Lack of association between hypoxia inducible factor-1 alpha gene polymorphisms and biopsy-proven giant cell arteritis

O. Torres[1*], R. Palomino-Morales[1*], T.R. Vazquez-Rodriguez[2], C. Gamallo[3], I.C. Morado[4], J.A. Miranda-Filloy[2], E. Amigo-Diaz[2], J.L. Callejas-Rubio[5], B. Fernandez-Gutierrez[4], S. Castañeda[3**], J. Martin[1**], M.A. Gonzalez-Gay[6**]

[1]Instituto de Parasitología y Biomedicina López Neyra, Consejo Superior de Investigaciones Cientificas (CSIC), Granada, Spain; [2]Division of Rheumatology, Hospital Xeral-Calde, Lugo, Spain; [3]Departments of Pathology and Rheumatology, Hospital de la Princesa, Universidad Autónoma, Madrid, Spain; [4]Rheumatology Service, Hospital Clínico San Carlos, Madrid, Spain; [5]Hospital Clínico San Cecilio, Granada, Spain; [6]Division of Rheumatology, Hospital Universitario Marques de Valdecilla, Santander (Cantabria), Spain.

[*]Drs. Torres and Palomino-Morales made an equal contribution to the study.
[**]Drs. Gonzalez-Gay, Martin and Castañeda share senior authorship in this study.

Orlando Torres, MD
Rogelio Palomino-Morales, PhD
Javier Martin, MD, PhD
Tomas R. Vazquez-Rodriguez, MD
José A. Miranda-Filloy, MD
Encarnacion Amigo-Diaz, MD
Carlos Gamallo, MD, PhD
Santos Castañeda, MD, PhD
Inmaculada C. Morado, MD
Benjamin Fernandez-Gutierrez, MD, PhD
José L. Callejas-Rubio, MD, PhD
Miguel A. Gonzalez-Gay, MD, PhD

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Please address correspondence and reprint requests to: M.A. Gonzalez-Gay, MD, PhD, Rheumatology Division, Hospital Universitario Marques de Valdecilla, 39008, Santander (Cantabria), Spain.
E-mail: miguelaggay@hotmail.com

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ABSTRACT

Background and objective: *Since the transcription factor hypoxia-inducible factor 1 (HIF-1) is a key early mediator of the response to ischemia and giant cell arteritis (GCA) is a polygenic disease leading to severe ischemic complications, in the present study we analysed for first time the implication of two HIF-1α gene polymorphisms in the susceptibility to and clinical expression of GCA.*

Methods: *Two hundred and fifteen biopsy-proven GCA patients and 470 matched controls were assessed. DNA from patients and controls was obtained from peripheral blood. Samples were genotyped for two single nucleotide polymorphisms, rs11549465 (C/T) and rs11549467 (G/A), 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 PRIM 7900 sequence.*

Results: *The HIF-1α, rs11549465 TT genotype was extremely uncommon in both GCA patients (2.3%) and controls (2.1%). Although the frequency of individuals carrying the CT or TT genotypes was increased in GCA patients (25.1%) compared to controls (20.4%) the difference was not statistically significant (OR 1.30 [95% CI: 0.89- 1.91]; p=0.17). Also, all GCA patients and most controls (98.9%) were homozygous for the rs11549467 GG genotype. GCA patients carrying the rs11549465 CT or TT genotypes had a slight increased risk of developing visual ischemic complications (33.1%) compared to the remaining GCA patients (22.8%); OR 1.60 (95% CI: 0.81- 3.16); p=0.18.*

Conclusion: *Our results do not confirm an implication of HIF-1α gene polymorphisms in the susceptibility to and clinical expression of GCA.*

Introduction

Giant cell arteritis (GCA), the most common vasculitis in the elderly in European countries and North-America (1, 2), 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 (1-3).

The inflammation of the arterial wall and vessel occlusion through fast and concentric intimal hyperplasia leads to the severe ischemic complications observed in patients with this vasculitis (4, 5). These ischemic events might be partially compensated by neovascularisation. Angiogenesis, the formation of new blood vessels, has been proposed to have a dual role in vasculitis. First, new vessel formation may compensate for ischemia and by the other hand, could have a proinflammatory role since neovessels are the main sites where adhesion molecules for leukocytes are expressed in GCA (6). Inflammation-induced angiogenic activity seems to counteract the GCA ischemic complications (7).

