Association of HLA-DPB1*0301 With IDDM in Mexican-Americans

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Susceptibility to IDDM has been associated with specific alleles at the HLA class II loci in a variety of human populations. Previous studies among Mexican-Americans, a group ancestrally derived from Native Americans and Hispanic whites, showed that the DR4 haplotypes (DRB1*0405-DQB1*0302 and DRB1*0402-DQB1*0302) and the DR3 haplotype (DRB1*0301-DQB1*0201) were increased among patients and suggested a role for both DR and DQ alleles in susceptibility and resistance. Based on the analysis of 42 Mexican-American IDDM families and ethnically matched control subjects by polymerase chain reaction/sequence-specific oligonucleotide probe typing, we report an association of IDDM with the DPB1 allele, *0301 (relative risk = 6.6; P = 0.0012) in this population. The analysis of linkage disequilibrium patterns in this population indicates that the observed increased frequency in DPB1*0301 among patients cannot be attributed simply to linkage disequilibrium with high-risk DR-DQ haplotypes. These data suggest that in addition to alleles at the DRB1 and DQB1 loci, polymorphism at the DPB1 locus may also influence IDDM risk. *Diabetes* 610–614, 1996

IDDM is a chronic autoimmune disease involving destruction of the pancreatic islet β-cells. Specific HLA class II alleles and haplotypes, defined either serologically (1–3) or by DNA typing (4–10), have been associated with IDDM in different ethnic groups. The HLA class II region (DR, DQ, and DP) consists of α-chain loci (e.g., DQA1) and β-chain loci (e.g., DQB1); these glycopeptide chains form cell-surface heterodimers that bind peptide fragments and present them to the antigen-specific T-cell receptor on $CD4^+$ T-cells. The peptide binding groove formed by the class II α/β heterodimer consists of a β-sheet floor and two α-helical walls and is encoded by the second exon of these class II loci. The second exon of the β-chain loci (DRB1, DQB1, and DPB1) and the DQA1 locus is highly polymorphic; and specific DNA-defined alleles and haplotypes are associated with IDDM. In particular, DRB1*0301, DQB1*0201, and a subset of DR4, DQB1*0302 (e.g., DRB1*0402, *0405, *0401) haplotypes are more frequent among patients and thus are thought to confer susceptibility (8,9). In addition, the haplotypes DRB1*1501–DQB1*0602, DRB1*1402–DQB1*0301, and DRB1*1101–DQB1*0301 appear to confer protection from IDDM, as they are significantly less frequent among patients (9,11). Other non-HLA genes within the major histocompatibility complex (MHC) region have also been proposed to influence IDDM predisposition (12,13). In addition, genes mapping outside the MHC region may also play a role in IDDM susceptibility (14–17). A recent genome-wide search for human IDDM susceptibility genes identified several other candidate regions, but concluded that the major determinant of genetic susceptibility mapped to the MHC (15).

The involvement of DRB1 and DQB1 polymorphism in a variety of autoimmune diseases is well known (6); however, the role of DPB1 alleles in disease susceptibility is less well documented. Since the development of a simple polymerase chain reaction (PCR)/sequence-specific oligonucleotide (SSO) probe typing system (18,19), a number of recent studies have reported an association of specific DPB1 alleles with several different diseases (20–22). In principle, these observed DPB1 disease associations could merely reflect linkage disequilibrium with disease-associated DRB1 and/or DQB1 alleles. Alternatively, however, specific DPB1 alleles could contribute independently to disease susceptibility. The analysis of families allows the determination of DR, DQ, and DP haplotypes and, consequently, the opportunity to evaluate the role of linkage disequilibrium in DP disease associations. In addition, different ethnic populations have different haplotypic combinations of HLA class II alleles; thus, HLA/disease studies among populations can often clarify which alleles (or combinations of alleles) on disease-associated haplotypes are likely to be responsible for susceptibility or protection.

