Lack of association between *IFNGR1* gene polymorphisms and biopsy-proven giant cell arteritis

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ABSTRACT

Background and objective. *Since IFN-gamma plays a pivotal role in the pathogenesis of giant cell arteritis (GCA), a polygenic primary systemic vasculitis involving elderly people from Western countries, in the present study we analysed for first time the implication of three IFN-gamma receptor (IFNGR) 1 gene variants in the susceptibility to and clinical expression of GCA.*

Methods. *Two hundred and sixteen biopsy-proven GCA patients and 460 matched controls were assessed. DNA from patients and controls was obtained from peripheral blood. Samples were genotyped for three single nucleotide polymorphisms (SNPs) rs1327474 (-611A/G), rs11914 (+189G7C) and rs7749390 (+95C/T) of the IFNGR1 gene using a pre-designed TaqMan allele discrimination assay. Post PCR, the genotype of each sample was attributed automatically by measuring the allelic specific fluorescence on the ABI PRISM 7900 sequence.*

Results. *No significant differences in the genotype or allele distribution between GCA patients and controls for the three IFNGR1 gene variants were found. Furthermore, no significant differences in the genotype distribution were observed when GCA patients were stratified according to the presence of specific clinical features of the disease such as polymyalgia rheumatica or severe ischemic complications including visual ischemic manifestations.*

Conclusion. *Our results do not show an implication of IFNGR1gene polymorphisms in the susceptibility to and clinical expression of GCA.*

Introduction

Giant cell arteritis (GCA) is the most common systemic vasculitis in the elderly in European countries and North America (1-3). GCA is a large and medium-sized blood vessel granulomatous vasculitis characterised by systemic inflammatory response and the involvement of the aorta and especially its cranial branches (1, 2, 4). This is an antigen-driven disease with local T-cell and macrophage activation in the vessel wall with an important role of proinflammatory cytokines (5). Activated T cells experience clonal expansion and are stimulated to produce interferon-gamma (IFN-γ). This leads to the differentiation and migration of macrophages and the formation of giant cells. IFN-γ specifically seems to play a pivotal role in the pathogenesis of GCA (5), and in the clinical expression of this vasculitis (6). High transcription of IFN-γ mRNA was associated with the formation of giant cells and with the evidence of cranial ischemic symptoms in GCA patients (6). IFN-γ may dictate the functional properties of other cell populations in the vascular infiltrates and guide the response-to-injury reaction of the artery (6).

GCA is a complex polygenic disease and a number of gene polymorphisms have been reported to be implicated in both disease susceptibility and the presence of specific clinical patterns of this vasculitis (7, 8). In this regard, a microsatellite polymorphism consisting of a dinucleotide cytosine-adenine (CA) repeat in the first intron of the *IFN-γ* gene was associated with some clinical differences between biopsy-proven GCA and isolated polymyalgia rheumatica (PMR) (9). Moreover, in assessing this microsatellite polymorphism, we observed an association between a 126 base pair allele (high IFN-γ production) and GCA patients with visual ischemic manifestations, and an inverse correlation with the 128 base pair allele (low IFN-γ producer) (9).

IFN-γ receptors (IFNGRs) are present on virtually all cells. They consist of two transmembrane chains that are required for activation. The IFN-γ receptor 1 (IFNGR1) chain has an important role as it binds the functional IFN-γ ligand (10). The *IFNGR1* gene is located on chromosome 6q23 (10). An association of the polymorphism of IFNGR1 gene with systemic lupus erythematosus was reported (11). However, to the best of our knowledge, no information on the potential association of *IFNGR1* gene polymorphisms with primary systemic vasculitides has been reported.

Taking all these considerations together, in the present study we aimed to establish for first time whether three single nucleotide polymorphisms (SNPs) of the *IFNGR1* gene: rs1327474 (-611A/G), rs11914 (+189G/C) and rs7749390 (+95C/T) may be implicated in the susceptibility to GCA and the clinical spectrum of this vasculitis.

