Autoimmune disease-associated *CD226* gene variants are not involved in giant cell arteritis susceptibility in the Spanish population

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ABSTRACT

Objectives. CD226 *genetic variants have been associated with a number of autoimmune diseases. The aim of this study was to investigate the potential implication of the* CD226 *loci in the susceptibility to and main clinical manifestations of giant cell arteritis (GCA).*

Methods. *A Spanish Caucasian cohort of 455 patients diagnosed with biopsy-proven GCA and 1414 healthy controls were included in the study. Three* CD226 *polymorphisms, rs727088, rs34794968 and rs763361, were genotyped using the TaqMan® allelic discrimination technology. PLINK software was used for the statistical analyses.*

Results. *No significant association between the* CD226 *polymorphisms and susceptibility to GCA was found (rs727088: p=0.92, OR=1.01, CI 95% 0.86–1.18; rs34794968: p=0.61, OR=1.04, CI 95% 0.89–1.22; rs763361: p=0.88, OR=0.99, CI 95% 0.84–1.16). Similarly, when patients were stratified according to the specific clinical features of GCA such as polymyalgia rheumatica, visual ischaemic manifestations or irreversible occlusive disease, no association was observed either between the case subgroups and the control set or between GCA patients with and without the specific features of the disease. Furthermore, the haplotype analysis revealed no significant association with the clinical manifestations of the disease.*

Conclusion. *Our results show that the three* CD226 *polymorphisms analysed do not play a relevant role in the susceptibility to GCA and clinical manifestations of this vasculitis.*

Introduction

CD226, also known as DNAX accessory molecule 1 (DNAM-1), PTA1, and TLiSA1, is a 67KDa type I membrane intracellular adhesion protein that belongs to the immunoglobulin superfamily (IgSF). It has only two immunoglobulin-like extracellular domains and it is constitutively expressed on the majority of natural killer cells, $CD4^+$ and $CD8^+$ T-cell, monocytes, platelets and a subset of B-cells (1). *CD226* plays an important role in T-cell activation, differentiation and cytotoxicity, and is involved in adhesion of platelets and megakaryocytic cells to vascular endothelial cells (2, 3). *CD226* is involved in LFA-1-mediated costimulatory signals for triggering naive T-cell differentiation and proliferation (4). In addition, DNAM-1 is a tyrosinephosphorylated signal-transducing molecule that participates in primary adhesion during cytotoxic T-lymphocyte (CTL)-mediated cytotoxicity (5).

The *CD226* gene has been associated with multiple autoimmune diseases (6). A non-synonymous variant of *CD226* in exon 7, rs763361, predisposes to type 1 diabetes (T1D), coeliac disease (CED), multiple sclerosis (MS), autoimmune thyroid disease, rheumatoid arthritis (RA), Wegener's granulomatosis (WG), and, recently, systemic sclerosis (SSc) (7-10). Moreover, a three-variant haplotype in *CD226* comprising rs763361, rs34794968, and rs727088 (ATC), in the last exon of this gene, have been recently found to be associated with systemic lupus erythematosus (SLE) (11).

Giant cell arteritis (GCA), also called temporal arteritis or Horton's arteritis, is a systemic vasculitis that affects vessels of medium and large calibre with a predisposition for the involvement of cranial arteries that supply the aortic arch especially extracranial territories, mainly branches of the external carotid (12, 13). GCA is the most common

Table I. Main clinical features of 455 Spanish Caucasian patients with biopsy-proven GCA.

