Basic and translational research

CONCISE REPORT

Influence of the *IL17A locus* in giant cell arteritis susceptibility

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ABSTRACT

Objective Different lines of evidence have highlighted the role of IL-17A in the inflammatory process occurring in giant cell arteritis (GCA). The aim of the present study was to assess whether the *IL17A locus* influences GCA susceptibility and its clinical subphenotypes.

Methods We carried out a large meta-analysis including a total of 1266 biopsy-proven GCA patients and 3779 healthy controls from four European populations (Spain, Italy, Germany and Norway). Five *IL17A* polymorphisms (rs4711998, rs8193036, rs3819024, rs2275913 and rs7747909) were selected by tagging and genotyped using TaqMan assays. Allelic combination and dependency tests were also performed.

Results In the pooled analysis, two of the five analysed polymorphisms showed evidence of association with GCA (rs2275913: P_{MH}=1.85E−03, OR=1.17 (1.06–1.29); rs7747909: P_{MH}=8.49E−03, OR=1.15 (1.04–1.27)). A clear trend of association was also found for the rs4711998 variant (P_{MH}=0.059, OR=1.11 (1.00–1.23)). An independent effect of rs2275913 and rs4711998 was evident by conditional regression analysis. In addition, the haplotype harbouring the risk alleles better explained the observed association than the polymorphisms independently (likelihood p value $<10^{-05}$).

Conclusions Polymorphisms within the *IL17A locus* show a novel association with GCA. This finding supports the relevant role of the Th17 cells in this vasculitis pathophysiology.

INTRODUCTION

Giant cell arteritis (GCA) is a systemic vasculitis with specific involvement of the aorta and external carotid arteries and their branches. Lesions are characterised by appearance of multinucleated giant cells, fragmented internal elastic lamina and cellular infiltrates, mainly macrophages and T lymphocytes.[1] GCA affects predominantly women and people generally older than 50 years, representing the most frequent vasculitis in elderly individuals from Western countries.[2]

Traditionally, GCA has been considered as a Th1-mediated disease based on granuloma formation and high levels of IFN-γ; however, Th17 cells are also present in GCA lesions.[3] Th17 cells are characterised by the secretion of IL-17, a cytokine leading to proinflammatory responses that participate in the pathogenic mechanisms of several auto-immune diseases.[4] Interestingly, a recent study has showed that the number of Th17 lymphocytes is significantly increased in patients with GCA, resulting in an imbalance between Th17 and regulatory T cells.[5] In addition, an increased expression of IL-17A[3 6] and several cytokines that participate in Th17 differentiation and maintenance has been detected in temporal artery samples from patients with GCA.[7 8] On the other hand, studies in animal models have shown that mice deficient in interferon regulatory factor 4 (IRF4) binding protein, which inhibits IL-17A production, develop a large-vessel vasculitis due to an inappropriate production of this cytokine.[9] These observations indicate that Th17 cells play a critical role in the inflammatory process occurring in GCA.

In recent years, several genes have been proposed to influence GCA susceptibility,[10] some of which have highlighted the relevant role of T cells in its predisposition, such as *HLA-DRB1* or *PTPN22*.[10 11] However, the overall genetic component of this condition remains unknown yet.

Considering this proposed crucial role of Th17 cells in GCA, we aimed to assess whether polymorphisms at the *IL17A* gene are involved in the genetic predisposition to this vasculitis and its clinical subgroups.

