HLA Class II (DR and DQ) Antigen Associations in Idiopathic Dilated Cardiomyopathy
Validation Study and Meta-Analysis of Published HLA Association Studies

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We previously reported antigen frequency differences for HLA-DR4 and HLA-DRw6 between idiopathic dilated cardiomyopathy (IDC) patients and healthy controls in a pilot study. To confirm these findings, we undertook an independent study with a prospective hypothesis regarding the frequencies of DR4 and DRw6; typing for a second family of class II antigens (HLA-DQ) was included because of the proximity of the DQ loci to the DR loci and the strong linkage disequilibrium between some of the DR and DQ alleles. Comparing a new consecutive series of IDC patients (n=41) and healthy blood bank controls (n=53), we confirmed an increase of DR4 antigen frequency in patients (49% versus 21%, p<0.005). A trend toward decreased expression of DRw6 among patients was also noted (10% of patients versus 23% of controls). HLA-DQw4 was significantly elevated in patients compared with controls (27% versus 6%, p<0.005; relative risk, 6.1; etiologic fraction, 0.22). We identified the combined DR4-DQw4 haplotype in five of 41 Caucasian IDC patients (12%) and none of 53 controls (p<0.007). A comparison of specific antigen frequencies between the preliminary and validation studies did not reveal significant differences; therefore, the data from the two studies were examined in combination. For the combined studies, DR4 was elevated (51% versus 27% in controls, p<0.001), and DRw6 was decreased (9% versus 24% in controls, p<0.01). The relative risk for DR4 was 2.8, and the etiologic fraction was 0.33. In this study, the DR4-DQw4 haplotype bears an indeterminately high risk for disease; the presence of DR4, DQw4, or both antigens was found in 26 of 41 IDC cases (63%) compared with 14 of 53 controls (26%) (p<0.001).

Meta-analysis of the exploratory and validation studies in combination with all reported studies comparing the frequency of HLA-DR antigens in IDC patients and controls was performed. This overview indicated that for all five studies, there is a significant increase in the frequency of HLA-DR4 in cases of IDC (overall odds ratio, 2.06; 98% confidence interval, 1.61–2.65; p<0.0001). In conclusion, we have verified HLA-DR4 involvement in IDC and suggested an additional association with DQw4. The meta-analysis of reported studies confirms that the DR4 association can be replicated in several different patient populations. Thus, in a portion of IDC cases, predisposing genetic factors linked to immunoregulatory loci appear to be present. (Circulation 1991;83:515–522)

Idiopathic dilated cardiomyopathy (IDC) is a disease of uncertain origin for which immune abnormalities, possibly in conjunction with a viral infection, are suspected but unproven. Abnormalities suggestive of dysfunctional immune responses and/or regulatory mechanisms include decreased natural killer cell activity (an antiviral defense mechanism), functional deficit in suppressor cell activity, and humoral and cellular autoimmune reactivities against myocytes. Autoantibodies with

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tissue specificity for an ADP/ATP transport protein that cross-react with synthetic peptides of coxsackie virus B have also been recently described in patients with IDC\textsuperscript{6} as well as antibodies capable of inhibiting ligand binding to cardiac β-receptors.\textsuperscript{7} Thus, several immunological factors may contribute to disease etiology or progression.

Immune responses are regulated at the cellular and molecular levels by the products of genes located within the major histocompatibility locus (MHC). The class II MHC antigens are restriction elements for the interaction of the CD4+ (T helper) subpopulation of lymphocytes with foreign antigen. Processed antigen must physically associate with class II molecules to be recognized by the CD4+ lymphocytes.\textsuperscript{8–10} Only after this MHC-restricted antigen recognition can CD4+ lymphocytes perform their spectrum of activities (i.e., induction or suppression of antibody synthesis).\textsuperscript{11–13} assistance in the clonal expansion of cytotoxic CD8+ lymphocytes, and mediation of delayed-type hypersensitivity responses through the production of IL-2 and other cytokines).\textsuperscript{14,15} In some cases, direct CD4+ lymphocyte-mediated cytotoxicity against class II antigen–bearing target cells has been observed.\textsuperscript{16}