Feature	Variable
Age at diagnosis, years, median (IQR)	75 (69–79)
Women	313 (68.8)
Men	142 (31.2)
Headache	359 (78.9)
Abnormal temporal artery on examination	265 (58.2)
Polymyalgia rheumatica	215 (47.3)
Jaw claudication	190 (41.8)
Arm-leg claudication	27 (5.9)
Visual ischaemic manifestations*	116 (25.5)
Permanent visual loss	49 (10.8)
Stroke	19 (4.2)
Severe ischaemic manifestations**	227 (49.9)
Irreversible occlusive disease***	83 (18.2)

IQR: interquartile range.
*Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia[6].
**At least one of the following features: visual manifestations, cerebrovascular accidents (stroke and/or transient ischaemic attacks), jaw claudication, or limb claudication.
***At least one of the following features: permanent visual loss, stroke and/or occlusive disease in the extremities.

vasculitis in elderly individuals and it is suffered mainly by women. It is also more prevalent in Caucasians, especially in populations of Scandinavian descent (14, 15).

The inflammatory activity of vascular lesions in GCA is mediated by adaptive immune responses, with $CD4^+$ T-cells undergoing clonal expansion in the vessel wall and releasing interferon (IFN)-γ (16). Cellular immune responses involving T-cells, antigen-presenting cells, and macrophages are fundamental elements in GCA. At least two distinct CD4 T-cell subsets promote vascular inflammation in GCA. In early disease, antigen presenting cells promote differentiation of Th17 as well as Th1 cells (17). Vascular lesions contain mainly T-cells that are activated, macrophages and multinucleated giant cells, so it was hypothesised that *CD226*, which is involved in adhesion and costimulation of T-cells, may play a key role in this process (18).

Taking this into account, along with the fact that shared immunological pathways have been proposed for the basis of the pathology of different autoimmune diseases (19), the aim of this study was to investigate the possible genetic association of *CD226* with the susceptibility to GCA.

Patients and methods

Study population

Four hundred and fifty-five patients diagnosed with biopsy-proven GCA and 1414 unrelated healthy controls were included in this study, after obtaining informed written consent and approval from the local ethical committees.

The age at diagnosis of the biopsy-proven patients (median [IQR]) was 75 (69–79) years. Women with biopsy-proven GCA (313 [68.8%]) outnumbered men (142 [31.2%]). The median age of controls was 74 years (67% women). The main clinical features of this series of biopsy-proven GCA are shown in Table I.

All patients fulfilled the 1990 American College of Rheumatology criteria for the classification of GCA (20). Inclusion criteria (presence of pathological findings of biopsy-proven GCA in a temporal artery biopsy) (21) and clinical features of the patient population including headache, polymyalgia rheumatica (PMR), jaw claudication, peripheral arteriopathy manifested by arm or leg claudication, visual ischaemic manifestations (VIM), permanent visual loss, stroke, severe ischaemic manifestations (SIM – encompassing visual manifestations, cerebrovascular accidents, jaw claudication or limb claudication of recent onset), and the presence of irreversible occlusive disease (IOD – if patients experienced at

Table II. Genotype and allele distribution of *CD226* rs727088, rs34794968 and rs763361 in biopsy-proven GCA patients and healthy controls.