The transcription factor hypoxia-inducible factor 1 (HIF-1) is a key early mediator of the response to ischemia. HIF-1 plays a central physiological role in oxygen and energy homeostasis (8). An increased activity is recognised in the majority of clinical relevant hypoxic and ischemic episodes and also in human cancers. The heterodimer of HIF-1α and β subunits is a potent transcription factor that promotes cell survival and angiogenesis (8). The transcriptional activity is primarily controlled by the oxygen-regulated breakdown of the α subunit (9). HIF-1 is activated during hypoxia by stabilisation of the subunit HIF-1α.

Susceptibility to autoimmune disorders may be the result of the interaction of multiple genetic factors that regulate the threshold of autoreactivity. GCA is a complex polygenic disease (10). A number of gene polymorphisms have been reported to be implicated in GCA susceptibility (11-16). In addition, other gene polymorphisms have been found to be associated with severe ischemic manifestations in patients with this vasculitis (17, 18).

The *HIF-1α* gene is located at chromosome 14q21-q24. Two single nucleotide polymorphisms in the *HIF-1α*, HIF1A.2- rs11549465 (C/T) (exon 12, position C1772T) and HIF1A.50-rs11549467 (G/A) (exon 12, position G1790A), which result in proline to serine (P582S) and alanine to threonine (A588T) amino acid substitutions, respectively, were found within the minimal N-terminal transactivation domain (19, 20). The presence of these polymorphic variants was shown to cause a significantly higher transcriptional activity than the activity of the wild type (21). *HIF-1α* gene polymorphisms have been associated with cancer; however, to the best of our knowledge, no information on the potential association of *HIF-1α* gene variants and primary systemic vasculitides has been reported.

There is no information regarding studies on the association of *HIF-1α* gene polymorphisms with GCA. Since the *HIF-1α* P582S and A588T mutations, the most commonly studied *HIF-1α* gene polymorphisms, result in higher transcriptional activity, suggesting that there are functional differences related to these alleles, we selected these two single nucleotide polymorphisms P582S and A588T of the *HIF-1α* gene to assess for first time whether some *HIF-1α* gene polymorphisms may be implicated in the susceptibility to biopsy-proven GCA. Moreover, we aimed to determine if these two functional *HIF-1α* gene polymorphisms might be implicated in the risk of developing severe ischemic complications, in particular visual ischemic events, in biopsy-proven GCA patients.

Patients and methods

Patients

Two hundred and fifteen patients diagnosed with biopsy-proven GCA between 1991 and 2007 were included in this study. Most of them (n=127) 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=470) from the corresponding cities (2.2 controls per case) matched by age ±2 years, and sex and ethnicity with GCA patients was also studied. All biopsy-proven GCA patients and controls were white Caucasians subjects living in northwestern (Lugo), middle (Madrid) and southern (Granada) Spain.

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 the presence of giant cells (22).

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 (23) and visual ischemic events have also been reported to occur within the first 48-72 hours after the onset of corticosteroid therapy (24). 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 polymyalgia rheumatica (PMR) manifestations if they had severe bilateral ache and pain involving the neck, the shoulder and or the pelvic girdles, associated with morning stiffness (25,26). 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 (27). Severe ischemic manifestations were considered to be present if GCA patients suffered at least one of the following complications: visual ischemic manifestations, strokes and/or transient ischemic attacks, jaw claudication, or large-artery stenosis of the extremities that caused signs of occlusive manifestations (28).

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 also obtained.

