Essential Hypertension and Histocompatibility Antigens
An Association Study

Maria Gerbase-DeLima, Maria Antonia Ladalardo, Jose Jayme Galvão DeLima, Hélio Bernardes Silva, Giovanni Bellotti, and Fábio Pileggi

Data from a previous study concerning the distribution of human leukocyte antigen (HLA) haplotypes in siblings with essential hypertension suggested that at least one of the genes responsible for the genetic susceptibility to this disease is located in or near the HLA complex. The objective of the present study was to investigate if a given HLA-A, B, or DR gene could represent a marker for susceptibility to essential hypertension at the population level. Thus, the frequencies of HLA antigens were determined in Caucasian patients with essential hypertension (HLA-A and B antigens were determined in 89 cases, 85 of which were also typed for HLA-DR antigens). The results showed an increased frequency ($p=0.00064$) of HLA-DR4, which was present in 34% of the patients and in 16% of local ethnically matched control subjects. We conclude that HLA-DR4 may represent a marker for susceptibility to essential hypertension in the Brazilian Caucasian population. (Hypertension 1992;19:400-402)

KEY WORDS • essential hypertension • HLA antigens • immunogenetics • histocompatibility antigens

Several studies have indicated that a large proportion of the phenotypic variation in blood pressure is genetically determined. The number and nature of the genes have not been elucidated, although it is accepted that this genetic control is polygenic in nature, i.e., variability in blood pressure is the result of multiple gene effects. Considering that essential hypertension is probably not a homogenous disease, it is conceivable that different sets of genes involved in the control of blood pressure may act as leading genes for susceptibility to essential hypertension in different patients.1-4

There is evidence that one of the genes that may confer susceptibility to essential hypertension in some patients is located within or near the HLA (human leukocyte antigens) complex. In a recent study,5 we observed a statistically significant excess of HLA haplotypes in common among 96 siblings with essential hypertension who were from 31 Brazilian families (18 Caucasian, 12 black, and 1 Oriental). The linkage between essential hypertension and the inheritance of HLA haplotypes was apparently stronger in Caucasian than in black families. In this same study, an increased frequency of the HLA antigens DR2 and DR4 was observed among the probands of the 18 Caucasian families evaluated, suggesting that these antigens may act as genetic markers of susceptibility to essential hypertension. In contrast to our findings, Sengar et al6 found an excess of HLA-DR7 among Canadian hypertensive patients. Studies concerning HLA-A and B antigens have been published by several authors, but no clear associations have been consistently demonstrated.6-16

The objective of the present investigation was to further study the relation between HLA antigens and essential hypertension and specifically to test if the associations with HLA-DR2 and DR4 antigens suggested by our previous work could be confirmed in a larger sample of patients.

Methods

The patient sample comprised 89 (35 men and 54 women) unrelated Brazilian Caucasian individuals with essential hypertension followed up at the outpatient clinic of Instituto do Coração, Faculdade de Medicina, Universidade de São Paulo. The criteria for classification of an individual as hypertensive were systolic blood pressure 160 mm Hg or higher or diastolic blood pressure 95 mm Hg or higher (average of at least two determinations on separate occasions). All patients underwent clinical and laboratory evaluation to exclude malignant or secondary hypertension, as well as systemic disease. Our study only included nonobese patients whose age at the time of diagnosis was under 45 years. The age at the time of diagnosis ranged from 15 to 43 years, with a mean of 33.8 and a median of 34 years. The weight(kg)/height(m)$^2$ index recorded on the occasion of blood collection for the present study ranged from 18.6 to 35.5, with a median index of 26.4. Among the 69 patients who provided information about familial occurrence of hypertension, 74% indicated that...
they had relatives who had hypertension, 10% indicated suggestive familial cases, and 16% were not aware of any case in the family. Informed consent to participate in this study was obtained from all patients.

The local control population for HLA antigenic frequencies consisted of unrelated apparently healthy Brazilian Caucasian individuals (potential organ donors, individuals typed for paternity tests, and laboratory personnel). HLA-A and B antigens were determined in 979 of the control population, 266 of whom were also typed for HLA-DR antigens. Fewer individuals, however, were used to assess the frequencies of the HLA A30 and A31 (n=222) and DRw6 (n=143) antigens since reliable antisera to define these specificities were not available for all the control population. Blood pressure was not recorded in the control individuals.