METHODS
Study population

A total of 1266 patients with GCA and 3779 unrelated controls from four European cohorts were analysed, comprising a discovery set from Spain (931 cases and 1845 controls) and three independent sets from Italy (178 cases and 1175 controls), Germany (74 cases and 480 controls) and Norway (83 cases and 279 controls). Online supplementary table S1 summarises the main characteristics of the analysed cohorts. Case/control sets were matched by geographical origin and ethnicity. All patients had a positive temporal artery biopsy and fulfilled the 1990 American College of Rheumatology

classification criteria.[12] Patients were stratified according to the presence or absence of polymyalgia rheumatica, visual ischaemic manifestations and irreversible occlusive disease, as previously described.[11]

Genotyping methods

Genomic DNA was extracted from peripheral white blood cells using standard procedures. We analysed five single-nucleotide polymorphisms (SNPs) located within *IL17A*, which tag over 86% of the variability of this *locus* as described elsewhere.[13] The rs4711998, rs8193036, rs3819024, rs2275913 and rs7747909 SNPs were genotyped using TaqMan allelic discrimination assays on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, California, USA).

Statistical analysis

Online supplementary table S2 shows the overall statistical power of the analysis (http://www.sph.umich.edu/csg/abecasis/CaTS/). Plink (V.1.07) (http://pngu.mgh.harvard.edu/purcell/plink/) and StatsDirect V.2.6.6 (StatsDirect Ltd, Cheshire, UK) were used to perform 2×2 contingency tables and χ^2 test. ORs and 95% CIs were obtained according to Woolf's method. Permutation test (10 000 permutations) was performed to estimate empirical p values (P_{EMP1}, uncorrected p value; P_{EMP2}, corrected for multiple testing) as implemented in Plink. p Values <0.05 were considered statistically significant. Dependency of association between SNPs was determined by conditional logistic regression analysis as implemented in Plink and the allelic combinations were tested using Haploview (V.4.2). The goodness of fit of both haplotype and independent SNP models was compared using Plink. The analysis of the combined data from all populations was performed using Plink and StatsDirect. Breslow-Day (BD) test was used to estimate the homogeneity among populations. Pooled analyses were performed by Mantel–Haenszel (MH) test under fixed effects.

RESULTS

Genotypic frequencies did not deviate from Hardy-Weinberg equilibrium (p>0.01), and the genotype success rate was >95%.

Allele test

First, we analysed the *IL17A* polymorphisms in the Spanish cohort (table 1). The case/control analysis yielded an association between rs2275913 (p=0.011, OR=1.17 (1.04–1.31)) and rs7747909 (p=0.042, OR=1.14 (1.01–1.29)) and GCA. A clear trend of association was also observed for rs4711998 (p=0.055, OR=1.13 (1.00–1.28)) and rs8193036 (p=0.063, OR=1.13 (0.99–1.29)). Permutation testing did not significantly affect the observed results (P_{EMP1}=0.055, P_{EMP1}=0.057, P_{EMP1}=0.429, P_{EMP1}=0.011 and P_{EMP1}=0.041, for rs4711998, rs8193036, rs3819024, rs2275913 and rs7747909, respectively). After applying the more stringent corrected permutation p values, rs2275913 remained significantly associated with GCA (P_{EMP2}=0.041). Subsequently, no specific genetic association with any of the analysed clinical forms was detected (data not shown).

To better explore the results observed in the Spanish cohort, *IL17A* polymorphisms were then analysed in three independent cohorts of European ancestry (see online supplementary table S3). Data from the discovery and replication cohorts were combined in order to elucidate the role of *IL17A* in GCA (BD test: p>0.05). As shown in table 2, the overall meta-analysis showed a clear association of rs2275913*A (P_{MH}=1.85E–03, OR=1.17 (1.06–1.29)) and rs7747909*A (P_{MH}=8.49E–03, OR=1.15 (1.04–1.27)) with GCA. A trend of association was also observed for the rs4711998*A variant (P_{MH}=0.059, OR=1.11 (1.00–1.23)).

Conditional logistic regression

As shown in table 3, the association observed for rs7747909 was dependent of rs2275913, maybe due to the moderate linkage disequilibrium between them ($r^2 \sim 0.59$) (see online supplementary figure S1). However, for rs4711998, a trend of association was still evident after conditioning by the other two polymorphisms.

