Brief Genetics Report

The HLA Class II Locus DPB1 Can Influence Susceptibility to Type 1 Diabetes

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HLA-DPB1 genotypes were determined for samples from 269 multiplex Caucasian families from the Human Biological Data Interchange. DRB1 and DQB1 loci were also characterized, allowing assignment of DPB1 alleles to haplotypes and calculation of linkage disequilibrium values. Frequencies for several DPB1 alleles differed significantly between patients and affected family–based control subjects. Some differences were attributable to linkage disequilibrium with DR and DQ alleles, whereas others were not. DPB1*0301 and DPB1*0202 alleles are predisposing for type 1 diabetes in these data, not only in analyses of individual alleles, but also in genotype analyses. DPB1*0402 appears protective; however, stratification analysis indicates that its protective effect is specific for DR3 haplotypes. A protective role for DPB1*0401 is suggested by genotype analysis. For increased statistical power, DPB1 alleles were pooled into three categories: susceptible, neutral, and protective after removal of effects due to linkage disequilibrium with DR-DQ. Analysis of these pools suggests that DPB1 primarily affects susceptibility to, rather than protection from, type 1 diabetes in a dominant fashion. This effect is more apparent in patients with genotypes other than the highest risk DR3/DR4-DQB1*0302 genotype. These data support a role for the DPB1 locus in conferring susceptibility to type 1 diabetes. *Diabetes* 49:121–125, 2000

Until recently, 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.

Table 1 shows a significant difference ($P < 0.00005$) in the distribution of DPB1 allele frequencies in patients compared with affected family–based control subjects (AFBACs), with the largest contribution to the overall χ^2 coming from increased patient frequencies of DPB1*0301 and DPB1*0202. DPB1*0402, DPB1*1701, and DPB1*1101 are significantly decreased in patients.

Data from the DRB1 and DQB1 loci in the AFBACs (not shown) were used to determine DPB1-DQB1-DRB1 haplotypes with significant linkage disequilibrium (Table 2). Some differences in DPB1 frequencies between patients and control subjects probably result from these haplotypes. For example, the apparent protective effects of DPB1*1101 and DPB1*1701 may be explained by their significant linkage disequilibrium with DRB1*0701-DQB1*02, a protective haplotype.

Conversely, although DPB1*0301 shows significant linkage disequilibrium with the mildly predisposing and rare DRB1*0801-DQB1*0402 haplotype (sampled three times), this common allele shows little evidence overall for significant linkage disequilibrium with predisposing DR-DQ haplotypes. Whereas the formal possibility exists that undetected linkage disequilibrium between DPB1*0301 and a susceptibility locus could cause the apparent predisposing effect of that allele, the fact that DPB1*0301 is observed on many different DR-DQ haplotypes in both transmitted and AFBAC data argues against this. The data strongly suggest that linkage disequilibrium with DR-DQ haplotypes does not completely account for the dramatic increase of DPB1*0301 in patients.

Linkage disequilibrium data must be interpreted with care, however, 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 simply 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

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AFBAC, affected family–based control subject; HBDI, Human Biological Data Interchange; IBD, identity by descent.

TABLE 1
Patient and control DPB1 allele frequencies

Allele	AFBAC frequency [% (*n*)]	Transmitted frequency [% (*n*)]	Odds ratio	χ^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
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
DPB1*3401	0.3 (1)	0.0 (0)	0.00	1.42	NS
DPB1*5901	0.0 (0)	0.2 (1)		0.70	NS
Total (*n*)	378	538		57.42	0.00005

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 nonindependence 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 were 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 are significantly less predisposing than DR3 haplotypes carrying other DPB1 alleles.

TABLE 2
DRB1-DQB1-DPB1 haplotypes showing significant linkage disequilibrium

DRB1	DQB1	DPB1	Counts	D′	*P* <
0404	0302	0601	3	0.586	2.8×10^{-12}
0301	02	0101	9	0.447	2.0×10^{-9}
1501	0602	0401	42	0.546	1.5×10^{-7}
0701	02	1101	6	0.551	4.1×10^{-7}
1104	0301	0402	6	0.449	7.5×10^{-7}
0701	02	1701	5	0.579	2.0×10^{-6}
0701	02	1501	3	0.720	3.4×10^{-5}
0401	0302	0401	11	0.734	0.0016
0701	02	0401	8	–0.536	0.002
0801	0402	0301	3	0.370	0.002
1301	0603	0201	7	0.252	0.009

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

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

TABLE 3
DPB1*0402 on DR3 haplotypes in patients and control subjects

Haplotype			Patients		Control subjects			
DRB1	DQB1	DPB1	Observed	Expected	Observed	Expected	χ^2	*P* value
0301	02	0402	3	6.57	5	1.43	10.86	0.001

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

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

*X = 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-DPB1 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.

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 χ^2 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-

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

Genotype	Expected frequency (%)	Observed frequency (%)	*Z*	*P* value
DPB1*0401, 0402	6.8	2.7	2.16	0.03
DPB1*0401, 0401	22.1	15.2	2.02	0.034

TABLE 6
Stratification analysis applied to pooled DPB1 allele categories

DPB1 category* (Patient group)	Control subjects		Patients		χ^2	P value
	Observed	Expected	Observed	Expected		
P (DR3/DR4)	65	61.37	17	20.63	0.853	0.356
P (not DR3/DR4)	65	56.89	25.5	33.62	3.117	0.077
N (DR3/DR4)	257	246.66	136.5	146.84	1.162	0.281
N (not DR3/DR4)	257	251.7	204	209.32	0.247	0.619
S (DR3/DR4)	36	40.94	45.5	40.56	1.198	0.274
S (not DR3/DR4) (threshhold = 1%)	36	48.49	69	56.52	5.973	0.015

*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).

quency 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 7
IBD distribution of genotypes of pooled DPB1 alleles

	IBD = 2	IBD = 1	IBD = 0	
Susceptible	64	33	3	$P < 0.03$
Protective	54	39	7	NS
All genotypes	56	38	6	

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/P, and P/P. 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:

$$\mathrm{E}\left[f\left(B_k|B_l\right)\right]_{patients} = \frac{1}{T}\sum_{i,\,j}\mathrm{E}\left[f\left(A_i B_k|A_j B_l\right)\right]_{patients}$$

$$\mathrm{E}\left[f\left(A_i B_k|A_j B_l\right)\right] = \delta_{ijkl}x_{i\,k}x_{j\,l}w_{i\,j}$$

$\delta_{ijkl} = 1$ if $i = j$ and $k = l$

$\delta_{ijkl} = 2$ otherwise

$$w_{i\,j} = \frac{f\left(A_i|A_j\right)_{patients}}{f\left(A_i|A_j\right)_{controls}}$$

$$x_{i\,k} = f\left(A_i B_k\right)_{controls}$$

$$T = \sum_{k,\,l}\sum_{i,\,j}\mathrm{E}\left[f\left(A_i B_k|A_j B_l\right)\right]_{patients}$$

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

$$\mathrm{E}\left[f\left(B_i|B_{\cdot}\right)\right]_{patients} = \sum_{j}\mathrm{E}\left[f\left(B_i|B_j\right)\right]_{patients}$$

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.

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