Vascular endothelial growth factor haplotypes are associated with severe ischaemic complications in giant cell arteritis regardless of the disease phenotype

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Abstract

Objective

*To determine whether functional vascular endothelial growth factor (*VEGF*) polymorphisms influence the expression of the clinical phenotype of giant cell arteritis (GCA). We also evaluated whether* VEGF *polymorphism is associated with the development of severe ischaemic manifestations in patients with GCA regardless of the clinical phenotype, classic cranial GCA or predominantly extracranial GCA large-vessel vasculitis (LVV).*

Methods

VEGF *rs833061 T/C, rs2010963 G/C and rs3025039 C/T polymorphisms were genotyped in 185 patients with biopsy-proven cranial GCA, 105 with extracranial LVV-GCA and 490 healthy controls. Allelic combinations (haplotypes) of* VEGF *were carried out. Comparisons were performed between patients with GCA and healthy controls as well as between patients with GCA stratified according to the clinical phenotype and the presence of severe ischaemic manifestations.*

Results

No significant differences in genotype, allele, and haplotype frequencies of VEGF *were found between patients with GCA and healthy controls as well as between GCA patients with the classic cranial pattern and the extracranial LVV-GCA pattern of the disease. However, the* VEGF *CGC haplotype (OR= 1.63 [1.05-2.53]) and the CGT haplotype (OR= 2.55 [1.10-5.91]) were significantly more frequent in GCA patients with severe ischaemic complications compared to those patients without these complications.*

Conclusion

VEGF *haplotypes seem to play a role in the development of severe ischaemic manifestations in GCA patients, regardless of the clinical phenotype of expression of the disease.*

Key words

giant cell arteritis, large-vessel vasculitis, vascular endothelial growth factor, genetics, haplotypes

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Introduction

Giant cell arteritis (GCA) is the most common vasculitis among individuals over the age of 50 of North European descent which is characterised by granulomatous inflammation of large and medium-sized vessels (1). The clinical spectrum of GCA involves two different phenotypes that can overlap: the classic cranial GCA pattern and the extracranial large-vessel (LVV)-GCA pattern (2–6). Classically, GCA has been related to the affection of cranial vessels with a particular predilection for the temporal arteries being responsible of the classic cranial manifestations of GCA such as headache, scalp tenderness or jaw claudication (7). This is the reason why this vasculitis was classically called temporal arteritis (7). However, progress in imaging techniques has contributed to identify a subgroup of patients with predominant extracranial manifestations (8-10). These patients are usually younger at the time of disease diagnosis and present more commonly as refractory polymyalgia rheumatica (PMR) often associated with constitutional symptoms and/or fever of unknown-origin (8, 9, 11-13).

Severe ischaemic complications can occur in the setting of GCA as a result of perpetuated inflammation of the vessel wall, particularly in patients with the classic cranial GCA pattern (14, 15). In the absence of early glucocorticoid therapy, severe inflammation of cranial arteries can lead to intimal hyperplasia and luminal occlusion (14, 15). Visual manifestations, including amaurosis fugax and irreversible vision loss, necrosis of the scalp or tongue, and ischaemic strokes are the major feared complications (16). Patients with predominant extracranial LVV involvement can also develop severe vascular complications as a consequence of the structural inflammatory damage in the vessel wall of the aorta and its main branches (17, 18). Patients with extracranial LVV-GCA have an increased risk of developing peripheral arterial disease that can manifest as claudication of the extremities (19).

Vascular endothelial growth factor (VEGF), which is a pivotal mediator of angiogenesis, seems to be implicated in GCA pathogenesis (20-22). In this regard, high levels of circulating VEGF have been found in patients with GCA and PMR which decrease in response to glucocorticoid therapy (23, 24). VEGF modulates the formation of new vessels which may compensate the ischaemic complications in GCA (25). However, it also plays a proinflammatory role by inducing the expression of leucocyte adhesion molecules on the endothelial cells (26, 27). An association of some *VEGF* functional polymorphism with the susceptibility to several systemic inflammatory conditions has been reported (28, 29), including classic cranial GCA (30-32). In this regard, in a previous study (30), we found that *VEGF* rs2010963 (-634G/C) polymorphism was associated with the development of severe ischaemic manifestations in biopsy-proven GCA patients with predominant classic cranial manifestations. However, the genetic role of *VEGF* in extracranial LVV GCA remains unknown.