Furthermore, it has been demonstrated in mice that during the fetal development of the immune system, the expression of a particular class II allele is a controlling factor in the elimination of reactive T cell clones specific for that particular allele. The end result of the elimination of these T cell clones is self-tolerance in the adult animal.\textsuperscript{17}

Because of the importance of these immunoregulatory and tolerogenic roles of the class II antigens, they have been studied for possible relations with diseases that are characterized by an immunoregulatory dysfunction (i.e., autoimmune diseases). A recent review\textsuperscript{18} lists known diseases for which there is strong evidence of linkage to antigens of the HLA class II family. A few of the more notable diseases are rheumatoid arthritis (associated with DR4 and DR1), myasthenia gravis (DR3 and DR7), multiple sclerosis (DR2), systemic lupus erythematosus (DR2 and DR3), and the most extensively studied disease, insulin-dependent diabetes mellitus (DR3 and DR4).

In keeping with the evidence for abnormal regulatory mechanisms in IDC, we previously undertook a preliminary investigation of the frequencies of HLA antigens in patients compared with healthy controls.\textsuperscript{19} In this exploratory investigation, no single haplotype could account for most cases, but uneven antigen distributions were noted. HLA-DR typing revealed two differences: the percentage of patients expressing DR4 was increased compared with controls (54% versus 32%, \textit{p}<0.02), and DRw6 was underrepresented (9% versus 26%, \textit{p}<0.04). Despite the apparent association between these antigens and IDC, problems of interpretation are present because in an exploratory study with a large number of specific antigen comparisons such as this study, the possibility of chance associations (type 1 errors) is high. It has been suggested that this type of error can be avoided by multiplying the nominal probability values obtained by \textit{χ}² analysis by the number of antigen comparisons.\textsuperscript{20}

Another approach is to use the exploratory study to generate hypotheses for a second, prospective study with an a priori hypothesis.\textsuperscript{21} In keeping with this approach and to independently confirm the findings in our initial pilot study, we have undertaken a second, prospective study to reexamine HLA frequencies with specific hypotheses regarding the relative frequencies of DR4 and DRw6. We also included typing for HLA-DQ, a locus of recent interest that codes for distinct class II antigens and is located close to the DR locus. Many of the DR and DQ antigens are in linkage disequilibrium. Thus, it was hoped that typing for these antigens would assist in showing a genetic contribution of the MHC region to disease etiology and to help locate more precisely the specific chromosomal site(s) containing the actual involved gene(s).

Finally, we used a statistical approach, meta-analysis\textsuperscript{22–24} to provide an overview of the frequency of HLA-DR antigens in IDC by combining the results of all known reported investigations that have examined the occurrence of these antigens in patients and healthy controls.

**Methods**

**Patient Selection**

Consecutively identified patients fulfilling previously established diagnostic criteria for IDC\textsuperscript{25} were entered into the study. These criteria included a dilated left ventricular chamber of idiopathic cause with an ejection fraction of less than 45%. Controls consisted of consecutively tested healthy blood bank donors. The genetic heritage of our population base is largely Northern European (Anglo-Scandinavian). The study was approved by the institutional review board, and all subjects were entered into the study after informed consent was obtained.

**Lymphocyte Isolation**

Lymphocytes were obtained by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) density gradient centrifugation. Heparinized blood (approximately 20 ml) was diluted 1:1 with McCoy’s 5A tissue culture medium overlaid on 10–15 ml Ficoll-Hypaque in a conical centrifuge tube. The mixture was centrifuged at 400g for 40 minutes. The mononuclear cell population was removed from the plasma–Ficoll-Hypaque interface and washed three times.

