A case-control study suggests that the CCR6 locus is not involved in the susceptibility to giant cell arteritis

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ABSTRACT

Objectives. Polymorphisms of the CC chemokine receptor 6 (CCR6) gene have been recently reported to be associated with a number of autoimmune diseases. We aimed to investigate the possible influence of CCR6 rs3093024 gene variant in the susceptibility to and clinical expression of GCA.

Methods. The CCR6 polymorphism rs3093024 was genotyped in a total of 463 Spanish patients diagnosed with biopsy-proven GCA and 920 healthy controls using a TaqMan® allelic discrimination assay. PLINK software was used for the statistical analyses.

Results. No significant association between this CCR6 variant and GCA was observed (p=0.42, OR=0.94, CI95% 0.79-1.10). Similarly, when patients were stratified according to the specific clinical features of GCA such as polymyalgia rheumatica, visual ischaemic manifestations or irreversible occlusive disease, no statistical significant difference was detected either between the case subgroups and the control set or between GCA patients with and without the specific features of the disease.

Conclusion. Our results suggest that the CCR6 rs3093024 polymorphism may not play a relevant role in the GCA pathophysiology.

Introduction

CCR6 encodes a member of the beta chemokine receptor family, which is predicted to be a seven transmembrane protein similar to the G protein-coupled receptors. This gene encodes an important receptor involved in the regulation of several aspects of the immunity, including the ability to mediate the recruitment of immature and mature dendritic cells (DCs), and professional antigen presenting cells (APCs) to the sites of epithelial inflammation. The ligand of this receptor is the macrophage inflammatory protein 3 alpha (MIP-3 alpha) (1). CCR6 has been shown to be important for B-lineage maturation and antigen-driven B-cell differentiation, and it may regulate the migration and recruitment of dendritic and T cells during inflammatory and immunological responses. Alternatively spliced transcript variants have been described for this gene. It is likely that the IL23 produced by DC acts to sustain dermal CCR6-expressing Th17 cells, which then produce IL22.

Recent studies have focused on the involvement of CCR6 in the pathogenesis of several autoimmune diseases such as Crohn’s disease (2), Graves’ disease (3), psoriasis (4), and rheumatoid arthritis (RA) (5). Giant cell arteritis (GCA), also known as temporal arteritis and Horton’s syndrome, is a systemic vasculitis affecting medium and large size arteries with a predisposition for the involvement of cranial arteries that supply the aortic arch especially extracranial territories, mainly branches of the external carotid (6, 7). The fundamental inflammatory lesion is composed of T cells and macrophages that have infiltrated through all layer of the arterial wall and the appearance of multinucleated giant cells (8). GCA is the most common type of vasculitis affecting people aged ≥50 years and it is suffered mainly by women. It is more frequent in Caucasians, with a higher prevalence in populations of Scandinavian origin (9, 10). The inflammatory activity of vascular lesions in GCA emphasises the role of the adaptive immunity. At least two separate lineages of CD4 T cells may participate in the vascular inflammation undergoing clonal expansion in the
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vessel walls and releasing interferon (IFN-γ) (11). Additionally, recent studies have described a distinctive population of DCs localised at the adventitia media border of normal medium sized arteries that appear to play a critical role in the initiation of this vasculitis. Cellular immune responses implicating T cells and APCs are crucial elements in GCA. At the disease onset, APCs contribute to differentiation of Th17 as well as Th1 cells (12). On the other hand, the vascular lesions contain mainly activated T cells, macrophages and multinucleated giant cells (13, 14).

It is known that chemokines and chemokine receptors play key roles in the early inflammatory response, as these molecules are directly involved in the chemoattractant process that recruit T cells and phagocytes to inflammation sites (15, 16). On the other hand, it has been proposed that shared immunological pathways may constitute the basis of the pathology of different autoimmune diseases. In this regard, like RA, GCA is also a complex polygenic disease in which more than 1 genetic locus is likely to contribute to disease susceptibility and clinical expression. Taking these considerations into account, since CCR6 single-nucleotide-polymorphism (SNP) rs3093024 was previously found associated with susceptibility to RA (17), in the present study we sought to determine whether this CCR6 gene variant may be associated with susceptibility to GCA. We also aimed to determine if this gene polymorphism might influence the expression of the main clinical manifestations of GCA in a large series of biopsy-proven GCA patients.

Patients and methods

Study population

After obtaining informed written consent and authorisation from the local ethical committees, we studied 463 patients diagnosed with biopsy-proven GCA and 920 unrelated healthy controls recruited in the same geographic regions and matched by age, gender, and ethnicity with the GCA patients. The main clinical characteristics of the analysed cohort are shown in Table 1.

Definitions for specific features of the disease including headache, polymyalgia rheumatica (PMR), jaw claudication, peripheral arteriopathy manifested by arm or leg claudication, visual ischaemic manifestations (VIM), permanent visual loss, stroke, severe ischaemic manifestations (SIM- encompassing visual manifestations, cerebrovascular accidents, jaw claudication or limb claudication of recent onset) and the presence of irreversible occlusive disease (IOD- if patients experienced at least one of the following complications: permanent visual loss, stroke or limb claudication of recent onset), were also previously described (18-22).

All patients fulfilled the 1990 American College of Rheumatology criteria for the classification of GCA (23). 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 (24).

