

Role of the rs6822844 gene polymorphism at the *IL2-IL21* region in biopsy-proven giant cell arteritis

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ABSTRACT

Objectives. To assess the influence of the interleukin (IL)2-IL21 rs6822844 G/T polymorphism in the susceptibility to biopsy-proven giant cell arteritis (GCA) and in the clinical spectrum of manifestations of this vasculitis.

Methods. Two hundred and seventy-two biopsy-proven GCA patients were included in this study. DNA from patients and matched controls (n=791) was obtained from peripheral blood. Samples were genotyped for the rs6822844 polymorphism using a pre-designed TaqMan allele discrimination assay and by polymerase chain reaction amplification.

Results. No significant differences in the allele and genotype frequencies between biopsy-proven GCA patients and controls were observed. However, the stratification of GCA patients disclosed some differences according to gender and ischemic manifestations of the disease. In this regard, the frequency of the minor allele T was increased in males (14.8%) compared to females (8.4%) (odds ratio-OR:1.89 (95% confidence interval-CI: 1.09–3.28); p=0.02; Bonferroni adjustment p=0.12). Also, minor allele T frequency was increased in GCA patients with severe ischemic complications (12.8%) compared to those without severe ischemic complications (7.7%) (OR:1.72 (95% CI: 0.97–3.05); p=0.05; Bonferroni adjustment p=0.30), and specifically in patients with jaw claudication (13.7% versus 8.2% in those without jaw claudication; OR:1.76 (95% CI: 1.02–3.04); p=0.04; Bonferroni adjustment p=0.24).

Conclusion. *IL2-IL21* rs6822844 polymorphism does not appear to be a genetic risk factor for susceptibility to biopsy-proven GCA. However, this gene polymorphism may contribute to the different phenotypic expression of

this vasculitis, in particular in the development of ischemic complications of the disease.

Introduction

GCA is a complex polygenic disease (1, 2) characterised by vasculitis affecting large and medium-sized blood vessels with predisposition to the involvement of cranial arteries derived from the carotid artery (3, 4). Previous studies have shown the familiar clustering of this condition and the strong association of this vasculitis with genes located within the major histocompatibility complex (MHC) class I (5) and class II (6, 7) regions. 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 (8-27).

Several genetic studies have shown that susceptibility genes/loci are commonly shared by many autoimmune diseases. In this regard, the interleukin (*IL*)2-*IL21* region at 4q27 has shown increasing evidence of association with a number of autoimmune diseases including Crohn's disease (28, 29), ulcerative colitis (30), type 1 diabetes mellitus (31-33), rheumatoid arthritis (33-35), juvenile idiopathic arthritis (36), psoriasis and psoriatic arthritis (37), systemic lupus erythematosus (38) and multiple sclerosis (39, 40). Among the different polymorphisms located in this intergenic region, the *IL2-IL21* rs6822844 G/T was found to be the most significantly associated with autoimmune disease susceptibility. This variant has been studied in Behcet's disease, another type of systemic vasculitis, and was not found to have a significant association with this condition (33).

Due its pleiotropic actions, *IL-21* has been implicated in several inflamma-

tory and auto-immune diseases. This cytokine controls a complex range of immune processes through both positive and negative regulatory effects on both lymphoid and myeloid target cells (41). IL-21 effects seem to be specific to the stage of the immune response, as well as to the cytokine environment (42). It is mainly produced by CD4⁺T cells, both Th1, Th2 and Th17 cells (43). However, neither the balance between CD4⁺ and CD8⁺ T-cells nor the balance between Th1 and Th2 cells seems to be affected by IL-21 (42). *In vitro* studies have shown that this cytokine can both induce or reduce interferon- γ production by Th1 cells (42). IL-21 inhibits angiogenesis *in vitro* and *in vivo* through reduction of the expression of growth factors produced by endothelial cells (44). This cytokine can also stimulate the production of matrix metalloproteinases by fibroblasts (45). Furthermore, on dendritic cells, IL21 is able to maintain an immature phenotype, inhibiting the upregulation of co-stimulatory molecules and down-regulating T-cell responses (41).

