A Nonsynonymous Functional Variant of the *ITGAM* Gene Is Not Involved in Biopsy-proven Giant Cell Arteritis

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ABSTRACT. ***Objective.*** To investigate whether a functional integrin alpha M (*ITGAM*) variant is involved in susceptibility to and clinical manifestations of giant cell arteritis (GCA).

Methods. A Spanish cohort of 437 white patients with biopsy-proven GCA and 1388 healthy controls were genotyped using the TaqMan allele discrimination technology.

Results. No association was observed between *ITGAM* rs1143679 and GCA (p = 0.80, OR 0.97). Similarly, subphenotype analyses did not yield significant differences between the case subgroups and the control set or between GCA patients with or without the main specific features of GCA.

Conclusion. Our results suggest that the *ITGAM* rs1143679 variant does not play an important role in the pathophysiology of GCA. (J Rheumatol First Release Oct 1 2011; doi:10.3899/jrheum.110685)

Key Indexing Terms:
GIANT CELL ARTERITIS TEMPORAL ARTERITIS
SINGLE-NUCLEOTIDE POLYMORPHISM ITGAM rs1143679

Integrin alpha M (*ITGAM*) encodes the alpha subunit of the αMß2-integrin. A nonsynonymous single-nucleotide polymorphism (SNP) at exon 3 of the *ITGAM* gene, rs1143679, produces a functional modification in the protein by changing the 77th amino acid residue arginine to histidine (R77H). This genetic variant has been reported to be one of the signals most highly associated with systemic lupus erythematosus (SLE), and the most likely causal polymorphism within the *ITGAM* region in several populations of different ethnicity[1,2]. There is evidence suggesting that *ITGAM* rs1143679 is also involved in the pathogenesis of systemic sclerosis (SSc)[3].

Giant cell arteritis (GCA) is a complex polygenic autoimmune disease characterized by inflammatory lesions of blood vessels, mainly involving medium- and large-size arteries. It

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Supported by Fondo de Investigaciones Sanitarias, grants PI06-0024 and PS09/00748 (Spain), and by RETICS Program RD08/0075 (RIER) from Instituto de Salud Carlos III (ISCIII). F.D. Carmona was supported by Consejo Superior de Investigaciones Científicas (CSIC) through the JAE-DOC program.

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Accepted for publication July 21, 2011.

represents the most common vasculitis in elderly individuals from Western countries[4].

Accumulation of polyclonal T lymphocytes in the arterial lesions has been observed in patients with GCA, and it was hypothesized that integrin-type adhesion receptors may play a key role in this process[5]. Our objective was to determine whether the *ITGAM* functional polymorphism rs1143679 is implicated in the genetic susceptibility and specific clinical features of GCA.

MATERIALS AND METHODS

Study population. A Spanish cohort of 437 white patients with GCA and 1388 unrelated healthy controls recruited in the same geographic areas and matched by age, sex, and ethnicity was analyzed. All patients had a positive temporal artery biopsy[6] and fulfilled the 1990 American College of Rheumatology classification criteria for GCA[7]. Table 1 shows their main clinical characteristics, as defined[6,8].

Written informed consent from subjects and approval of the local ethical committees were obtained.

Genotyping methods. DNA was extracted from peripheral blood cells using standard procedures. All participants were genotyped for the *ITGAM* variant rs1143679 using the TaqMan allele discrimination assay technology (assay ID: C___2847895_1_) in a 7900HT Fast Real-time polymerase chain reaction system, both from Applied Biosystems (Foster City, CA, USA).

Statistical analysis. The overall statistical power of the analysis was 70% to detect associations, with OR = 1.3 at the 5% significance level, according to Power Calculator for Genetic Studies 2006 software (Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA; Website: http://www.sph.umich.edu/csg/abecasis/CaTS/). Plink (v1.07; Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; Website: http://pngu.mgh.harvard.edu/purcell/plink/) was used to perform 2×2 contingency tables and chi-square test and/or Fisher's exact test. OR and 95% CI were obtained according to Woolf's method. P values < 0.05 were considered statistically significant.

Table 1. Main clinical features of 437 white Spanish patients with biopsy-proven giant cell arteritis (GCA). The control group included in the analysis with this GCA cohort was composed of 825 women (59.4%) and 563 men (40.6%).

Feature	Number (%)
Age at diagnosis, yrs, median (IQR)	75 (69–79)
Women	288 (65.8)
Men	149 (34.2)
Headache	348 (83.9)
Abnormal temporal artery on examination	256 (63.7)
Polymyalgia rheumatica	208 (48.6)
Jaw claudication	184 (43.6)
Arm-leg claudication	26 (6.2)
Visual ischemic manifestations*	111 (26.1)
Permanent visual loss	49 (11.6)
Stroke	19 (4.6)
Severe ischemic manifestations**	217 (51.3)
Irreversible occlusive disease†	82 (19.7)

* Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia[6]. ** At least one of the following: visual manifestations, cerebrovascular accidents (stroke and/or transient ischemic attacks), jaw claudication, or limb claudication[6]. † At least one of the following: permanent visual loss, stroke, and/or occlusive disease in the extremities[8]. IQR: interquartile range.

RESULTS

No divergence from Hardy-Weinberg equilibrium was observed ($p < 0.05$) in controls or cases, and control allele frequencies were similar to those reported in the white European population[9].

Table 2 shows the genotype and minor allele frequencies of the control cohort and the different case sets. No significant differences were detected between the allele frequencies of the patients with biopsy-proven GCA and the control group ($p = 0.80$, OR 0.97). To test for a possible gender-specific association, the female patients with GCA (n = 288) were compared with female controls (n = 825). No association of this polymorphism was observed in women ($p = 0.65$, OR 1.06).

