Concise Report

Contribution of MHC class I region to genetic susceptibility for giant cell arteritis


Objective. The aim of this study was to assess the potential contribution of HLA-class I MICA and HLA-B gene polymorphisms towards the pathogenesis of giant cell arteritis (GCA).

Methods. Ninety-eight biopsy-proven GCA patients and 225 ethnically matched controls from Lugo, Northwest Spain, were genotyped for the MICA-TM microsatellite polymorphism using a polymerase chain reaction (PCR)-based method. Genotyping of HLA-B was performed using PCR and detection with a reverse sequence-specific oligonucleotide (SSO) probes system.

Results. A significant difference in the distribution of the alleles of MICA between patient and control groups (P = 0.005) was found. This was due to an increased frequency of the MICA A5 allele in GCA patients compared with controls (26 vs 13.6%; P = 0.0001; PC = 0.0005; OR 2.2, 95% CI 1.4–3.4). In addition, the HLA-B*15 allele showed a higher frequency in GCA patients compared with controls (P = 0.004; PC = 0.04; OR 2.7, 95% CI 1.3–5.7). Interestingly, the association observed with the MICA A5 allele seems to be independent of linkage disequilibrium with HLA-B, as well as independent of that previously described with HLA-DRB1*04. Remarkably, simultaneous presence of MICA A5 and HLA-B*15 or HLA-DRB1*04 genetic markers leads to an increase in the OR obtained for each individual genetic marker (MICA A5 + B*15 OR 3.2; MICA A5 + DRB1*04 OR 5.8).

Conclusions. Our results provide the first evidence that the MICA and HLA-B genes are independently associated with the genetic susceptibility to GCA, and suggest that several genes within the MHC might have independent effects in the susceptibility to this systemic vasculitis.

Key words: Giant cell (temporal) arteritis, Genetics, MICA, HLA-B, HLA-DRB1, Susceptibility.
[10]. Potential stimulation by antigens of unknown nature, probably infectious, could trigger heat shock protein expression in the artery tissue inducing MICA expression.

In the present study we have assessed the potential contribution of MICA and HLA-B gene polymorphisms towards the pathogenesis of GCA.

Patients and methods

Study population

For the purpose of the present study, only GCA patients who had a positive temporal artery biopsy showing disruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without giant cells were included.

The study group comprised 98 patients (56% women; age 60–92 years, median age 75 years) diagnosed with biopsy-proven GCA at the Division of Rheumatology of the Hospital Xeral-Calde (Lugo, Northwest Spain) and 226 ethnically matched controls from the same region. All GCA patients fulfilled the 1990 American College of Rheumatology criteria for the classification of GCA [11]. Samples were obtained from subjects after they had given written informed consent [12, 13]. The study was approved by all local ethical committees of the corresponding hospital.

MICA genotyping

Genomic DNA was isolated from anticoagulant-treated peripheral blood mononuclear cells using standard methods. Genotyping of the MICA–TM microsatellite polymorphism was performed using a polymerase chain reaction (PCR)-based method as previously described [9]. Briefly, primer sequences were MICA 5′-CTT TTT TTT TTT CAG GGA AAG TGC-3′ and MICA 3′-ACC TAC CAT CTC CAG AAA CTG C-3′. The reverse primer was labelled at the 5′ end with 6-FAM. PCR products were electrophoresed in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and their sizes were determined using the Genescan 672 software (Applied Biosystems, Foster City, CA, USA).

HLA genotyping

Genotyping for HLA-B and HLA-DRB1 was carried out using a reverse dot blot kit with sequence-specific oligonucleotide (SSO) probes (Dynal RELITM SSO HLA-B and DRB1 typing kits, Dynal Biotech, Bromborough, UK).

Statistical analysis

Allelic and phenotypic frequencies of the two HLA-class I markers studied were obtained by direct counting. Statistical analysis to compare distributions was performed by chi-square test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated according to Woolf’s method. The software used was Statcalc program (Epi Info 2002; Centers for Disease Control and Prevention, Atlanta, GA, USA). Results were shown as OR and 95% CI. P-values below 0.05 were considered statistically significant.

As a test for association of MICA independent of HLA-class I, we compared the distribution of HLA-B15– haplotypes carrying the MICA A5 allele in GCA patients and controls with the distribution of haplotypes not carrying the MICA A5 allele [14]. Additionally, a conditional case-control test implemented in the UNPHASED software was used as an overall test for independent association of HLA-class I [15]. The same tests were applied to test for MICA independence of DRB1*04 HLA-class II alleles.

