Influence of *IL2RA* rs2104286 Polymorphism in the Risk of Biopsy-proven Giant Cell Arteritis

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ABSTRACT. *Objective.* To assess the influence of the *IL2RA* rs2104286 A>G polymorphism on susceptibility to and clinical spectrum of manifestations of biopsy-proven giant cell arteritis (GCA).

Methods. Our study included 318 patients with biopsy-proven GCA. DNA from patients and healthy controls was obtained from peripheral blood. Samples were genotyped for the *IL2RA* rs2104286 A>G polymorphism using a predesigned TaqMan allele discrimination assay and by PCR amplification.

Results. Although GCA patients showed a higher frequency of the minor allele homozygote of *IL2RA* rs2104286 (GG) compared to controls (5.1% vs 2.8%, respectively; $p = 0.06$, odds ratio 1.84, 95% confidence interval 0.91–3.70), the allele distribution showed no significant differences between GCA patients and controls. Stratification of GCA patients according to sex or polymyalgia rheumatica, jaw claudication, visual ischemic manifestations, or other severe ischemic complications did not yield significant differences in the allele or genotype frequencies of the *IL2RA* rs2104286 polymorphism.

Conclusion. *IL2RA* rs2104286 polymorphism does not appear to be a genetic risk factor for susceptibility to biopsy-proven GCA. Also, this polymorphism does not seem to be implicated in the clinical expression of this vasculitis. (First Release September 1 2010; J Rheumatol 2010;37:2331–3; doi:3899/jrheum.100388)

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Giant cell arteritis (GCA) is an inflammatory vasculopathy affecting medium-size and large arteries, with inflammatory lesions extending throughout all layers of the arterial wall. Vascular infiltrate is composed of T cells (the dominant cell population), highly activated macrophages and, in some cases, multinucleated giant cells¹. CD4 T cells undergo clonal proliferation, confirmed by the isolation of identical clones from separate and independent parts of the same artery, and from both temporal arteries of the same patient¹. This phenomenon supports an antigen-specific trigger within the affected arteries that induces production of interleukin 2 (IL-2) and interferon- γ ¹.

IL-2 has a central role for T cell responses, by controlling the magnitude of clonal expansion, development of effector cells, and subsequent contraction of antigen-specific T cells². Also, this cytokine plays a role in activation-induced cell death and in the regulatory T cell homeostasis².

IL-2 receptor (IL-2R) is composed of 3 different subunit complexes, CD25, CD122, and CD132³. CD25 is mostly limited to activated T cells, including regulatory T cells³.

IL2RA rs2104286 (A>G) polymorphism has been associated with susceptibility to various autoimmune diseases, such as multiple sclerosis^{4,5}, rheumatoid arthritis⁶, and type 1 diabetes⁵. This polymorphism has shown no association with other autoimmune diseases such as systemic lupus ery-

thematosus or antineutrophil cytoplasmic antibody-associated vasculitis⁷.

GCA is a complex polygenic disease in which more than 1 genetic locus is likely to contribute to disease susceptibility and severity⁸.

Taking these considerations together, the aim of our study was to assess the potential association between *IL2RA* rs2104286 polymorphism and GCA in a large series of patients with biopsy-proven disease. We also studied whether this polymorphism may be implicated in the clinical spectrum of manifestations of GCA.

MATERIALS AND METHODS

Patients. We recruited 318 Spanish patients who fulfilled the 1990 American College of Rheumatology classification criteria for GCA⁹. All patients had a positive temporal artery biopsy¹⁰. Subjects were from Departments of Rheumatology or Internal Medicine from 5 Spanish cities: Lugo, Madrid, L'Hospitalet de Llobregat, Sabadell, and Granada. A control population of 867 healthy controls from corresponding cities matched with GCA patients was also assessed. Approval from the local ethical committees and written informed consent from patients and controls were obtained.

Clinical manifestations including polymyalgia rheumatica, jaw claudication, visual ischemic manifestations, cerebrovascular accidents (including stroke and/or transient ischemic attacks), severe ischemic manifestations, and the presence of a "true" occlusive disease were assessed^{10,11,12}.

Genotyping methods. DNA from patients and controls was obtained from peripheral blood, using standard methods. Samples were genotyped for *IL2RA* rs2104286 A>G polymorphism using a TaqMan 5' allele discrimination assay (Applied Biosystems, Foster City, CA, USA), following the manufacturer's specifications.

Statistical analysis. Power of the study was assessed using Quanto v1.2.3. 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 (CI) were calculated according to Woolf's method using the Statcalc program (Epi-Info 2002; US Centers for Disease Control, Atlanta, GA, USA).

RESULTS

Clinical features of patients with GCA are summarized in Table 1. No evidence of departure from Hardy-Weinberg equilibrium was observed in controls. The case:control ratio was 1:2.7. The power of our study to find a difference between GCA patients and controls with an estimated OR between 1.5 and 2.0, a type I error rate of 0.05, a dominant inheritance mode, and 0.0001% of population risk was 71%–99%.

