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

A. Serrano[1], F.D. Carmona[1], S. Castañeda[2], J.A. Miranda-Filloy[3], I.C. Morado[4], C. Gomez-Vaquero[5], R. Solans[6], B. Sopeña[7], R. Blanco[8], A. Unzurrunzaga[9], N. Ortego-Centeno[10], B. Marí-Alfonso[11], A. Hidalgo-Conde[12], J. Hernández-Rodríguez[13], M.C. Cid[13], J. Martín[1], M.A. González-Gay[8]

[1]Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Granada; [2]Dept. of Rheumatology, Hospital de la Princesa, IIS-Princesa, Madrid; [3]Dept. of Rheumatology, Hospital Xeral-Calde, Lugo; [4]Dept. of Rheumatology, Hospital Clínico San Carlos, Madrid; [5]Dept. of Rheumatology, Hospital Universitario de Bellvitge-IDIBELL, L'Hospitalet de Llobregat, Barcelona; [6]Dept. of Internal Medicine, Hospital Vall d'Hebron, Barcelona; [7]Thrombosis and Vasculitis Unit-Internal Medicine Department, Complejo Hospitalario Universitario de Vigo; [8]Dept. of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV, Santander; [9]Dept. of Internal Medicine, Hospital de Galdakano, Vizcaya; [10]Dept. of Internal Medicine, Hospital Clínico San Cecilio, Granada; [11]Dept.of Internal Medicine, Corporació Sanitaria Parc Taulí, Instituto Universitario Parc Taulí, UAB, Sabadell, Barcelona; [12]Dept. of Internal Medicine, Hospital Universitario Virgen de la Victoria, Málaga; [13]Vasculitis Research Unit, Dept. of Autoimmune and Systemic Diseases, Hospital Clínic, University of Barcelona, (IDIBAPS), Barcelona, Spain.

Aurora Serrano, F. David Carmona, Santos Castañeda, José A. Miranda-Filloy, Inmaculada C. Morado, Carmen Gomez-Vaquero, Roser Solans, Bernardo Sopeña, Ricardo Blanco, Ainhoa Unzurrunzaga, Norberto Ortego-Centeno, Begoña Marí-Alfonso, Ana Hidalgo-Conde, José Hernández-Rodríguez, Maria C. Cid, Javier Martín, Miguel A. González-Gay

Please address correspondence to:
Javier Martin, MD, PhD,
Instituto de Parasitología y Biomedicina López-Neyra, IPBLN-CSIC, Consejo Superior de Investigaciones Científicas, Parque Tecnológico Ciencias de la Salud, Avenida del Conocimiento s/n, 18100 Armilla (Granada), Spain.
E-mail: martin@ipb.csic.es

Received on March 22, 2012; accepted in revised form on June 13, 2012.

Clin Exp Rheumatol 2013; 31 (Suppl. 75): S5-S8.

Key words: GCA, temporal arteritis, SNP, *CCR6*, rs3093024

Competing interests: none declared.

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

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 I.

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, 2x2 contingency tables and χ^2 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 I. Main clinical features of 463 Spanish Caucasian patients with biopsy-proven GCA.

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

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.

Table II. Genotype and allele distribution of *CCR6* rs3093024 in biopsy-proven GCA patients and healthy controls.

SNP	Subgroup (n)	Genotype, n (%)			MAF (%)	Allele test	
		AA	AG	GG		*p*-value*	OR [95%CI]**
rs3093024	Controls (n=866)	195 (22.52)	420 (48.50)	251 (28.98)	46.77		
	GCA (n=429)	92 (21.45)	203 (47.32)	134 (31.24)	45.10	0.425	0.94 [0.79–1.10]
	PMR+ (n=206)	50 (24.27)	90 (43.69)	66 (32.04)	46.12	0.812	0.97 [0.79–1.21]
	VIM+ (n=110)	23 (20.91)	60 (54.55)	27 (24.55)	48.18	0.692	1.06 [0.80–1.40]
	SIM+ (n=211)	44 (20.85)	106 (50.24)	61 (28.91)	45.97	0.769	0.97 [0.78–1.20]
	IOD+ (n=84)	20 (23.81)	44 (52.38)	20 (23.81)	50.00	0.423	1.14 [0.83–1.56]

*All *p*-values have been calculated for the allelic model. **Odds ratio for the minor allele. MAF: minor allele frequency; GCA: giant cell arteritis; PMR: polymyalgia rheumatica; VIM: visual ischaemic manifestations; SIM: severe ischaemic manifestations; IOD: irreversible occlusive disease.

Table III. Genotype distribution and minor allele frequency (MAF) of *CCR6* rs3093024 in biopsy-proven GCA patients according to the presence (with) or absence (without) of specific manifestations of the disease.