Results

Allelic and genotypic frequencies of MICA microsatellite in biopsy-proven GCA patients and controls

Table 1 shows the distribution of the MICA alleles in 98 biopsy-proven GCA patients and 225 controls. Accordingly with previous results in other Caucasian populations [9, 16], five different MICA alleles, A4, A5, A5.1, A6 and A9, were found in Northwestern Spanish individuals. In both patient and control groups, the MICA allele displaying the highest frequency was A6. Interestingly, a significant difference in the distribution of MICA alleles was observed when comparing patient and control groups ($P=0.005$ by chi-square test on a 5 × 2 contingency table). This was due to a statistically significant increased frequency of MICA A5 allele (26 vs 13.6%; $P=0.0001$; $P_C=0.0005$; OR 2.2, 95% CI 1.4–3.4) among biopsy-proven GCA patients compared with controls (Table 1). Moreover, homozygosity for MICA A5 allele was associated with an increased risk of developing GCA (MICA A5/A5 genotype: 19.4% in GCA patients vs 4.2% in controls. $P=0.000015$; $P_C=0.0001$; OR 5.2, 95% CI 2.2–12.5).

HLA-B allelic frequencies in biopsy-proven GCA patients and controls

The most frequent allele in both GCA and control populations was HLA-B*44 (14 and 18%, respectively) according to data previously reported for other Caucasian populations (Table 2) [17, 18]. The overall comparison of HLA-B allelic frequencies between GCA patients and controls showed a statistically significant deviation ($P=0.04$). Furthermore, an interesting finding was that the HLA-B*15 allele showed a higher frequency in the GCA patients group than in controls ($P=0.004$, $P_C=0.04$; OR 2.7, 95% CI 1.3–5.7) (Table 1).

Analysis of MICA A5 allele in relation with HLA-class I and II alleles

Due to the strong linkage disequilibrium existing within the MHC region, we investigated whether the MICA A5 contribution to GCA susceptibility was independent or attributed to linkage with HLA-B. Interestingly, we observed that the MICA A5 allele was associated with GCA susceptibility in HLA-B*15

<table>
<thead>
<tr>
<th>MICA allele</th>
<th>GCA patients</th>
<th>Controls</th>
<th>$P$</th>
<th>$P_C$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4</td>
<td>20 (10.2)</td>
<td>52 (11.6)</td>
<td>NS</td>
<td>NS</td>
<td>0.8 (0.5–1.5)</td>
</tr>
<tr>
<td>A5</td>
<td>51 (26.0)</td>
<td>61 (13.6)</td>
<td>0.0001</td>
<td>0.0005</td>
<td>2.2 (1.4–3.4)</td>
</tr>
<tr>
<td>A5.1</td>
<td>45 (23.0)</td>
<td>99 (22.0)</td>
<td>NS</td>
<td>NS</td>
<td>0.6 (0.7–1.6)</td>
</tr>
<tr>
<td>A6</td>
<td>62 (31.6)</td>
<td>169 (37.6)</td>
<td>NS</td>
<td>NS</td>
<td>0.7 (0.5–1.1)</td>
</tr>
<tr>
<td>A9</td>
<td>23 (9.2)</td>
<td>69 (15.3)</td>
<td>NS</td>
<td>NS</td>
<td>0.7 (0.4–1.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HLA-B allele</th>
<th>GCA patients</th>
<th>Controls</th>
<th>$P$</th>
<th>$P_C$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7</td>
<td>15 (8.2)</td>
<td>25 (7)</td>
<td>0.7</td>
<td>NS</td>
<td>1.2 (0.6–2.3)</td>
</tr>
<tr>
<td>B8</td>
<td>13 (7.1)</td>
<td>20 (5.6)</td>
<td>0.6</td>
<td>NS</td>
<td>1.2 (0.6–2.6)</td>
</tr>
<tr>
<td>B14</td>
<td>13 (7.1)</td>
<td>19 (5.3)</td>
<td>0.7</td>
<td>NS</td>
<td>1.2 (0.6–2.6)</td>
</tr>
<tr>
<td>B15</td>
<td>20 (11)</td>
<td>15 (4.2)</td>
<td>0.004</td>
<td>0.004</td>
<td>2.7 (1.3–5.7)</td>
</tr>
<tr>
<td>B18</td>
<td>7 (3.8)</td>
<td>20 (5.6)</td>
<td>0.5</td>
<td>NS</td>
<td>0.6 (0.3–1.6)</td>
</tr>
<tr>
<td>B35</td>
<td>13 (7.1)</td>
<td>39 (11)</td>
<td>0.2</td>
<td>NS</td>
<td>0.3 (0.6–1.2)</td>
</tr>
<tr>
<td>B40</td>
<td>12 (6.5)</td>
<td>12 (3.4)</td>
<td>0.2</td>
<td>NS</td>
<td>1.8 (0.7–4.2)</td>
</tr>
<tr>
<td>B44</td>
<td>27 (14.7)</td>
<td>64 (18)</td>
<td>0.4</td>
<td>NS</td>
<td>0.7 (0.4–1.2)</td>
</tr>
<tr>
<td>B51</td>
<td>18 (9.8)</td>
<td>25 (7)</td>
<td>0.3</td>
<td>NS</td>
<td>1.4 (0.7–2.7)</td>
</tr>
<tr>
<td>B57</td>
<td>10 (5.4)</td>
<td>10 (2.8)</td>
<td>0.2</td>
<td>NS</td>
<td>2.0 (0.8–4.9)</td>
</tr>
<tr>
<td>Others*</td>
<td>36 (19.6)</td>
<td>107 (30.1)</td>
<td>0.01</td>
<td>0.1</td>
<td>0.57 (0.4–0.9)</td>
</tr>
</tbody>
</table>

