Rheumatic Fever and the HLA Complex
A Cosegregation Study

M. Gerbase-DeLima, MD; L.C.N. Scala, MD; J. Temin, MSc; D.V. Santos, MD; P.A. Otto, MD

Background Considering the controversial results published in the literature concerning associations between human leukocyte antigens (HLA) and rheumatic fever (RF), the purpose of the present study was to investigate by means of cosegregation analysis the participation of HLA genes in susceptibility to RF.

Methods and Results The sample reported here was composed of 51 affected and 66 healthy individuals belonging to 22 genetically informative families. The comparison \( (x^2\) goodness-of-fit test) of the observed numbers of identical-by-descent (IBD) HLA haplotypes among all affected individuals (siblings, cousins, and uncle/nephew and grandparent/grandchild type of pairs) with the expected ones under the assumption of independent segregation of HLA alleles and the presumptive RF susceptibility gene gave a value of \( P=0.088\).

It is generally believed that factors involved in the pathogenesis of rheumatic fever (RF) include group A \( Streptococcus\) infection, environmental conditions (such as crowding, poor medical care, undernutrition), and hereditary factors.\(^1\)\(^-\)\(^3\) At least part of the genetic susceptibility could be accounted for by human leukocyte antigen (HLA) alleles, as suggested in a number of association studies. These investigations, however, have failed to disclose a single HLA marker for susceptibility to RF, irrespective of the genetic composition of the population in which the study was undertaken. Association with HLA-DR4 was reported in Caucasian populations both from the United States\(^4\),\(^5\) and Saudi Arabia\(^6\); DR1 was found associated in black populations of Martinique\(^7\) and South Africa,\(^\) whereas DR2 was implicated in susceptibility to RF in black American patients.\(^8\) HLA-DR7 and DR53 had high frequencies in Brazilian patients of admixed genetic background.\(^9\) In patients from India, a positive association with HLA-DQ2 and a negative association with HLA-DR2\(^10\) were also reported. This array of data could best be interpreted as indicating that the real susceptibility gene for RF is not represented by any of the above-mentioned markers themselves but instead by another gene present in the HLA complex that could display, in different populations, various linkage disequilibrium patterns with HLA-DR or DQ alleles.

Since the number of subjects studied was relatively small and the rejection level obtained was near the usual .05 significance level, we calculated the expected HLA IBD scores in the 13 pairs of affected sibs of our sample for all possible frequencies of the presumptive RF susceptibility gene. This analysis allowed clear rejection of a recessive mode, considering susceptibility gene frequencies lower than 20%, whereas the observed values fitted very well a dominant mode of inheritance, with penetrance (K) values varying between 0.5 and 0.9 and a frequency of the susceptibility gene of at least 1%.

Conclusions The present data support the hypothesis of an RF susceptibility gene within or very near the HLA complex. (Circulation. 1994;89:138-141.)

Key Words • rheumatic heart disease • leukocytes • antigens • genetics

Alternatively, the lack of consistency among the different studies concerning the associated HLA allele could indicate that HLA genes do not play a significant role in susceptibility to RF.

To examine the question of participation of the HLA complex in susceptibility to RF independently from any specific HLA allele, we selected multiple-case families, comparing the observed numbers of identical-by-descent (IBD) HLA haplotypes among affected relatives with the expected ones under the assumption of independent segregation of HLA alleles and the presumptive RF susceptibility gene.

Methods

Case Study

The 22 multiple-case families (13 of Caucasian and 9 of Negroid extraction) included in this study were selected at the Cardiology Department of Escola Paulista de Medicina. First, a questionnaire was given to 709 patients currently being treated, who were queried about the existence of other affected family members. All available relatives of 58 of 99 patients who referred familial cases were examined, and 32 families with at least one other individual with RF were detected. Since 10 of these families were represented by noninformative affected parent/child pairs, they were excluded from the analysis reported here. Sixteen of the probands were females and 6 were males, ages 9 to 67 years (mean, 34.4 years); the affected relatives were represented by 17 females and 12 males, ages 6 to 62 years (mean, 30.3 years). Rheumatic heart disease (RHD) was present in 20 of the 22 probands and in 23 of the 29 affected relatives. The remaining cases presented with acute rheumatic fever (ARF) at the time of examination or in the past. The diagnosis of ARF was made according to the modified Jones criteria.\(^11\) The diagnosis of RHD was based on medical history, physical examination, available previous medical records, chest x-ray, ECG, and Doppler echocardiogram and included anatomic pathological studies of the valves in the patients who underwent surgery. In

