Analysis of two autoimmunity genes, *IRAK1* and *MECP2*, in giant cell arteritis

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ABSTRACT

Objective. *The Xq28 region, containing* IRAK *and* MECP2*, represent a common susceptibility locus for a high number of autoimmune diseases. Our aim in the present study was to evaluate the influence of the* IRAK1 *and* MECP2 *autoimmunity-associated genetic variants in the giant cell arteritis (GCA) susceptibility and its clinical subphenotypes.*

Methods. *We analysed a total of 627 female biopsy-proven GCA patients and 1,520 female healthy controls of Spanish Caucasian origin. Two polymorphisms, rs1059702 and rs17345, located at* IRAK1 *and* MECP2*, respectively, were genotyped using TaqMan® allelic discrimination assays.*

Results. *No association with any of the analysed polymorphisms was evident when genotype and allele frequencies were compared between GCA patients and controls (rs1059702: allelic p-value=0.699, OR=0.96, CI 95% 0.80-1.17; rs17435: allelic p-value=0.994, OR=1.00, CI 95% 0.84-1.19). Likewise, the subphenotype analysis yield similar negative results.*

Conclusion. *We have assessed for the first time the possible role of* IRAK1 *and* MECP2 *autoimmune disease-associated polymorphisms in GCA. Our data suggest that* IRAK1 *rs1059702 and* MECP2 *rs17435 genetic variants do not play a significant role in GCA susceptibility or severity.*

Introduction

Giant cell arteritis (GCA) is a chronic vasculitis characterised by an inflammation of large- and medium-sized blood vessels (1). Most patients with this condition present several complications such as stroke, aortic aneurysm, myocardial infarction, and, the most severe, visual loss (2, 3); although, no increased mortality risk for patients has been demonstrated (4). GCA affects predominantly women, with a female to male ratio around 2-3:1, and people aged over 50 years, with highest incidence rates in the eight decade of life (5).

In the last few years, it has been proposed that several genes play a role in the development of this pathology; however, only a few have been consistently associated with GCA so far (6). Interestingly, most of them represent common risk factors in autoimmunity.

As GCA, most autoimmune disorders show a female preponderance; therefore it is reasonable to presume that genes located on the X chromosome may play a role on their susceptibility. In this sense, several genetic variants at Xq28 region, harbouring two strong candidate genes for autoimmunity, methyl CpG binding protein 2 (*MECP2*) and interleukin-1 receptor-associated kinase 1 (*IRAK1*), have been associated with a number of autoimmune conditions, specifically primary Sjögren's syndrome (pSS) (7), systemic lupus erythematosus (SLE) (8), rheumatoid arthritis (RA) (9) and systemic sclerosis (SSc) (10). *IRAK1* encodes a serine/threonine protein kinase with a key role in the IL-1 receptor/Toll like receptor (TLR)-mediated signal transduction processes (11) and *MECP2* acts as a key transcription regulator (12). Recently, a fine mapping of this region identified the *IRAK1* non-synonymous polymorphism rs1059702 (Phe196Ser), which 196Phe allele has been reported

to confer increased NF-κB activity *in vitro* (13), as the likely causal variant predisposing to SLE susceptibility (8). The SLE-risk genotype of rs1059702 was associated with lower mRNA levels of *MECP2*, thus suggesting that both *IRAK1* and *MECP2* are SLE risk genes. In addition, an independent role of these two genes has been described in SSc, with the functional genetic variant influencing pulmonary fibrosis development and the rs17435 polymorphism, located in *MECP2*, conferring risk to diffuse cutaneous SSc (10). This same *MECP2* variant has also been associated with pSS (7).

Based on this, we decided to analyse the role of the disease associated *IRAK1/MECP2* polymorphisms in both predisposition to and the clinical subphenotypes of GCA.

