytokines has been shown to participate in MS pathogenesis via T cell mediated destruction of myelin sheath[3]. In addition, the role of adhesion molecules in the pathophysiology and treatment of MS has been noted for more than a decade[4,5]. More specifically, α4β1 integrin, also known as Very Late Antigen 4 (VLA4) has been shown to assist in migration of leukocytes across the blood brain barrier. In addition, elevated expression of α4 integrin has been shown in MS patients compared with controls, while its suppression has been associated with response to immunomodulatory treatments[6]. The association between single nucleotide polymorphisms (SNPs) in the gene encoding α4 integrin (ITGA4) and susceptibility to MS has been assessed previously[6].

Among its genetic variants is the rs1143676 which leads to an arginine (CGG) to glutamine (CAG) transversion at amino acid position 844 in exon 24[7]. This SNP has been shown to be associated with MS risk in Slovaks[7] but not in Italian MS patients[8]. Considering the putative role of ITGA4 in the pathogenesis of MS as well as patients' response to current treatments, we aimed at evaluation of the association between an SNP within its coding region and MS risk in an Iranian population.

Materials and methods

Patients

The current case-control association study enrolled 410 unrelated patients with sporadic RRMS from Tehran Hospitals and MS society of Iran and 477 healthy matched controls. Control group consisted of healthy volunteers without MS or other inflammatory demyelinating diseases that were matched with patients in the terms of sex, age-distribution and ethnic background. RRMS has been diagnosed in patients by specialized neurologists based on the revised McDonald criteria[9]. Written informed consent was acquired from participants. Patients' clinical and demographic data such as age, sex, disease duration, age at onset and disease severity based on the Expanded Disability Status Scale (EDSS)[10] have been collected through questionnaires, interviews and physical examinations. The local ethical committee approved the study.

Genotyping

Genotyping of rs1143676 was carried out using tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR). PCR was performed in a FlexCycler (Analytik Jena, Germany) system using Taq 2x red master mix (Ampliqon, Denmark) and specific primers whose sequences are listed in Table 1. Primers were designed using the primer 1 software[11]. The PCR program consisted of an initial denaturation at 95 ºC for 5 minutes, and subsequent 35 cycles of 95 ºC for 45 seconds, 57 ºC for 35 seconds, and 72 ºC for 45 seconds, with the final extension of 72 ºC for 5 minutes. Ten percent of samples were sequenced by using ABI 3730xl DNA analyzer (Macrogen, Korea) to evaluate T-ARMS-PCR results.

Statistical analysis

Statistical Package for Social Science (SPSS) software version 20 (SPSS Inc., Chicago, IL) was used for statistical analyses. The $\chi 2$ test was applied for evaluation of goodness of fit to the Hardy-Weinberg equilibrium in each study group as well as comparison of genotype and allele frequencies between patients and healthy subjects. The relative risk conferred by each genotype was calculated by odds ratio (OR) and 95% confidence intervals (CI) in SNPStats online programme[12]. Differences were regarded as significant when $P<0.05$.

Primer sequence	T_m	Annealing temperature	PCR product size (bp)
Forward inner primer (G allele): ATGCAGACCTTGAAAGG-CATAGTACG	65 °C	57 °C	200 bp (G allele)
Reverse inner primer (A allele): AGCCTCTTATCAGTCTTG-GACAAGACCT	65 °C		298 bp (A allele)
Forward outer primer: GTTTCTGCCACAGACATT-TAATCACTCC	65 °C		444 bp (two outer primers)
Reverse outer primer: TGTTCCTTCCACGCAA-GAATACTACCTA	65°C		

Table 1: Primer sequences and PCR conditions.

Results

The number, gender, age and disease characteristics of all study participants are shown in Table 2. The control group included 477 healthy blood donors who were age and gender-matched with patients. The genotype distribution rs1143676 polymorphism in the controls and patients were in accordance with Hardy-Weinberg equilibrium ($P>0.05$). The genotyping results using T-ARMS-PCR were in complete agreement with the sequencing results (data not shown). The allele and genotype frequencies of the rs1143676 polymorphism for the MS patients and control group are presented in Table 3. The rs1143676 polymorphism showed significant differences in allele and genotype frequencies between the MS patients and healthy subjects. In addition, genotypes were risk associated in dominant model (P value= 0.0082 and odds ratio (95% CI) = 0.69 (0.53-0.91)) and codominant model (P value= 0.02 and odds ratio (95% CI)= 0.72 (0.54-0.95) for AG genotype and P value= 0.05 and odds ratio (95% CI)= 0.63 (0.39-1.00) for GG genotype).

