In recent years, polymorphism in the DP molecule has been largely overlooked in studies of HLA-associated disease susceptibility. Now, however, DNA-based typing methods have allowed identification of more than 60 alleles at the DPB1 locus, allowing the study of DPB1 association with diseases (1). For type 1 diabetes, reports of DPB1 association vary in their conclusions. Some reports suggest association reflecting direct involvement in disease susceptibility (2-5); however, other studies attribute DPB1 association primarily to linkage disequilibrium of DPB1 alleles with predisposing or protective DRB1-DQB1 haplotypes (6,7). This report is an expansion of our previous work (4) and focuses exclusively on the DPB1 locus. The results reported here indicate that DPB1 alleles can affect susceptibility to type 1 diabetes.

Linkage disequilibrium data must be interpreted with caution, as evidenced by the fact that the DPB1*0202 allele is not represented in Table 2, even though it is found almost exclusively on DR3 haplotypes. The data in Table 2 are based on AFBACs. DPB1*0202-DR3 haplotypes are transmitted to patients in all Human Biological Data Interchange (HBDI) families in which they are present; therefore, they are not present in the AFBACs. Although the predisposing effect of DPB1*0202 might be attributed to linkage disequilibrium with DR3, its 100% rate of transmission suggests that it may be contributing disease predisposition. Our data suggest that DR3 haplotypes carrying DPB1*0202 may be more predisposing than other DR3 haplotypes; however, this trend...
TABLE 1

Patient and control DPB1 allele frequencies

| Allele       | AF BAC frequency [% (n)] | Transmitted frequency [% (n)] | Odds ratio | \( \chi^2 \) | P <  
|--------------|---------------------------|-----------------------------|------------|---------|------
| DPB1*0101   | 4.8 (18)                  | 7.3 (39.5)                  | 1.58       | 2.35    | NS   
| DPB1*0201   | 15.6 (59)                 | 12.4 (66.5)                 | 0.76       | 1.71    | NS   
| DPB1*0202   | 0.0 (0)                   | 2.8 (15)                   | *          | 10.54   | 0.001 
| DPB1*0301   | 9.3 (35)                  | 18.0 (97)                  | 2.16       | 11.85   | 0.001 
| DPB1*0401   | 42.1 (159)                | 38.9 (209.5)               | 0.88       | 0.54    | NS   
| DPB1*0402   | 9.3 (35)                  | 4.7 (25.5)                 | 0.49       | 6.87    | 0.009 
| DPB1*0501   | 3.2 (12)                  | 1.4 (7.5)                  | 0.43       | 3.31    | NS   
| DPB1*0601   | 1.3 (5)                   | 3.3 (18)                   | 2.58       | 3.62    | 0.057 
| DPB1*0901   | 0.8 (3)                   | 0.4 (2)                    | 0.47       | 0.72    | NS   
| DPB1*1001   | 1.9 (7)                   | 1.4 (7.5)                  | 0.75       | 0.29    | NS   
| DPB1*1101   | 2.6 (10)                  | 0.9 (5)                    | 0.35       | 3.99    | 0.046 
| DPB1*1301   | 1.9 (7)                   | 1.8 (9.5)                  | 0.95       | 0.01    | NS   
| DPB1*1401   | 1.9 (7)                   | 1.2 (6.5)                  | 0.65       | 0.62    | NS   
| DPB1*1501   | 1.1 (4)                   | 2.0 (10.5)                 | 1.86       | 1.12    | NS   
| DPB1*1601   | 0.5 (2)                   | 0.6 (3)                    | 1.05       | 0.00    | NS   
| DPB1*1701   | 2.1 (8)                   | 0.5 (2.5)                  | 0.22       | 5.28    | 0.022 
| DPB1*1801   | 0.0 (0)                   | 0.1 (0.5)                  | 0.35       | NS      | NS   
| DPB1*1901   | 0.5 (2)                   | 0.8 (4.5)                  | 1.59       | 0.30    | NS   
| DPB1*2001   | 0.5 (2)                   | 1.0 (5.5)                  | 1.94       | 0.66    | NS   
| DPB1*2301   | 0.5 (2)                   | 0.2 (1)                    | 0.35       | 0.80    | NS   
| DPB1*2601   | 0.0 (0)                   | 0.1 (0.5)                  | 0.35       | NS      | NS   
| DPB1*3401   | 0.3 (1)                   | 0.0 (0)                    | 0.00       | 1.42    | NS   
| DPB1*5901   | 0.0 (0)                   | 0.2 (1)                    | 0.70       | NS      | NS   
| Total (n)   | 378                      | 538                        | 57.42      | 0.0005  |      

