Lack of Association Between the rs6920220 (G/A) Polymorphism of the 6q23 Region and Biopsy-proven Giant Cell Arteritis

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ABSTRACT. ***Objective.*** Recently, 2 independent studies have identified an association between several single-nucleotide polymorphisms (SNP) located in the 6q23 chromosomal region and rheumatoid arthritis (RA). Like RA, giant cell arteritis (GCA) is also a complex polygenic disease in which more than 1 genetic locus is likely to contribute to disease susceptibility and clinical expression. We analyzed the involvement of the rs6920220 (G/A) polymorphism from the *6q23/TNFAIP3* gene region in susceptibility to GCA.

Methods. Two hundred twenty patients with biopsy-proven GCA and 490 matched controls were assessed. DNA from patients and controls was obtained from peripheral blood. Samples were genotyped for the 6q23 region rs6920220 using a TaqMan allele discrimination assay and by polymerase chain reaction (PCR) amplification. After PCR, the genotype of each sample was attributed automatically by allelic-specific fluorescence using the ABI Prism 7900 sequence detection system.

Results. No significant differences in the genotype distribution between patients with GCA and controls for the rs6920220 (G/A) polymorphism were found. No significant differences were observed when patients with GCA were stratified according to the presence of specific clinical features of the disease such as polymyalgia rheumatica or severe ischemic manifestations or specific visual ischemic complications.

Conclusion. Our results show no involvement of this *6q23/TNFAIP3* gene region SNP in the susceptibility to or clinical expression of GCA. (J Rheumatol First Release March 15 2010; doi:10.3899/jrheum.091142)

Key Indexing Terms:
GIANT CELL ARTERITIS DISEASE SUSCEPTIBILITY *6q23/TNFAIP3*
GENETIC STUDIES GENE POLYMORPHISM

Giant cell arteritis (GCA) is the most common systemic vasculitis in people older than 50 years from Western countries[1]. This granulomatous systemic vasculitis of the large- and medium-size blood vessels is characterized by the involvement of the aorta and especially its cranial branches[1]. Inflammation of the arterial wall and vessel occlusion through fast and concentric intimal hyperplasia leads to the severe ischemic complications observed in patients with this vasculitis[2]. Although there have been advances in the genetic and immunologic understanding of the underlying artery pathogenesis of the disease, the exact etiology of the condition remains unclear.

Genetic contributions to GCA have been established and several genes have been associated with either disease susceptibility[3] or a higher risk of severe ischemic complications[4] in patients with this vasculitis. However, the genetics of GCA, like those of other autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), represent a complex situation in which more than 1 genetic locus is likely to contribute to disease susceptibility and clinical expression[5].

In a recent study, The Wellcome Trust Case Control Consortium (WTCCC) identified several single-nucleotide

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Supported by a grant from Fondo de Investigaciones Sanitarias PI06-0024 (Spain) and in part by RETICS Program, RD08/0075 (RIER) from Instituto de Salud Carlos III, within the VI PN de I+D+I 2008-2011.

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Accepted for publication December 28, 2009.

polymorphisms (SNP) associated with RA ($p = 1 \times 10^{-5}$ to $p = 5 \times 10^{-7}$) in a genome-wide association screen[6]. One of them, the rs6920220 (G/A), was in strong association with RA susceptibility ($p = 5 \times 10^{-6}$) and it was unequivocally replicated (trend $p = 1.1 \times 10^{-8}$) in a validation study[7]. Moreover, in an independent study, Plenge, *et al* disclosed an association of RA with this SNP (rs6920220)[8]. In addition, in a recent replication study, Perdigones, *et al* showed that this SNP rs6920220 was also associated with RA in a Spanish cohort[9]. Similarly, this SNP has also been associated with SLE and type 1 diabetes[10-12]. This SNP maps to 6q23, between the genes oligodendrocyte lineage transcription factor 3 (*OLIG3*) and tumor necrosis factor-α-induced protein 3 (*TNFAIP3*).