On the other hand, IL-2 is essential for the correct function of the immune system. This cytokine is a key factor for T-cell activation and immune function (46). Abnormal control of IL-2 production impacts a number of immune cell functions, especially the development and homeostasis of CD4⁺CD25⁺ regulatory T-cells (47).

Taking all these considerations into account, in the present study we aimed to assess the potential association between the *IL2-IL21* rs6822844 G/T polymorphism and GCA. We also studied whether this polymorphism may be implicated in the clinical spectrum of manifestations of this vasculitis, in particular whether an association between the *IL2-IL21* rs6822844 G/T polymorphism and the development of ischemic complications in the setting of this vasculitis might exist. For this purpose we studied a large series of biopsy-proven GCA patients.

Material and methods

Patients

Two hundred and seventy-two Spanish patients that fulfilled the 1990 American College of Rheumatology classifi-

cation criteria for GCA were included in the present study (48). All patients were required to have 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 (49). Subjects were recruited from the Departments of Rheumatology or Internal Medicine from 5 Spanish cities: Lugo (Hospital Xeral-Calde), Madrid (Hospital Clínico San Carlos and Hospital de la Princesa), L'Hospitalet de Llobregat (Hospital Universitari de Bellvitge), Sabadell (Corporació Sanitària Parc Taulí) and Granada (Hospital Clínico San Cecilio).

A control population composed of 791 healthy controls from the corresponding cities, matched with GCA patients, was also evaluated.

To encompass the clinical spectrum of GCA manifestations observed at the time of disease diagnosis, clinical features found from the onset of the disease to 1 month after the initiation of the corticosteroid treatment were assessed (50, 51). These features were considered to be present based on previously established definitions and included the presence of polymyalgia rheumatica (PMR) (52, 53), jaw claudication (54, 55), visual ischemic manifestations including permanent visual loss (54, 55), and severe ischemic manifestations (55, 56).

Of the GCA patients in whom full clinical information was available, 184/272 (67.7%) were females. The median (range) age at the time of disease diagnosis was 75 (58–93) years, 126/271 (46.5%) had polymyalgia rheumatica, 41.7% (113/271) had jaw claudication, 62/271 (22.9%) had visual manifestations, 20/271 (7.4%) experienced permanent visual loss, 13/271 (4.8%) had a stroke and 148/271 (54.6%) suffered severe ischemic manifestations.

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

DNA from patients and controls was obtained from peripheral blood, using standard methods. Samples were geno-

typed for the *IL2-IL21* rs6822844 polymorphism using a TaqMan 5' allele discrimination assay (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labelled with the fluorescent dyes VIC and FAM. Polymerase chain reaction (PCR) was carried out in a total reaction volume of 4 μ l with the following amplification protocol: denaturation at 95°C for 10min, followed by 45 cycles of denaturation at 92°C for 15s and finished with annealing and extension at 60°C for 1min. 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 (Applied Biosystems) (57). 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). A Bonferroni correction was applied to adjust for multiple testing; *p*-values <0.05 were considered statistically significant.

Results

As previously reported (54), the vast majority of patients had an erythrocyte sedimentation greater than 50mm/1st hour at the time of the disease diagnosis.

No evidence of departure from Hardy-Weinberg equilibrium was observed in controls. The case: control ratio was 1:2.7. The power of this study for finding a difference between GCA patients and healthy control 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 52% to 94%.

Influence of IL2-IL21 rs6822844 polymorphism in susceptibility to GCA
GCA patients and healthy controls

showed a similar distribution of genotypes and allele frequencies (Table I). With respect to this, the frequency of the minor allele T was slightly lower in GCA patients than in healthy controls but the difference did not reach statistical significance (10.5% in patients compared to 11.1% in controls, $p=0.71$). Likewise, the genotype distribution did not show significant differences between GCA patients and healthy controls ($p=0.55$).