In the AFBAC frequency and transmitted frequency columns, numbers in parentheses represent actual counts. Numbers of transmitted alleles were divided by two to account for the non-independence of the sibs in a sib pair. P values shown in the table are uncorrected for multiple comparisons. Because of the large number of alleles (23), the Bonferroni inequality was deemed overly conservative for these data. However, if this correction was applied, P values for DPB1*0202 and DPB1*0301 would remain significant (P < 0.023). *Adding 0.5 to the allele counts in every cell in the table (not shown) creates an odds ratio of 22.58 for the allele DPB1*0202.

did not quite reach statistical significance (P < 0.063, Fisher's exact test). If DR3-DPB1*0202 haplotypes are, as they seem from these data, more predisposing than other DR3 haplotypes, the additional risk could be attributed to either the DPB1 allele itself or to another susceptibility locus in strong linkage disequilibrium with it.

Heterogeneity of transmission of DR3 haplotypes to type 1 diabetes patients has been previously reported (8). Polymorphism at the DPB1 locus may help explain that observation. The hypothesis that the predisposing effect of DR3 haplotypes can be modulated by DPB1 alleles is supported by the result in Table 3, which shows that DR3 haplotypes carrying DPB1*0402 on DR3 haplotypes in patients and control subjects.

TABLE 3

DPB1*0402 on DR3 haplotypes in patients and control subjects

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>DRB1</th>
<th>DQB1</th>
<th>DPB1</th>
<th>Patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
<td></td>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>0301</td>
<td>02</td>
<td>0402</td>
<td></td>
<td>3</td>
<td>6.57</td>
</tr>
</tbody>
</table>

In the counts column, haplotypes sampled fewer than three times are not included.

DPB1*0402 are significantly less predisposing than DR3 haplotypes carrying other DPB1 alleles.

To further test whether the DPB1 locus has an effect on disease susceptibility in addition to that of DR-DQ, stratified contingency table analysis was applied to all of the DR-DQ haplotypes on which a given DPB1 allele was found. This analysis showed that DPB1*0202 is significantly predisposing (P = 0.046). DPB1*0301 shows a strong predisposing trend, which, in this analysis, does not reach statistical significance (P = 0.08) (data not shown).

Predisposing effects of both DPB1*0301 (P < 0.002) and DPB1*0202 (P < 0.03) were also observed in analysis of patient genotype frequencies. Expected genotype frequencies were derived under the null hypothesis that DP is not directly involved in disease predisposition. The model used for these calculations takes linkage disequilibrium between DR-DQ and DP into account (see RESEARCH DESIGN AND METHODS). Table 4 shows the results of application of this test to genotypes carrying at least one copy of a specific allele. This type of analysis is useful because it can reveal dominant effects of low-frequency alleles, which may not be detectable in allele frequency analyses. DPB1*1901 is such an allele in these data, suggesting that further examination of this allele in larger data sets, or in populations in which its frequency is higher, is warranted.

Application of this analysis to individual genotypes resulted in only two specific genotypes (DPB1*0401, 0402, and DPB1*0401, 0401) that varied significantly from expected values (Table 5). These data suggest a weak, but statistically significant, protective effect of DPB1*0401. That the effect is observed only when the other allele in the genotype is either the apparently protective allele DPB1*0402 or a second copy of DPB1*0401 suggests that, if type 1 diabetes
protection can be mediated by DPB1 alleles, this protection may be recessive. Further studies will be required to confirm this result.