*MICA genotypes with frequency less than 5%.
negative individuals. The MICA A5+;HLA-B*15— haplotypes were significantly increased among GCA patients compared with controls than MICA A5—;HLA-B*15— haplotypes (P = 0.003 OR: 2.13 95% CI 1.2–3.7) (Table 2). In addition, it is worth noting that the conditional extended case-control analysis for MICA conditioned on the HLA-B locus reached a significant P-value of 0.01.

Furthermore, we assessed the possibility that the observed association of MICA A5 allele with GCA susceptibility could be due to linkage disequilibrium with HLA-DRB1*04, which was previously reported as a genetic risk factor for GCA predisposition in our population [3, 6]. Similar to what has been observed for HLA-class I alleles, the MICA A5+;DRB1*04— were statistically significantly more represented among GCA patients compared with controls than the MICA A5—;DRB1*04— haplotypes (P = 0.001; OR: 2.7, 95% CI 1.4–5) (Table 2). Likewise, in this case, the conditional extended case-control analysis for MICA and HLA-class II alleles reached a significant P-value of 0.02 (Table 2).

All these findings suggest that the association of MICA A5 allele with GCA susceptibility seems to be independent of linkage disequilibrium with both HLA-class I and HLA-class II alleles.

Furthermore, we investigated the additive effect of these risk alleles in GCA susceptibility. The presence of MICA A5 together with HLA-B*15 or HLA-DRB1*04 risk alleles at the same time leads to an increase in the OR (MICA A5+;DRB1*04 OR 5.8; MICA A5+B*15 OR 3.1), while the absence of these risk factors confers a potent protective effect (OR 0.4 for both MICA A5+;DRB1*04— and MICA A5+;B*15—) (Table 2).

Discussion

This study constitutes the first attempt to assess the potential implication of the HLA-class I region genes MICA and HLA-B in genetic susceptibility to GCA.

Interestingly, we demonstrated an association of the MICA A5 allele with GCA, which was markedly stronger in homozygous individuals for MICA A5 allele. Additionally, we observed that HLA-B gene contributes towards GCA genetic predisposition. Moreover, our results suggest that the contribution of these two GCA genetic risk factors seems to be independent in spite of the strong linkage disequilibrium described within this region.

The possible contribution of HLA-B to GCA pathogenesis is clearly based on their role of presenting peptides to T cells. Nevertheless, this explanation is less clear in the case of the MICA microsatellite polymorphism although it has been associated with susceptibility to connective-tissue diseases and other vasculitis such as Behçet’s disease [16, 19]. Interestingly, some epidemiological studies emphasize the presence of a cyclic pattern in incidence rates of GCA over time supporting the hypothesis of an infectious origin for this vasculitis [20]. Infections might lead to GCA development through various mechanisms including interactions between microbial ligands and endogenous molecules, impairment of pathogen clearance, molecular mimicry, modification of self-epitopes into neoantigens or failure to down-regulate the alloimmune response [21]. These infectious stimuli might also induce MICA expression in GCA. Accordingly, polymorphisms of MICA gene might up-regulate immune response against foreign antigens in these patients. MICA expression might promote antigen presentation or macrophage/NK cell effector functions, each of which could separately influence the pathogenesis of GCA.

Although an involvement of MICA molecules in the susceptibility to GCA is possible, little is known about the functional role of the MICA–TM variants. To date only the MICA A5.1 allele has been observed to have putative functional relevance [22]. However, for the MICA A5 allele associated with GCA susceptibility no functional implication has been as yet identified. Different MICA alleles without putative functional relevance have been associated with several autoimmune conditions, such as rheumatoid arthritis (RA) or type I diabetes (TID) [23–26]. Accordingly with our findings, the implication of MICA–TM alleles in RA and T1D susceptibility was shown to be independent of HLA-class I and II linkage disequilibrium [23–26]. On this basis, it could be speculated that both MICA A5 and HLA-B*15 alleles might be positional markers in strong linkage disequilibrium with the real disease causing variants located in or close to the HLA-class I region but independent of the previously reported association with the class II region.

Our results provide evidence that the MICA and HLA-B genes are independently associated with the genetic susceptibility to GCA, and suggest that two regions within the MHC might have independent effects in the susceptibility to this systemic vasculitis. Replication of these findings in other populations would be of interest.

Acknowledgements

This work was supported by grant SAF03-3460 from Plan Nacional de I+D+I, and in part by Junta de Andalucía, grupo CTS-180.

The authors have declared no conflicts of interest.
References