Role of rs1343151 *IL23R* and rs3790567 *IL12RB2* Polymorphisms in Biopsy-proven Giant Cell Arteritis

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ABSTRACT. ***Objective.*** To assess the potential association between the rs1343151 *IL23R* and the rs3790567 *IL12RB2* polymorphisms and giant cell arteritis (GCA). We also studied whether these polymorphisms might influence the phenotypic expression of GCA.

Methods. In total, 357 Spanish patients with biopsy-proven GCA and 574 matched controls were assessed. DNA from patients and controls was obtained from peripheral blood. Samples were genotyped for the rs1343151 *IL23R* and the rs3790567 *IL12RB2* polymorphisms using a predesigned TaqMan allele discrimination assay and by polymerase chain reaction amplification.

Results. Regarding the rs1343151 *IL23R* polymorphism, no significant differences in the genotype or allele frequencies between GCA patients and healthy controls were observed. The frequency of the minor allele A of the rs3790567 *IL12RB2* variant was increased in GCA patients compared with controls (30.1% vs 25.7%, respectively; p = 0.039, OR 1.25, 95% CI 1.01–1.54). An increased frequency of subjects carrying the minor allele A (GA+AA genotypes) of the rs3790567 *IL12RB2* polymorphism was found among GCA patients compared with controls (52.8% vs 44.4%; p = 0.013, OR 1.40, 95% CI 1.06–1.85). Although a higher frequency of the combination of minor alleles (A-A) in the subgroup of patients with visual ischemic complications compared with the combination of both major alleles (G-G; p = 0.029) or with the other allelic combinations (p = 0.035) was found, logistic regression analysis showed that this association was no longer significant after adjustment for potential confounding factors (A-A vs G-G: OR 2.10, 95% CI 0.88–5.04, p = 0.096).

Conclusion. Our results support a potential influence of the rs3790567 *IL12RB2* polymorphism in the pathogenesis of GCA. (First Release Feb 1 2011; J Rheumatol 2011;38:889–92; doi:10.3899/jrheum.101046)

Key Indexing Terms:

GIANT CELL ARTERITIS | TEMPORAL ARTERY BIOPSY | GENETICS
IL12RB2 | *IL23R* | POLYMORPHISM

Giant cell arteritis (GCA) is the most common primary systemic vasculitis in the elderly in Western countries[1]. It is also a complex polygenic disease[2]. CD4 T cells are the dominant cell population in the vascular lesions[3], with Th1 and Th17 cells playing a role in pathogenesis of GCA[4]. IL-12 and IL-23 drive the development of naïve T cells into

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either one of those Th cells. Both molecules share a receptor subunit (IL-12Rß1). For proper signaling, IL-12 also has to bind to IL-12Rß2 and IL-23 to IL-23R[5,6], leading to different cellular responses. Both receptors are expressed in T cells; therefore, the responsiveness to either cytokine is determined by their respective expression[6].

The coding genes for both these receptor subunits are located in chromosome 1, less than 50 kb apart. Both have been associated with different autoimmune and inflammatory diseases[7,8,9].

The aim of our study was to analyze the potential influence of 2 polymorphisms, the rs1343151 variant in the *IL23R* gene and the rs3790567 variant in the *IL12RB2* gene, isolated or in combination, in susceptibility to biopsy-proven GCA and in the risk of ischemic GCA-related manifestations.

MATERIALS AND METHODS

Patients. We recruited 357 Spanish patients with a positive temporal artery biopsy[10] who fulfilled the 1990 American College of Rheumatology classification criteria for GCA[11] from Departments of Rheumatology or Internal Medicine from 5 Spanish cities: Lugo, Madrid, L'Hospitalet de Llobregat, Sabadell, and Granada. A control population of 574 healthy controls from the corresponding cities matched by age and sex with GCA patients was also assessed. Approval from the local ethical committees and written informed consent from patients and controls were obtained.

Clinical manifestations were assessed as reported[12,13,14].

Genotyping methods. DNA from patients and controls was obtained from peripheral blood, using standard methods. Samples were genotyped for the rs1343151 *IL23R* and the rs3790567 *IL12RB2* polymorphisms using a TaqMan 5' allele discrimination assay (Applied Biosystems, Foster City, CA, USA) following manufacturer's instructions.