Haplotype analysis

Two haplotypes were associated with GCA (see online supplementary figure S1 and table S4), a risk haplotype harbouring the minor alleles of the analysed polymorphisms (rs4711998*A/rs8193036*C/rs3819024*G/rs2275913*A/rs7747909*A: p=0.021, OR=1.38 (1.06–1.79)) and a protective haplotype containing the major alleles

Table 1 Genotype and allele distribution of the *IL17A* genetic variants in Spanish biopsy-proven GCA patients and healthy controls

| SNP | 1/2 | Subgroup (N) | Genotype, N (%) | | | MAF (%) | Allele test | | |
			1/1	1/2	2/2		p Value*	P_{EMP2}†	OR (95% CI)‡
rs4711998	A/G	Controls (n=1769)	127 (7.18)	688 (38.89)	954 (53.93)	26.63			
		GCA (n=916)	79 (8.62)	375 (40.94)	462 (50.44)	29.09	0.0547	0.196	1.13 (1.00 to 1.28)
rs8193036	C/T	Controls (n=1814)	82 (4.52)	647 (35.67)	1085 (59.81)	22.35			
		GCA (n=923)	47 (5.09)	360 (39.00)	516 (55.90)	24.59	0.0631	0.224	1.13 (0.99 to 1.29)
rs3819024	G/A	Controls (n=1827)	197 (10.78)	815 (44.61)	815 (44.61)	33.09			
		GCA (n=915)	100 (10.93)	425 (46.45)	390 (42.62)	34.15	0.4301	0.894	1.05 (0.93 to 1.18)
rs2275913	A/G	Controls (n=1815)	197 (10.85)	801 (44.13)	817 (45.01)	32.92			
		GCA (n=908)	116 (12.78)	429 (47.25)	363 (39.98)	36.40	**0.0107**	**0.041**	1.17 (1.04 to 1.31)
rs7747909	A/G	Controls (n=1820)	137 (7.53)	709 (38.96)	974 (53.52)	27.01			
		GCA (n=920)	73 (7.93)	399 (43.37)	448 (48.70)	29.62	**0.0416**	0.153	1.14 (1.01 to 1.29)

Significant p values are shown in bold.
*All p values have been calculated for the allelic model.
†p Values based on 10 000 permutations and corrected for multiple testing.
‡OR for the minor allele.
GCA, giant cell arteritis; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

Table 2 Combined analysis of the allele frequencies of the *IL17A* genetic variants in biopsy-proven patients with GCA and healthy controls from Spain, Germany, Italy and Norway

			Genotype, N (%)				Allele test	
SNP	1/2	Subgroup (N)	1/1	1/2	2/2	MAF (%)	p Value*	OR (95% CI)†
rs4711998	A/G	Controls (n=3654)	249 (6.81)	1385 (37.90)	2020 (55.28)	25.77		
		GCA (n=1238)	103 (8.32)	494 (39.90)	641 (51.78)	28.27	0.0591	1.11 (1.00 to 1.23)
rs8193036	C/T	Controls (n=3702)	196 (5.29)	1315 (35.52)	2191 (59.18)	23.06		
		GCA (n=1234)	65 (5.27)	472 (38.25)	697 (56.48)	24.39	0.2289	1.07 (0.96 to 1.19)
rs3819024	G/A	Controls (n=3719)	455 (12.23)	1667 (44.82)	1597 (42.94)	34.65		
		GCA (n=1242)	148 (11.92)	586 (47.18)	508 (40.90)	35.51	0.1434	1.08 (0.98 to 1.19)
rs2275913	A/G	Controls (n=3676)	432 (11.75)	1618 (44.02)	1626 (44.23)	33.76		
		GCA (n=1235)	172 (13.93)	571 (46.23)	492 (39.84)	37.04	**1.85E−03**	1.17 (1.06 to 1.29)
rs7747909	A/G	Controls (n=3733)	264 (7.07)	1423 (38.12)	2046 (54.81)	26.13		
		GCA (n=1252)	102 (8.15)	526 (42.01)	624 (49.84)	29.15	**8.49E−03**	1.15 (1.04 to 1.27)

Significant p values are shown in bold.
*All p values have been calculated for the allelic model.
†OR for the minor allele.
GCA, giant cell arteritis; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

of these SNPs (rs4711998*G/rs8193036*T/rs3819024*A/rs2275913*G/rs7747909*G: p=0.048, OR=0.91 (0.83–1.00)).