HIF-1α gene genotyping

DNA was obtained from peripheral blood mononuclear cells, using standard methods. The genotyping of the *HIF-1α* gene rs11549465 (C/T) and rs11549467 (G/A) polymorphisms (18) was performed using a pre-designed TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labeled with the fluorescent dyes VIC and FAM, respectively. The polymerase chain reaction (PCR) was carried out in a total reaction volume of 5 μl, containing 50 ng genomic DNA as template, 2.5 μl of TaqMan genotyping master mix, 0.25μl of 20× assay mix, and ddH2O up to 5 μl of final volume. The amplification protocol used was the following: initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 92°C for 15 s, and annealing/extension at 60°C for 1 min. Post-PCR, the genotype of each sample was attributed automatically by measuring the allelic-specific fluorescence on the ABI PRIM 7900 Sequence Detection Systems using SDS 2.3 software for allelic discrimination (Applied Biosystems).

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 fifteen biopsy-proven GCA patients were enrolled. Most of them were women (n=145) (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 171 (80%) had headache, 135 (63%) abnormal temporal artery on physical examination, 100 (47%) experienced PMR manifestations, 86 (40%) jaw claudication and 53 (25%) visual ischemic manifestations. In addition, 22 (10%) experienced irreversible (permanent) visual loss, 10 (5%) had strokes and 116 (54%) fulfilled the definitions for severe ischemic manifestations. Furthermore, as previously reported (29), most patients (n=211; 98%) had an erythrocyte sedimentation rate higher than 40 mm/1st hour (Table I).

Table I. Main epidemiologic and clinical features of 215 patients with biopsy-proven GCA.

	Variable
Age (years) at the time of diagnosis	
Median	74
Range	52-93
Women: Men	145: 70
Headache	171 (80)
Abnormal temporal artery on physical examination	135 (63)
Polymyalgia rheumatica	100 (47)
Jaw claudication	86 (40)
Visual ischemic manifestations*	53 (25)
Permanent visual loss	22 (10)
Stroke	10 (5)
Severe ischemic manifestations**	116 (54)
ESR mm/1hour greater than 40 mm/1st hour	211 (98)

Number in parenthesis indicates the total percentage of patients with a particular variable.
*If they had any of the following visual ischemic complications: transient visual loss including *amaurosis fugax*, permanent visual loss, or diplopia.
**If they experienced at least one of the following features: visual manifestations, cerebrovascular accidents (stroke and/or transient ischemic attacks), jaw claudication, or limb claudication of recent onset.

Influence of HIF-1α gene polymorphisms in the susceptibility to GCA

The case: control ratio achieved was 1:2.2. The estimated power of this study for an estimated OR between 1.5 and 2.0 was 62–98% for a type I error rate of 0.05.

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

In our study, no significant differences in the genotype and allele frequencies on the *HIF-1α* 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 *HIF-1α* gene variants was similar in controls from the 3 different Spanish regions (data not shown).

Table II shows the genotype and allele frequencies of the *HIF-1α* gene polymorphisms in GCA patients and control subjects. No significant differences in the genotype distribution between GCA patients and controls for both *HIF-1α* gene variants were observed (Table II). With respect to the *HIF-1α*, rs11549465 (C/T), TT genotype was extremely uncommon in both GCA patients (2.3%) and controls (2.1%) (Table II). Moreover, although the frequency of individuals carrying the T allele (CT plus TT genotypes) was increased in the group of biopsy-proven GCA patients (25.1%) compared to the controls (20.4%) the difference was not statistically significant (OR 1.30 [95% CI: 0.89-1.91]; *p*=0.17). Likewise, when the *HIF-1α*, rs11549467 (G/A) variant was assessed we found that all GCA patients and the vast majority of controls (465 of 470; 98.9%) were homozygous for the GG genotype (Table II). Due to this, the genotype distribution for the *HIF-1α*, rs11549467 (G/A) variant did not show significant differences between biopsy-proven GCA patients and controls (*p*=0.13).

Table II. *HIF-1α* gene polymorphisms in biopsy-proven GCA patients and healthy controls.