Genotyping methods and statistical analysis

DNA was obtained from peripheral blood mononuclear cells, using standard methods. All samples were genotyped for the CCR6 single-nucleotide-polymorphism (SNP) rs3093024 by using predesigned TaqMan® allelic discrimination assay technology in a 7900HT Real-Time polymerase chain reaction (PCR) System, from Applied Biosystems (Foster City, CA, USA). The statistical power of the analysis was 82% to detect associations with odds ratios (ORs)=1.2 at the 5% significant level, according to Power Calculator for Genetic Studies 2006 software (25). Plink software (v1.07; http://pngu.mgh.harvard.edu/purcell/plink/) (26) was used for the statistical analyses. To test for individual population association, 2×2 contingency tables and χ² or Fisher’s exact test, when appropriate, were performed. Odds ratios (OR) and 95% confidence intervals (CI) were obtained according to Woolf’s method. p-values lower than 0.05 were considered as statistically significant.

Results

The genotyping success rate was higher than 90% in both GCA patients and controls. After genotyping, no evidence of departure from Hardy-Weinberg equilibrium was observed in either case or control populations at the 5% significance level.

Table II shows the genotype and minor allele frequencies (MAFs) of the control cohort and the different case sets analysed. No significant differences were detected between the allele frequencies of the GCA patients and controls.

Table 1. Main clinical features of 463 Spanish Caucasian patients with biopsy-proven GCA.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, years, median (IQR)</td>
<td>75 (69–79)</td>
</tr>
<tr>
<td>Women</td>
<td>321 (69.3)</td>
</tr>
<tr>
<td>Men</td>
<td>142 (30.6)</td>
</tr>
<tr>
<td>Headache</td>
<td>366 (79.0)</td>
</tr>
<tr>
<td>Abnormal temporal artery on examination</td>
<td>272 (58.7)</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
<td>217 (46.8)</td>
</tr>
<tr>
<td>Jaw claudication</td>
<td>191 (41.3)</td>
</tr>
<tr>
<td>Arm-leg claudication</td>
<td>28 (6.0)</td>
</tr>
<tr>
<td>Visual ischaemic manifestations*</td>
<td>120 (25.9)</td>
</tr>
<tr>
<td>Permanent visual loss</td>
<td>53 (11.4)</td>
</tr>
<tr>
<td>Stroke</td>
<td>20 (4.3)</td>
</tr>
<tr>
<td>Severe ischaemic manifestations**</td>
<td>231 (49.9)</td>
</tr>
<tr>
<td>Irreversible occlusive disease***</td>
<td>87 (18.7)</td>
</tr>
</tbody>
</table>

IQR: interquartile range. Rest of values are expressed as number and percentages (%).

*Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia.

**At least one of the following features: visual manifestations, cerebrovascular accidents (stroke and/or transient ischaemic attacks), jaw claudication, or limb claudication.

***At least one of the following features: permanent visual loss, stroke and/or occlusive disease in the extremities.
To examine whether the *CCR6* locus might influence the clinical manifestations of the disease, GCA patients were subdivided according to the presence of PMR, VIM, SIM and IOD. However, no significant differences were found in the analyses between the different case subgroups and the control population (Table II). Moreover, similar negative results were also observed when GCA patients with and without specific clinical features of the disease were compared (Table III).

**Discussion**

*CCR6* is believed to be involved in leukocyte recruitment and inflammatory response (27). There are data suggesting that inappropriate activation, maturation and retention of DCs in the adventitia constitutes one of the earliest steps in the pathogenesis of GCA and that subsequent events are dependent on DC-induced T cell activation (28, 29). GCA and RA are complex autoimmune diseases in which both environmental and genetic factors contribute to their etiology (9, 30). These inflammatory conditions share some genetic associations, including HLA-DRB1*04 alleles (31). An increasing number of common susceptibility loci among different autoimmune diseases have been identified by genome-wide association studies (GWAS) in the last years (32-35), thus suggesting a shared underlying immunological mechanisms. In this regard, polymorphisms within the *CCR6* gene have been associated with different immune-mediated diseases including RA (5, 17).

Taken together all these considerations, we aimed to determine whether *CCR6* might also be a susceptibility locus for GCA. However, in this study that was the first attempt to determine the potential influence of the *CCR6* genetic variant rs3093024 in both GCA susceptibility and phenotypic expression of this vasculitis, no association between this gene variant and GCA was found. These results are in line with former studies of our group that did not disclose association between polymorphisms in inflammatory pathway genes and biopsy-proven GCA (36-38). Nevertheless, a possible association between GCA and other *CCR6* gene variants different from that analysed here may not be discarded.

Finally, although we recruited the largest series of biopsy-proven GCA patients included in a genetic study on this disease so far, the statistical power was still moderate to detect a low signal, and this could be considered a limitation of this study.

In conclusion, our results indicate no evidence for a contribution of the analysed *CCR6* gene variant in the susceptibility to or clinical manifestations of GCA. Further studies in populations with different genetic backgrounds may be necessary to fully exclude the contribution of *CCR6* gene polymorphisms in the pathogenesis of GCA.

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**Authors’ contributions**

A. Serrano and F.D. Carmona contributed equally to this work. J. Martín and M.A. González-Gay share senior authorship.

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