Of the more than 60 known DPB1 alleles, 23 are represented in these data. Despite the large data set, the enormous number of DPB1-DQB1-DRB1 haplotypes (229) present creates very small sample sizes for most haplotypes. To increase our statistical power, we have pooled the DPB1 alleles into three broad categories (Table 6). Alleles were classified on the basis of the difference of observed patient values to expected patient values calculated by stratified contingency table analysis. Although assignments for some rare alleles may not be accurate, most alleles should be properly assigned. Moreover, any misclassification of rare alleles should not affect the overall results.

Stratification analysis of the pooled DPB1 categories shows significant deviation of their distribution in patients and control subjects from that expected under the null hypothesis (P < 0.01, data not shown). Table 6 illustrates the results of this stratification when the patients with the highest-risk DR-DQ genotypes (DR3/DR4) are analyzed separately from other patients. When the DR3/DR4 patients are excluded from the analysis, a significant predisposing effect is seen for the "susceptible" category of DPB1 alleles (P = 0.015), whereas a trend toward protection is seen in the "protective" category (P = 0.077). These effects disappear completely when only the DR3/DR4 patients are considered in the comparison with control subjects. Two conclusions may be drawn: First, the effect of the DPB1 locus on disease susceptibility comes primarily from predisposing, rather than protective, alleles. Second, the effects of the DPB1 locus to disease susceptibility are most apparent in patients who do not have the highest-risk DR-DQ genotypes.

Further evidence of the effect of the "susceptible" category of DPB1 alleles comes from analyzing the identity-by-descent (IBD) distributions of alleles in the sibling pairs. Table 7 indicates an excess in sharing of genotypes that contain at least one allele from the susceptible category (S/S, S/N, and S/P) compared with those genotypes that do not contain at least one susceptible allele (P/P, P/N, and N/N). However, the converse is not observed. Genotypes with at least one protective allele (P/P, P/N, and P/S) do not exhibit a decrease in sharing when compared with genotypes with no protective alleles (S/S, S/N, and N/N). Like the genotype analysis in Table 5, this suggests that DPB1-associated predisposition to disease may be dominant and that protection may be recessive.

The effect of the DPB1 locus on susceptibility to type 1 diabetes is certainly less apparent than that of the DR and DQ loci. Conclusions about the involvement of DPB1 in disease susceptibility may vary due to allele or haplotype frequencies in a given population or due to study design. The data presented here, based on a large set of Caucasian multiplex families, argue in favor of a role for DPB1 in susceptibility to type 1 diabetes.

Further analysis of these data, though beyond the scope of this report, supports this view. Specifically, the marker association segregation χ² test (9) was applied to test the hypothesis that the DR and DQ loci alone can account for the IBD distribution in the sibling pairs. The hypothesis was rejected; however, when the two DPB1 alleles that have the strongest apparent effect on disease susceptibility (DPB1*0301 and DPB1*0202) were added to the analysis, the data could fit the hypothesis. This suggests that at least these two DPB1 alleles, in addition to alleles at DR and DQ loci, must be considered to account for the observed IBD distribution (A.M.V., J.A.N., E. Genin, F. Clerget-Darpoux, H.A.E., and G.T., unpublished observations). This result does not, however, preclude the existence of additional susceptibility factors on the chromosome.

Several published reports argue in favor of a role for DPB1 in susceptibility to type 1 diabetes; all reports point to DPB1*0301 as a susceptibility allele. These include an analysis of a population of Mexican-American families (2), an analysis of age of onset (5), and a restriction fragment length polymorphism–based case versus control subject study (3).

Even those studies that conclude that HLA-DP has little or no effect on type 1 diabetes susceptibility show increased fre-

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### TABLE 4
Genotype analysis: patient frequencies of genotypes carrying a given DPB1 allele