Variables	MS patients	Controls
Female/Male [no. (%)]	308(75%)/102(25%)	350(73.37%)/127(26.6%)
Age (mean ± SD, Y)	37.4 ± 5.2	36.9 ± 4.6
Age range (Y)	17-69	19-63
Age of onset (mean ± SD, Y)	31.36 ± 2.4	-
Relapsing-remitting course (no. %)	410 (100%)	-
Disease duration (mean ± SD, Y)	6.1 ± 3.1	-
EDSS[a] (mean ± SD)	2.72 ± 2.3	-

Table 2: Demographic and clinical profiles of MS patients and healthy controls.
a; Expanded disability status scale of Kurtzke.

Model	Genotype	Cases	Controls	ORa (95% CI)	P value
Allele	A vs. G	598 (73%)	640 (67%)	0.76 (0.62-0.93)	0.008
		222 (27%)	314 (33%)		
Codominant	A/A	223 (54.4%)	217 (45.5%)	1.00	
	A/G	152 (37.1%)	206 (43.2%)	0.72 (0.54-0.95)	0.02
	G/G	35 (8.5%)	54 (11.3%)	0.63 (0.39-1.00)	0.05
Dominant	A/A	223 (54.4%)	217 (45.5%)	1.00	0.008
	A/G-G/G	187 (45.6%)	260 (54.5%)	0.69 (0.54-0.91)	
Recessive	A/A-A/G	375 (91.5%)	423 (88.7%)	1.00	0.17
	G/G	35 (8.5%)	54 (11.3%)	0.73 (0.47-1.14)	
Overdominant	A/A-G/G	258 (62.9%)	271 (56.8%)	1.00	0.064
	A/G	152 (37.1%)	206 (43.2%)	0.77 (0.59-1.01)	

Table 3: Genotype and allele frequencies are significantly different between MS patients and healthy controls.
[a]OR: odds ratio

Discussion

In the present study we have shown the association between a single polymorphism in ITGA4 gene and MS risk in an Iranian population. ITGA4 codes for one of the α4β1 integrin subunits, which participates in leukocyte adhesion and migration via the blood-brain barrier[13]. Consequently, it might be involved in the pathogenesis of neuroinflammatory disorders such as MS. The association between other ITGA4 SNPs and MS has been assessed previously in other populations. Ďurmanova et al. have shown rs1143676 as an independent genetic risk factor for MS development in Slovak patients[7].

They have demonstrated higher frequencies of the AG genotype in MS patients compared to the healthy subjects[7]. However, Andreoli et al. did not observe any significant difference in rs1143676 variants between MS patients and healthy subjects[8]. In the current study although we determined significant difference in allele and genotype frequencies between MS patients and healthy subjects, A/G genotype was presented lower in MS patients compared with controls. O'Doherty et al. have analyzed association of 13 SNPs in this gene (not including rs1143676) with the susceptibility to MS in two distinctive populations from the Basque Country in northern Spain and Nordic countries and have shown a weak association between the ITGA4 promoter SNP rs1449263 and MS[6]. In addition, SNP rs6721763 of the ITGA4 has been shown to considerably affect disease severity in Cypriot and Greek MS patients[14]. The role of ITGA4 in the pathogenesis of MS has been further highlighted by Miller et al. study that have shown the efficacy of Natalizumab, a recombinant anticlonal antibody against α4 integrins in the treatment of MS[15].

Using the HaploReg v4 software which is a tool for detection of annotations of the noncoding genomic variants[16], we found that rs1143676 is a missense variant changing the binding site for Nuclear respiratory factor 1 (NRF1). NRF1 is as a transcription factor that induces the expression of a multiple nuclear genes necessary for mitochondrial biogenesis and function, such as mitochondrial respiratory complex subunits, heme biosynthetic enzymes, and regulatory factors participated in the replication and transcription of mitochondrial DNA[17]. Abnormal regulation of NRF1 and its targets has been suggested as a possible underlying mechanism for human neurodegenerative diseases which can be exerted via dysregulation of various mitochondrial and extra-mitochondrial functions[17]). So the participation of rs1143676 variant in the pathogenesis of MS might be linked with its function in changing the affinity for this transcription factor. In addition, the rs1143676 G variant has been suggested to change the α4 subunit conformations resulting in greater affinity for its ligand VCAM-1[7]. Functional studies are needed to evaluate the role of this variant in the pathogenesis of MS.

In brief, data regarding the contribution of the rs1143676 variants in the pathogenesis of MS is controversial in different populations. The presence of numerous non-replicated results in the molecular

genetics of MS has been noted previously. This fact might demonstrate both diverse frequencies in diverse populations and genetic heterogeneity[8]. Consequently, future studies are needed in other ethnic groups to evaluate the association this variant and the risk of developing MS.

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