Because of the strong association of the SNP rs6920220 (G/A) in the *6q23/TNFAIP3* gene region with susceptibility to several autoimmune diseases, we analyzed for the first time the potential involvement of this polymorphism in the susceptibility of patients with biopsy-proven GCA or in the clinical spectrum of manifestations to this systemic vasculitis.

MATERIALS AND METHODS

Patients. A total of 220 patients diagnosed with biopsy-proven GCA between 1991 and 2007 were initially included in this study. Most of them (n = 128) were diagnosed in the Division of Rheumatology of the Hospital Xeral-Calde (Lugo, Spain). The remaining patients were diagnosed in 2 centers in Madrid (Hospital Clínico San Carlos and Hospital de la Princesa; n = 82) and Granada (Hospital Clínico San Cecílio; n = 10). A control population (n = 490) from the corresponding cities matched by age, sex, and ethnicity with patients with GCA was also studied. Patients and controls gave written informed consent. Ethical committee approval was obtained.

All patients with GCA had a positive temporal artery biopsy showing disruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without giant cells[13].

Severe ischemic complications, mainly strokes in the vertebrobasilar territory, may occur after the onset of corticosteroid therapy. In this regard, strokes have been observed within the first month after GCA diagnosis[14] and visual ischemic events have also been reported to occur within the first 48–72 hours after the onset of corticosteroid therapy[15]. However, severe ischemic complications related to the disease are uncommon in patients treated with corticosteroids for at least 1 month. To encompass the whole spectrum of clinical manifestations directly attributed to GCA, we assessed all the clinical manifestations that occurred in the period from the onset of GCA symptoms to 1 month after the onset of corticosteroid therapy.

Patients with GCA were considered to have polymyalgia rheumatica (PMR) manifestations if they had severe bilateral ache and pain involving the neck, the shoulder, and/or the pelvic girdle, associated with morning stiffness[16]. Patients were considered to have visual ischemic manifestations in the context of GCA if they experienced at least 1 of the following ocular complications: transient visual loss including amaurosis fugax, permanent visual loss, or diplopia[17]. Severe ischemic manifestations were considered to be present if patients with GCA had at least 1 of the following complications: visual ischemic complications, strokes and/or transient ischemic attacks, jaw claudication, or large-artery stenosis of the extremities that caused signs of occlusive manifestations[18].

There were no significant differences in the demographic and clinical features between patients from Lugo with biopsy-proven GCA and those from Madrid or Granada (data not shown).

SNP genotyping. DNA was obtained from peripheral blood mononuclear cells using standard methods. The genotyping of the *6q23/TNFAIP3* gene region G/A (rs6920220) polymorphism was performed using a TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labeled with the fluorescent dyes FAM and VIC. The polymerase chain reaction (PCR) was carried out in a total reaction volume of 5 μl, containing 50 ng genomic DNA as template, 2.5 μl of TaqMan genotyping master mix, 0.25 μl of 20× assay mix, and ddH_2O up to 5 μl of final volume. The amplification protocol used was initial denaturation at 95˚C for 10 min followed by 40 cycles of denaturation at 92˚C for 15 s, and annealing/extension at 60˚C for 1 min. After the PCR, the genotype of each sample was attributed automatically by measuring the allelic-specific fluorescence on the ABI Prism 7900 Sequence Detection System using SDS 2.3 software for allelic discrimination (Applied Biosystems).

Statistical analysis. We used the chi-square test for assessment of Hardy-Weinberg equilibrium. Genotype and allele frequencies were also analyzed by chi-square test. Odds ratios and 95% CI were calculated according to Woolf's method using the Statcalc program (Epi Info 2002, Centers for Disease Control and Prevention, Atlanta, GA, USA). P values < 0.05 were considered statistically significant.