Briefly, the G allele of the rs1327474 at -611 position conferred significantly higher transcription activity in human cell lines, resulting in higher expression of the protein (12). The rs7749390 (+95C/T) *IFNGR1*gene polymorphism resides in intron 1 and it is close to a mRNA splicing site. Nevertheless, the role of this polymorphism in IFNGR1 mRNA splicing is not clear and the influence of this *IFNGR1*gene variant has been related with the fact that this polymorphism is a perfect proxy of -56C/T IFNGR1 (13), which has been documented to affect IFNR1 promoter activity distinctly in various cellular contexts conferring a functional role to this polymorphism (12, 14). The rs11914 (+189) at exon7 of *IFNGR1*gene is close to a mRNA splicing site and although its functional relevance in the IFNGR1 regulation is not elucidated yet, it is plausible to think that this variant could affects IFNGR1 mRNA splicing conferring a functional role to this polymorphism.

Patients and methods

Patients

A total of 216 patients diagnosed with biopsy-proven GCA were included in this study. Most of them (n=128) were diagnosed in the Division of Rheumatology of the Hospital Xeral-Calde (Lugo, northwest Spain). The remaining patients were diagnosed in two centers from Madrid (Hospital Clínico San Carlos and Hospital de la Princesa; n=78) and Granada (Hospital Clínico San Cecílio; n=10). A control population (n= 460) from the corresponding cities matched by age, and sex and ethnicity with GCA patients was also studied.

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 (15).

Severe ischemic complications, mainly strokes in the vertebrobasilar territory, may occur after the onset of corticosteroid therapy. In this regard, strokes have been observed within the first month after GCA diagnosis (16) and visual ischemic events have also been reported to occur within the first 48-72 hours after the onset of corticosteroid therapy (17). However, severe ischemic complications related to the disease are uncommon in corticosteroid-treated patients for at least 1 month. Due to this, to encompass the whole spectrum of clinical manifestations directly attributed to GCA, we assessed all the clinical manifestations that occurred in the period of time from the onset of GCA symptoms to 1 month after the onset of corticosteroid therapy.

GCA patients were considered to have PMR manifestations if they had severe bilateral ache and pain involving the neck, the shoulder and or the pelvic girdles, associated with morning stiffness (18, 19). Patients were considered to have visual ischemic manifestations in the setting of GCA if they experienced at least one of the following ocular manifestations: transient visual loss including amaurosis fugax, permanent visual loss, or diplopia (20). Severe ischemic complications were considered to be present if GCA patients suffered at least one of the following complications: visual ischemic complications, strokes and/or transient ischemic attacks, jaw claudication, or large-artery stenosis of the extremities that caused signs of occlusive manifestations (21).

There were no significant differences in the demographic and clinical features between biopsy-proven GCA patients from Lugo and those from Madrid or Granada (data not shown).

Patients and controls were included in this study after written informed consent. Ethical committee approval was obtained.

IFNGR1 gene genotyping

DNA was obtained from peripheral blood mononuclear cells, using standard methods. Briefly, samples were genotyped for *IFNGR1* rs1327474, *IFNGR1* rs11914 and IFNGR1 rs7749390 SNPs using a pre-developed TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labelled with the fluorescent dyes VIC and FAM, respectively. PCR reaction was carried out in a total reaction volume of 5 μl with the following amplification protocol: denaturation at 95ºC for 10 min, followed by 40 cycles of denaturation at 92ºC for 15 sec and finished with annealing and extension at 60ºC for 1 min. Post PCR, the genotype of each sample was attributed automatically by measuring the allelic specific fluorescence on the ABI PRISM 7900 Sequence Detection Systems using the SDS 2.3 software for allelic discrimination (Applied Biosystems, Foster City, CA, USA). Duplicate samples and negative controls were included to ensure accuracy of genotyping.

Statistical analysis

We used the χ^2 test for assessment of Hardy-Weinberg equilibrium. Genotype and allele frequencies were also analysed using χ^2 test. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated according to Woolf's method using the Statcalc program (Epi Info 2002, Centers for Disease Control and Prevention, Atlanta, GA, USA). *P*-values less than 0.05 were considered statistically significant.