**HLA Typing**

Mononuclear cells were separated from heparinized peripheral blood by centrifugation on a Ficoll-Hypaque gradient and the HLA antigens were determined by the standard microlymphocytotoxicity method. For HLA-DR typing, we used nylon-wool-separated, B-cell-enriched populations. The battery of antisera included 140 selected sera for HLA-A and B and 58 selected sera for HLA-DR typing, capable of defining 12 HLA-A, 17 HLA-B, and 7 HLA-DR specificities.

**Statistical Analysis**

The significance of the association was tested using the χ² test with Yates’ correction. The degree of association was assessed using the odds ratio as an approximation to estimate the relative risk. The logarithm (to the base e) of the calculated odds ratio and its standard error were used to construct the 95% confidence interval.

**Results**

Data on the frequencies of HLA-A, B, and DR antigens in patients and control group are presented in Tables 1 and 2, respectively. The HLA-DR4 antigen was the only antigen showing a statistically higher prevalence among patients than among the controls (34%×16%; p=0.00064). The calculated odds ratio for the presence of essential hypertension among Brazilian Caucasian individuals with the HLA-DR4 antigen compared with individuals from the same population without this marker was 2.68, with a 95% confidence interval between 1.49 and 4.85.

The frequency of the HLA-B16 antigen was lower in the patient group (1.1%×9.5%, p=0.013), but this difference was no longer significant when the probability value was corrected for the number of antigens tested (p×36). Furthermore, the frequency of the HLA-B16 antigen in the patient group was not statistically different when compared with a subgroup of 170 individuals from the local control population who were typed with the same battery of sera as that used to type the patients.

**Discussion**

In the present study we confirmed in a sample of 85 Brazilian Caucasian patients with essential hypertension the increased frequency of the HLA antigen DR4, which had previously been indicated from data obtained in a pilot study involving a very small sample. In the previous study, HLA-DR4 frequency among 18 Caucasian patients was 40% (versus 14.8% in the control group); in the present study we found a frequency of 34%, which was significantly different (p=0.00064) from that observed in the local control population (16.2%).

**Table 1. HLA-A and B Phenotypic Frequencies in Patients With Essential Hypertension and in Local Ethnically Matched Controls**

<table>
<thead>
<tr>
<th>HLA</th>
<th>Patients (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>24.7</td>
<td>19.2</td>
</tr>
<tr>
<td>A2</td>
<td>34.8</td>
<td>42.3</td>
</tr>
<tr>
<td>A3</td>
<td>19.1</td>
<td>15.9</td>
</tr>
<tr>
<td>A9</td>
<td>21.3</td>
<td>26.3</td>
</tr>
<tr>
<td>A10</td>
<td>6.7</td>
<td>12.9</td>
</tr>
<tr>
<td>A11</td>
<td>18.0</td>
<td>12.5</td>
</tr>
<tr>
<td>A28</td>
<td>5.6</td>
<td>11.7</td>
</tr>
<tr>
<td>A29</td>
<td>6.7</td>
<td>5.1</td>
</tr>
<tr>
<td>A30</td>
<td>5.6</td>
<td>4.5*</td>
</tr>
<tr>
<td>A31</td>
<td>7.8</td>
<td>8.1*</td>
</tr>
<tr>
<td>A32</td>
<td>7.9</td>
<td>6.4</td>
</tr>
<tr>
<td>Aw33</td>
<td>4.4</td>
<td>2.0</td>
</tr>
</tbody>
</table>

| B5  | 14.6         | 19.3         |
| B7  | 14.6         | 12.9         |
| B8  | 9.0          | 7.9          |
| B12 | 29.2         | 24.5         |
| B13 | 5.6          | 4.1          |
| B14 | 9.0          | 11.0         |
| B15 | 5.6          | 6.8          |
| B16 | 1.1          | 9.6          |
| B17 | 13.5         | 10.0         |
| B18 | 9.0          | 6.5          |
| B21 | 5.6          | 6.2          |
| Bw22| 2.3          | 2.6          |
| B27 | 4.5          | 4.3          |
| B35 | 24.7         | 23.8         |
| B37 | 1.1          | 0.7          |
| B40 | 10.1         | 7.9          |
| Bw41| 1.1          | 1.7          |

* p=0.00064.

