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


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 autoimmune-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; rs17345: 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 rs17345 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, methy 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 committee 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). 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.

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 χ² 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<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
activation of NF-κB (17). On the other hand, it is well accepted that both geneti
cic and environmental factors are involved in GCA appearance and progres
sion, thus suggesting that epige
netic alterations, which are thought
to mediate the relationship between the genome and the environment, are
probably involved in the pathogenesis of this vasculitis. MEC2P acts a tran
scription repressor that exerts its effects by two epigenetic mechanisms, DNA
methylation and histone deacetylation, leading to a chromatin configuration in
accessible for transcription and, there
fore, silencing gene expression (18).
Recent evidence indicates that MEC2P
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 susceptibil
ity to GCA. However, our results evi
denced no association of any analysed IRAK1 and MEC2P genetic variants
with either GCA or its clinical subphe
notypes. Our analysis had enough sta
tistical power to detect a possible mod
erate 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 con
sequence of a reduced cohort size.
It should be noted that tumor necrosis
factor (TNF) receptor-associated fac
tor 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 find
ing, coupled with the fact that the func
tional IRAK1 Phe196Ser polymorphism
(supposed to increase the NF-κB activ
ation and decrease the mRNA levels of
ME2P2) is not associated with this vasculitis in our study, suggests that geneti
cic variants located in genes involved
in these molecular pathways are not im
plicated in the GCA susceptibility.
Several factors have been proposed to
explain the sex bias observed in most
autoimmune diseases, such as sex hor
mones, gender differences in the im
mune system or fetal microchimerism.
However, these factors do not com
pletely explain the female preponder
ance and hence, in recent years, efforts
have focused on analysing the contribu
tion of sex-linked genes to the sexual
dimorphism. Our study does not sup
port a role of IRAK1 and MEC2P 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 im
plication of IRAK1 and MEC2P 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/ME2P2-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, might
not be ruled out.

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Table II. Genotype and allele distribution of IRAK1 rs1059702 and MEC2P rs17435 in Spanish female biopsy-proven GCA patients and healthy controls.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Locus</th>
<th>1/2 Subgroup (N)</th>
<th>Genotype, n (%)</th>
<th>MAF (%)</th>
<th>p-value*</th>
<th>OR [CI 95%]**</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1059702</td>
<td>IRAK1</td>
<td>A/G Controls (n=1449)</td>
<td>39 (2.69)</td>
<td>344 (23.74)</td>
<td>1066 (73.57)</td>
<td>14.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCA (n=617)</td>
<td>23 (3.73)</td>
<td>126 (20.75)</td>
<td>466 (75.53)</td>
<td>14.10 0.6993 0.96 (0.80-1.17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PMR+ (n=283)</td>
<td>7 (2.47)</td>
<td>62 (21.91)</td>
<td>214 (75.62)</td>
<td>13.43 0.4818 0.91 (0.70-1.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIM+ (n=166)</td>
<td>8 (4.82)</td>
<td>36 (21.99)</td>
<td>122 (73.19)</td>
<td>15.66 0.5913 1.09 (0.80-1.49)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IOD+ (n=103)</td>
<td>1 (0.97)</td>
<td>25 (24.27)</td>
<td>77 (74.76)</td>
<td>13.11 0.5662 0.89 (0.58-1.34)</td>
</tr>
<tr>
<td>rs17435</td>
<td>MEC2P</td>
<td>T/A Controls (n=1423)</td>
<td>56 (3.94)</td>
<td>412 (28.95)</td>
<td>955 (67.11)</td>
<td>18.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCA (n=608)</td>
<td>29 (4.77)</td>
<td>166 (27.30)</td>
<td>413 (67.93)</td>
<td>18.42 0.9944 1.00 (0.84-1.19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PMR+ (n=281)</td>
<td>11 (3.91)</td>
<td>84 (29.89)</td>
<td>186 (66.19)</td>
<td>18.86 0.8020 1.03 (0.82-1.30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VIM+ (n=165)</td>
<td>9 (5.45)</td>
<td>49 (29.70)</td>
<td>107 (64.85)</td>
<td>20.30 0.4033 1.13 (0.85-1.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IOD+ (n=100)</td>
<td>2 (2.00)</td>
<td>34 (33.00)</td>
<td>64 (64.00)</td>
<td>19.00 0.8358 1.05 (0.67-1.70)</td>
</tr>
</tbody>
</table>

*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.
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Acknowledgements
The authors thank Sofía Vargas and Sonia García for their excellent technical assistance, and all the patients and healthy controls for kindly accepting their essential collaboration.
Banco Nacional de ADN (University of Salamanca, Spain) is thanked for supplying part of the control material.

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