<table>
<thead>
<tr>
<th>DPB1 genotype</th>
<th>Expected frequency (%)</th>
<th>Observed frequency (%)</th>
<th>Z</th>
<th>P value</th>
<th>Observed/expected ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0202,X*</td>
<td>2.3</td>
<td>5.8</td>
<td>-2.03</td>
<td>0.03</td>
<td>2.54</td>
</tr>
<tr>
<td>0301,X</td>
<td>21.3</td>
<td>32.9</td>
<td>-2.96</td>
<td>0.002</td>
<td>1.55</td>
</tr>
<tr>
<td>0402,X</td>
<td>13.3</td>
<td>9.5</td>
<td>1.34</td>
<td>NS</td>
<td>0.72</td>
</tr>
<tr>
<td>0401,X</td>
<td>70.3</td>
<td>62.1</td>
<td>1.96</td>
<td>0.05</td>
<td>0.88</td>
</tr>
<tr>
<td>1901,X</td>
<td>0.1</td>
<td>1.8</td>
<td>-2.01</td>
<td>0.04</td>
<td>24.87</td>
</tr>
<tr>
<td>1701,X</td>
<td>1.2</td>
<td>1.0</td>
<td>0.27</td>
<td>NS</td>
<td>0.80</td>
</tr>
<tr>
<td>0601,X</td>
<td>4.7</td>
<td>7.0</td>
<td>-1.10</td>
<td>NS</td>
<td>1.48</td>
</tr>
<tr>
<td>1101,X</td>
<td>1.3</td>
<td>1.9</td>
<td>-0.59</td>
<td>NS</td>
<td>1.51</td>
</tr>
</tbody>
</table>

*Χ = any DPB1 allele. In the expected frequency column, because some DPB1 alleles were present in patients but not present among AFBACs, frequencies of three-locus DRB1-DQB1-DRB1 haplotypes that were not present in AFBACs but that were present among nontransmitted haplotypes were added. This correction was made only if the two-locus DR-DQ haplotype was present in AFBACs. The corrected frequencies are nearly identical to the original AFBAC ones.

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### TABLE 5
Genotype analysis: individual DPB1 genotypes showing significant deviation from expected frequency

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Expected frequency (%)</th>
<th>Observed frequency (%)</th>
<th>Z</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPB1*0401, 0402</td>
<td>6.8</td>
<td>2.7</td>
<td>2.16</td>
<td>0.03</td>
</tr>
<tr>
<td>DPB1*0401, 0401</td>
<td>22.1</td>
<td>15.2</td>
<td>2.02</td>
<td>0.034</td>
</tr>
</tbody>
</table>
frequency of DPB1*0301 in patients relative to control subjects (6,7). In a study of Norwegian patients and control subjects limited to only DR3/4 and DR4/4 genotypes, Lie et al. (7) observed no independent association between DP alleles and disease susceptibility. However, the frequency of DPB1*0301 was increased, at least slightly, overall and in every subset of genotypes examined (with the exception of DRB1*0404 homozygotes, which were quite rare). The HBDI data suggest that the effect of DPB1 is most pronounced in individuals with non-DR3/DR4 genotypes. Consequently, the study design of Lie et al. may have excluded those patients in whom the contribution of the DP locus to disease susceptibility should be most evident.

Parental origin of transmitted haplotypes was examined to look for significant differences in maternal versus paternal transmission of individual DPB1 alleles. Only a few marginally significant differences were seen (data not shown). Of note, the increased maternal transmission of the DR3-DPB1*0101 haplotype, reported in the analysis of a subset of these families (4), was not observed in these data.

In summary, HLA region-based susceptibility to type 1 diabetes results from the alleles at multiple genetic loci within the region, including, but not necessarily limited to, the genes encoding HLA DR and DQ molecules. The data presented here argue for a role of the DPB1 locus in disease risk.

### Table 6
Stratification analysis applied to pooled DPB1 allele categories

<table>
<thead>
<tr>
<th>DPB1 category* (Patient group)</th>
<th>Control subjects</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
</tr>
<tr>
<td>P (DR3/DR4)</td>
<td>65</td>
<td>61.37</td>
</tr>
<tr>
<td>P (not DR3/DR4)</td>
<td>65</td>
<td>56.89</td>
</tr>
<tr>
<td>N (DR3/DR4)</td>
<td>257</td>
<td>246.66</td>
</tr>
<tr>
<td>N (not DR3/DR4)</td>
<td>257</td>
<td>251.7</td>
</tr>
<tr>
<td>S (DR3/DR4)</td>
<td>36</td>
<td>40.94</td>
</tr>
<tr>
<td>S (not DR3/DR4)</td>
<td>36</td>
<td>48.49</td>
</tr>
<tr>
<td>(threshold = 1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Protective (P) alleles include 0402, 0501, 0901, 1001, 1701, 2301, and 3401; susceptible (S) alleles include 0202, 0301, 1801, 1901, 2001, and 5901. All other alleles are classified as neutral (N).