Association of IL2RA rs2104286 polymorphism with GCA. Allele frequencies in controls were similar to those reported⁶. We found a nonsignificant trend towards a higher frequency of homozygotes for the minor allele G among GCA patients compared to controls (GG in GCA patients 5.1% vs 2.8% in controls; p = 0.06, OR 1.84, 95% CI 0.91–3.70; Table 2). However, no significant differences were found in the allelic distribution between GCA patients and controls.

Genotype and allele frequencies of IL2RA rs2104286 polymorphism according to patients' clinical manifestations. No significant differences in the genotypic or allelic frequencies

Table 1. Main clinical features of 318 patients with biopsy-proven giant cell arteritis Data in parentheses are % unless otherwise indicated.

Feature	Variable
Age at diagnosis, yrs, median (IQR)	75 (70–79)
Women	216 (68)
Headache	267 (84)
Abnormal temporal artery on examination	196 (62)
Polymyalgia rheumatica	151 (48)
Jaw claudication	130 (41)
Visual ischemic manifestations*	68 (21)
Stroke	14 (4)
Severe ischemic manifestation**	169 (53)
True occlusive disease***	44 (14)

* Transient visual loss including amaurosis fugax, permanent visual loss, diplopia. ** At least one of: visual manifestations, cerebrovascular accident (stroke and/or transient ischemic attacks), jaw claudication, limb claudication of recent onset. *** At least one of: permanent visual loss, stroke, limb claudication of recent onset.

Table 2. Genotype and allele frequencies of IL2RA rs2104286 polymorphism in healthy controls and patients with biopsy-proven GCA.

	Controls, n = 867 (%)	GCA, n = 318 (%)	p	OR (95% CI)
AA	559 (64.5)	202 (63.5)		1 = reference
AG	284 (32.8)	100 (31.4)	0.86	0.97 (0.73–1.30)
GG	24 (2.8)	16 (5.1)	0.06	1.84 (0.91–3.70)
AA + AG	843 (97.3)	302 (94.9)		1 = reference
GG	24 (2.8)	16 (5.1)	0.06	1.86 (0.93–3.70)
A	1402 (80.9)	504 (79.2)		1 = reference
G	332 (19.1)	132 (20.8)	0.38	1.11 (0.88–1.39)

were found when GCA patients were stratified according to sex or presence or absence of specific features of the disease (Table 3).

DISCUSSION

We examined for the first time the contribution of the *IL2RA* rs2104286 polymorphism to GCA susceptibility in a large series of histologically confirmed patients. However, our results do not confirm a role of the *IL2RA* rs2104286 polymorphism in susceptibility to GCA or in the clinical expression of this vasculitis. In this regard, only a nonsignificant increased frequency of homozygotes for the minor allele G among GCA patients compared to healthy controls was found.

IL2RA rs2104286 polymorphism has been associated with other autoimmune diseases, especially with multiple sclerosis^{4,5}. We aimed to establish the influence of this polymorphism in GCA because of the role that IL-2 has in CD4+ T cell regulation. Although IL-2 is dispensable for the generation of effective T cell-mediated immunity, several studies indicate that a failure in the production of CD4+CD25+ regulatory T cells is the underlying cause of autoimmunity

Table 3. Minor allele G frequency (%) of *IL2RA* rs2104286 polymorphism in GCA patients according to gender and the presence (With) or absence (Without) of specific clinical features of the disease.

Feature	With, %	Without, %	p	OR (95% CI)
Gender [†]	20.4	21.6	0.73	0.93 (0.61–1.43)
Polymyalgia rheumatica	21.5	20.2	0.68	1.08 (0.73–1.62)
Jaw claudication	21.5	20.3	0.71	1.08 (0.72–1.62)
Visual ischemic manifestations*	19.1	21.3	0.58	0.87 (0.53–1.44)
Stroke	17.9	21.0	0.69	0.82 (0.24–2.26)
Severe ischemic complications**	21.9	19.6	0.47	1.15 (0.77–1.72)
True occlusive disease***	21.6	20.7	0.85	1.06 (0.59–1.88)

[†] With: females; Without: males. * Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia. ** At least one of: visual manifestations, cerebrovascular accident (stroke and/or transient ischemic attacks), jaw claudication, or limb claudication of recent onset. *** At least one of: permanent visual loss, stroke, or limb claudication of recent onset.

in the absence of IL-2¹³. In GCA patients, the peripheral blood count of CD4+CD25+ regulatory T cells is not significantly different from that observed in healthy subjects¹³.

IL2RA rs2104286 polymorphism has no effect on the percentage of CD4+CD25+ regulatory T or memory CD4+CD25+ T cells. However, it is associated with a lower percentage of CD4+ naive cells expressing CD25+ on the cell surface¹⁴; it is also associated with a lower proportion of CD69+CD4+ naive T cells that upregulate CD25 upon T cell activation¹⁴. As a consequence, it is possible that this polymorphism might cause decreased production of IL-2¹⁴, which would impair CD4+CD25+ regulatory T cell function¹⁵, taking into account that these cells themselves do not secrete IL-2² and this cytokine seems to function as a T cell growth factor for them.

Our results do not confirm a mayor contribution of *IL2RA* rs2104286 polymorphism in GCA.

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