RESULTS

Two hundred twenty patients with biopsy-proven GCA were enrolled. Most were women (n = 148; median age at disease diagnosis 74 yrs; range 52–93 yrs). From the onset of GCA symptoms to 1 month after the onset of corticosteroid therapy, 173 (78.6%) had headache, 103 (46.8%) experienced PMR manifestations, 88 (40.0%) had jaw claudication, and 54 (24.5%) had visual ischemic manifestations. Also, 23 (10.4%) experienced irreversible (permanent) visual loss, 11 (5.0%) had strokes, and 119 (54.1%) fulfilled the definitions for severe ischemic manifestations. As reported[17], most patients (n = 216; 98.2%) had an erythrocyte sedimentation rate > 40 mm/hour.

Influence of the 6q23/TNFAIP3 G/A SNP susceptibility to GCA. A genotyping success rate > 98% in patients with GCA and controls was achieved in this study. No evidence of departure from Hardy-Weinberg equilibrium was observed in controls. Control ratio achieved was 1:2.27. The estimated power of this study for an estimated OR between 1.5 and 2.0 was 60%–98%, for a type I error rate of 0.05.

No significant differences in the genotype and allele frequencies on the *6q23/TNFAIP3* G/A SNP were observed when patients with GCA from Lugo were compared with those from Madrid or Granada. Moreover, the allele and genotype distribution of the polymorphism was similar in controls from the 3 different regions (data not shown).

Table 1 shows the allele and genotype frequencies of the *6q23/TNFAIP3* G/A gene region polymorphism in patients with biopsy-proven GCA and controls. No significant differences in the genotype distribution between patients with GCA and controls for the *6q23/TNFAIP3 G/A* polymorphism were observed.

Influence of 6q23/TNFAIP3 G/A polymorphism in the clinical spectrum of GCA. To determine whether polymorphism of the *6q23/TNFAIP3* gene region might influence the clinical spectrum and severity of GCA, we stratified patients

Table 1. Allele and genotype frequencies of the *6q23/TNFAIP3* gene region polymorphism in patients with biopsy-proven GCA and healthy controls.

6q23 rs6920220	Patients with GCA, n = 216 (%)	Controls, n = 490 (%)	p	OR (95% CI)
A/A	8 (3.7)	20 (4.1)	0.81	0.90 (0.36–2.21)
A/G	67 (31.0)	157 (32.0)	0.79	0.95 (0.67–1.37)
G/G	141 (65.3)	313 (63.9)	0.72	1.06 (0.75–1.51)
A	83 (19.2)	197 (20.1)	0.70	0.95 (0.70–1.27)
G	349 (80.8)	783 (79.9)	0.70	1.06 (0.79–1.42)

GCA: giant cell arteritis.

with biopsy-proven GCA according to the presence/absence of PMR, visual ischemic manifestations, or severe ischemic manifestations. As shown in Table 2, no significant differences in the genotype or allele frequency were found. Moreover, the genotypic distribution did not differ when we excluded from the category of severe ischemic manifestations the patients who presented only jaw claudication but no other severe ischemic complications (data not shown).

DISCUSSION

Our study is the first to determine the potential influence of the rs6920220 (G/A) polymorphism of the *6q23/TNFAIP3* gene region in the susceptibility and phenotypic expression of biopsy-proven GCA. Our data show no association between this polymorphism and disease susceptibility or with specific features of this vasculitis.

The SNP rs6920220 maps to an intergenic region of 6q23, between the *OLIG3* and *TNFAIP3* genes. *OLIG3* is involved in the development and differentiation of neuronal cells[19]. Conversely, the *TNFAIP3* gene encodes a ubiquitin-editing enzyme, A20, that acts as a negative regulator of nuclear factor-κB (NF-κB) in response to TNF and Toll-like receptor, but not interleukin 1ß-induced activation[20,21], and is required for preventing spontaneous inflammation[22]. In contrast, *TNFAIP3*-deficient mice evidence chronic inflammation, cachexia, and premature death[22].