Purified B lymphocytes for DR and DQ typing were obtained by treatment of the mononuclear cells with B Quick B-lymphocyte isolation reagent (One Lambda, Inc., Los Angeles). The B lymphocytes were adjusted to a final concentration of 1−2×10⁶/ml in serum-free McCoy’s.
TABLE 1. 2x2 Contingency Table Used for Statistical Comparison of Patient and Control Groups and for Calculations of Relative Risk and Etiologic Fraction

<table>
<thead>
<tr>
<th>Individuals (n)</th>
<th>Antigen-positive</th>
<th>Antigen-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Controls</td>
<td>c</td>
<td>d</td>
</tr>
</tbody>
</table>

Relative risk (RR) = \((a/b)/(c/d)\).
Etiologic fraction = \((RR-1/RR)/(a/a+b)\).

**Cytotoxicity Assay**

HLA-DR and HLA-DQ typings were done by the microcytotoxicity method of Terasaki et al. Briefly, 1 μl of a lymphocyte suspension was added to each well of a typing tray (One Lambda, Inc.) and incubated at 37°C for 60 minutes. After the primary incubation, 5 μl of rabbit complement was added to each well, and the tray was incubated at room temperature for 2 hours. Dead cells were stained by adding 2 μl eosin Y (5%) and fixing in formalin. The percent viability was visually assessed with an inverted phase-contrast microscope.

**Haplotype Assignments**

Where possible, HLA-DR and HLA-DQ alleles were presumptively assigned to specific haplotypes using known DR and DQ associations.

**Presentation and Statistical Analysis of HLA-DR and HLA-DQ Typing Results**

Results are reported as both the percentage of patients positive for each specificity and the number of times a particular antigenic specificity was identified. The sum of all antigens identified may be slightly less than the number of antigen-bearing haplotypes in the study (twice the number of patients) because of untypeable or “blank” specificities or homozygosity for a particular allele. Comparison between patients and control subjects used 2x2 contingency tables as illustrated in Table 1. The strength of an association between an antigen and disease is estimated by the relative incidence ratio or relative risk. This is the calculated ratio of the cross product of the four entries in the 2x2 table as illustrated in Table 1. The etiologic fraction was calculated by the method of Bengtsson and Thompson and is also illustrated in Table 1.

**Selection of Studies for Meta-Analysis**

All known studies examining the frequencies of HLA-DR antigens in association with IDC in the cardiovascular and related literature were included in the meta-analysis. A Medline search was used to assist in complete identification of studies. All studies used standard criteria for the diagnosis of IDC and compared antigen frequencies with those of healthy controls selected from the same geographic locality. The studies included in the meta-analysis are those of Zerbe et al (University of Pittsburgh), Arbustini et al (Pavia University), Limas and Limas (University of Minnesota), and Komajda et al (CHU Pitie-Salpetriere, Paris).

**Meta-Analysis**

The meta-analysis of HLA-DR4 typing combining the results from the present study and four other centers was performed using the method of combining the logarithms of the odds ratios as described by Fleiss and DerSimonian and Laird. In this procedure, the odds are defined as the ratio of the DR4 positive to the DR4 negative subjects, and the odds ratio is the ratio of the odds obtained for the patient sample to the odds obtained for the control group. For the meta-analysis, the log of the odds ratio, the standard error of the log odds ratio, and a weighting factor (based on the inverse of the standard error) are obtained for each individual study. Then, the weighted averages of the log odds ratio and log standard error are obtained, and these measures are used to construct a test for homogeneity of the odds ratios obtained for the different studies. These measures are also used to construct a global test of association for the weighted average of the odds ratio. The test of association assesses the significance of the departure from the null hypothesis (odds ratio, 1) and a 95% confidence interval for the odds ratio.