### Table 7
IBD distribution of genotypes of pooled DPB1 alleles

<table>
<thead>
<tr>
<th>IBD = 2</th>
<th>IBD = 1</th>
<th>IBD = 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>64</td>
<td>33</td>
</tr>
<tr>
<td>Protective</td>
<td>54</td>
<td>39</td>
</tr>
<tr>
<td>All genotypes</td>
<td>56</td>
<td>38</td>
</tr>
</tbody>
</table>

Data are % or P. Susceptible genotypes are defined as those that contain at least one copy of an allele from the susceptible category, i.e., genotypes S/S, S/N, and S/P. For determination of significance, frequencies of these genotypes are compared with frequencies of genotypes that contain zero copies of a susceptible allele, i.e., genotypes N/N, N/S, and S/S. Protective genotypes are defined as those that contain at least one copy of an allele from the protective group, i.e., genotypes P/P, P/N, and P/S. For determination of significance, frequencies of these genotypes are compared with frequencies of genotypes that contain zero copies of a protective allele, i.e., genotypes N/N, N/S, and S/S.

### RESEARCH DESIGN AND METHODS

DNA samples from 269 Caucasian multiplex families were obtained from the collection of the HBDI (Philadelphia). Molecular HLA typing data were generated by means of previously described polymerase chain reaction/sequence-specific oligonucleotide probe methods (10,11). Control haplotypes were determined with the AFBAC method (12). AFBAC haplotypes are defined as those haplotypes never transmitted to the affected sib pair. Sample size for the IBD distribution analysis was reduced to 257 families due to exclusion of recombinant families, families in which DPB1 alleles could not be unambiguously assigned to haplotypes, and families in which the haplotypes in a parent were indistinguishable.

Tests for differences in predisposition effects of HLA alleles, haplotypes, and genotypes were described previously (4). Expected DPB1 genotype frequencies were derived under the null hypothesis that the only predisposing genes in the region are DR and DQ (locus A) and that DPB1 (locus B) is neutral with respect to type 1 diabetes. The following formula was used:

\[
E \left( f(B_i|B_j) \right)_{\text{patients}} = \frac{1}{T} \sum_{i,j,k,l} E \left( f(A_i,B_j|A_k,B_l) \right)_{\text{patients}}
\]

\[
E \left( f(A_i,B_j|A_k,B_l) \right) = \delta_{i,j} X_k X_l w_{ij}
\]

\[
\delta_{i,j} = 1 \text{ if } i = j \text{ and } k = l
\]

\[
\delta_{i,j} = 2 \text{ otherwise}
\]

\[
w_{ij} = \frac{f(A_i|A_j)_{\text{patients}}}{f(A_i|A_j)_{\text{controls}}}
\]

\[
X_k = f(A_k|B_k)_{\text{controls}}
\]

\[
T = \sum_{i,j,k,l} E \left( f(A_i,B_j|A_k,B_l) \right)_{\text{patients}}
\]

The expected frequencies of genotypes carrying a specific allele (B_i) were obtained by adding overall genotypes carrying that allele:

\[
E \left( f(B_i|B_j) \right)_{\text{patients}} = \sum f(B_i|B_j)_{\text{patients}}
\]

Comparison of observed with expected genotype frequencies was carried out with use of a two-tailed Z test of proportions (13). For haplotype analysis, some observed values were zero, and some expected frequencies were quite small, making the use of asymptotic results questionable, even for large sample sizes (14,15). To address small cell counts, DPB1 alleles were classified into three pooled categories on the basis of their expected to observed frequency ratios derived by stratification analysis.

### ACKNOWLEDGMENTS

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