Lack of Association Between STAT4 Gene Polymorphism and Biopsy-proven Giant Cell Arteritis

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ABSTRACT. Objective. To investigate the potential implication of the STAT4 gene polymorphism rs7574865 in the predisposition to or the clinical expression of giant cell arteritis (GCA).

Methods. A total of 212 patients diagnosed with biopsy-proven GCA were studied. DNA from patients and controls matched by age, sex, and ethnicity was obtained from peripheral blood. Samples were genotyped for STAT4 rs7574865 polymorphism.

Results. No statistically significant differences in the allele frequencies for the STAT4 rs7574865 polymorphism were observed between patients and controls. Although we observed an increased frequency of the T/T genotype in GCA patients (6.0%) compared to healthy controls (3.9%), this difference did not achieve statistical significance (OR 1.57, 95% CI 0.72–3.41). No statistically significant differences in allele or genotype frequencies were observed when patients were stratified according to the presence of typical disease features such as polymyalgia rheumatica, severe ischemic manifestations, and visual ischemic complications in the setting of this vasculitis.

Conclusion. Our results do not support a major role of the STAT4 rs7574865 gene polymorphism in susceptibility to or clinical manifestations of GCA. (First Release April 1 2009; J Rheumatol 2009; 36:1021–5; doi:10.3899/jrheum.081060)

Key Indexing Terms:
Giant Cell Arteritis
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Giant cell arteritis (GCA) is the most common systemic vasculitis in individuals over the age of 50 years in Western countries. It is characterized by the granulomatous involvement of large and medium-size blood vessels of the aorta with predilection for the extracranial branches of the carotid artery. Cranial ischemic events, blindness in particular, constitute the most feared complications of this vasculitis. These severe ischemic complications are the result of inflammation of the arterial wall, which leads to intimal hyperplasia, fragmentation of internal elastic laminae, and luminal occlusion.

GCA is a complex polygenic disease. Observations of familial clustering of GCA support this genetic component, and there is a strong association of this vasculitis with genes within the major histocompatibility complex (MHC). With respect to this, besides an association with HLA-DRB1*04 and TNF microsatellite polymorphisms, we recently reported an independent association of MICA and HLA-B genes with the genetic susceptibility to GCA in Northwestern Spain, suggesting that several genes within the MHC may have independent effects in the susceptibility to GCA.

Moreover, many other studies have shown the implication of genetic variants in key components of immune and inflammatory pathways in GCA susceptibility or clinical expression of this vasculitis.

An important step forward in our understanding of the pathogenesis of autoimmune diseases may be to establish the presence of common (shared) mechanisms that may lead to a variety of very different complex autoimmune diseases. In this regard, the Janus kinase and signal transducer and activator of transcription (Jak-STAT) pathway is the signaling target of a multitude of cytokines that are thought to have biologically significant roles in autoimmunity.
STAT4 transmits signals induced by interleukin 12 (IL-12), IL-23, and type 1 interferons. A major action of IL-12 is to promote the differentiation of naïve CD4+ T cells into T-helper (Th1) cells, which produce interferon-γ (IFN-γ). On the other hand, Th1 cells are thought to drive the chronic autoimmune response, and IFN-γ is known to have a key role in the pathogenesis of GCA. Also, STAT4 is important for the development of the recently identified IL-17-secreting Th cells in response to IL-23.

As discussed for GCA, rheumatoid arthritis (RA) is also a polygenic disease, and the role of genes within the MHC in RA susceptibility accounts for only one-third to one-half of the total genetic contribution. Interestingly, a RA linkage peak in chromosome 2q was found in North American families of European ancestry. Also, a followup study identified several polymorphisms in the third intron of the STAT4 gene as the markers responsible for the signal in 2q. Four polymorphisms in tight linkage disequilibrium (rs11889341, rs7574865, rs8179673, and rs10181656, r^2 > 0.97 in Caucasians) form a susceptible haplotype tagged by the T allele of rs7574865 that showed the most significant association with RA and systemic lupus erythematosus (SLE). This association of STAT4 with RA has recently been replicated in a series of European patients with RA that included individuals from Spain. Moreover, the STAT4 rs7574865 polymorphism has recently been found to be implicated in the predisposition to other complex autoimmune diseases such as type 1 diabetes mellitus and inflammatory bowel disease in the Spanish population, suggesting the involvement of STAT4 gene in the pathogenesis of both diseases. Recent data also support an association of the STAT4 rs7574865 gene polymorphism with susceptibility to type 1 diabetes mellitus in another Southern European population.