**Table 2. HLA-DR Phenotypic Frequencies in Patients With Essential Hypertension and in Local Ethnically Matched Controls**

<table>
<thead>
<tr>
<th>HLA</th>
<th>Patients (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>9.4</td>
<td>16.9</td>
</tr>
<tr>
<td>DR2</td>
<td>21.2</td>
<td>22.6</td>
</tr>
<tr>
<td>DR3</td>
<td>12.9</td>
<td>20.7</td>
</tr>
<tr>
<td>DR4</td>
<td>34.1*</td>
<td>16.2</td>
</tr>
<tr>
<td>DR5</td>
<td>30.6</td>
<td>27.8</td>
</tr>
<tr>
<td>DRw6</td>
<td>11.8</td>
<td>13.3†</td>
</tr>
<tr>
<td>DR7</td>
<td>24.7</td>
<td>30.8</td>
</tr>
</tbody>
</table>

* p=0.00064.
† n=142.
It is well known that studies of association between HLA antigens and diseases may produce false-positive associations mainly because patients and controls are subjected to multiple comparisons. To circumvent this problem, two approaches are recommended: one is to correct the probability value by multiplying it by the number of different antigens tested; the other is to perform a second study on a new group of patients and then look for associations with antigens that were found to be significantly increased (or decreased) at the value of \( p \leq 0.05 \) in the first study. If the values in the second study are again \( p \leq 0.05 \), then those antigens are considered to be significantly associated with the disease.20

Considering that a higher frequency of DR4 among the patients was one of our a priori hypotheses (the other being increased frequency of HLA-DR2), there was no need to perform the correction of the probability value (\( p_n \)) for the number of antigens tested.20 It should be pointed out, however, that the increase of HLA-DR4 remained significant (\( p = 0.023 \)) even after this correction was made. Another possible cause of error in association studies is represented by any systematic error in HLA antigen assignment in either the patient or the control population. We do not believe that this type of factor interfered with our results since the definition of HLA-DR4 was clear with the antisera used in the present study. Furthermore, the increased frequency of DR4 in the present patients was also significant when compared with those obtained for samples of Caucasian populations reported at the IV Latin American Histocompatibility Workshop21 (DR4, 15.4%; \( p = 0.00002 \)) and at the IX International Histocompatibility Workshop22 (DR4, 23.8%; \( p = 0.04 \)).

To evaluate the relative risk associated with the presence of HLA-DR4 in our population, i.e., to estimate the probability for the development of hypertension in an individual positive for HLA-DR4 versus an individual without this marker, we calculated the odds ratio. The value obtained was 2.68, with a 95% confidence interval between 1.49 and 4.85.

In the present study, we did not confirm the association with HLA-DR2 that was suggested by data from our previous study; this further emphasizes the need for confirmatory studies before any assumption of HLA with any disease can be considered likely.

Our results concerning association of HLA-DR4 with essential hypertension are at variance with the only other study6 known to us concerning HLA-DR antigens in patients with this disease. In that study, conducted by Sengar et al.,6 61 Caucasian patients from Ottawa, Canada, were analyzed, and an increased HLA-DR7 frequency was observed among the patients as compared with a local control population (34% \( \times \) 21%; \( p < 0.05; p_n \) not significant). To what extent this discrepancy could stem from differences in the ethnic background of the Brazilian and Canadian populations is a matter open to conjecture. In this context, it is perhaps interesting to add that when we compared the frequencies of HLA-DR4 and DR7 obtained for our control population (DR4, 16.2% and DR7, 30.8%) and for the Canadian one (DR4, 34% and DR7, 21%) we found statistically significant differences (\( p = 0.00006 \) for DR4 and \( p = 0.014 \) for DR7). Different associations of HLA antigens with a particular disease in different ethnic groups are relatively frequent and are usually interpreted as a reflection of different types of linkage disequilibrium between the disease susceptibility gene and the HLA marker.25 Since there are no arguments in favor of a possible role of the HLA molecules themselves in the control of blood pressure, we believe that the putative gene for susceptibility to essential hypertension, located in or near the HLA complex, is represented by a not yet identified gene different from the known HLA genes. In the Brazilian Caucasian population, this gene appears to be in linkage disequilibrium with HLA-DR4.

References

**Essential hypertension and histocompatibility antigens. An association study.**
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