We report the analysis of HLA-DPB1 alleles in Mexican-American families ascertained via at least one IDDM patient as well as in unrelated Mexican-American control subjects. Because the ancestors of this population are both Native Americans and Hispanic whites, population studies of these two groups can be used to identify specific HLA class II haplotypes among Mexican-Americans as either Amerindian or European in origin (9). These Mexican-American families and control subjects have been typed recently by restriction fragment length polymorphism (RFLP) (10) and PCR/SSO probe methods for DRB1 and DQB1 (9). The white haplotypes, DRB1*0301–DQB1*0201, DRB1*0402–DQB1*0302, and DRB1*0405–DQB1*0302, conferred IDDM susceptibility, while the most protective haplotype was the Native American,

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AFBAC, affected family-based control; MHC, major histocompatibility complex; OR, odds ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RR, relative risk; SSO, sequence-specific oligonucleotide.

DRB1*1402–DQB1*0301 (9). Here we report that, based on the results of DPB1 typing in this sample of Mexican-American IDDM patients, DPB1*0301 is also associated with disease susceptibility.

RESEARCH DESIGN AND METHODS

PCR HLA DPB1 typing. Frozen cells (~1 × 10^6) from Epstein-Barr virus–transformed lymphoblastoid cell lines were resuspended in distilled water at a concentration of 200 cells · $\mu l^{-1} \cdot 0.5$ μl^{-1} or an equivalent of 1,000 cells were lysed in Chelex. Then 30 μl supernatant consisting of 150–200 lysed cells were PCR-amplified (PECI-Thermal cycler) and HLA-DPB1–typed with horseradish peroxidase–labeled oligonucleotide probes according to previously reported procedures (16,17,23).

Statistical analysis. The *G*-test for heterogeneity (24) was used to compare haplotype and genotype frequencies in patients and control subjects. The odds ratio (OR) was calculated from the numbers of patients and control subjects with and without the HLA marker. The calculation of linkage disequilibrium and the definition of the normalized linkage disequilibrium parameter *D′* were described in Trachtenberg et al. (25,26).

Haplotype assignment. HLA-DR-DQ-DP haplotypes were determined by segregation analysis in the families and inferred in the unrelated individuals based on known patterns of linkage disequilibrium and by computer analysis using the procedure of Baur and Danilovs (27). Haplotypes observed within IDDM families that were not transmitted to IDDM patients were included as affected family-based control (AFBAC) haplotypes. Analysis using the *G*-test for heterogeneity revealed no significant differences between the family-matched control subjects and unrelated control data sets.

RESULTS

Pedigree analysis and linkage disequilibrium. PCR-based DPB1 typing was carried out on 41 IDDM families, including 167 nuclear family members and 57 second- and third-degree relatives, and on 60 unrelated Mexican-American control individuals. IDDM was defined as determined by the National Diabetes Data Group (28). In one of these families, pedigree analysis revealed a crossover between the DQB1 and DPB1 loci. The recombination frequency (0.6%) between these two loci, calculated from the one recombinant haplotype and the total number of informative meioses (n = 165) in these 41 families, is consistent with the estimate of 1% based on our previous analysis of the Centre d'Etude de Polymorphism Humain families (29).

For the analysis of linkage disequilibrium in this population of Mexican-Americans, only the unrelated control subjects and, in the families, only the haplotypes not transmitted to affected family members (see METHODS) were considered. The DRB1-DQB1-DPB1 haplotypes derived from analyzing segregation in the family pedigrees and from computer analysis of the unrelated control subjects that displayed significant linkage disequilibrium are shown in Table 1. Although several DRB1-DQB1-DPB1 haplotypes exhibit significant linkage disequilibrium, the IDDM-associated DPB1 allele, *0301 (see below), is not in linkage disequilibrium with any particular DRB1-DQB1 haplotype. The two most frequent haplotypes exhibiting strong linkage disequilibrium are of Amerindian origin, and the calculated *D′* values probably reflect a population admixture.