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addition to the 51 affected individuals, 66 healthy family members were included in our study to provide information for the IBD HLA haplotype assignments. The protocol was approved by the Ethical Committee of Escola Paulista de Medicina, and informed consent to participate in the study was obtained from all participants.

**HLA Typing**

HLA antigens were determined by the standard complement-dependent microlymphocytotoxicity method using a battery of 140 anti-HLA class I sera (local, Biotest, and Pel-Freez sera) capable of recognizing 12 HLA-A (HLA A1, 2, 3, 9, 10, 11, 28, 29, 30, 31, 32, 33) and 17 HLA-B (HLA B5, 7, 8, 12, 13, 14, 15, 16, 17, 18, 21, 22, 27, 35, 37, 40, 41) specificities. HLA class II specificities were determined with a battery composed of 70 to 120 antisera (local, Biotest, and Pel-Freez sera) capable of recognizing the specificities HLA-DR 1, 2, 3, 4, 5, 6, 7, 52, 53, and DQ1, 2, and 3.

IBD HLA haplotypes were assigned by comparing the HLA phenotypes among family members.

**Statistical Analysis**

The numbers of shared HLA haplotypes among pairs or trios of affected individuals were compared with those expected under the assumption of independent segregation of HLA and RF susceptibility genes by the usual $\chi^2$ goodness-of-fit test.

Applying the general method proposed by Suarez to our data of 13 pairs of affected sibs (5 pairs with 2 IBD; 7 pairs with 1 IBD; 1 pair with 0 IBD), we calculated the expected numbers of shared haplotypes by varying the gene frequency $q$ from 0 to 1 under two different hypotheses: (1) recessive, in which case the penetrance vector is given by $(f_1, f_2, f_3)=(0, 0, 1)$, where $f_1$, $f_2$, and $f_3$ are, respectively, the probabilities of dominant homozygotes, heterozygotes, and recessive homozygotes presenting the recessive phenotype; and (2) dominant, in which case the penetrance vector is given by $(f_1, f_2, f_3)=(1-0.01K^2, K, 0)$, where $K$ is the penetrance value in heterozygotes and $f_1$, $f_2$, and $f_3$ are the probabilities of dominant homozygotes, heterozygotes, and recessive homozygotes presenting the dominant phenotype. The penetrance value in homozygotes is assumed to be a function of $K$ and has the form $1-(1-K)^2$, an expression that takes into account the probability of penetrance in one or the other or both of the genes in homozygosis.

Using the expected numbers of shared haplotypes calculated for each value of gene frequency in the range (0, 1) in both hypotheses, we also determined the corresponding $\chi^2$ values, which are plotted in Figs 1 and 2. In Fig 2 (dominant hypothesis), these values were calculated for the following values of $K$: 0.9, 0.8, 0.7, 0.6, and 0.5.

This strategy enabled us to verify whether, for some acceptable range of the frequency of the susceptibility gene, our data are in accordance with one of the two genetic hypotheses.

**Results**

The observed and expected numbers of shared IBD HLA haplotypes among members of the 22 families studied are presented in the Table. A $\chi^2$ test yielded for these data a value of $P=.088$. Since we considered that this probability value did not enable us to completely rule out the participation of HLA genes in susceptibility to RF, we decided to separately analyze the cosegregation of RF and HLA haplotypes in the 13 pairs of sibs. In this group, we observed 5, 7, and 1 pairs, respectively, with 2, 1, and 0 IBD HLA haplotypes. These observed values were then compared with the expected ones by $\chi^2$ tests, taking into consideration frequencies of the putative susceptibility gene ranging from 0 to 1 and recessive and dominant modes of inheritance. As Fig 1 clearly shows, under the recessive hypothesis, the fit of the observed numbers to the expected ones was good only for gene frequencies larger than 20%. In contrast, as we observe in Fig 2, under the dominant model, with penetrance ($K$) values varying between 0.5 and 0.9 and a frequency of the susceptibility gene of at least 1%, all the $\chi^2$ values are below the critical figure at the 5% significance level for two degrees of freedom (5.99).