**Results**

**Results of Validation Study of DR Antigen Frequencies in Idiopathic Dilated Cardiomyopathy**

The results for the validation study for IDC patients and concurrent controls are shown in Table 2. In the validation study, the association of DR4 with IDC was confirmed. For IDC patients, 49% were positive for DR4 compared with 21% of the controls (p<0.005). The frequency of the DRw6 antigen was...
10% for patients versus 23% for controls, a trend that did not reach significance (p=0.14) for the sample size. In this sample, the frequency of DR5 was also nominally increased in the patient sample compared with controls (37% versus 15%, p<0.02, uncorrected), but after correction of the probability value for multiple comparisons, the difference was not significant (p=0.2).

**HLA-DQ Antigen Frequencies and Idiopathic Dilated Cardiomyopathy**

Results of HLA-DQ antigen typing in IDC patients and controls are presented in Table 3.

An association between IDC and one HLA-DQ specificity, DQw4, was noted. The antigen frequency of DQw4 was 27% in patients versus 6% in controls (p<0.005, uncorrected; p<0.03, corrected). A slight decrease in DQw2 was also found in patients (29% versus 49%), but this was not significant (p<0.06, uncorrected; p<0.3, corrected). It is noteworthy that five of 41 patients (12%) had haplotypes containing the DR4 and DQw4 antigens. This combination was not identified in any of the 54 control subjects (p<0.009).

**Combined Studies**

Because the distribution of DR4 and DRw6 antigen frequencies in patient and controls in the validation and exploratory studies were generally similar, the results from both studies were combined, and the results of the combined series are presented in Table 4. The overall antigen frequency for DR4 was 27% for controls and 51% for patients (p<0.001). (This difference is significant even if a further correction for multiple comparisons is made; p<0.01). The antigen frequency of DRw6 was 24% for controls and 9% for patients (p<0.01, uncorrected; p<0.1, corrected). DR1 and DRw8 were reduced compared with controls (13% versus 24% and 7% versus 11%, respectively) in the combined series, but these differences were not significant.

**Table 3. Frequency of HLA-DQ Antigens in Patients and Controls**

<table>
<thead>
<tr>
<th>HLA-DQ type</th>
<th>Positive tests (n)</th>
<th>Patients positive (n=41) (%)</th>
<th>Controls Positive tests (n)</th>
<th>Patients positive (n=53) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>w1</td>
<td>25</td>
<td>61</td>
<td>37</td>
<td>70</td>
</tr>
<tr>
<td>w2</td>
<td>12</td>
<td>29</td>
<td>26</td>
<td>49</td>
</tr>
<tr>
<td>w3</td>
<td>24</td>
<td>59</td>
<td>29</td>
<td>55</td>
</tr>
<tr>
<td>w7*</td>
<td>18</td>
<td>44</td>
<td>24</td>
<td>45</td>
</tr>
<tr>
<td>w4</td>
<td>11</td>
<td>27†</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

*DQw7 is a split of DQw3.
†p<0.0055 (corrected, p<0.03).

**Table 4. HLA-DR Antigen Frequencies in Combined Exploratory and Validation Studies**

<table>
<thead>
<tr>
<th>HLA-DR type</th>
<th>Positive tests (n)</th>
<th>Patients positive (n=76) (%)</th>
<th>Controls Positive tests (n)</th>
<th>Patients positive (n=135) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>13</td>
<td>33</td>
<td>24</td>
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<td>2</td>
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<td>27</td>
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<td>3</td>
<td>18</td>
<td>24</td>
<td>34</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>51*</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>35</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>w6</td>
<td>7</td>
<td>9†</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>30</td>
<td>28</td>
<td>21</td>
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<tr>
<td>w8</td>
<td>5</td>
<td>7</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>w10</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

*p<0.0005 uncorrected (p<0.005 corrected).
†p<0.01 uncorrected (p<0.1 corrected).