HIF-1α (rs11549465)	GCA patients n=215 (%)	Controls n=470 (%)	*p*-value	OR (95% CI)
C/C	161 (74.9)	374 (79.6)	0.17	0.77 (0.51 – 1.14)
C/T	49 (22.8)	86 (18.3)	0.17	1.32 (0.87 – 1.99)
T/T	5 (2.3)	10 (2.1)	1.00	1.10 (0.32 – 3.53)
C	371 (86.3)	834 (88.7)	0.20	0.80 (0.56 – 1.14)
T	59 (13.7)	106 (11.3)	0.20	1.25 (0.88 – 1.78)
HIF-1α (rs11549467)	GCA patients n=215 (%)	Controls n=470 (%)		
G/G	215 (100)	465 (98.9)		
G/A	0 (0.0)	5 (1.1)		
A/A	0 (0.0)	0 (0.0)		
G	430 (100)	935 (99.5)		
A	0 (0.0)	5 (0.5)		

In addition, these variants did not form haplotypes, since these polymorphisms were Taq SNPs of different haplotype blots and the linkage disequilibrium was very low.

Influence of HIF-1α gene variants in the clinical spectrum of GCA

To determine whether polymorphisms of the *HIF-1α* 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 III, no statistically

Table III. Association between *HIF-1α* genotypes and typical disease features in biopsy-proven GCA patients.

	HIF-1α (rs11549465)	With n (%)	Without n (%)	*p*-value	OR (95% CI)
PMR	CC	75 (75.0)	86 (74.8)	0.97	1.01 (0.52 – 1.96)
	CT	23 (23.0)	26 (22.6)	0.95	1.02 (0.51 – 2.03)
	TT	2 (2.0)	3 (2.6)	1.00	0.76 (0.09 – 5.74)
	C	173 (86.5)	198 (86.1)	0.90	1.04 (0.58 – 1.86)
	T	27 (13.5)	32 (13.9)	0.90	0.97 (0.54 – 1.73)
Visual manifestations	CC	36 (67.9)	125 (77.2)	0.18	0.63 (0.30 – 1.31)
	CT	15 (28.3)	34 (21.0)	0.27	1.49 (0.69 – 3.18)
	TT	2 (3.8)	3 (1.8)	0.60	2.08 (0.24 – 15.8)
	C	87 (82.1)	284 (87.7)	0.15	0.64 (0.34 – 1.22)
	T	19 (17.9)	40 (12.3)	0.15	1.55 (0.82 – 2.92)
Severe ischemic manifestations	CC	85 (73.3)	76 (76.8)	0.56	0.83 (0.43 – 1.62)
	CT	27 (23.3)	22 (22.2)	0.85	1.06 (0.53 – 2.12)
	TT	4 (3.4)	1 (1.0)	0.38	3.50 (0.36 – 83.6)
	C	197 (84.9)	174 (87.9)	0.37	0.78 (0.43 – 1.40)
	T	35 (15.1)	24 (12.1)	0.37	1.29 (0.71 – 2.34)
	HIF-1α (rs11549467)	With n (%)	Without n (%)		
PMR	GG	100 (100)	115 (100)		
	AG	0 (0.0)	0 (0.0)		
	AA	0 (0.0)	0 (0.0)		
	G	200 (100)	230 (100)		
	A	0 (0.0)	0 (0.0)		
Visual manifestations	GG	53 (100)	162 (100)		
	AG	0 (0.0)	0 (0.0)		
	AA	0 (0.0)	0 (0.0)		
	G	106 (100)	324 (100)		
	A	0 (0.0)	0 (0.0)		
Severe ischemic manifestations	GG	116 (100)	99 (100)		
	AG	0 (0.0)	0 (0.0)		
	AA	0 (0.0)	0 (0.0)		
	G	232 (100)	198 (100)		
	A	0 (0.0)	0 (0.0)		

significant differences for both *HIF-1α* gene variants were found.

In this regard, as discussed for disease susceptibility, biopsy-proven GCA patients carrying the T allele (CT or TT genotype) had a slight increased risk of developing visual ischemic complications (32.1%) compared to the remaining biopsy-proven GCA patients (22.8%); OR 1.60 (95% CI: 0.81- 3.16); *p*=0.18.

Likewise, no significant differences were found when patients were stratified according to the presence/absence of severe ischemic manifestations of this vasculitis (Table III). 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

All patients included in this genetic study have GCA confirmed histopathologically which contrasted with similar genetic studies from other groups. Also, the number of biopsy-proven GCA patients included in this study is high considering the relatively low prevalence of this type of systemic vasculitis.