Taken all these considerations into account, we aimed to assess for the first time if *VEGF* functional polymorphisms are associated with distinct clinical phenotypes of GCA. In addition, we also aimed to evaluate if *VEGF* functional polymorphisms influence the development of severe ischaemic manifestations in both patients with the cranial and the extracranial LVV-GCA pattern.

Methods

Patients and controls

The study group comprised 185 patients with biopsy-proven cranial GCA, 105 with LVV-GCA and 490 healthy controls. All patients and controls were Spanish of European ancestry. They were recruited in ten collaborative centres: Hospital Universitario Marqués de Valdecilla (Santander, Spain), Hospital Universitario de Basurto (Bilbao, Spain), Hospital de León (León, Spain), Hospital Universitario de La Princesa (Madrid, Spain), Hospital Universitario y Politécnico La Fe (Valencia, Spain), Hospital Universitario Virgen del Rocío (Sevilla, Spain), Hospital Universitario de Pontevedra

Table I. Main clinical features of patients with classic cranial GCA and extracranial LVV-GCA pattern.

	Classic cranial GCA pattern n=185	LVV-GCA pattern n=105	*p*
Age at diagnosis (mean ± SD)	74.0 ± 10.4	67.5 ± 9.8	<0.01
Women, n (%)	122 (65.9%)	76 (72.4%)	0.24
Positive TAB, n (%)	185 (100%)	3/37 (8.1%)	<0.01
Headache, n (%)	145 (79.2%)	0 (0%)	<0.01
Abnormal temporal artery on physical examination, n (%)	110 (60.1%)	0 (0%)	<0.01
Jaw claudication, n (%)	73 (39.5%)	0 (0%)	<0.01
Polymyalgia rheumatica, n (%)	74 (40.0%)	86 (81.9%)	<0.01
Visual manifestations, n (%)	47 (25.4%)	0 (0%)	<0.01
Permanent visual loss, n (%)	21 (11.4%)	0 (0%)	<0.01
Peripheral arteriopathy, n (%)	0 (0%)	12 (11.4%)	<0.01
Stroke, n (%)	8 (4.3%)	0 (0%)	0.05
ESR > 40 mm/1st h. at diagnosis, n (%)	182 (98.4%)	84 (80%)	<0.01
Positive imaging test for extracranial LVV-GCA	-	105 (100%)	-
18F-FDG PET/CT scan	-	92 (87.6%)	-
MRI-A	-	7 (6.7%)	-
CT-A	-	6 (5.7%)	-

CT-A: computed tomography angiography; ESR: erythrocyte sedimentation rate; 18F-FDG PET/CT: 18Fluorodeoxyglucose positron emission tomography; GCA: giant cell arteritis; LVV: large-vessel vasculitis; MRI-A: magnetic resonance imaging-angiography; SD: standard deviation; TAB: temporal artery biopsy.

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The study was approved by the Ethics Committee of clinical research of Cantabria for Hospital Universitario Marqués de Valdecilla as well as by the remaining participant centres mentioned above. All subjects provided informed written consent before being enrolled in the study. The procedures followed were in accordance with the ethical standards of the approved guidelines and regulations, according to the Declaration of Helsinki.

Patients with classic cranial phenotype of GCA

A total of 185 patients with the cranial phenotype of GCA were included in our study. GCA diagnosis was based on the American College of Rheumatology (ACR) 1990 classification criteria (33). In addition, the diagnosis of GCA was confirmed in all patients by a positive temporal artery biopsy showing the typical histopathologic findings of this vasculitis. None of them presented clinical symptoms suggesting peripheral arterial involvement.

Patients with extracranial LVV-GCA phenotype

A set of 105 ethnically matched patients with the extracranial LVV-GCA phenotype were also included in our study. LVV-GCA diagnosis was established by experienced rheumatologists based on confirmatory imaging techniques, such as 18F-fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT), angiographic magnetic resonance (MRI-A) and/or computed tomography angiography (CT-A).

For the present study, in an attempt to evaluate a well-defined group of LVV-GCA patients who were clinically different from those with classic cranial GCA, patients with LVV-GCA presenting symptoms of cranial GCA were excluded from the analysis (Table I). Patients with other underlying inflammatory conditions, infections or neoplastic diseases that could present with LVV involvement were also excluded.

