HLA class II alleles of susceptibility and protection in Brazilian and Dutch pemphigus foliaceus

de Sena Nogueira Maehara, L; De-Souza-Santana, F C; Porro, A M; Marcos, E V C; Ura, S; Nolte, I M; Pas, H H; Jonkman, M F; Tomimori, J

Published in:
The British journal of dermatology

DOI:
10.1111/bjd.16022

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Dear Editor, Pemphigus foliaceus (PF) is a worldwide chronic autoimmune bullous disease targeting desmoglein 1 on the epithelial cell surface of skin and mucous membranes, causing lesions in skin but sparing mucosa, as the latter contain compensating desmoglein 3. Several environmental triggers for the autoimmune reaction have been imputed, such as drugs, ultraviolet radiation and mercury compounds. HLA-DRB4 alleles (also known as HLA-DR4) have been linked to the disease in Ashkenazi Jews and other populations. For endemic PF in Brazil, named fogo selvagem (FS), HLA-DRB1*04 has been shown to have a relative risk >14 for the disease. In this study, we analysed HLA (major histocompatibility complex, also known as human leucocyte antigens) alleles in patients with FS in Brazil and PF in the Netherlands.

We selected 59 patients: 42 with FS from two institutions in Brazil (Federal University of São Paulo and Lauro de Souza Lima Institute) and 17 with PF from the Groningen Centre for Blistering Diseases in the Netherlands. In the latter sample, only white people were included in order to obtain a homogeneous population. In the Brazilian sample all patients were included as the population is of mixed ethnicity. DNA was extracted from blood samples of the patients with FS and PF in both countries and for controls from Brazil (n = 478). Diagnosis of FS or PF was confirmed by clinical, histopathological and immunological tests, when necessary. Control groups were obtained from the same geographic region and ethnic background. For HLA-DQB1 447 Dutch controls were used and for HLA-DRB1 two Dutch control groups were obtained: 447 from different provinces and 6559 from North Netherlands, where the expertise centre is located, in order to validate the first group (available at http://www.allelefrequencies.net/hla6006a.asp and in the literature).

DNA samples were obtained using the salting-out technique from coagulated blood with a Gentra Puregene kit (Qiagen, Venlo, the Netherlands). The HLA class II (locus DRB1* and DQB1*) alleles were determined in low resolution by polymerase chain reaction-sequence-specific oligonucleotide probe hybridization with a Labtype-SSO kit (One Lambda, CA, U.S.A.). The study followed the three institutes ethic committees’ recommendations. Statistical analysis comparing allele frequencies between patients and controls was performed using Graph Pad (GraphPad Software, La Jolla, CA, U.S.A.), with P-values calculated with Fisher’s exact test or χ²-test and multiple-testing; corrected P-values (Pc) by Bonferroni’s correction for 13 common broad DRB1 alleles and five common broad DQB1 alleles. Allele frequencies were considered significantly different if Pc ≤ 0.05. Odds ratios (OR) were calculated with a 95% confidence interval to determine the ratio for susceptibility or protection conferred by the respective HLA allele.

Table 1 HLA alleles associated with susceptibility or protection in patients in Brazil with fogo selvagem and in the Netherlands with pemphigus foliaceus

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency in patients, %</th>
<th>Frequency in controls, %</th>
<th>P</th>
<th>Pc</th>
<th>Odds ratio (95% CI)</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>DRB1*04</td>
<td>39-3</td>
<td>9-1</td>
<td>1.02 × 10⁻¹⁶ 1.33 × 10⁻¹⁵</td>
<td>6.46 (3.96–10.55)</td>
<td>Susceptibility</td>
</tr>
<tr>
<td></td>
<td>DRB1*07</td>
<td>2-4</td>
<td>13-4</td>
<td>0.0016 0.045</td>
<td>0.16 (0.04–0.65)</td>
<td>Protection</td>
</tr>
<tr>
<td></td>
<td>DRB1*16</td>
<td>11-9</td>
<td>3-8</td>
<td>0.0025 0.033</td>
<td>3.45 (1.65–7.23)</td>
<td>Susceptibility</td>
</tr>
<tr>
<td></td>
<td>DQB1*02</td>
<td>3-6</td>
<td>19-8</td>
<td>5.07 × 10⁻⁵ 0.0012</td>
<td>0.15 (0.05–0.48)</td>
<td>Protection</td>
</tr>
<tr>
<td></td>
<td>DQB1*05</td>
<td>42-9</td>
<td>20-1</td>
<td>1.32 × 10⁻⁶ 6.59 × 10⁻⁶</td>
<td>2.98 (1.88–4.73)</td>
<td>Susceptibility</td>
</tr>
<tr>
<td>Netherlands</td>
<td>DRB1*04</td>
<td>38-2</td>
<td>16.4±</td>
<td>0.0006 0.0058</td>
<td>3.17 (1.58–6.33)</td>
<td>Susceptibility</td>
</tr>
<tr>
<td></td>
<td>DRB1*04</td>
<td>38-2</td>
<td>14-9±</td>
<td>0.0010 0.0031</td>
<td>3.54 (1.73–7.25)</td>
<td>Susceptibility</td>
</tr>
</tbody>
</table>