To the best of our knowledge, there are no previous studies aimed to determine the potential implication of the *HIF-1α* gene polymorphisms in the susceptibility to GCA. Therefore, this study constitutes the first attempt to determine the potential influence of two functional polymorphisms of the *HIF-1α* gene in the susceptibility and phenotypic expression of biopsy-proven GCA. However, this study does not support a major role for the two functional *HIF-1α* gene polymorphisms in GCA susceptibility, a fact clearly related to the extremely low prevalence of the TT genotype (2%). In this regard, genetic polymorphisms affecting less than 10% of patients with systemic autoimmune diseases are rarely associated with clinical expression, due to the heterogeneity of these diseases.

In line with the above, we did not observe any association between these variants of the *HIF-1α* gene with specific features of GCA.

In GCA the hypoxic environment subsequent to stenosis or occlusion of the vascular lumen is a potent signal for the generation of new blood vessels. Angiogenesis may be a compensatory response to ischemia (4). Hernandez-Rodriguez *et al*. disclosed a correlation between tissue production of pro-inflammatory cytokines and the intensity of the acute-phase response (30). GCA patients with ischemic complications were found to have lower tissue expression and circulating levels of interleukin-6 (IL-6) than those GCA patients with no ischemic events (31). Hernandez-Rodriguez et al also showed that IL-6 activated a functional program related to angiogenesis that may compensate for ischemia in patients with GCA (31). The same group of investigators examined the clinical relevance of neovascularisation in 31 patients with GCA. Patients without ischemic complications had significantly higher tissue angiogenesis scores than the patients with ischemic events. Angiogenesis was more severe in GCA patients with a strong acute phase response compared with those with a weak systemic inflammatory response (6). According to all these evidences, inflammation-induced angiogenic activity plays a compensatory role for ischemia in GCA patients (6, 30, 31).

In line with the above, we found an association between the G allele of the vascular endothelial growth factor (VEGF) -634 G→C promoter polymorphism with the risk of severe ischemic manifestations and, additionally, a higher risk of developing irreversible severe ischemic complications was observed for VEGF -634 GG homozygous individuals with biopsy-proven GCA (18). Since functional studies have demonstrated that VEGF -634 G allele is associated with lower circulating VEGF levels *in vivo*, reduced VEGF transcription and less IRES-mediated VEGF expression (32), GCA patients carrying the VEGF –634 G allele might undergo an inhibition of angiogenesis, and therefore an exacerbation of the ischemic phenomena.

Polymorphisms in *HIF-1α* gene including the HIF1A.2- rs11549465 (C/T) were associated with development of stable exertional angina rather than acute myocardial infarction as the initial clinical presentation of coronary artery disease (20). Resar *et al*. examined whether polymorphisms in *HIF-1α* gene were correlated with the presence of coronary artery collaterals (33). They studied a series of 100 consecutive patients who had either stable or unstable angina and angiographically documented coronary artery disease. As we have done in our study, they also assessed both *HIF-1α*, HIF1A.2- rs11549465 (C/T) and HIF1A.50-rs11549467 (G/A) gene polymorphisms. They found that the minor allele of *HIF-1α*, HIF1A.2-rs11549465 was more common in patients without coronary collaterals (25%) than in patients with coronary collaterals (7%) (33). Although it may be difficult to compare their findings with our data, it is important to highlight that in our series of biopsy-proven GCA patients we also observed an increased risk of developing visual ischemic complications (32.1%) in patients carrying the minor *HIF-1α*, HIF1A.2-rs11549465 T allele compared to the remaining biopsy-proven GCA patients (22.8%). However, the difference was not statistically significant.

In conclusion, our results do not support an evidence for a major role contribution of two functional *HIF-1α* gene polymorphisms in the susceptibility to GCA in Spanish individuals. However, we cannot exclude that other polymorphisms located within the *HIF-1α* gene might account for susceptibility to GCA. Further studies in biopsy-proven GCA patients from different genetic backgrounds are needed to fully exclude an implication of the *HIF-1α* gene in the susceptibility to and clinical spectrum of this vasculitis.

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