			Genotype, n (%)				Allele test	
SNP	1/2	Subgroup (n)	1/1	1/2	2/2	MAF (%)	*p*-value#	OR [CI 95%]§
rs727088	G/A	Controls (n=1356)	301 (22.20)	661 (48.75)	394 (29.06)	46.57		
		GCA (n=402)	86 (21.39)	204 (50.75)	112 (27.86)	46.77	0.922	1.01 [0.86–1.18]
		PMR+ (n=194)	40 (20.62)	100 (51.55)	54 (27.84)	46.39	0.976	1.00 [0.75–1.32]
		VIM+ (n=104)	26 (25.00)	48 (46.15)	30 (28.85)	48.08	0.547	1.10 [0.80–1.52]
		SIM+ (n=198)	48 (24.24)	99 (50.00)	51 (25.76)	49.24	0.091	1.28 [0.96–1.69]
		IOD+ (n=73)	19 (26.03)	34 (46.58)	20 (27.40)	49.32	0.402	1.17 [0.81–1.67]
rs34794968	A/C	Controls (n=1377)	230 (16.70)	644 (46.77)	503 (36.53)	40.09		
		GCA (n=398)	71 (17.84)	185 (46.48)	142 (35.68)	41.08	0.615	1.04 [0.89–1.22]
		PMR+ (n=189)	31 (16.40)	89 (47.09)	69 (36.51)	39.95	0.669	0.94 [0.71–1.25]
		VIM+ (n=100)	19 (19.00)	46 (46.00)	35 (35.00)	42.00	0.620	1.09 [0.78–1.51]
		SIM+ (n=199)	40 (20.10)	93 (46.73)	66 (33.17)	43.47	0.079	1.30 [0.97–1.73]
		IOD+ (n=74)	13 (17.57)	35 (47.30)	26 (35.14)	41.22	0.798	1.05 [0.73–1.51]
rs763361	T/C	Controls (n=1342)	298 (22.21)	640 (47.69)	404 (30.10)	46.05		
		GCA (n=399)	83 (20.80)	199 (49.87)	117 (29.32)	45.74	0.877	0.99 [0.84–1.16]
		PMR+ (n=193)	39 (20.21)	97 (50.26)	57 (29.53)	45.34	0.984	1.00 [0.75–1.32]
		VIM+ (n=101)	24 (23.76)	46 (45.54)	31 (30.69)	46.53	0.659	1.08 [0.78–1.48]
		SIM+ (n=194)	49 (25.26)	91 (46.91)	54 (27.84)	48.71	0.047*	1.33 [1.00–1.78]
		IOD+ (n=73)	19 (26.03)	32 (43.84)	22 (30.14)	47.95	0.441	1.15 [0.80–1.66]

*Benjamini and Hochberg (1995) step-up FDR correction: p=0.091.
#All p-values have been calculated for the allelic model. §Odds ratio for the minor allele. MAF: minor allele frequency; GCA: giant cell arteritis; PMR: polymyalgia rheumatica; VIM: visual ischaemic manifestations; SIM: severe ischaemic manifestations; IOD: irreversible occlusive disease.

Table III. Genotype distribution and minor allele frequency (MAF) of *CD226* rs727088, rs34794968 and rs763361 in biopsy-proven GCA patients according to the presence (with) or absence (without) of specific manifestations of the disease.

		With		Without		Test statistic	
		Genotypic frequencies	MAF (%)	Genotypic frequencies	MAF (%)	*p*-value[#]	OR [95% CI] [§]
rs727088	Polymyalgia rheumatica	40/100/54	46.39	43/100/57	46.5	0.976	1.00 [0.75–1.32]
	Visual ischaemic manifestations	26/48/30	48.08	57/148/82	45.64	0.547	1.10 [0.80–1.52]
	Severe ischaemic manifestations	48/99/51	49.24	35/95/61	43.19	0.091	1.28 [0.96–1.69]
	Irreversible occlusive disease	19/34/20	49.32	62/157/90	45.47	0.402	1.18 [0.81–1.67]
rs34794968	Polymyalgia rheumatica	31/89/69	39.95	38/89/72	41.46	0.669	0.94 [0.71–1.25]
	Visual ischaemic manifestations	19/46/35	42.00	50/128/107	40.00	0.620	1.09 [0.78–1.51]
	Severe ischaemic manifestations	40/93/66	43.47	29/79/76	37.23	0.079	1.30 [0.97–1.73]
	Irreversible occlusive disease	13/35/26	41.22	54/134/114	40.07	0.798	1.05 [0.73–1.51]
rs763361	Polymyalgia rheumatica	39/97/57	45.34	41/96/59	45.41	0.984	1.00 [0.75–1.32]
	Visual ischaemic manifestations	24/46/31	46.53	56/143/86	44.74	0.659	1.08 [0.78–1.48]
	Severe ischaemic manifestations	49/91/54	48.71	31/96/63	41.58	0.047*	1.33 [1.00–1.78]
	Irreversible occlusive disease	19/32/22	47.95	59/152/93	44.41	0.441	1.15 [0.80–1.66]

*Benjamini and Hochberg (1995) step-up FDR correction: p=0.091.
[#]All p-values have been calculated for the allelic model. [§]Odds ratio for the minor allele; MAF: minor allele frequency; GCA: giant cell arteritis.

least one of the following complications: permanent visual loss, stroke or limb claudication of recent onset), were described previously (22-26).