Taking all these considerations together, our aim was to determine whether the STAT4 polymorphism rs7574865 may be associated with susceptibility to biopsy-proven GCA.

**MATERIALS AND METHODS**

**Patients.** A total of 212 patients diagnosed with biopsy-proven GCA were included in this study. Most (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 = 73) and Granada (Hospital Clínico San Cecilio; n = 11). A control population from the corresponding cities matched by age, sex, and ethnicity with GCA patients was also assessed; 371 healthy controls were included (226 from Lugo, 125 from Madrid, 20 from Granada). All GCA patients 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. All met the 1990 American College of Rheumatology criteria for the classification of GCA.

Patients with GCA were considered to have an associated polymyalgia rheumatica (PMR) if they had severe bilateral ache and pain involving the neck, the shoulder, and/or the pelvic girdle, associated with morning stiffess. Patients were considered to have severe ischemic manifestations if they experienced at least one of the following complications: visual manifestations (transient visual loss including amaurosis fugax, permanent visual loss, or diplopia), cerebrovascular accidents (stroke and/or transient ischemic attacks), jaw claudication, or large-artery stenosis of the extremities that caused signs of occlusive manifestations (limb claudication) of recent onset.

Patients and controls were included in this study after giving written informed consent. We obtained approval for the study from the local ethical committees.

**Genotyping methods.** DNA from patients and controls was obtained from peripheral blood, using standard methods. Samples were genotyped for STAT4 rs7574865 polymorphism using a TaqMan 5’ allele discrimination assay. Allele-specific probes were labeled with the fluorescent dyes VIC and FAM. 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 the allelic-specific fluorescence on ABI Prism 7900 Sequence Detection Systems using SDS 2.3 software for allelic discrimination. Duplicate samples and negative controls were included to ensure accuracy of genotyping.

**Statistical analysis.** We used the chi-square test and Fisher exact test for Hardy-Weinberg equilibrium and statistical analysis to compare allelic and genotypic distributions. Odds ratios (OR) and 95% confidence intervals (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**

The study comprised 142 women and 70 men (median age at disease diagnosis 74 yrs, range 52–93). Headache and an abnormal temporal artery on physical examination were the most common features, observed in 170 (80%) and 134 (63%) patients, respectively. From the onset of GCA symptoms until 1 month after the onset of steroid therapy, 97 (46%) had manifestations of PMR. Severe ischemic complications were found in 112 (53%) patients. Visual ischemic complications were present in 52 (25%). Twenty (9%) patients experienced permanent visual loss. Eighty-five (40%) had jaw claudication and 10 (5%) suffered a stroke. Also, 207 (98%) had an erythrocyte sedimentation rate > 40 mm/h.

**Genotype and allele frequencies of rs7574865 STAT4 polymorphism.** A genotyping rate of 95% was achieved in this series of GCA patients. Information on the STAT4 rs7574865 polymorphism was available in 201 patients and 357 matched controls (Table 1). The case:control ratio was 1:1.8. The estimated power of the study for an estimated OR between 1.5 and 2.0 was 77%–99.5% for a type I error rate of 0.05.

No evidence of departure from Hardy-Weinberg equilibrium was observed in patients and controls; Table 1 shows allele and genotype frequencies of the STAT4 rs7574865 polymorphism. Although the mutated allele T was more commonly observed in patients (20.6%) than in controls (17.8%), comparisons of allele frequencies between patients and controls showed no significant differences (Table 1). In
addition, investigation of STAT4 genotype polymorphism showed that G/T or T/T genotypes were more common in patients (35.3%) than in controls (31.6%). This was especially true for the mutant T/T genotype that was almost twice as common in patients (6.0%) as in controls (3.9%). However, the difference for the T/T genotype did not achieve statistical significance (OR 1.57, 95% CI 0.72–3.41).