GCA patients with severe ischaemic manifestations

As previously reported (30, 34), patients were considered to have severe ischaemic manifestations if they had at least one of the following complications: visual manifestations (transient visual loss including amaurosis fugax, permanent visual loss, or diplopia), cerebrovascular strokes, jaw claudication, or large-artery stenosis of the extremities that caused signs of occlusive manifestations (limb claudication) of recent onset.

Healthy controls

A cohort of 490 ethnically matched unaffected control subjects, without history of vasculitis or any other autoimmune disease, constituted by blood donors from National DNA Bank Repository (Salamanca, Spain), were also included in this study.

VEGF polymorphisms genotyping

Genomic DNA was extracted from peripheral blood using the REALPURE "SSS" kit (RBME04, REAL, Durviz SL, Valencia, Spain), as previously described (35).

All patients and healthy controls were genotyped for *VEGF* rs833061 T/C, rs2010963 G/C, and rs3025039 C/T by TaqMan assays. Negative controls and duplicate samples were included to check the accuracy of the genotyping. Genotyping was performed in a QuantStudio™ 7 Flex real-time polymerase chain reaction system (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

All genotype data were checked for deviation from Hardy-Weinberg equilibrium (HWE).

First, comparisons were performed considering each *VEGF* polymorphism independently. Both genotype and allele frequencies of rs833061, rs2010963 and rs3025039 were calculated and compared between patients with GCA and healthy controls as well as between patients with GCA stratified according to the clinical phenotype (cranial and extracranial LVV-GCA) and the presence of severe ischaemic manifestations by chi-square or Fisher tests when necessary (expected values below 5). Strength of association was estimated using odds ratios (OR) and 95% confidence intervals (CI).

Subsequently, allelic combinations

(haplotypes) of *VEGF* were carried out. Haplotype frequencies were calculated by the Haploview v4.2 software (http:// broad. mit. edu/ mpg/ haplo view) and then compared by chi-square or Fisher tests between the groups mentioned above. Strength of associations was estimated by OR and 95% CI.

P-values lower than 0.05 were considered as statistically significant. All analyses were performed with the STATA statistical software 12/SE (Stata Corp., College Station, TX, USA).

Results

Genotyping quality control

The rs833061, rs2010963, and rs3025039 genotype distribution was in HWE. Genotype and allele frequencies were in agreement with the data of the 1000 Genomes Project for Europeans.

Association of VEGF with classic cranial GCA and extracranial LVV-GCA

Genotype, allele, and haplotype frequencies of *VEGF* were compared between the whole cohort of patients with GCA and healthy controls. As shown in Table II, no statistically significant genetic differences were found between these groups. This was also the case when patients with the classic cranial GCA pattern and patients with the extracranial-LVV GCA pattern were compared to healthy controls (Supplementary Tables S1 and S2). We also examined whether differences in the genotype, allele and haplotype frequencies of *VEGF* could exist between patients with GCA stratified according to the clinical phenotype of GCA. However, no statistically significant differences were found in this regard between GCA patients with the classic cranial pattern and the LVV-GCA extracranial pattern (Table III).

Association of VEGF with severe ischaemic manifestations

In an additional step, we also evaluated the differences in *VEGF* frequencies among the entire cohort of the GCA patient group (both cranial and extracranial LVVGCA) stratified according to the presence or absence of severe ischaemic complications. Severe is-

Table II. Genotype, allele, and haplotype frequencies of *VEGF* in all patients with GCA and healthy controls.

Polymorphism	All patients with GCA % (n)	Healthy controls % (n)	*p*	OR [95% CI]
VEGF rs833061				
TT	30 (85)	28 (135)	-	Ref.
TC	48 (138)	48 (233)	0.73	0.94 [0.67-1.33]
CC	22 (64)	24 (122)	0.38	0.83 [0.55-1.25]
T	54 (308)	51 (503)	-	Ref.
C	46 (266)	49 (477)	0.37	0.91 [0.74-1.12]
VEGF rs2010963				
GG	42 (123)	48 (237)	-	Ref.
GC	46 (133)	40 (197)	0.10	1.30 [0.95-1.77]
CC	12 (34)	12 (56)	0.52	1.17 [0.72-1.89]
G	65 (379)	68 (671)	-	Ref.
C	35 (201)	32 (309)	0.20	1.15 [0.93-1.43]
VEGF rs3025039				
CC	72 (210)	75 (368)	-	Ref.
CT	27 (78)	23 (110)	0.21	1.24 [0.89-1.74]
TT	1 (2)	2 (12)	0.11	0.29 [0.06-1.32]
C	86 (498)	86 (846)	-	Ref.
T	14 (82)	14 (134)	0.80	1.04 [0.77-1.40]
Haplotype*				
TGC	34 (198)	33 (326)	-	Ref.
CGC	26 (149)	29 (288)	0.24	0.85 [0.65-1.11]
TCC	15 (86)	13 (132)	0.67	1.07 [0.78-1.48]
CCC	10 (60)	10 (100)	0.95	0.99 [0.69-1.42]
CGT	4 (25)	5 (51)	0.41	0.81 [0.48-1.34]
TCT	4 (22)	4 (39)	0.79	0.93 [0.53-1.61]
CCT	6 (32)	3 (38)	0.20	1.39 [0.84-2.29]