Genotype and allele frequencies of the IL2-IL21 rs6822844 polymorphism in biopsy-proven GCA patients stratified according to gender and clinical manifestations of the disease

To assess whether the *IL2-IL21* rs6822844 polymorphism might influence the phenotypic expression of this vasculitis, biopsy-proven GCA patients were stratified according to gender, and the presence or absence of PMR and ischemic complications of the disease (Table II). Interestingly, some differences according to gender and ischemic manifestations were observed. In this regard, the frequency of the minor allele T was increased in males (14.8%) compared to females (8.4%) ($p=0.02$; Bonferroni adjustment $p=0.12$). Also, we found a trend towards a higher frequency of the minor allele T among patients with severe ischemic complications (12.8%) compared to those without severe ischemic complications (7.7%) ($p=0.05$; Bonferroni adjustment $p=0.30$). Differences in T allele frequency were also present when we specifically compared patients with and without jaw claudication (13.7% versus 8.2% respectively; OR: 1.76 (95% CI: 1.02–3.04); $p=0.04$; Bonferroni adjustment $p=0.24$). Moreover, an increased frequency of the minor allele T was observed in the subgroup of patients who experienced permanent (irreversible) visual loss (17.5%) compared to the remaining biopsy-proven GCA patients (10%) (OR: 2.09 (95% CI: 0.90–4.86)). However, the difference did not achieve a statistically significant difference ($p=0.13$; Bonferroni adjustment $p=0.78$) probably due to the relatively low number

Table I. Genotype and allele frequencies of the *IL2-IL21* rs6822844 G/T polymorphism in healthy controls and patients with biopsy-proven GCA.

	Controls n=791 (%)	GCA n=272 (%)	p-value	OR (95% CI)
<i>Genotype</i>				
GG	629 (79.5)	217 (79.8)	0.93	1.01 (0.72–1.42)
GT	149 (18.8)	53 (19.5)	0.81	1.05 (0.74–1.49)
TT	13 (1.6)	2 (0.7)	0.27	0.62 (0.16–2.39)
<i>Allele (2n)</i>				
G	1407 (88.9)	487 (89.5)		1
T	175 (11.1)	57 (10.5)	0.71	0.95 (0.69–1.30)

of patients who suffered irreversible visual loss. Finally, no genotype or allele differences were observed when GCA patients were stratified according to the presence of classic cardiovascular risk factors (data not shown).

Discussion

In the present study we analysed for first time the potential implication of the *IL2-IL21* rs6822844 G/T polymorphism in the susceptibility to GCA in a large series of histologically confirmed patients with this vasculitis. Our results do not support a role of this polymorphism in the susceptibility to GCA. However, we observed a higher frequency of the minor allele T in men and among patients with severe ischemic complications. It is possible that certain autoimmune diseases may have common underlying physiopathologic mechanisms sharing several susceptibility genes/loci implicated in the inflammatory response, despite having different target organs. In this regard, the *IL2-IL21* rs6822844 G/T polymorphism has been found to be associated with several autoimmune diseases such as Crohn's disease (28, 29), ulcerative colitis (30), type 1 diabetes mellitus (31–33), rheumatoid arthritis (33–35), juvenile idiopathic arthritis (36), psoriasis and psoriatic arthritis (37), systemic lupus erythematosus (38) and multiple sclerosis (39, 40). The absence of association of the *IL2-IL21* rs6822844 G/T polymorphism with GCA may reflect a different physiopathology for susceptibility to GCA.

To the best of our knowledge, rs6822844 is in a noncoding poly-

morphism located between *IL21* (upstream) and *IL2* (downstream) with no molecular function identified. However, this polymorphism may play a role in autoimmunity by modulating the gene expression of these two genes or by being in linkage disequilibrium with a causative mutation. Interestingly, the neighbouring sequences up and downstream rs6822844 show strong homology with mature microRNA (58, 59). MicroRNAs are post-transcriptional regulators that bind to complementary sequences in the three prime unrelated regions (3' UTRs) of target messenger RNA transcripts (mRNAs), usually resulting in gene silencing inhibiting their translation (60). The major allele G of the *IL2-IL21* rs6822844 polymorphism is conserved in all microRNA-precursor hairpin structures. Therefore, it is possible that the mutation might abolish microRNA production, altering the expression of the genes regulated by this micro-RNA.