Methods

Study population

Since the *IRAK1/MECP2* genes are located in a sex-linked region, only women were included in the study. A total of 627 female biopsy-proven GCA patients and 1,520 female unrelated healthy controls, both of Spanish Caucasian ancestry, were included in this study. Case and control sets were matched by geographical origin and ethnicity. This is a well characterised cohort included in previous studies (14); its main characteristics are shown in Table I. Informed written consent from all participants and approval from the local ethical committees were obtained in accordance with the tenets of the Declaration of Helsinki. All patients had a positive temporal artery biopsy (disruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without multinucleated giant cells) and fulfilled the 1990 American College of Rheumatology classification criteria for GCA (15). In the subphenotype analysis, the patients were stratified according to manifestations of polymyalgia rheumatica (PMR) and the presence or absence of visual ischaemic manifestations (VIM; if they experienced transient visual loss including amaurosis fugax, permanent visual loss, or diplopia) and irreversible occlusive disease (IOD; if they had at least one of the following features: permanent visual loss, stroke or occlusive disease in the upper or lower extremities).

Table I. Main clinical features of the female giant cell arteritis patients included in the study.

Feature	Number (%)
Age at diagnosis, years, median (IQR)	75 (70-80)
Headache	509 (81.2)
Abnormal temporal artery on examination	413 (65.9)
Polymyalgia rheumatica	289 (46.1)
Jaw claudication	283 (45.1)
Arm-leg claudication	36 (5.7)
Visual ischaemic manifestations*	170 (27.1)
Permanent visual loss	96 (15.3)
Stroke	32 (5.1)
Severe ischaemic manifestations**	265 (42.3)
Oclussive Vascular Disease***	105 (16.7)

IQR, interquartile range.

*Transient visual loss including amaurosis fugax, permanent visual loss, or diplopia.
**At least one of the following features: visual manifestations, cerebrovascular accidents (stroke and/or transient ischaemic attacks), jaw claudication, or limb claudication.
***Whether permanent visual loss or stroke is present.

Genotyping methods

Genomic DNA was extracted from peripheral white blood cells using standard procedures. Two single-nucleotide polymorphisms (SNPs), rs1059702 and rs17435, located within *IRAK1* and *MECP2*, respectively, were genotyped using the TaqMan® allelic discrimination assay technology on a 7900HT Fast Real-Time PCR System, both from Applied Biosystems (Foster City, California, USA).

Statistical analysis

The overall statistical power of the analysis was calculated using Power Calculator for Genetic Studies 2006 software (http://www.sph.umich.edu/csg/abecasis/CaTS/). Plink (v1.07) (http://pngu.mgh.harvard.edu/purcell/plink/) was used to perform 2x2 contingency tables and χ^2 test and/or Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (CI) were obtained according to Woolf's method. *p*-values lower than 0.05 were considered statistically significant. The allelic combinations were tested using Plink and Haploview (V. 4.2).

Results

The genotyping success rate was higher than 95%. No statistically significant deviation from Hardy-Weinberg equilibrium ($p \leq 0.01$) was observed in the control set.

As shown in Table II, when genotype and allele frequencies for the analysed *IRAK1* and *MECP2* genetic variants were compared between GCA patients and controls, no association with the global disease susceptibility was observed for any of the analysed polymorphisms (rs1059702: allelic *p*-value=0.699, OR=0.96, CI 95% 0.80-1.17; rs17435: allelic *p*-value=0.994, OR=1.00, CI 95% 0.84-1.19).

Subsequently, to analyse the possible influence of these SNPs on clinical subphenotypes of GCA, patients were stratified according to the presence of PMR, VIM and IOD. Likewise, this subphenotype analysis yielded negative results (Table II).

In addition, we also studied the possible additive effect of the two studied polymorphisms in the global disease by allelic combination analysis. The comparisons of the different detected haplotypes between cases and controls did not show significant results (data not shown).

Discussion

Although the pathophysiology of GCA is still unknown, an implication of both the innate and adaptive immune systems has been clearly demonstrated (16). IRAK1 plays a key role in both type of immunity, through transcriptional regulation of selected genes via

Table II. Genotype and allele distribution of *IRAK1* rs1059702 and *MECP2* rs17435 in Spanish female biopsy-proven GCA patients and healthy controls.