Results

Two hundred and sixteen biopsy-proven GCA patients were enrolled. Most of them were women (n=146) (median age at disease diagnosis 74 years; range: 52-93 years). From the onset of GCA

symptoms to 1 month after the onset of corticosteroid therapy 172 (79.6%) had headache, 136 (63.0%) abnormal temporal artery on physical examination, 101 (46.8%) experienced PMR manifestations, 86 (39.8%) jaw claudication and 53 (24.5%) visual ischemic manifestations. In addition, 22 (10.2%) experienced irreversible (permanent) visual loss, 10 (4.6%) had strokes and 118 (54.6%) fulfilled the definitions for severe ischemic manifestations. Furthermore, as previously reported (22), most patients (n=212; 98.1%) had an erythrocyte sedimentation rate higher than 40 mm/1st hour.

Influence of the IFNGR1 *gene variants in the susceptibility to GCA*

We did not find evidence of departure from Hardy-Weinberg equilibrium in patients and controls.

No significant differences in the genotype and allele frequencies on the IFNGR1 gene variants were observed when GCA patients from Lugo were compared with those from Madrid or Granada. Moreover the allele and genotype distribution of the IFNGR1 gene variants was similar in controls from the 3 different Spanish regions (data not shown).

Table I shows the genotype and allele frequencies of the *IFNGR1* gene polymorphisms in GCA patients and control subjects. No significant differences in the genotype or allele distribution between GCA patients and controls for the three *IFNGR1* gene variants were found (Table I). In addition, these gene variants did not form haplotypes, since these polymorphisms were Taq SNPs of different haplotype blots and the linkage disequilibrium was very low ($r^2 \leq 0.3$).

Table I. *IFNGR1* gene polymorphisms in biopsy-proven GCA patients and healthy controls.

IFNGR1 (rs7749390)	GCA patients n=216 (%)	Controls n=460 (%)	*p*-value	OR (95% CI)
A/A	72 (33.3)	136 (29.6)	0.32	1.19 (0.83 – 1.71)
A/G	96 (44.4)	220 (47.8)	0.41	0.87 (0.62 – 1.22)
G/G	48 (22.2)	104 (22.6)	0.08	0.70 (0.47 -1.06)
A	240 (55.6)	492 (53.5)	0.48	1.09 (0.86 – 1.38)
G	192 (44.4)	428 (46.5)	0.48	0.92 (0.73 – 1.16)
(rs11914)				
T/T	142 (65.7)	303 (65.9)	0.97	0.99 (0.70 – 1.42)
G/T	64 (29.6)	139 (30.2)	0.88	0.97 (0.67 – 1.41)
G/G	10 (4.6)	18 (3.9)	0.66	1.19 (0.50 – 2.78)
T	348 (80.6)	745 (81.0)	0.85	0.97 (0.72 – 1.31)
G	84 (19.4)	175 (19.0)	0.85	1.03 (0.76 – 1.39)
(rs1327474)				
A/A	72 (33.3)	158 (34.3)	0.79	0.96 (0.67 – 1.36)
A/G	110 (50.9)	218 (47.4)	0.39	1.15 (0.82 – 1.61)
G/G	34 (15.7)	84 (18.3)	0.42	0.84 (0.53 – 1.32)
A	254 (58.8)	534 (58.0)	0.79	1.03 (0.81 – 1.31)
G	178 (41.2)	386 (42.0)	0.79	0.97 (0.76 – 1.23)

Table II. Lack of association between *IFNGR1* genotypes and typical disease features in biopsy-proven GCA patients.

