Lack of Association Between TRAF1/C5 Gene Polymorphisms and Biopsy-proven Giant Cell Arteritis

ORLANDO TORRES, ROGELIO PALOMINO-MORALES, TOMAS R. VAZQUEZ-RODRIGUEZ, SANTOS CASTAÑEDA, INMACULADA C. MORADO, JOSE A. MIRANDA-FILLOY, NORBERTO ORTEGO-CENTENO, ISIDORO GONZALEZ-ALVARO, BENJAMIN FERNANDEZ-GUTIERREZ, JAVIER MARTIN, and MIGUEL A. GONZALEZ-GAY

ABSTRACT. Objective. A novel association with a 100-kb region on chromosome 9 that contains the tumor necrosis factor receptor-associated factor 1 (TRAF1) and C5 genes has been observed in some autoimmune rheumatic diseases, in particular in rheumatoid arthritis. We analyzed the influence of 2 single-nucleotide polymorphisms (SNP) from the TRAF1/C5 region in susceptibility to giant cell arteritis (GCA).

Methods. We assessed 220 patients with biopsy-proven GCA and 410 matched controls. DNA from patients and controls was obtained from peripheral blood. Samples were genotyped for the rs10818488 and rs2900180 TRAF1/C5 gene polymorphisms by polymerase chain reaction, using a predesigned TaqMan allele discrimination assay.

Results. A genotyping rate of 95% was achieved in this series of GCA. No significant differences in the genotype distribution between GCA patients and controls were found for the 2 SNP. GCA patients exhibited a reduced frequency of TRAF1/C5 AA homozygosity (7.6%) compared to controls (12.7%) but the difference was only marginally significant (OR 0.58, 95% CI 0.30–1.11, p = 0.07). The frequency of minor allele T of TRAF1/C5 rs2900180 was also slightly reduced in patients (24.3%) compared to controls (27.8%) (p = 0.19). No significant differences were observed when patients were stratified according to the presence of specific clinical disease features.

Conclusion. Our results showed no influence of rs10818488 and rs2900180 TRAF1/C5 gene polymorphisms in susceptibility to and clinical expression of GCA. (First Release Nov 15 2009; J Rheumatol 2010;37:131–5; doi:10.3899/jrheum.090646)

Key Indexing Terms:
GIANT CELL ARTERITIS
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Giant cell arteritis (GCA) is the most common systemic vasculitis in people older than 50 years from Western countries1. This granulomatous systemic vasculitis of large and medium-size blood vessels is characterized by involvement of the aorta and especially its cranial branches1. Inflammation of the arterial wall and vessel occlusion through rapid and concentric intimal hyperplasia lead to the severe ischemic complications observed in patients with this vasculitis2. Susceptibility to autoimmune disorders may be the result of the interaction of multiple genetic factors that regulate the threshold of autoreactivity. In this regard, GCA is a polygenic disease3 and a number of gene polymorphisms have been associated with either disease susceptibility3 or higher risk of severe ischemic complications4.5. Matrix metalloproteinase-9 and toll-like receptor gene polymorphisms have recently been implicated in the pathogenesis of GCA6,7.

Both GCA and rheumatoid arthritis (RA) are complex polygenic diseases3,8, and several genetic risk factors of susceptibility to RA, such as HLA-DRB1 alleles, tumor necrosis factor (TNF) microsatellites, or interleukin 6 (IL-6) polymorphisms, have also been associated with susceptibility to GCA or to specific clinical features of it9-11. A genome-wide study identified a novel association with a 100-kb region on chromosome 9q33–34 that contains the TNF receptor-associated factor 1 (TRAF1) and C5 genes linked with RA12. The
The strongest association in this locus was seen for SNP rs3761847\(^{12}\). A fine mapping approach published in the same study noted that a polymorphism, rs2900180, could explain the majority of the association signal across the locus. As well, an independent candidate-gene study identified another SNP, rs10818488, showing almost total linkage disequilibrium with rs3761847, associated with RA\(^{12,13}\).

TRAF1 gene encodes an intracellular protein that mediates signal transduction through TNF receptors 1 and 2 and through CD40, and TRAF1 acts as a negative regulator of these signaling pathways\(^{14}\). Complement activation occurs through multiple pathways (classical, alternative, and lectin-binding) in the circulation, each of which produces C5a, a central component of the complement pathway\(^{15}\). Both molecules are potent immune mediators involved in the development of the acute-phase response, which in turn, is implicated in the pathogenesis of GCA\(^{16,17}\).

An association of the TRAF1/C5 locus with RA has been described in 2 large case-control series from populations of European descent\(^{18}\). This was also the case for systemic lupus erythematosus (SLE), implying that this region may be relevant to multiple autoimmune diseases\(^{19}\). However, to our knowledge, there are no data on the potential association of common genetic variants at the TRAF1–C5 locus with systemic vasculitides. Thus, we analyzed for the first time the influence of SNP from the TRAF1/C5 region on the susceptibility to biopsy-proven GCA or in the clinical spectrum of manifestations of this systemic vasculitis.