CI: confidence interval; GCA: giant cell arteritis; OR: odds ratio.
*The polymorphism order was rs833061, rs2010963 and rs3025039.

Table III. Genotype, allele, and haplotype frequencies of *VEGF* in patients with LVV-GCA pattern and classic cranial GCA pattern.

Polymorphism	LVV-GCA pattern % (n)	Classic cranial GCA pattern % (n)	*p*	OR [95% CI]
VEGF rs833061				
TT	31 (32)	29 (53)	-	Ref.
TC	45 (46)	50 (92)	0.51	0.83 [0.47-1.46]
CC	24 (24)	21 (40)	0.990.99	[0.51-1.94]
T	54 (110)	54 (198)	-	Ref.
C	46 (94)	46 (172)	0.93	0.98 [0.70-1.39]
VEGF rs2010963				
GG	48 (50)	39 (73)	-	Ref.
GC	44 (46)	47 (87)	0.32	0.77 [0.47-1.28]
CC	8 (9)	14 (25)	0.14	0.53 [0.23-1.22]
G	70 (146)	63 (233)	-	Ref.
C	30 (64)	37 (137)	0.11	0.75 [0.52-1.07]
VEGF rs3025039				
CC	71 (75)	73 (135)	-	Ref.
CT	29 (30)	26 (48)	0.67	1.13 [0.66-1.92]
TT	0 (0)	1 (2)	-	-
C	86 (180)	86 (318)	-	Ref.
T	14 (30)	14 (52)	0.94	1.02 [0.63-1.66]
Haplotype*				
TGC	37 (76)	33 (122)	-	Ref.
CGC	27 (55)	25 (94)	0.78	0.94 [0.61-1.46]
TCC	12 (24)	17 (62)	0.09	0.62 [0.36-1.08]
CCC	10 (20)	11 (40)	0.48	0.80 [0.44-1.47]
CGT	4 (9)	4 (16)	0.82	0.90 [0.38-2.15]
TCT	4 (9)	4 (13)	0.82	1.11 [0.45-2.72]
CCT	5 (10)	6 (22)	0.44	0.73 [0.33-1.72]

CI: confidence interval; GCA: giant cell arteritis; LVV: large-vessel vasculitis; OR: odds ratio.
*The polymorphism order was rs833061, rs2010963 and rs3025039.

chaemic complications included visual manifestations, cerebrovascular strokes or jaw claudication in patients with the classic cranial GCA phenotype and, exclusively large-artery stenosis of the extremities that caused signs of peripheral arteriopathy in those with the extracranial LVV-GCA pattern. 161 severe ischaemic complications were reported in 109 GCA patients (12 with peripheral arteriopathy in the LVV-GCA group and 73 with jaw claudication, 47 with visual manifestations, 21 with permanent vision loss and 8 with stroke in the classic cranial GCA group).

No statistically significant differences in the genotype and allele frequencies of each *VEGF* polymorphism were disclosed between them, except for rs2010963 GC genotype which showed a decreased frequency in patients with ischaemic manifestations (Table IV).

Nevertheless, when *VEGF* haplotypes were analysed, we found that the CGC haplotype (OR= 1.63 [1.05-2.53]) and the CGT haplotype (OR= 2.55 [1.10-5.91]) were significantly more frequent among GCA patients with severe ischaemic complications compared to those patients without these complications (Table IV).

Table IV. Genotype, allele, and haplotype frequencies of *VEGF* in patients with GCA according to the presence of severe ischaemic manifestations.