The present data based on the stratification of biopsy-proven GCA patients according to the presence of ischemic complications suggest that the *IL2-IL21* rs6822844 G/T polymorphism might be implicated in the development of ischemic events in the setting of this vasculitis. Recent studies have disclosed that a severe inflammation-induced angiogenic activity may counteract the risk of ischemic complications in patients with GCA (61). In keeping with these observations, we described that a functional variant of vascular endothelial growth factor (*VEGF*) gene was associated with severe ischemic complications in biopsy-proven GCA patients (19). The frequency of *VEGF* alleles associated with lower circulat-

Table II. Genotype and allele frequencies of the *IL2-IL21* rs6822844 G/T polymorphism in biopsy-proven GCA patients stratified according to gender (males) or the presence (yes) or absence (no) of specific clinical features of the disease.

	yes %	no %	p-value	OR (95% CI)	#p-value
Gender (males)					
GG	63 (71.6)	154 (83.7)		1	
GT	24 (27.3)	29 (15.8)	0.025	2.02 (1.09–3.74)	0.30
TT	1 (1.1)	1 (0.5)	0.53	2.44 (0.15–39.69)	
G	150 (85.2)	337 (91.6)		1	
T	26 (14.8)	31 (8.4)	0.02	1.89 (1.09–3.28)	0.12
Polymyalgia rheumatica					
GG	98 (77.8)	120 (81.1)		1	
GT	27 (21.4)	27 (18.2)	0.51	1.22 (0.67–2.22)	
TT	1 (0.8)	1 (0.7)	0.89	1.22 (0.08–19.83)	
G	223 (88.5)	264 (90.4)		1	
T	29 (11.5)	28 (9.6)	0.48	1.21 (0.70–2.09)	
Jaw claudication					
GG	84 (73.7)	134 (83.8)		1	
GT	29 (25.4)	25 (15.6)	0.045	1.85 (1.02–3.37)	0.54
TT	1 (0.9)	1 (0.6)	0.74	1.60 (0.10–25.85)	
G	195 (86.3)	292 (91.8)		1	
T	31 (13.7)	26 (8.2)	0.04	1.76 (1.02–3.04)	0.24
Visual ischemic manifestations*					
GG	47 (75.8)	171 (80.7)		1	
GT	14 (22.6)	40 (18.9)	0.49	1.27 (0.64–2.54)	
TT	1 (1.6)	1 (0.5)	0.36	3.64 (0.22–59.27)	
G	108 (87.1)	379 (90.2)		1	
T	16 (12.9)	41 (9.8)	0.32	1.40 (0.76–2.58)	
Permanent visual loss					
GG	14 (70.0)	204 (80.3)		1	
GT	5 (25.0)	49 (19.3)	0.47	1.49 (0.51–4.32)	
TT	1 (5.0)	1 (0.4)	0.063	14.57 (0.86–245.49)	0.76
G	33 (82.5)	452 (90.0)		1	
T	7 (17.5)	50 (10.0)	0.13	2.09 (0.90–4.86)	0.78
Severe ischemic complications**					
GG	112 (75.7)	104 (84.6)		1	
GT	34 (23.0)	19 (15.5)	0.11	1.66 (0.89–3.09)	
TT	2 (1.4)	0 (0.00)	-	-	
G	258 (87.2)	227 (92.3)		1	
T	38 (12.8)	19 (7.7)	0.05	1.72 (0.97–3.05)	0.30

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

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

#Bonferroni adjusted p-value.

ing VEGF levels was significantly increased in the subgroup of GCA patients with severe ischemic complications (19).

Taking together all these observations, it is possible that the mutant *IL2-IL21* rs6822844 T allele might alter compensatory physiologic mechanisms aimed to counteract ischemic complications of GCA. This fact might lead to an increased risk of ischemic complications in patients with GCA. However, replication studies are needed to support a role of the *IL2-IL21* rs6822844 polymorphism in the development of ischemic complications of this vasculitis.

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