		With		Without		Test Statistic	
		Genotypic frequencies	MAF (%)	Genotypic frequencies	MAF (%)	*p*-value*	OR [95%CI]**
rs3093024	PMR	50/90/66	46.12	40/109/64	44.37	0.611	1.07 [0.82–1.41]
	VIM	23/60/27	48.18	67/138/101	44.44	0.340	1.16 [0.85–1.58]
	SIM	44/106/61	45.97	46/90/67	44.83	0.741	1.05 [0.80–1.38]
	IOD	20/44/20	50.00	70/148/105	44.58	0.209	1.24 [0.88–1.75]

*All *p*-values have been calculated for the allelic model. **Odds ratio for the minor allele. MAF: minor allele frequency; GCA: giant cell arteritis; PMR: polymyalgia rheumatica; VIM: visual ischaemic manifestations; SIM: severe ischaemic manifestations; IOD: irreversible occlusive disease.

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.

Acknowledgements

The authors thank Gema Robledo, Sofía Vargas and Sonia García for their excellent technical assistance, and all the patients and healthy controls for kindly giving their essential collaboration.

Authors' contributions

A. Serrano and F.D. Carmona contributed equally to this work.

J. Martín and M.A. González-Gay share senior authorship.

Funding

This study was supported by 'Fondo de Investigaciones Sanitarias' through

grants PI06-0024 and PS09/00748 (Spain), and partially supported by RETICS Program RD08/0075 (RIER) from 'Instituto de Salud Carlos III' (ISCIII). FDCL was supported by Consejo Superior de Investigaciones Científicas (CSIC) through the programme JAE-DOC.