We assessed the potential association of 2 candidate gene SNPs with GCA. We selected the TRAF1/C5 rs10818488 and TRAF1/C5 rs2900180 SNPs because they were found to be associated with susceptibility to RA\(^{13}\). Moreover, TRAF1/C5 rs10818488 was found to predispose to anti-body-positive RA compared to controls\(^{13}\), and carriers of the minor allele A of this SNP were associated with more severe course and disease progression of RA determined by radiographic damage over time compared to non-A carriers (allele G)\(^{13}\).

### MATERIALS AND METHODS

**Patients.** A total of 220 patients diagnosed with biopsy-proven GCA were initially included in this study. Most (n = 128) were diagnosed in the Division of Rheumatology, Hospital Xeral-Calde, Lugo, Spain. The remaining patients were diagnosed in 2 centers from Madrid, Hospital Clínico San Carlos and Hospital de la Princesa (n = 82), and Granada, Hospital Clínico San Cecilio (n = 10). A control population (n = 410) was selected from the corresponding cities, matched with GCA patients by age, sex, and ethnicity, was also studied.

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\(^{20}\).

GCA patients were considered to have manifestations of polymyalgia rheumatica (PMR) if they had severe bilateral pain and pain involving the neck, shoulder, and/or pelvic girdle, associated with morning stiffness\(^{21,22}\). Patients were considered to have visual ischemic complications in the context of GCA if they experienced transient visual loss including amaurosis fugax, permanent visual loss, or diplopia\(^{23}\). Severe ischemic manifestations were considered to be present if patients had at least one of the following: visual manifestations, strokes and/or transient ischemic attacks, jaw claudication, or large-artery stenosis of the extremities that caused signs of occlusive manifestations\(^{24}\).

We found no significant differences between populations when we compared clinical features in the group of GCA patients or genotype and allele frequencies in the TRAF1/C5 variants assessed among the different groups of patients or controls (data not shown). As well, 410 controls from the corresponding centers in the study were enrolled for the genetic study. All patients and controls provided written informed consent, and ethical committee approval was obtained for the study.

TRAF1/C5 genotyping. DNA samples were obtained from peripheral blood mononuclear cells, using standard methods. Genotyping of TRAF1/C5 rs10818488 and TRAF1/C5 rs2900180 SNP was performed as described\(^{13,19}\), using a TaqMan 5’ allele discrimination assay (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labeled with VIC and FAM fluorescent dyes, respectively. Polymerase chain reaction (PCR) was carried out in a total reaction volume of 4 μl with the following amplification protocol: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s, and finished with annealing and extension at 60°C for 1 min. Post-PCR, the genotype of each sample was attributed automatically by measuring allele-specific fluorescence on the ABI Prism 7900 sequence detection system using the SDS 2.3 software for allelic discrimination (Applied Biosystems). Duplicate samples and negative controls were included to ensure accuracy of genotyping.

**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% confidence intervals were calculated according to Woolf’s method using the Statacal program (EpiInfo 2002; US 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 = 150), of median age at disease diagnosis of 74 years (range 52–93 yrs). From the onset of GCA symptoms to 1 month after the start of corticosteroid therapy, 173 (78.6%) patients had headache, 103 (46.8%) experienced PMR manifestations, 88 (40.0%) jaw claudication, and 54 (24.5%) visual ischemic manifestations. As well, 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\(^{25}\), most patients (n = 216; 98.2%) had an erythrocyte sedimentation rate > 40 mm/h.

**Influence of TRAF1/C5 gene polymorphisms on susceptibility to GCA.** A genotyping success rate > 95% in GCA patients and controls was achieved. No evidence of departure from Hardy-Weinberg equilibrium was observed in controls. The case:control ratio was 1:1.95. 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.

Table 1 shows the allele and genotype frequencies of the TRAF1/C5 gene polymorphisms in patients and controls. No significant differences in the genotype distribution for the 2 SNP were found between patients and controls. In this regard, the minor allele A of the TRAF1/C5 rs10818488 was decreased in patients compared to controls (31.0% vs 34.3%, respectively; p = 0.24). Moreover, GCA patients...
exhibited a reduced frequency of TRAF1/C5 AA homozygosity (7.6%) compared to controls (12.7%), but the difference was only marginally significant (OR 0.58, 95% CI 0.30–1.11, p = 0.07; Table 1). Similarly, the frequency of the minor allele T of the TRAF1/C5 rs2900180 was also slightly reduced in patients (24.3%) compared to controls (27.8%), but the difference remained insignificant (OR 0.82, 95% CI 0.62–1.10, p = 0.19; Table 1).