Peripheral expression of tumor necrosis factor-α (TNF-α) has been found to play an important role in the pathogenesis of GCA[23]. Although A20 has a potent antiinflammatory role because it is required for the termination of both TNF and Toll-like receptor-induced NF-κB signaling[20,21], our results, which are based on the largest series of patients with biopsy-proven GCA assessed for genotype analyses, do not confirm an association between the genetic polymorphism of the *6q23/TNFAIP3* gene region and this vasculitis.

It has been postulated that a variety of inflammatory and autoimmune diseases may share a common genetic background. Similarly to RA, environmental and genetic factors seem to contribute to GCA etiology[1,3]. Both conditions are associated with high inflammatory response and share an association with HLA-DRB1*04 alleles[3,24]. Thus the reasons for a negative association with GCA of this 6q23 region SNP (rs6920220), reported to be associated with RA, are unknown[6-9]. Despite having similarities in terms of HLA class II association with RA, the immune-mediated mechanisms characterized by granulomatous infiltrates leading to the vasculitic damage in GCA are different from those observed in RA[2,24].

The lack of association of this *6q23/TNFAIP3* gene region polymorphism with susceptibility to GCA is in keeping with a recent study reported by Dieguez-Gonzalez, *et al*, which did not disclose a significant association between the rs6920220 polymorphism and RA in Spanish patients[25]. Similarly, Scherer, *et al* found no influence of genotypes of this polymorphism on RA severity in Dutch patients[26].

Table 2. Association between 6q23 rs6920220 genotypes and typical disease features in patients with GCA.

Disease Features	6q23 rs6920220	With, n (%)	Without, n (%)	p	OR (95% CI)
PMR	AA	4 (3.9)	4 (3.5)	1.00	1.12 (0.23–5.51)
	AG	32 (31.4)	35 (30.7)	0.92	1.03 (0.56–1.91)
	GG	66 (64.7)	75 (65.8)	0.87	0.95 (0.52–1.74)
	A	40 (19.6)	43 (18.9)	0.84	1.05 (0.63–1.74)
	G	164 (80.4)	185 (81.1)	0.84	0.95 (0.57–1.58)
Visual manifestations	AA	1 (1.9)	7 (4.3)	0.68	0.42 (0.02–3.51)
	AG	18 (33.3)	49 (30.2)	0.67	1.15 (0.57–2.34)
	GG	35 (64.8)	106 (65.4)	0.93	0.97 (0.49–1.95)
	A	20 (18.5)	63 (19.4)	0.83	0.94 (0.52–1.70)
	G	88 (81.5)	261 (80.6)	0.83	1.06 (0.59–1.93)
Severe ischemic manifestations	AA	4 (3.4)	4 (4.0)	1.00	0.84 (0.17–4.13)
	AG	36 (30.8)	31 (31.3)	0.93	0.97 (0.53–1.81)
	GG	77 (65.8)	64 (64.6)	0.85	1.05 (0.58–1.92)
	A	44 (18.8)	39 (19.7)	0.81	0.94 (0.57–1.57)
	G	190 (81.2)	159 (80.3)	0.81	1.06 (0.64–1.76)

GCA: giant cell arteritis; PMR: polymyalgia rheumatica.

An association of SNP rs6920220 with RA has been demonstrated in several studies with an overall OR of 1.23[6-8]. Our study had enough power (60%–98% for p = 0.05) to detect an effect size (OR) between 1.5 and 2.0. However, we found a weaker effect (OR = 0.95) than in other studies. Based on these data, we cannot rule out the possibility that this SNP might exert a weak effect that requires larger sample numbers for detection. Moreover, further investigation in other populations with different genetic backgrounds is needed to fully exclude a role of this polymorphism of 6q23 region in the susceptibility to GCA. In addition, we cannot exclude that other polymorphisms located within the 6q23 region locus might account for susceptibility to GCA.

Our results do not support a major contribution of the rs6920220 polymorphism to the susceptibility to or clinical manifestations of GCA.

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