Duplicate samples and negative controls were included. The genotyping success rate in the healthy control group was 100% for both polymorphisms, in the GCA group, 98.6% (352/357) for the rs1343151 *IL23R* polymorphism and 97.5% (348/357) for the rs3790567 *IL12RB2* polymorphism.

Statistical analysis. Genotype data were checked for deviation from Hardy-Weinberg equilibrium using the program http://ihg.gsf.de/cgi-bin/hw/hwa1.pl (Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK). Linkage disequilibrium (LD) values (D', r^2) and allelic combinations were generated using UNPHASED software[15]. Only those allelic combinations with a frequency in healthy controls higher than 5% were analyzed. The chi-square or Fisher tests were used to compare allelic, genotypic, or allelic combination distributions. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated according to Woolf's method. Multiple logistic regression was used to adjust the association between GCA-related ischemic manifestations and alleles, genotypes, or allelic combinations of both polymorphisms, by sex, age at GCA diagnosis, and traditional cardiovascular risk factors as potential confounders.

RESULTS

Clinical features of patients with GCA are summarized in Table 1. No evidence of departure from Hardy-Weinberg equilibrium was observed in controls or GCA patients. The power of this study for finding 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 84.5% to 99.9% for the rs3790567 *IL12RB2* and 81.6% to 99.7% for the rs1343151 *IL23R* variant. No differences in genotype or allele distribution, ethnicity, or age at diagnosis were observed among the GCA patients and controls of the different study centers. However, a higher percentage of females with biopsy-proven GCA were recruited in Madrid compared with the other centers (83% vs 61%, respectively; $p < 0.01$). The 2 polymorphisms were not in LD (D' = 0.21, $r^2 = 0.033$).

Table 1. Main clinical features of 357 patients with biopsy-proven giant cell arteritis. Data in parentheses are percentages unless otherwise indicated.

Feature	Value
Age at diagnosis, yrs, median (IQR)	75.0 (70.0–79.0)
Women	247/357 (69)
Headache	264/323 (82)
Abnormal temporal artery on examination	188/308 (61)
Polymyalgia rheumatica	167/343 (49)
Jaw claudication	143/343 (42)
Visual ischemic manifestations*	74/343 (22)
Stroke	14/343 (4)
True occlusive disease **	51/343 (15)

* Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia. ** At least one of the following manifestations: permanent (irreversible) visual loss, stroke, or limb claudication of recent onset.

Influence of rs1343151 IL23R and rs3790567 IL12RB2 polymorphisms in susceptibility to GCA. Regarding the rs3790567 *IL12RB2* polymorphism, a higher frequency of the minor allele A (30.1% vs 25.7%; p = 0.039, OR 1.25, 95% CI 1.01–1.54) and carriers of the minor allele (GA+AA: 52.8% vs 44.4%, p = 0.013, OR 1.40, 95% CI 1.06–1.85) was observed in GCA patients compared with controls, respectively (Table 2). No significant differences were observed in the distribution of the rs1343151 *IL23R*.

Influence of rs1343151 IL23R and rs3790567 IL12RB2 polymorphisms in the risk of GCA-related ischemic manifestations. As shown in Table 3, a higher frequency of the combination of minor alleles (A-A) in the subgroup of patients with visual ischemic complications compared with the combination of both major alleles (G-G; p = 0.029, OR 2.10, 95% CI 1.01–4.35) or with the other allelic combinations (p = 0.035, OR 1.99, 95% CI 0.99–3.96) was found. However, no significant differences were observed in the adjusted analysis. In this regard, the association between the allelic combination and visual ischemic manifestations remained out of the range of significance (A-A vs G-G: OR 2.10, 95% CI 0.88–5.04, p = 0.096).

DISCUSSION

In this study we assessed for the first time the contribution of the rs1343151 *IL23R* and rs3790567 *IL12RB2* polymorphisms, isolated and in combination, in susceptibility to biopsy-proven GCA. Our results support a potential role of the rs3790567 *IL12RB2* polymorphism, but not the rs1343151 *IL23R* variant, in susceptibility to this condition.

Table 2. Genotype, allele, and allelic combination frequencies of the rs3790567 *IL12RB* and rs1343151 *IL23R* gene polymorphisms in patients with biopsy-proven GCA and healthy controls.