**Relative Risk, Etiologic Fraction, and Preventive Fraction**

The relative risk and etiologic and preventive fractions were calculated for DR4, DRw6 (combined studies), DQw4, and DR4/DQw4 (validation study) as described in “Methods” and given in Table 5. The relative risk (increased probability of developing the disease in the presence of compared with the absence of a particular allele) for DR4 was 2.8, and the etiologic fraction (the percentage of all cases of the disease attributable to the presence of a particular allele) was 0.33. For DQw4, the relative risk was 6.1, and the etiologic fraction was 0.22. The combination of DR4 and DQw4 on a single haplotype (n=5) was associated with an indeterminately (“infinitely”) high risk for disease in that this haplotype was not identified in any control subjects. The etiologic fraction of DR4/DQw4, however, was only 0.12. The relative risk for DRw6 was 0.31, indicating that the presence of this allele was associated with a decreased risk for the disease compared with DRw6-negative individuals. The

**Table 5. Calculated Relative Risks and Etiologic Fractions Associated With DR and DQ Antigens**

<table>
<thead>
<tr>
<th>HLA type</th>
<th>Patients positive (%)</th>
<th>Relative risk</th>
<th>Etiologic fraction</th>
<th>Preventive fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR4*</td>
<td>51</td>
<td>27</td>
<td>2.8</td>
<td>0.33</td>
</tr>
<tr>
<td>DRw6*</td>
<td>9</td>
<td>24</td>
<td>0.3</td>
<td>...†</td>
</tr>
<tr>
<td>DQw4</td>
<td>27</td>
<td>6</td>
<td>6.1</td>
<td>0.22</td>
</tr>
<tr>
<td>DR4/DQw4</td>
<td>12</td>
<td>0</td>
<td>...‡</td>
<td>0.12</td>
</tr>
</tbody>
</table>

IDC, idiopathic dilated cardiomyopathy.
*Combined series.
†Does not apply.
‡DR4/DQw4 shows an indeterminately high association with disease in this study.
TABLE 6. Comparison of All Studies Examining Frequencies of HLA-DR Antigens in Idiopathic Dilated Cardiomyopathy

<table>
<thead>
<tr>
<th>HLA-DR Type</th>
<th>Salt Lake City</th>
<th>Pittsburgh</th>
<th>Minneapolis</th>
<th>Pavia, Italy</th>
<th>Paris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients %</td>
<td>Controls %</td>
<td>Patients %</td>
<td>Controls %</td>
<td>Patients %</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>10</td>
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<tr>
<td>w6</td>
<td>9</td>
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<td>24</td>
<td>33</td>
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<tr>
<td>w7</td>
<td>30</td>
<td>23</td>
<td>21</td>
<td>28</td>
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<tr>
<td>w8</td>
<td>7</td>
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<td>11</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>w10</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>76</td>
<td>135</td>
<td>84</td>
<td>208</td>
<td>102</td>
</tr>
</tbody>
</table>

calculated preventive fraction for DRw6 was 0.16. In all, DR4, DQw4, or both antigens in combination were found in 26 of 41 disease cases (63%) compared with 14 of 53 controls (26%) (p<0.001).

Combined Statistical Analysis (Meta-Analysis) of All Comparable Studies

The findings for our combined studies are compared with findings from similar studies (Table 6). The results of the meta-analyses of five studies, representing 361 IDC patients, are shown in Tables 7 and 8. The odds ratios and the 95% confidence intervals of the odds ratios of the HLA-DR4 frequencies (comparing IDC patients with controls) are presented for our study and the other four studies in Table 7. These data show a range of sample odds ratios of 1.05–3.23; combining all studies (Table 8), the odds of having a DR4 allele is 2.06 (95% confidence intervals, 1.61–2.65; p<0.0001) in patients with IDC. A test for homogeneity indicated that the results from the different studies were similar, with the exception of the Zerbe et al29 study. Thus, the meta-analysis was performed both with and without inclusion of the Zerbe et al data (Table 8). In both cases, the test of association was significant beyond the 0.001 level, indicating that the overview of studies verifying an increase in the frequency of HLA-DR4 associated with IDC compared with healthy local controls can be replicated over a broad geographical range in populations of European extraction. No other associations of IDC with other HLA-DR alleles emerged in the overview.