Part of the control samples were obtained from the Spanish National Bank of DNA, and the rest from different areas of Spain (including Madrid, Barcelona, Lugo and Granada), with the same criteria for sex and region of origin.

Genotyping methods and statistical analysis

DNA was extracted from peripheral blood cells using standard procedures. All participants were genotyped for the *CD226* single-nucleotide-polymorphisms (SNP), rs763361 and rs34794968, located in exon 7, and rs727088 in the 3'UTR region. The SNPs were analysed using the TaqMan® allelic discrimination assay technology in a 7900HT Real-Time polymerase chain reaction (PCR) System from Applied Biosystems (Foster City, CA, USA).

The statistical power of the analysis was >93% to detect associations with OR=1.3 at 5% significant level for the three SNPs, according to Power Calculator for Genetic Studies 2006 software (27).

PLINK (v1.07) software (http://pngu.mgh.harvard.edu/purcell/plink/) (28) was used for individual population association tests (significance was calculated by 2x2 contingency tables and Fisher's exact test or χ^2 when appropriate), and haplotype analysis. Odds ratios (OR) and 95% confidence intervals (CI) were obtained according to Woolf's method. p-values lower than 0.05 were considered as statistically significant. Benjamini and Hochberg False Discovery Rate Method correction (FDR) was applied for multiple test correction (29).

We also analysed the *CD226* rs727088-rs34794968-rs763361 haplotype using PLINK and Haploview (v.4.2) (30). Allelic combinations with a frequency lower than 5% in control groups were excluded from the analysis.

Results

After genotyping, no evidence of departure from Hardy-Weinberg equilibrium was observed in either case or control populations at the 5% significance level.

Table II shows the genotype and minor allele frequencies (MAF) of the control cohort and the different case sets analysed. No significant differences were detected between the allele frequencies of the GCA patients and controls for the 3 SNPs.

To examine whether the *CD226* locus might influence the clinical manifestations of the disease or not, GCA patients were subdivided according to the presence of PMR, VIM, SIM and IOD. However, no significant differences were found for the analysis between the different case subgroups and the control population (Table II). Although no significant differences were found, there was a trend when we compared SIM+ *versus* controls in the three SNPs analysed/studied.

Moreover, similar negative results were also observed when GCA patients with and without specific clinical features of the disease were compared (Table III).

Considering the *CD226* haplotype block association described in SLE (11), we decided to analyse the possible effect of this haplotype in GCA patients. However, in this analysis, we did not observe significant association with GCA susceptibility (Table IV).

Discussion

CD226 gene polymorphisms have been correlated with an increasing number of autoimmune diseases that often share some common pathogenic pathways (31). *CD226* plays an important role in T-cell activation, differentiation and cytotoxicity, and it is involved in adhesion of platelets and megakaryocytic cells to vascular endothelial cells (2, 3). Interestingly, the inflammatory activity of vascular lesions in GCA is mediated by adaptive immune responses, with $CD4^+$ T-cells (16, 18). There is an immune-mediating factor in the process of GCA, and both the innate immune system and the adaptive, are involved

Table IV. Pooled-analysis of rs727088-rs34794968-rs763361 allelic combinations according to disease.