References

1. SCHUTYSER E, STRUYF S, VAN DAMME J: The CC chemokine CCL20 and its receptor CCR6. *Cytokine Growth Factor Rev* 2003; 14: 409-26.
2. BARRETT JC, HANSOUL S, NICOLAE DL *et al*.: Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008 Aug; 40: 955-62.
3. CHU X, PAN CM, ZHAO SX *et al*.: A genome-wide association study identifies two new risk loci for Graves' disease. *Nat Genet* 2011; 43: 897-901.
4. MABUCHI T, CHANG TW, QUINTER S, HWANG ST: Chemokine receptors in the pathogenesis and therapy of psoriasis. *J Dermatol Sci* 2012; 65: 4-11.
5. STAHL EA, RAYCHAUDHURI S, REMMERS EF *et al*.: Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat Genet* 2010; 42: 508-14.
6. SALVARANI C, CANTINI F, BOIARDI L, HUNDER GG: Polymyalgia rheumatica and giant-cell arteritis. *N Engl J Med* 2002; 347: 261-71.
7. GONZALEZ-GAY MA, VAZQUEZ-RODRIGUEZ TR, LOPEZ-DIAZ MJ *et al*.: Epidemiology of giant cell arteritis and polymyalgia rheumatica. *Arthritis Rheum* 2009; 61:1454-61.
8. LIE JT: Illustrated histopathologic classification criteria for selected vasculitis syndromes. American College of Rheumatology Subcommittee on Classification of Vasculitis. *Arthritis Rheum* 1990; 33: 1074-87.
9. GONZALEZ-GAY MA, GARCIA-PORRUA C: Epidemiology of the vasculitides. *Rheum Dis Clin North Am* 2001; 27: 729-49.
10. GONZALEZ-GAY MA, MIRANDA-FILLOY JA, LOPEZ-DIAZ MJ *et al*.: Giant cell arteritis in northwestern Spain: a 25-year epidemiologic study. *Medicine* (Baltimore) 2007; 86: 61-8.
11. WEYAND CM, MA-KRUPA W, PRYSHCHEP O, GROSCHEL S, BERNARDINO R, GORONZY JJ: Vascular dendritic cells in giant cell arteritis. *Ann N Y Acad Sci* 2005; 1062: 195-208.
12. WEYAND CM, YOUNGE BR, GORONZY JJ: IFN-gamma and IL-17: the two faces of T-cell pathology in giant cell arteritis. *Curr Opin Rheumatol* 2011; 23: 43-9.
13. CID MC, CAMPO E, ERCILLA G *et al*.: Immunohistochemical analysis of lymphoid and macrophage cell subsets and their immunologic activation markers in temporal arteritis. Influence of corticosteroid treatment. *Arthritis Rheum* 1989; 32: 884-93.
14. ANDERSSON R, JONSSON R, TARKOWSKI A, BENGTSSON BA, MALMVALL BE: T cell subsets and expression of immunological activation markers in the arterial walls of patients with giant cell arteritis. *Ann Rheum Dis* 1987; 46: 915-23.
15. ROSSI D, ZLOTNIK A: The biology of chemokines and their receptors. *Annu Rev Immunol* 2000; 18: 217-42.
16. YOSHIE O, IMAI T, NOMIYAMA H: Chemokines in immunity. *Adv Immunol* 2001; 78: 57-110.
17. KOCHI Y, OKADA Y, SUZUKI A *et al*.: A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility. *Nat Genet* 2010; 42: 515-9.
18. PALOMINO-MORALES R, TORRES O, VAZQUEZ-RODRIGUEZ TR *et al*.: Association between toll-like receptor 4 gene polymorphism and biopsy-proven giant cell arteritis. *J Rheumatol* 2009; 36: 1501-6.
19. GONZALEZ-GAY MA, GARCIA-PORRUA C, VAZQUEZ-CARUNCHO M: Polymyalgia rheumatica in biopsy proven giant cell arteritis does not constitute a different subset but differs from isolated polymyalgia rheumatica. *J Rheumatol* 1998; 25: 1750-5.
20. GONZALEZ-GAY MA, BARROS S, LOPEZ-DIAZ MJ, GARCIA-PORRUA C, SANCHEZ-ANDRADE A, LLORCA J: Giant cell arteritis: disease patterns of clinical presentation in a series of 240 patients. *Medicine* (Baltimore) 2005; 84: 269-76.
21. GONZALEZ-GAY MA, LOPEZ-DIAZ MJ, BARROS S *et al*.: Giant cell arteritis: laboratory tests at the time of diagnosis in a series of 240 patients. *Medicine* (Baltimore) 2005; 84: 277-90.
22. RUEDA B, LOPEZ-NEVOT M, LOPEZ-DIAZ M *et al*.: A functional variant of vascular endothelial growth factor is associated with severe ischemic complications in giant cell arteritis. *J Rheumatol* 2005; 32: 1737-41.
23. HUNDER GG, BLOCH DA, MICHEL BA *et al*.: The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum* 1990; 33: 1122-8.
24. GONZALEZ-GAY MA, GARCIA-PORRUA C, LLORCA J, GONZALEZ-LOUZAO C, RODRIGUEZ-LEDO P: Biopsy-negative giant cell arteritis: clinical spectrum and predictive factors for positive temporal artery biopsy. *Semin Arthritis Rheum* 2001; 30: 249-56.
25. SKOL A, SCOTT L, ABECASIS G, BOEHNKE M: Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 2006; 38: 209-13.
26. PURCELL S, NEALE B, TODD-BROWN K *et al*.: PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559-75.
27. PERKINS EA, LANDIS D, CAUSEY MPH ZL *et al*.: Association of single nucleotide polymorphisms (SNPs) in CCR6, TAGAP and TNFAIP3 with rheumatoid arthritis in African Americans. *Arthritis Rheum* 2011; 64: 1355-8.
28. KRUPA WM, DEWAN M, JEON MS *et al*.: Trapping of misdirected dendritic cells in the granulomatous lesions of giant cell arteritis. *Am J Pathol* 2002; 161: 1815-23.
29. MA-KRUPA W, JEON MS, SPOERL S, TEDDER TF, GORONZY JJ, WEYAND CM: Activation of arterial wall dendritic cells and breakdown of self-tolerance in giant cell arteritis. *J Exp Med* 2004; 199: 173-83.
30. OROZCO G, RUEDA B, MARTIN J: Genetic basis of rheumatoid arthritis. *Biomed Pharmacother* 2006; 60: 656-62.
31. GONZALEZ-GAY MA, AMOLI MM, GARCIA-PORRUA C, OLLIER WE: Genetic markers of disease susceptibility and severity in giant cell arteritis and polymyalgia rheumatica. *Semin Arthritis Rheum* 2003; 33: 38-48.
32. CHO JH, GREGERSEN PK: Genomics and the multifactorial nature of human autoimmune disease. *N Engl J Med* 2011; 365: 1612-23.
33. ZENEWICZ LA, ABRAHAM C, FLAVELL RA, CHO JH: Unraveling the genetics of autoimmunity. *Cell* 2010; 140: 791-7.
34. COTSAPAS C, VOIGHT BF, ROSSIN E *et al*.: Pervasive sharing of genetic effects in autoimmune disease. *PLoS Genet* 2011; 7: e1002254.
35. ZHERNAKOVA A, VAN DIEMEN CC, WIJMENGA C: Detecting shared pathogenesis from the shared genetics of immune-related diseases. *Nat Rev Genet* 2009; 10: 43-55.
36. TORRES O, PALOMINO-MORALES R, VAZQUEZ-RODRIGUEZ T *et al*.: Lack of association between IFNGR1 gene polymorphisms and biopsy-proven giant cell arteritis. *Clin Exp Rheumatol* 2010; 28: 31-4.
37. RODRIGUEZ-RODRIGUEZ L, CASTANEDA S, VAZQUEZ-RODRIGUEZ TR *et al*.: Role of the rs6822844 gene polymorphism at the IL2-IL21 region in biopsy-proven giant cell arteritis. *Clin Exp Rheumatol* 2011; 29 (Suppl. 64): S12-16.
38. TORRES O, PALOMINO-MORALES R, VAZQUEZ-RODRIGUEZ TR *et al*.: Lack of association between hypoxia inducible factor-1 alpha gene polymorphisms and biopsy-proven giant cell arteritis. *Clin Exp Rheumatol* 2010; 28 (Suppl. 57): 40-5.