		PMR		Visual ischemic manifestations		Severe ischemic complications	
		With n (%)	Without n (%)	With n (%)	Without n (%)	Without n (%)	With n (%)
IFNGR1							
rs7749390	AA	32 (31.7)	40 (34.8)	17 (32.1)	55 (33.7)	33 (37.3)	39 (33.1)
	AG	45 (44.6)	51 (44.3)	29 (54.7)	67 (41.1)	44 (44.9)	52 (44.1)
	GG	24 (23.8)	24 (20.9)	7 (13.2)	41 (25.2)	21 (21.4)	27 (22.9)
	A	109 (54.0)	131 57.0)	63 (59.4)	177 (54.3)	110 (56.1)	130 (55.1)
	G	93 (46.0)	99 (43.0)	43 (40.6)	149 (45.7)	86 (43.9)	106 (44.9)
rs11914	TT	70 (69.3)	72 (62.6)	36 (67.9)	106 (65.0)	71 (72.4)	71 (60.2)
	GT	27 (26.7)	37 (32.2)	15 (28.3)	49 (30.1)	24 (24.5)	40 (33.9)
	GG	4 (4.0)	6 (5.2)	2 (3.8)	8 (4.9)	3 (3.1)	7 (5.9)
	T	167 (82.7)	181 78.7)	87 (82.1)	261 (80.1)	166 (84.7)	182 (77.1)
	G	35 (17.3)	49 (21.3)	19 (17.9)	65 (19.9)	30 (15.3)	54 (22.9)
rs1327474	AA	30 (29.7)	42 (36.5)	15 (28.3)	57 (35.0)	35 (35.7)	37 (31.4)
	AG	55 (54.5)	55 (47.8)	30 (56.6)	80 (49.1)	46 (46.9)	64 (54.2)
	GG	16 (15.8)	18 (15.7)	8 (15.1)	26 (16.0)	17 (17.3)	17 (14.4)
	A	115 (56.9)	139 (60.4)	60 (56.6)	194 (59.5)	116 (59.2)	138 (58.5)
	G	87 (43.1)	91 (39.6)	46 (43.4)	132 (40.5)	80 (40.8)	98 (41.5)

Influence of the IFNGR1 *gene variants in the clinical spectrum of GCA*

To determine whether polymorphisms of the *IFNGR1* gene might influence the clinical spectrum and the severity of the GCA, we stratified biopsy-proven GCA patients according to the presence/absence of PMR and visual ischemic manifestations. However, as shown in Table II, no significant differences for the three *IFNGR1* gene variants were found. It was also the case when patients were stratified according to the presence/absence of severe ischemic complications of this vasculitis (Table II). Moreover, the genotypic distribution did not differ when we excluded from the category of severe ischemic complications the patients that only presented jaw claudication but no other severe ischemic complications (data not shown).

Discussion

This study constitutes the first attempt to determine the potential influence of three polymorphisms of the *IFNGR1* gene in the susceptibility and phenotypic expression of biopsy-proven GCA. However, our data show no association between these variants of the *IFNGR1* gene with disease susceptibility or with specific features of GCA.

IFN-γ is a pleiotropic cytokine that has a number of activities including enhancement of the major histocompatibility complex expression on antigen-presenting cells, regulatory effects on T cells and increased expression of

intercellular adhesion molecule-1 on endothelial cells (23). GCA is a complex polygenic disease (7). In this large-vessel vasculitis both peripheral expression of IFN-γ and *IFN-γ* gene polymorphisms have been found to play an important role in both the pathogenesis of disease (5), and in the risk of developing severe ischemic complication, in particular blindness (6, 9). Although IFNGR1 chain binds the functional IFN-γ ligand (10), our results, which are based on the largest series of biopsy-proven GCA patients assessed for genotype analyses, do not confirm an association between genetic polymorphisms of the *IFNGR1* gene and this vasculitis.

The reasons for these negative associations are unknown. The lack of association of these *IFNGR1* gene variants with susceptibility to GCA is in keeping with a previous report that did not disclose a significant association between *IFNGR1*gene polymorphisms and multiple sclerosis (24). However, despite having found negative results, we cannot exclude that other polymorphisms located within the IFNGR1 locus might account for susceptibility to GCA. Moreover, since the different genetic backgrounds of the European populations have proved to influence differences in terms of gene association when genetic studies on GCA have been performed in different countries such as Italy or Spain (7), to fully exclude the contribution of these *IFNGR1* gene variants, an analysis on the potential implication of these *IFNGR1* gene polymorphisms should be performed in biopsy-proven GCA patients with different genetic backgrounds. Moreover, although the role of the rs7749390 (+95C/T) *IFNGR1* gene polymorphism has been related to the fact that this polymorphism is in strong linkage disequilibrium with the 56C/T *IFNGR1* gene polymorphism in some populations (13), so far there are no data available confirming that both polymorphisms may be surrogates in the Spanish population.

In conclusion, our results do not support an evidence for a major role contribution of the *IFNGR1* gene polymorphisms in the susceptibility or clinical manifestations of GCA.

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