Since our study was largely underpowered to detect modest effect sizes (OR ~1.2), and considering that allele frequencies of the rs10818488 polymorphism found in the controls from Lugo were similar to those in controls reported in previous studies assessing the influence of the rs10818488 polymorphism in susceptibility to other autoimmune diseases in the Spanish population19, we performed a new analysis including data of all the available controls that were assessed for the rs10818488 polymorphism. We found a total of 1214 healthy controls. However, although this combined dataset enhanced the power of the study, the lack of association between the rs10818488 polymorphism and biopsy-proven GCA remained unchanged (p = 0.20, OR 0.87, 95% CI 0.69–1.08). Unfortunately, there is no previous information available from the Spanish population for the rs2900180 polymorphism of TRAF1/C5 gene.

Influence of TRAF1/C5 gene polymorphisms in the clinical spectrum of GCA. To determine whether SNP of the TRAF1/C5 locus might influence the clinical spectrum and severity of GCA, we stratified patients with biopsy-proven GCA according to the presence of PMR, visual ischemic manifestations, or severe ischemic complications. However, no significant differences were found for the TRAF1/C5 rs10818488 and rs2900180 biallelic polymorphisms between GCA patients with or without PMR or visual ischemic complications (Table 2). Although the frequency of the minor allele A of TRAF1/C5 rs10818488 was increased in the subset of patients with visual ischemic complications (34.9% vs 29.6%, respectively; OR 1.27, 95% CI 0.78–2.09), the difference was not statistically significant (p = 0.30). This was also the case for association between the minor allele T of TRAF1/C5 rs2900180 polymorphism and the presence of visual ischemic complications (30.4% vs 22.3% in those without visual ischemic events; OR 1.52, 95% CI 0.90–2.58, p = 0.10). Moreover, no significant differences were observed when GCA patients were stratified according to the presence of any severe ischemic complications (data not shown).

In a further step, we investigated whether significant differences for the TRAF1/C5 rs10818488 and rs2900180 biallelic polymorphisms might exist in patients with PMR, visual ischemic manifestations, or severe ischemic complications compared to controls; no significant differences were found (data not shown).

DISCUSSION

This study constituted the first attempt to establish the potential influence of 2 TRAF1/C5 gene polymorphisms in the susceptibility and phenotypic expression of biopsy-proven GCA. Our data showed no association between

<table>
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<th>rs10818488</th>
<th>With, n (%)</th>
<th>Without, n (%)</th>
<th>p</th>
<th>OR (95% CI)</th>
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<td>0.95</td>
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<tr>
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<td>Without, n (%)</td>
<td>p</td>
<td>OR (95% CI)</td>
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<tr>
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<td>70 (22.3)</td>
<td>0.10</td>
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</table>
the TRAF1/C5 rs10818488 and rs2900180 biallelic polymorphisms with disease susceptibility or with specific features of GCA.

It has been postulated that a variety of inflammatory and autoimmune diseases may share a common genetic background. Recent studies have emphasized the influence of TRAF1/C5 loci with RA, the prototype of common inflammatory autoimmune disease, in different populations. More importantly, the significant association of the TRAF1/C5 locus in European patients with SLE highlights the potential role of this region as a target relevant to different autoimmune diseases.

Similarly to RA, environmental and genetic factors seem to contribute to the etiology of GCA. Both conditions are associated with increased inflammatory response and share an association with HLA-DRB1*04 alleles. The reasons for a negative association with GCA of these 2 TRAF1/C5 SNPs, reported to be associated with RA, are unknown. Despite having similarities in terms of HLA class II association with RA, the immune-mediated mechanisms characterized by granulomatous infiltrates leading to vasculitic damage in GCA are certainly different from those observed in RA. Moreover, studies have shown differences in genetic susceptibility between RA and GCA. In this regard, unlike observations in patients with RA, we did not observe an association of STAT4 gene polymorphism with biopsy-proven GCA in Spanish individuals.

However, taking into account the modest contribution of the TRAF1/C5 locus in other autoimmune diseases (providing an OR of around 1.1 in RA and 1.2 in SLE), our study, of a rare disease, was limited by the small sample size. For this population size and the minor allele frequency observed for these variants, it would be necessary for an OR of 1.6 to have enough power to detect an association at the 0.05 level of significance. Thus, further studies in other populations with different genetic backgrounds are needed to fully exclude an influence of the rs10818488 and rs2900180 TRAF1/C5 gene polymorphisms in GCA. A recent report from studies in Colombians failed to confirm an association of TRAF1/C5 gene polymorphisms with risk of developing RA or SLE, supporting a different influence of these variants among some populations. Moreover, we cannot rule out that other polymorphisms located within the TRAF1/C5 locus might account for susceptibility to GCA.

Our results provide no evidence for a contribution of the TRAF1/C5 rs10818488 and rs2900180 gene polymorphisms in susceptibility to or clinical manifestations of GCA. Further studies are needed to fully exclude the contribution of TRAF1/C5 gene polymorphisms in the pathogenesis of GCA.

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REFERENCES
pro-inflammatory cytokines (IL-1 beta, TNF alpha and IL-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. Rheumatology 2004;43:294-301.