	Allelic combination		
	ACC	GAT	GCT
GCA, n (%)	1321 (53.9)	962 (39.2)	169 (6.9)
Controls, n (%)	374 (53.4)	285 (40.7)	42 (6.0)
p-value	0.888	0.710	0.323
OR [95% CI]	0.98 [0.83–1.16]	1.06 [0.89–1.26]	0.88 [0.62–1.24]
PMR+, n (%)	184 (54.1)	136 (40.0)	20 (5.9)
Controls, n (%)	1321 (53.9)	962 (39.2)	169 (6.9)
p-value	0.717	0.923	0.373
OR [95% CI]	1.01 [0.80–1.27]	1.03 [0.82–1.30]	0.88 [0.55–1.41]
VIM+, n (%)	93 (52.0)	76 (42.5)	10 (5.6)
Controls, n (%)	1321 (53.9)	962 (39.2)	169 (6.9)
p-value	0.877	0.630	0.538
OR [95% CI]	0.93 [0.68–1.25]	1.15 [0.84–1.56]	0.87 [0.46–1.65]
SIM+, n (%)	178 (51.6)	148 (42.9)	19 (5.5)
Controls, n (%)	1321 (53.9)	962 (39.2)	169 (6.9)
p-value	0.277	0.177	0.646
OR [95% CI]	0.91 [0.73–1.14]	1.17 [0.93–1.46]	0.82 [0.51–1.33]
IOD+, n (%)	69 (52.7)	53 (40.5)	9 (6.9)
Controls, n (%)	1321 (53.9)	962 (39.2)	169 (6.9)
p-value	0.603	0.648	0.889
OR [95% CI]	0.95 [0.67–135]	1.06 [0.74–1.51]	1.09 [0.55–2.15]

GCA: giant cell arteritis; PMR: polymyalgia rheumatica; VIM: visual ischaemic manifestations; SIM: severe ischaemic manifestations; IOD: irreversible occlusive disease.

(32). Two pathogenic pathways mediated by Th17 and Th1 cells contribute to the systemic and vascular manifestations of GCA (33). Similar to T1D, MS, RA and WG, in which distinct T-cell alterations are also common, *CD226* polymorphisms could predispose to the altered T-cell response in GCA (9).

As reported in RA, a chronic inflammatory autoimmune disease in which joints also show predominance of $CD4^+$ Th1 cells, environmental and genetic factors seem to contribute to the etiology of GCA (14, 34). Both RA and GCA are associated with increased inflammatory response and share association with HLA-DRB1*04 alleles (35). Genome-wide association studies (GWAS) have identified shared non-HLA gene variants that influence susceptibility to unrelated to autoimmune diseases. Association of *CD226* rs763361 with RA has recently been described (6, 36).

Taking into account these considerations, an important step forward in our understanding of the pathogenesis of GCA might be to determine whether *CD226* is also a good susceptibility candidate locus to GCA or not. Therefore, we performed a case-control study to establish any genetic linkage of *CD226* with GCA. In this regard, our study constituted the first attempt to determine the potential influence of three *CD226* genetic variants (rs763361, rs34794968 and rs727088) in both GCA susceptibility and phenotypic expression of this vasculitis. However, our data showed no significant association between the analysed *CD226* polymorphisms with disease susceptibility or with specific features of GCA, although trends of association between the three genetic variants and SIM were suggested. Considering that the study had enough power to detect a possible moderate signal, it is unlikely that this gene might play a relevant role in GCA.

The reasons for this discrepancy in terms of genetic association between two CD4 T + cell-mediated inflammatory diseases like RA and GCA are unknown. However, despite having similar HLA-DRB1*04 association, former genetic studies did not disclose association of biopsy-proven GCA patients with non-*HLA* genes such as *PTPN22, STAT4* and *TRAF1/C5* that were associated with RA in independent Caucasians cohorts, including the Spanish population (37-43).

These negative results are in keeping with former studies of our group that did not disclose association between polymorphisms in inflammatory pathway genes and biopsy-proven GCA (44-46). However, we cannot exclude that association between GCA and other CD226 gene variants different from the polymorphisms assessed in the present study may exist.

In conclusion, although further studies in other populations with different genetic backgrounds are recommended to fully exclude an influence of the rs763361, rs34794968 and rs727088 *CD226* gene polymorphisms in GCA, our results suggest no evidence for a contribution of these *CD226* gene variants in the pathogenesis of GCA.

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Authors' contributions

Aurora Serrano and F. David Carmona contributed equally to this work. Javier Martín and Miguel A. González-Gay share senior authorship.

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