Pc, Corrected P-value; CI, confidence interval. *P-values calculated by a Fisher’s exact test or χ²-test; †control group with 6559 individuals; ‡control group with 447 individuals.

DOI: 10.1111/bjd.16022
In Brazil, the allele frequencies of HLA-DRB1*04, DRB1*16 and DQB1*05 were higher among patients than controls and hence implied susceptibility to FS, whereas DRB1*07 and DQB1*02 were less frequent alleles among patients and hence protected against the disease (Table 1). In the Dutch population, HLA-DRB1*04 was the only allele statistically significantly associated with PF compared with the two control groups (Table 1).

The carrier frequency of the HLA-DRB1*04 allele was 60% in the Brazilian patients (25/42), and 71% in the Dutch patients (12/17). Homozygosity for the HLA-DRB1*04 allele was more frequent in Brazil (8/25 vs. 1/12), but interestingly the allele frequencies were similar in the two populations (39% in Brazil and 38% in the Netherlands). Comparison of ORs between the Brazilian and Netherlands groups, showed that the risk conferred by HLA-DRB1*04 was higher in the Brazilian group ($P = 0.0498$). The calculated increased risk was because of the lower allele frequency of this allele in the general Brazilian population (9.1% vs. 16.4%).

The role of HLA-DRB1*04 in the pathogenicity of PF has been confirmed in many studies. Initially described in Xavante Indians, in Brazil, this allele conferred susceptibility to FS.5 Here we confirmed that HLA-DRB1*04 is the major HLA risk allele for FS in the Brazilian population, and extended its susceptibility for PF in Dutch patients. Our findings are in accordance with previous publications in French6 and Italian8 populations. The higher risk of HLA-DRB1*04 in the Brazilian population (OR 6.46) might suggest that FS in Brazil is determined more by genetic predisposition than PF in the Netherlands. Moreover, environmental triggers such as sun exposure and insect bites add an additional risk to the genetic one, making FS endemic in Brazil.5

The HLA-DQB1*05 allele also conferred susceptibility for FS in the Brazilian population (OR 2.98), and might be associated with the intense Italian colonization in the country, particularly in São Paulo state, as this allele has been described as a risk for Italian PF (DQB1*0503).6 Recently, Brochado et al. also associated this allele with susceptibility for FS in Brazil.7 The susceptibility observed for HLA-DRB1*16 might be caused by a strong linkage disequilibrium to HLA-DQB1*05, as the former allele does not contain the sequence LLEQRRAA, present in the HLA alleles of risk.7,11 This amino-acid sequence is present in the HLA class II beta-chain molecule and is shared by different alleles. In this region, there may be an alpha-helix structure12 that could therefore represent the portion that interacts with T-lymphocyte receptors.13 Ultimately, it is possible that this sequence is associated with antigen expression capable of stimulating an autoreactive T cell, targeting desmoglein, leading to disease. This hypothesis has been applied to pemphigus vulgaris and might be extrapolated to PF. The protection conferred by HLA-DQB1*02 in the present study had already been reported for FS in association with HLA-DRB1*07 in the Brazilian population, and specifically in Parana state.14,15

In conclusion, HLA-DRB1*04, DRB1*16, and DQB1*05 confer susceptibility to FS in Brazil, whereas only HLA-DRB1*04 was associated with susceptibility to PF in the Netherlands.

References


Funding sources: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)/Brazil (201591/2012-0) and Groningen University Institute for Drug Exploration (GUIDE)/the Netherlands provided a scholarship for L.d.S.N.M.

Conflicts of interest: none declared.

L.d.S.N.M. and F.C.D.-S.-S. contributed equally to the work.