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HLA class II alleles as markers of tuberculosis susceptibility and resistance
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ARTIGO ORIGINAL

HLA class II alleles as markers of tuberculosis susceptibility and resistance

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Abstract

Background: Not every individual exposed to Mycobacterium tuberculosis becomes infected. One host genetic factor, involved in modulating the immune response in many ethnic groups, is the association of human leukocyte antigens (HLA) with tuberculosis (TB).

Objective: To investigate the association between TB, HLA-DRB1 and HLA-DQB1 gene polymorphisms in Portuguese population.

Methods: HLA-DRB1 and HLA-DQB1 gene polymorphisms were analyzed by PCR-SSP in 92 TB patients and 82 healthy exposed healthcare professionals.

Results: HLA-DRB1*14 frequency is higher in the TB patients group (7% vs. 0%) than in healthy exposed individuals. Tuberculin skin test reaction (TST) was positive in 69 individuals (all over 15 mm) in the healthy exposed group (HE+) and negative in thirteen (HE−).

Conclusions: No clear genetic markers of disease susceptibility or resistance were identified in this study. However, HLA-DRB1*14 was more frequent in TB patients, suggesting it may be involved in the evolution of infection towards active TB in our population.
Introduction

Infection with *Mycobacterium tuberculosis* (MT) results in a variety of conditions ranging from asymptomatic infection to active tuberculosis (TB) with pulmonary or extrapulmonary involvement. In extreme cases, MT infection may be fatal. One third of the World’s population is infected with MT; however, only a minority ever develop clinical disease. In 90% of infected individuals, bacilli remain under control in a latent state (latent TB infection).

The various clinical features of TB result from cell-cell interactions that are promoted by cytokines produced by immune cells in response to MT infection. Studies of the diverse consequences of infection in twins and under similar exposure conditions in a familial context suggest the importance of genetics in susceptibility and/or resistance to TB. Several case-control studies have identified associations between TB disease and gene polymorphisms. Among the candidate genes potentially involved in the immune response to TB are the murine natural resistance-associated macrophage protein 1 (NRAMP1) gene, the vitamin D receptor (VDR) gene, and the human leukocyte antigen (HLA) genes.

Some hospitals were once TB sanatoriums, and for several years maintained an important tradition of inpatient TB treatment. Until recently, there were no special measures to prevent against nosocomial transmission, and isolation rooms have only become available in the last decade. However, after having worked in an inpatient setting with high TB exposure and without special conditions during the sanatorium phase, most are disease-free and/or uninfected.

The aim of this study was to evaluate allelic associations with outcome of TB exposure, particularly in healthcare professionals heavily exposed to TB in the past, focusing on the importance of HLA-DRB1 and HLA-DQB1 alleles.

Methods

Subjects

All individuals were vaccinated with bacillus Calmette-Guérin (BCG) at birth, according to the national guidelines.
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In the current study, there were no statistically significant differences of the distribution of HLA-DQB1 alleles in TB patients and the HE group (data not shown). When the HE group was stratified for the presence of infection, however, we found that the HLA-DRB1*14 allele was absent in the TB patients and the HE group (0/6 cases) (7% vs. 0; \( p = 0.038 \)) (Table 2).

### HLA-DQB1 alleles

There were no statistically significant differences between frequencies of the distribution of HLA-DQB1 alleles in TB patients and the HE group.

The allele frequencies for normal healthy individuals (HC) were not substantially different from those reported in the same region. 28

### Discussion

TB pathogenesis is very complex, with many factors influencing disease development and determining the outcome of infection. MT usually enters the respiratory route. Phagocytosis by macrophages is the first event in the host-pathogen interaction and may determine the

### Table 1  Characteristics of TB patients and healthy controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TB patients (n = 92)</th>
<th>HE— (n = 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>45.47 ± 15.40</td>
<td>42.57 ± 11.42</td>
</tr>
<tr>
<td>Range</td>
<td>21-82</td>
<td>25-72</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>33 (36%)</td>
<td>69 (84%)</td>
</tr>
<tr>
<td>Male</td>
<td>59 (64%)</td>
<td>0</td>
</tr>
<tr>
<td>Positive TST</td>
<td>92 (100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2  HLA-DRB1* alleles phenotype frequencies

<table>
<thead>
<tr>
<th>Alleles</th>
<th>TB patients (n = 92)</th>
<th>HE+ (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DRB1*</td>
<td></td>
<td></td>
</tr>
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</table>

HLA-DRB1 and HLA-DQB1 phenotypic frequencies were determined by direct count. Comparisons of HLA frequencies between patients and controls were performed using the Pearson \( \chi^2 \) Test with continuity correction or the Fisher's Exact Test when an expected absolute cell frequency was less than 5; \( p \) values \( \leq 0.05 \) were considered statistically significant.
Within 2 to 6 weeks of infection, cell-mediated immunity is developed and an influx of lymphocytes and activated macrophages appears in the lesion, resulting in granuloma formation. The bacilli are contained in the granuloma, where they may remain forever (latent TB infection), or become re-activated at a later date to proliferate and ultimately evolve to an active disease state.

Not all individuals exposed to MT become infected. Of those who do become infected, the course and duration of progression to active disease are highly variable. This lack of uniformity in disease manifestation among infected individuals may reflect a complex interaction between genetic and environmental factors. The relative weight of some risk factors, such as AIDS, diabetes, family income and nutritional status, are known, but host genetic factors may also influence susceptibility to infection and disease pathogenesis. Despite the compelling rationale for the involvement of host genetic influences, evidence for a genetic basis for TB susceptibility has been difficult to establish.

Some candidate genes have been reported, and a role for HLA, which is important in the immune response, has been recognized. In particular, a number of studies have reported an association between HLA Class II alleles and TB, but these associations are not consistent across different populations and the results remain controversial. A study by Ruggiero et al showed an increase in HLA-DR4 frequency in an Italian population. A significant increase in the frequency of HLA-DRB1*14 has been reported in Iranian patients, an association that was confirmed by Matrashkin et al in a Russian population. A case-control study published by Dubaniewicz et al reported a strong association of HLA-DRB1*16 with TB in a Polish population. More recently, this group confirmed and extended these results using a “high resolution” method, showing a high frequency of the HLA-DRB1*1601 allele and a low frequency of HLA-DQB1*0201 in Polish TB patients; this latter result suggests that the HLA-DQB1*0201 allele may be linked to TB resistance. A high frequency of the HLA-DQB1*0501 allele has been reported among North American Indian and Mexican TB patients, whereas the frequency of the HLA-DQB1*0502 was increased among TB patients in a Thai population. In a report from Cambodia, the HLA-DQB1*0503 allele was found to be associated with TB whereas the HLA-DQB1*0501 allele was not. These differences are likely attributable to the differing ethnic backgrounds of the studied populations, but it should be noted that many differences are likely attributable to the differing ethnic backgrounds of the studied populations.

Conflict of interest
Authors state that they don’t have any conflicts of interest.

References
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