	GCA	Controls	p	OR (95% CI)
rs3790567 *IL12RB*	n = 352 (%)	n = 574 (%)		
GG	166 (47.2)	319 (55.6)		1, Reference
GA	160 (45.4)	215 (37.5)	0.011	1.43 (1.07–1.91)
AA	26 (7.4)	40 (6.9)	0.41	1.25 ((0.71–2.18)
GA+AA	186 (52.8)	255 (44.4)	0.013	1.40 (1.06–1.85)
	2n = 704	2n = 1148		
G	492 (69.9)	853 (74.3)		1, Reference
A	212 (30.1)	295 (25.7)	0.039	1.25 (1.01–1.54)
rs1343151 *IL23R*	n = 348 (%)	n = 574 (%)		
GG	144 (41.4)	236 (41.1)		1, Reference
GA	159 (45.7)	260 (45.3)	0.99	1.00 (0.75–1.35)
AA	45 (12.9)	78 (13.6)	0.79	0.95 (0.61–1.47)
	2n = 696	2n = 1148		
G	447 (64.2)	732 (63.8)		1, Reference
A	249 (35.8)	416 (36.2)	0.84	0.98 (0.80–1.20)
IL12RB-IL23R	2n = 532	2n = 916		
G-G	280 (52.6)	502 (54.8)		1, Reference
A-G	122 (22.9)	235 (25.7)	0.59	0.93 (0.71–1.22)
G-A	81 (15.2)	114 (12.4)	0.14	1.27 (0.91–1.78)
A-A	49 (9.2)	65 (7.1)	0.14	1.35 (0.89–2.05)
D´	0.21			
r^2	0.033			

Table 3. Association between the allelic combinations of the rs3790567 *IL12RB* and the rs1343151 *IL23R* polymorphisms and specific clinical manifestations of GCA.

IL12RB - IL23R	Without, n (%)	With, n (%)	p	OR (95% CI)
Polymyalgia rheumatica				
G-G	143 (55.9)	119 (50.9)		1, Reference
A-G	60 (23.4)	52 (22.2)	0.86	1.04 (0.65–1.66)
G-A	30 (11.7)	40 (17.1)	0.082	1.60 (0.91–2.82)
A-A	23 (9.0)	23 (9.8)	0.57	1.20 (0.61–2.35)
Visual ischemic manifestations				
G-G	209 (55.0)	53 (48.2)		1, Reference
A-G	89 (23.4)	23 (20.9)	0.95	1.02 (0.57–1.82)
G-A	52 (13.7)	18 (16.4)	0.32	1.37 (0.70–2.63)
A-A	30 (7.9)	16 (14.5)	0.029	2.10 (1.01–4.35)
Jaw claudication				
G-G	149 (52.5)	113 (54.8)		1, Reference
A-G	64 (22.5)	48 (23.3)	0.96	0.99 (0.62–1.58)
G-A	41 (14.4)	29 (14.1)	0.80	0.93 (0.53–1.65)
A-A	30 (10.6)	16 (7.8)	0.29	0.70 (0.35–1.41)
True occlusive disease				
G-G	224 (53.9)	38 (51.4)		1, Reference
A-G	94 (22.6)	18 (24.3)	0.70	1.13 (0.61–2.08)
G-A	57 (13.7)	13 (17.6)	0.40	1.34 (0.67–2.69)
A-A	41 (9.9)	5 (6.8)	0.51	0.72 (0.27–1.93)

Although both Th1 and Th17 cells seem to play a role in GCA[4], our results may indicate a more important role of Th1 cells in initiation of this condition.

The potential association between allelic combination and visual ischemic complications observed in this study might suggest a potential collaborative effect of both Th1 and Th17 cells in the development of these manifestations. However, this association was lost after adjustment for demographic and classic cardiovascular risk factors.

Although our cohort encompassed the largest series of patients with biopsy-proven GCA assessed in a genetic study on GCA and this vasculitis is a relatively uncommon condition, we are aware of the potential limitation of the study related to the sample size. Therefore, further studies are required to fully characterize the clinical significance of our findings and the contribution of these polymorphisms to the pathogenesis of GCA.

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