**Discussion**

The basis of multiple-case family studies is to use HLA haplotypes as markers to trace the inheritance pattern of closely linked disease susceptibility genes. The present cosegregation analysis of RF and IBD HLA haplotypes supports the hypothesis that one of the genes responsible for susceptibility to RF is located near or within the HLA complex.

The affected members in the 22 families of this study were represented by the following sets of pairs: siblings, cousins, uncle/nephew, and grandparent/grandchild. The comparison of the numbers of observed and expected IBD HLA haplotypes, taking into account the values derived from all the affected individuals, gave a value of $P=.088$. Even considering that the usual 5%
Distribution of HLA Haplotype IBD Scores in Relatives Affected With Rheumatic Fever*

<table>
<thead>
<tr>
<th>Family</th>
<th>Affected Individuals</th>
<th>Obs</th>
<th>Exp</th>
<th>Obs</th>
<th>Exp</th>
<th>Obs</th>
<th>Exp</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>2 Sibs</td>
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<td>1</td>
<td>0.5</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>Aunt/niece</td>
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<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>Aunt/niece</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
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<td>0.5</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>Uncle/niece</td>
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<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
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<td>2 Sibs</td>
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<td>0.25</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>7</td>
<td>2 Sibs</td>
<td>0</td>
<td>0.25</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>8</td>
<td>2 Cousins</td>
<td>0</td>
<td>0</td>
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<td>0.25</td>
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<td>0.75</td>
</tr>
<tr>
<td>9</td>
<td>3 Sibs</td>
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<td>0</td>
<td>0.375</td>
<td>1</td>
<td>0.5625</td>
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</tr>
<tr>
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<td>0</td>
<td>0.5</td>
</tr>
<tr>
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<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>13</td>
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<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
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<td>2 First cousins, 1 double first cousin</td>
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<td>0.1094</td>
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<td>0.25</td>
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<td>0</td>
<td>0.5</td>
<td>0</td>
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<tr>
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<td>0.5</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>20</td>
<td>Grandmother/grandchild</td>
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<td>0</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
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<td>2 Sibs</td>
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<td>0</td>
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<td>0.25</td>
</tr>
<tr>
<td>22</td>
<td>2 First cousins</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.25</td>
<td>0</td>
<td>0.75</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
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<td>2.3281</td>
<td>14</td>
<td>9.5469</td>
<td>5</td>
<td>10.125</td>
</tr>
</tbody>
</table>

*Comparison between observed (Obs) and expected (Exp) numbers of shared haplotypes. HLA indicates human leukocyte antigen; and IBD, identical by descent.

$\chi^2$ goodness-of-fit test: $\chi^2 = 4.87 \ (2 \ df); \ P = .088.$

The frequency of susceptible individuals in the population would be 2% to 1.0% under K values ranging from 1 to 0.5, respectively.

One possible way through which an HLA-linked gene could render the host susceptible to RF would be by interfering with the immune response to the streptococcal infection. In this context, it is interesting to mention that a family study concerning the in vitro blastogenic response of lymphocytes to an extracellular antigen from group A streptococci suggested that high responsiveness was controlled by at least one dominant gene linked to the HLA complex. In another family study, increased avidity for adherence of RF-associated streptococcal strains to pharyngeal cells was present in rheumatic siblings and in some of their healthy siblings. Genetic analysis of these data suggested that increased adherence was related to a dominant HLA-linked gene.

In conclusion, the present analysis further supports the hypothesis of an HLA-linked RF susceptibility gene. A similar study performed in Egyptian families, published by Hafez et al, also provided evidence for an
HLA-linked RF susceptibility gene, although a recessive pattern was suggested. At the moment, we have no plausible explanation for this discrepancy.

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