We observed a statistically significant improvement of the goodness of fit of the allelic combination model compared with that including rs4711998 (likelihood p value=8.38×10^{-06}), rs2275913 (likelihood p value=3.60×10^{-05}) or rs7747909 (likelihood p value=3.49×10^{-05}) individually.

DISCUSSION

Our data show for the first time an association between polymorphisms at the *IL17A* gene and GCA. The pooled analysis of four European cohorts evidenced three SNPs (rs4711998, rs2275913 and rs7747909) conferring risk to GCA, with rs2275913 showing the strongest association. Interestingly, this polymorphism is located within a binding motif for the nuclear factor activated T cells (NFAT), a central regulator of the IL-17 promoter.[14] Consistent with our results, showing an increased frequency of the rs2275913*A allele in GCA patients compared with controls, it has been recently proposed that this allele leads to a higher affinity for NFAT that correlates with an increase of the IL-17A secretion.[15] This observation would agree with the elevated levels of IL-17A found in inflammatory lesions of patients with GCA.[3 6]

Our results are in line with those previously reported in rheumatoid arthritis (RA),[13] which show a stronger effect for rs2275913. Moreover, the effect size detected in our study is consistent with the weak effect described in patients with RA (OR=1.17). Due to the proposed functional role for

rs2275913, this polymorphism was considered as a putative candidate to be responsible of the detected association between *IL17A* and RA.[13] However, our results suggest the existence of several independent signals in GCA. Accordingly with this idea, several polymorphisms in *IL17A*, in addition to rs2275913, have been found to influence binding affinity with transcription factor complexes, IL-17A levels and methylation status of the *IL17A* promoter in inflammatory bowel disease patients,[16] thus supporting the possible presence of different genetic variants with independent functional consequences.

The immunopathogenesis of GCA is mainly driven by two separate lineages of CD4 T cells, Th1, which seem to play a more important role in chronic disease, and Th17, which appear to have a relevant implication in early disease.[8] Although the role of Th1 cytokines and their receptors, such as *IFN-γ*, *IL-18* and *IL12RB2*, in the susceptibility to GCA has been more clearly demonstrated,[10] evidences of the influence of cytokines involved in Th17 responses in this vasculitis predisposition have also emerged during the last years. Interestingly, a role of *IL6* and *IL21*, involved in promoting the differentiation of Th17 cells, has been reported in several genetic studies.[17 18] Our results, together with these previous findings, support a crucial involvement of Th17 cells in GCA and firmly implicate the IL6-IL17 cytokine cluster in its susceptibility.

On the other hand, it should be highlighted the important role of IL-17A as a proatherogenic factor.[19] It has been reported that pre-existing atherosclerosis represents a potential risk factor for ischaemic manifestations in GCA.[20] It is therefore plausible that this cytokine might also affect the severity of this vasculitis, thus representing an attractive therapeutic target for GCA.

In summary, our study provides clear evidence of the role of *IL17A* as a genetic risk *locus* for GCA, thus contributing to the advance in the knowledge of the genetic network underlying this vasculitis susceptibility. Functional studies are required in order to clarify the responsible variant/s of this association and its functional consequences.

Table 3 Conditional logistic regression analysis for the *IL17A* polymorphisms considering the four populations as covariates

	GCA vs Controls			
	p Value	p Value add to rs4711998	p Value add to rs2275913	p Value add to rs7747909
rs4711998	0.0591	N/A	0.080	0.064
rs2275913	**1.85E−03**	**8.30E−03**	N/A	**0.022**
rs7747909	**8.49E−03**	**0.042**	0.782	N/A

Significant p values are shown in bold.
GCA, giant cell arteritis.

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