## Table 7. Individual Odds Ratios and Confidence Intervals for Association of DR4 and Idiopathic Dilated Cardiomyopathy From Five Studies

<table>
<thead>
<tr>
<th>Study location</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt Lake City</td>
<td>2.79</td>
<td>2.34–3.33</td>
</tr>
<tr>
<td>Pittsburgh</td>
<td>1.05</td>
<td>0.68–1.64</td>
</tr>
<tr>
<td>Minneapolis</td>
<td>2.12</td>
<td>1.36–3.31</td>
</tr>
<tr>
<td>Pavia, Italy</td>
<td>3.23</td>
<td>1.61–6.46</td>
</tr>
<tr>
<td>Paris</td>
<td>2.03</td>
<td>1.14–3.61</td>
</tr>
</tbody>
</table>

## Discussion

IDC is undoubtedly a heterogeneous disease in which multiple factors may be contributory, including infectious, toxic, and genetic factors. In this regard, it is not surprising that we were not able to find evidence for genetic predisposition in all cases of IDC examined. However, our findings indicate that in a certain number of cases (approximately one third to one half of cases as suggested by etiologic fractions), genetically determined immunoregulatory factors associated with MHC antigens in our population of European (primarily Anglo-Scandinavian) descent may be involved in pathogenesis. Our validation study confirmed the involvement of DR4 in some cases as predicted by our preliminary study. The association with HLA-DR4 was further replicated by meta-analysis of other studies.

Meta-analysis is the statistical analysis of a collection of results in an effort to objectively combine the results of different studies. There are several advantages to the use of such an approach. An individual study may not be sufficiently large to warrant certain conclusions. This is particularly true for studies such as these in which multiple variables (i.e., antigen specificities) are analyzed simultaneously, thus increasing the probability of chance associations. Thus, the weight and thereby the significance of a finding are increased by the increased sample size obtained by combining studies. In addition, a finding is given particular significance if it is replicated by several independent observations. Finally,
this technique tends to minimize any population bias associated with a single study performed in a limited geographical location. A potential drawback to the use of such an analysis is that not all studies are performed in an identical manner. In our meta-analysis of combined studies, variations in diagnostic criteria for IDC and methodological variations in HLA antigen identification are potential limitations. However, these appear to be small based on the similarity of results reported by the different studies.

The increased frequency of HLA-DR4 in our study population as well as other populations does not preclude the participation in disease etiology of other genes in linkage with DR4. HLA-DR4 is a public specificity that is present on a number of different haplotypes. Individuals possessing the DR4 specificity may also express a variety of serologically detectable polypeptide products from the DQ and DP regions. At least five DR4-containing haplotypes have been identified by Dw (mixed lymphocyte reaction) typing and/or by serological typing of associated DQ alleles. The association of DR4 with disease risk may apply primarily to one or two of these subtypes.

A noteworthy feature of the class II region is the strong linkage disequilibrium present between some of the DR and DQ alleles. Therefore, we sought in this prospective study to type for additional haplotype markers (i.e., DQ) that would be of help in beginning to examine DR4-related subregions for further evidence for and localization of a genetic basis for IDC. The result of this study was the discovery of a significant association with HLA-DQw4, which remained even after correction for multiple comparisons. Moreover, the combined DR4-DQw4 haplotype was found in five of 41 IDC patients (12%) but in none of 53 controls. It is of interest that in large population studies, the DR4 allele is in linkage disequilibrium with DQw4 in Japanese but generally linked with the DQw3 specificity in Caucasian populations. Thus, in normal Caucasian populations, the DR4-DQw4 haplotype is expected to be relatively rare, as we confirmed for our control group.