No significant differences were observed when we compared the allele or genotype frequencies between controls from Lugo and the remaining controls. This was also the case when we analyzed allele or genotype frequencies between GCA patients from Lugo and the remaining GCA patients or when we specifically assessed possible allele or genotype differences between the 2 biggest groups of patients (Lugo and Madrid; data not shown).

Genotype and allele frequencies of STAT4 rs7574865 polymorphism according to patients’ clinical manifestations. In a further step we stratified patients according to the presence of PMR, severe ischemic manifestations, and visual ischemic complications. However, as shown in Table 2, no significant differences were observed when patients were compared according to these specific clinical disease features. This was also the case when patients were stratified according to the presence of anemia (hemoglobin < 12 g/dl) at the time of disease diagnosis or when GCA patients with PMR, severe ischemic complications, or visual ischemic manifestations were compared with the control group (data not shown).

DISCUSSION

GCA is a disease associated with a high inflammatory response, and we examined for the first time the contribution of the rs7574865 STAT4 polymorphism to susceptibility to GCA in a large series of histologically confirmed patients with this vasculitis. Our results do not support a role of the rs7574865 STAT4 polymorphism in susceptibility to GCA or in the clinical expression of this vasculitis.

It has been proposed that a variety of inflammatory and autoimmune diseases may share a common genetic background. Interestingly, a haplotype of STAT4 has been associated with increased risk for both RA and SLE in North American individuals of European descent, suggesting a shared pathway for these 2 rheumatic autoimmune diseases. Since we have recently confirmed an association of the rs7574865 STAT4 polymorphism in Spanish patients with RA, the presence of a potential implication of the rs7574865 STAT4 polymorphism in GCA might have further supported a role of STAT4 as a common risk factor for different complex rheumatic autoimmune diseases.

Although our results do not confirm an association with biopsy-proven GCA, it is clear that STAT4 plays an important role in several pathways involved in autoimmunity and inflammation. The lack of association of one single-nucleotide polymorphism (SNP) of the STAT4 gene with GCA does not rule out the potential association of a different SNP of the same gene with this vasculitis. Therefore, it is still possible that the STAT4 participates in the pathogenesis of GCA. However, the genetic predisposition, i.e., a SNP associated with either RA or GCA, could be different.

Our results do not support any evidence for a major role of the STAT4 rs7574865 gene polymorphism in the susceptibility to or clinical manifestations of GCA.

### Table 1. Genotypic and allelic frequencies of STAT4 rs7574865 polymorphism in patients with biopsy-proven GCA and healthy controls.

<table>
<thead>
<tr>
<th>STAT4 rs7574865 Polymorphism</th>
<th>Patients, n (%)</th>
<th>Controls, n (%)</th>
<th>p</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1417938 Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>130 (64.7)</td>
<td>244 (68.3)</td>
<td>0.37</td>
<td>0.85 (0.59–1.22)</td>
</tr>
<tr>
<td>G/T</td>
<td>59 (29.3)</td>
<td>99 (27.7)</td>
<td>0.68</td>
<td>1.09 (0.74–1.59)</td>
</tr>
<tr>
<td>T/T</td>
<td>12 (6.0)</td>
<td>14 (3.9)</td>
<td>0.27</td>
<td>1.57 (0.72–3.41)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>319 (79.4)</td>
<td>587 (82.2)</td>
<td>0.24</td>
<td>0.83 (0.61–1.13)</td>
</tr>
<tr>
<td>T</td>
<td>83 (20.6)</td>
<td>127 (17.8)</td>
<td>0.24</td>
<td>1.12 (0.89–1.64)</td>
</tr>
</tbody>
</table>

PMR: polymyalgia rheumatica.
REFERENCES


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