	Ischaemic manifestations			
Polymorphism	Yes % (n)	No % (n)	*p*	OR [95% CI]
VEGF rs833061				
TT	28 (31)	30 (54)	-	Ref.
TC	44 (48)	51 (90)	0.80	0.93 [0.53-1.63]
CC	28 (30)	19 (34)	0.20	1.54 [0.79-2.97]
T	50 (110)	56 (198)	-	Ref.
C	50 (108)	44 (158)	0.23	1.23 [0.88-1.73]
VEGF rs2010963				
GG	49 (53)	39 (70)	-	Ref.
GC	38 (41)	51 (92)	**0.04**	**0.59 [0.35-0.98]**
CC	13 (15)	10 (19)	0.92	1.04 [0.49-2.24]
G	67 (147)	64 (232)	-	Ref.
C	33 (71)	36 (130)	0.41	0.86 [0.60-1.23]
VEGF rs3025039				
CC	72 (78)	73 (132)	-	Ref.
CT	28 (31)	26 (47)	0.69	1.12 [0.66-1.90]
TT	0 (0)	1 (2)	-	-
C	86 (187)	86 (311)	-	Ref.
T	14 (31)	14 (51)	0.97	1.01 [0.62-1.64]
Haplotype*				
TGC	30 (66)	37 (132)	-	Ref.
CGC	31 (67)	23 (82)	**0.03**	**1.63 [1.05-2.53]**
TCC	17 (37)	14 (49)	0.12	1.51 [0.90-2.54]
CCC	8 (17)	12 (43)	0.47	0.79 [0.42-1.49]
CGT	6 (14)	3 (11)	**0.03**	**2.55 [1.10-5.91]**
TCT	3 (7)	4 (15)	0.89	0.93 [0.36-2.40]
CCT	5 (10)	6 (22)	0.82	0.91 [0.41-2.03]

CI: confidence interval; GCA: giant cell arteritis; OR: odds ratio.
*The polymorphism order was rs833061, rs2010963 and rs3025039.

Discussion

GCA patients can present different clinical phenotypes and outcomes that might be influenced by a different genetic susceptibility. *VEGF* seems to play an important role in the pathogenesis of GCA and the development of ischaemic complications. In this regard, we wondered if *VEGF* could influence the development of different GCA phenotypes and severity of the disease. We found no implication of *VEGF* polymorphisms in the genetic predisposition to cranial and extracranial LVV-GCA. However, two haplotypes of *VEGF* were associated with the development of severe ischaemic manifestations, regardless of the clinical phenotype of GCA.

Interestingly, in the present study the two *VEGF* haplotypes that were associated with severe ischaemic complications carried the G allele of rs2010963. In accordance, a previous study that included 103 biopsy-proven GCA patients with the classic cranial pattern of the disease showed that the G allele of rs2010963 (-634G/C) was significantly overrepresented in cranial GCA patients with ischaemic manifestations (30). Therefore, we confirm the possible implication of *VEGF* in the development of severe ischaemic manifestations in GCA patients with the classic cranial and the extracranial LVV-GCA pattern. Of note, the presence of the G allele of rs2010963 (-634G/C) has also been related to the development of vascular complications in other systemic vasculitis. In this regard, the frequency of the G allele was significantly higher in patients with Kawasaki disease with coronary artery lesions (36). Furthermore, a previous study observed that the G allele of rs2010963 (-634G/C) is associated with a reduced *VEGF* transcription and, consequently, with a lower production of VEGF (37), which may explain the increased risk of ischaemic complications.

Although previous studies observed an association of *VEGF* in GCA susceptibility (31, 32), we did not find an implication of *VEGF* rs833061, rs2010963 and rs3025020 in GCA genetic predisposition. Furthermore, we did not observe a role of *VEGF* in the development of different clinical phenotypes of GCA. Similarly, in former studies, we could not find differences in HLA class I and class II genetic predisposition between patients with the cranial and the extracranial LVV-GCA phenotype (38, 39). Thereby, more studies are needed to assess the possible implication of other genes that may explain the different clinical expression of GCA.

In conclusion, our study reveals that *VEGF* haplotypes may play a role in the development of severe ischaemic manifestations in GCA patients with both the cranial and the extracranial-LVV pattern.

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Competing interests

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The other authors have declared no competing interests.

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