The etiologic fraction is derived from the relative risk and theoretically represents the fraction of all disease cases that are etiologically associated with a particular HLA antigen. If the disease susceptibility is actually due to the presence of a gene sharing a haplotype with a particular HLA allele, then under certain strict conditions the etiologic fraction may be used to provide an estimate of the degree of linkage disequilibrium between the marker and the locus of the disease susceptibility gene. If we assume the presence of such a disease susceptibility gene located on chromosome 6, as suggested by available evidence, then calculating the etiologic fractions for DR4 and DQw4 gives values of similar magnitude (DR4, 0.32; DQw4, 0.25). Thus, the more specific location for the putative disease-causing gene is not readily suggested. However, several possibilities should be considered. First, the fact that the combined DR4-DQw4 haplotype was identified in approximately 13% of patients but was not found in healthy controls could indicate that the particular “disease locus” in question lies between the DR and DQ loci and that the particular disease-producing allele is in linkage disequilibrium with both DR4 and DQw4. In this case, it would appear in very high frequency on haplotypes carrying both the DR4 and DQw4 specificities, as was observed. It would fail to appear only when a crossover event occurred between two haplotypes, one bearing DR4 and the other DQw4, but each without the gene in question. Another possibility is that the “disease gene” is actually associated with a subtype of DR4 that is defined by its linkage with DQw4 (i.e., the DRw15 subtype). In this case, the disease gene would be linked to DR4, but the DR4 etiologic fraction we calculated would be an average of all DR4 subtypes—both those linked to the disease and those not associated with it. This would result in an artificially low calculated etiologic fraction for DR4, indicating a weaker degree of linkage than actually exists. Finally, the potential contribution of two genes in the pathogenesis of the disease must be considered. In this case, one associated with DR4 and one associated with DQw4 could be postulated. Such a situation has been described for insulin-dependent diabetes mellitus, in which the relative risks associated with the two alleles are each less than 1.0 when they occur separately. However, when they occur together on a single haplotype, the relative risk increases to 12.1.

The contribution of two class II alleles in the development of disease may involve the process of interisotypic pairing of class II peptides from different loci to form heterodimers. Lotteau and co-workers have recently described a human cell line in which DRα/DQβ heterodimers were expressed. In this system, the expression of the DRα/DQβ heterodimers was associated with a high ratio of DRα messenger RNA (mRNA) to DQβ mRNA. The extent of this type of interisotypic pairing or the implications for antigen presentation and disease associations are not known, but intriguing possibilities are presented.

At this time, it is unclear why specific antigen-containing haplotypes are associated with disease development, in this or other diseases with HLA class II associations. However, the class II antigens function in the presentation of foreign antigen to T helper lymphocytes, and it is conceivable that abnormal antigen presentation could lead to an autoin-
mune response. Consistent with this hypothesis, in IDC, investigators have identified in the sera of patients antibodies to human cardiac β-receptors that are capable of inhibiting ligand binding. Furthermore, the occurrence of these antibodies appears to correlate with DR4-containing haplotypes. These observations begin to focus attention on the actual pathogenetic mechanisms and may eventually explain the increased risk associated with certain haplotypes.

In conclusion, these studies provide evidence for an association between certain HLA antigens and IDC. The association between DR4 and disease observed in an exploratory study has been validated in this second, prospective study. This study also suggested the involvement of DQw4-carrying haplotypes in the pathogenesis of the disease. The relation of DRW6 to prevention of IDC is suggestive but deserves further confirmation. The strong statistical correlation of DR4 with IDC observed in an overview of all published studies lends further weight to the validity of this observation. Overall, these findings are consistent with the hypothesis that IDC may arise in part from abnormal immune regulation, perhaps in conjunction with an infectious process. Although the full extent of these antigen associations and the specific pathogenic mechanisms are at present unknown, the evidence thus far is sufficiently compelling to warrant further investigation.

References


**KEY WORDS** • cardiomyopathy • genetics • meta-analysis • HLA
HLA class II (DR and DQ) antigen associations in idiopathic dilated cardiomyopathy. Validation study and meta-analysis of published HLA association studies.
J F Carlquist, R L Menlove, M B Murray, J B O'Connell and J L Anderson

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