SNP	Locus	1/2	Subgroup (N)	Genotype, n (%)			MAF (%)	*p*-value*	OR [CI 95%]**
				1/1	1/2	2/2			
rs1059702	IRAK1	A/G	Controls (n=1449)	39 (2.69)	344 (23.74)	1066 (73.57)	14.56		
			GCA (n=617)	23 (3.73)	128 (20.75)	466 (75.53)	14.10	0.6993	0.96 [0.80-1.17]
			PMR+ (n=283)	7 (2.47)	62 (21.91)	214 (75.62)	13.43	0.4818	0.91 [0.70-1.18]
			VIM+ (n=166)	8 (4.82)	36 (21.69)	122 (73.49)	15.66	0.5913	1.09 [0.80-1.49]
			IOD+ (n=103)	1 (0.97)	25 (24.27)	77 (74.76)	13.11	0.5662	0.89 [0.58-1.34]
rs17435	MECP2	T/A	Controls (n=1423)	56 (3.94)	412 (28.95)	955 (67.11)	18.41		
			GCA (n=608)	29 (4.77)	166 (27.30)	413 (67.93)	18.42	0.9944	1.00 [0.84-1.19]
			PMR+ (n=281)	11 (3.91)	84 (29.89)	186 (66.19)	18.86	0.8020	1.03 [0.82-1.30]
			VIM+ (n=165)	9 (5.45)	49 (29.70)	107 (64.85)	20.30	0.4033	1.13 [0.85-1.50]
			IOD+ (n=100)	2 (2.00)	34 (34.00)	64 (64.00)	19.00	0.8358	1.04 [0.72-1.50]

*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; IOD: irreversible occlusive disease.

activation of NF-κB (17). On the other hand, it is well accepted that both genetic and environmental factors are involved in GCA appearance and progression, thus suggesting that epigenetic alterations, which are thought to mediate the relationship between the genome and the environment, are probably involved in the pathogenesis of this vasculitis. MECP2 acts a transcription repressor that exerts its effects by two epigenetic mechanisms, DNA methylation and histone deacetylation, leading to a chromatin configuration inaccessible for transcription and, therefore, silencing gene expression (18). Recent evidence indicates that MECP2 can also act as a transcription activator of a high number of genes (12).

Taking this into account, we considered that Xq28, harboring these two genes, could be involved in the susceptibility to GCA. However, our results evidenced no association of any analysed *IRAK1* and *MECP2* genetic variants with either GCA or its clinical subphenotypes. Our analysis had enough statistical power to detect a possible moderate signal (the statistical power of our study was higher than 80% to detect an OR>1.30). Consequently, it is unlikely that the observed lack of association might be due to a type II error as a consequence of a reduced cohort size.

It should be noted that tumor necrosis factor (TNF) receptor-associated factor 6 (*TRAF6*), which also participates in the activation of NF-κB by the IL-1R/TLR superfamily, has been recently reported to be associated with RA and SLE but not with GCA (19). This finding, coupled with the fact that the functional *IRAK1* Phe196Ser polymorphism (supposed to increase the NF-κB activation and decrease the mRNA levels of *MECP2*) is not associated with this vasculitis in our study, suggests that genetic variants located in genes involved in these molecular pathways are not implicated in the GCA susceptibility.

Several factors have been proposed to explain the sex bias observed in most autoimmune diseases, such as sex hormones, gender differences in the immune system or fetal microchimerism. However, these factors do not completely explain the female preponderance and hence, in recent years, efforts have focused on analysing the contribution of sex-linked genes to the sexual dimorphism. Our study does not support a role of *IRAK1* and *MECP2* in the GCA susceptibility but, presumably, other X-linked genes could be involved in its predisposition.

In summary, this study represents the first attempt to evaluate the possible implication of *IRAK1* and *MECP2* in the pathophysiology of GCA in a large and well-defined case/control cohort. The lack of association between the analysed genetic variants indicates that the *IRAK1/MECP2*-associated diseases share a common underlying mechanism that may not be relevant in the GCA pathogenesis. Nevertheless, a possible association between GCA and other Xq28 genetic variants, different from that analysed in the present study, may not be ruled out.

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