GCA patients were further subdivided into those positive for polymyalgia rheumatica (PMR), visual ischemic manifestations (VIM), severe ischemic manifestations (SIM), and irreversible occlusive disease (IOD). However, analysis of data stratified according to the different phenotypes of clinical expression of this vasculitis revealed no significant heterogeneity between the GCA subgroups and the control population (PMR-positive vs controls, $p = 0.71$, OR 0.95; VIM-positive vs controls, $p = 0.58$, OR 0.89; SIM-positive vs controls, $p = 0.75$, OR 0.95; IOD-positive vs controls, $p = 0.61$, OR 0.89). Similar negative results were also observed when GCA patients with and without specific clinical disease features were compared to one another (Table 3). Finally, no significant deviation in genotype frequencies was evident in any of the comparisons above (data not shown).

DISCUSSION

Shared immunological pathways have been proposed to underlie different autoimmune conditions[10]. *ITGAM* rs1143679 has been associated with other autoimmune diseases, including SLE and SSc[1,2,3]. Previous studies in SLE predicted that the R77H substitution caused by this nonsynonymous SNP alter the structure and function of this integrin, and may contribute to endothelial injury and impairment of immune complex clearance in patients with SLE[1,11,12]. Because integrin molecules have been proposed to be crucial in the vasculopathy of GCA[5], *ITGAM* represented a good susceptibility candidate locus for investigation. The power of this study and the differences in the effect magnitude between SSc and SLE make it difficult to draw definitive conclusions. However, considering the well-defined clinical cohort we analyzed, and that our statistical analysis had enough power to detect a possible moderate signal, it is unlikely that this gene may play a relevant role in GCA.

Recent studies in rheumatoid arthritis (RA) also described a lack of association with rs1143679[13]. It could be speculated that common pathways involving the αMß2-integrin were restricted to specific subgroups of patients with RA and/or GCA that share more similarity with SLE and SSc. For instance, variation in the *IRF5* gene has been associated with a subgroup of RA cases (not clearly delimited to date) with a

Table 2. Genotype and allele distribution of *ITGAM* rs1143679 in patients with biopsy-proven giant cell arteritis (GCA) and healthy controls.

Sample	Genotype, N (%) AA	GA	GG	MAF, %	Allelic Model p	OR (95% CI)
Controls, n = 1388	36 (2.59)	341 (24.57)	1011 (72.84)	14.88		
GCA, n = 437	8 (1.83)	111 (25.40)	318 (72.77)	14.53	0.801	0.97 (0.78–1.21)
PMR, n = 208	3 (1.44)	53 (25.48)	152 (73.08)	14.18	0.710	0.95 (0.70–1.27)
VIM, n = 111	1 (0.90)	28 (25.23)	82 (73.87)	13.51	0.582	0.89 (0.60–1.33)
SIM, n = 217	3 (1.38)	56 (25.81)	158 (72.81)	14.29	0.747	0.95 (0.71–1.27)
IOD, n = 82	1 (1.22)	20 (24.39)	61 (74.39)	13.41	0.608	0.89 (0.59–1.41)

p values and OR were obtained from comparison of the different disease groups versus controls. * OR for the minor allele. MAF: minor allele frequency; PMR: polymyalgia rheumatica; VIM: visual ischemic manifestations; SIM: severe ischemic manifestations; IOD: irreversible occlusive disease.

Table 3. Genotype distribution and minor allele frequency (MAF) of *ITGAM* rs1143679 in patients with biopsy-proven giant cell arteritis (GCA) according to presence or absence of specific disease manifestations.

Manifestation	With Manifestations Genotypic Frequencies	MAF, %	Without Manifestations Genotypic Frequencies	MAF, %	Allelic Model p	OR (95% CI)*
Polymyalgia rheumatica	3/53/152	14.18	5/57/158	15.23	0.67	0.92 (0.63–1.34)
Visual ischemic manifestations	1/28/82	13.51	7/81/226	15.13	0.56	0.88 (0.56–1.36)
Severe ischemic manifestations	3/56/158	14.29	5/52/149	15.05	0.75	0.94 (0.64–1.38)
Irreversible occlusive disease	1/20/61	13.41	7/88/239	15.27	0.55	0.86 (0.52–1.41)

* OR for the minor allele.

pattern of association very similar to that described in patients with SLE[14]; no association of *IRF5* rs2004640 and CGGGG insertion/deletion gene polymorphisms in the susceptibility to and clinical expression of GCA was described[15]. It should be noted that, despite not achieving statistical significance, all the OR that we observed were in the same direction; this might suggest a hypothetical role of *ITGAM* in a specific subgroup of patients that could not be categorized using the current definitions to describe the clinical features of GCA. However, the direction of the OR was the opposite of those reported for SLE and SSc[1,2,3] and thus the previous assumption does not seem feasible. Another possibility could be that this gene is only weakly involved in predisposition to GCA, and the lack of high power of our study (e.g., > 90% to detect associations with OR = 1.2) did not make it possible to observe statistical significance. Further studies are needed to clarify whether the nonsynonymous change caused by *ITGAM* rs1143679 is a key event in the development of autoimmunity or if it is only associated with some SLE-related manifestations.

ACKNOWLEDGMENT

The authors thank Sofía Vargas, Sonia García, and Gema Robledo for their excellent technical assistance, and all the patients and controls for kindly providing their essential collaboration. We thank Banco Nacional de ADN (University of Salamanca, Spain) for supplying part of the control material.

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