Association of DPB1*0301 with IDDM. The distribution of DPB1 alleles among IDDM patients is significantly different from the DPB1 allele distribution of control haplotypes based on the *G*-test for heterogeneity ($P < 0.001$) (Table 2). The allele frequencies in the three sources of control haplotypes, randomly sampled unrelated control subjects, AFBAC subjects (see METHODS), and control subjects from other non-nuclear family members, were not significantly different based on the *G*-test for heterogeneity, and so the samples were combined. The difference in the allelic frequency distribution between IDDM and combined control groups (Table 2) is due to an excess of DPB1*0301 in the IDDM patients (G = 16.7). (DPB1*0301 is also the source of significant heterogeneity in the comparison of patients and the AFBAC subjects.) The DPB1*0301 allele frequency among patients was 21.4% (18 of 84) compared with 4.4% (10 of 225) in the

TABLE 1
DR-DQ-DP haplotypes with significant linkage disequilibrium among Mexican-Americans

DRB1	DQB1	DPB1	n	D'	P value	Haplotype source
0101	0501	0401	7	0.29	0.0238	
0103	0501	0201	3	1.00	0.0008	
0301	0201	0202	4	0.61	0.0003	
0403	0302	0201	3	0.43	0.0131	
0407	0302	0402	9	0.58	0.0161	Native American
0408	0301	1501	2	1.00	0.0004	
0410	0302	0402	5	1.00	0.0102	
0802	0400	0402	21	0.42	0.0031	Native American

P values determined with Fisher's exact test. The DRB1-DQB1 haplotypes in significant positive disequilibrium with DPB1 are shown for the control population sample of 225 haplotypes. DR-DQ was treated as a single locus. *D′* is the normalized linkage disequilibrium parameter described by Trachtenberg et al. (25). Five different haplotypes sampled only once and showing nominally significant disequilibrium ($P < 0.05$) are not included in the table. Population admixture generates disequilibrium between two loci proportional to the difference in frequencies of the alleles at each of the loci in the founding populations. Two haplotypes from the table may derive a portion of their observed disequilibrium from the high frequency in Native American founding populations of DRB1*0407-DQB1*0302, DRB1*0802-DQB1*0400, and DPB1*0402 relative to that of the European Hispanic founders. The haplotypes 0407-0302-0402 and 0802-0400-0402 have significant positive *D′* values of 0.58 and 0.42, respectively.

TABLE 2
Distribution of DPB1 alleles among IDDM patients and combined controls

	DPB1	IDDM	Control	G	OR
1	0101	4.0	7.0	0.4	
2	0201	11.0	21.0	0.8	
3	0202	4.0	6.0	0.8	
4	0301	18.0	10.0	16.7	5.9
5	0401	16.0	46.0	0.1	
6	0402	19.0	90.0	5.7	0.4
7	0501	4.0	8.0	0.2	
8	0601	1.0	2.0	0.1	
9	0901	1.0	3.0	0.0	
10	1001	0.0	6.0	3.8	0.2
11	1101	2.0	3.0	0.4	
12	1301	1.0	3.0	0.0	
13	1401	1.0	4.0	0.1	
14	1501	2.0	3.0	0.4	
	1601*	0.0	2.0		
15	1701	0.0	8.0	5.1	0.2
	1901*	0.0	1.0		
	2301*	0.0	2.0		
16	Combined (3*)	0.0	5.0	3.2	0.3
Sum		84.0	225.0	37.8	

The *G*-test for heterogeneity indicates that the allele frequency distributions for the patient (84 alleles) and control (225 alleles) groups were significantly different, with the DPB1*0301 allele contributing most to the *G*-value. *Alleles sampled less than three times were combined into a single group for testing. df = 15. $P < 0.001$.

TABLE 3
HLA-DPB1*0301 and IDDM in Mexican-Americans

	RR (OR)	
	Patients	Unrelated control subjects
DPB1*0301	14	4
Not *0301	28	53
n	42	57

RR = 6.6; 95% CI = 3.2–13.0; $P = 0.0012$, Fisher's exact test. The P value corrected for the number of alleles compared with Table 2 is 0.018.

combined control haplotypes (OR = 5.9). The phenotype or marker frequency (i.e., presence of the allele) of DPB1*0301 among patients was 33% compared with 7% among unrelated control subjects; the relative risk (RR) (or OR) for individuals carrying DPB1*0301 was 6.6 ($P = 0.0012$, using Fisher's exact test) (Table 3).

The observed increase in DPB1*0301 among patients could, in principle, simply reflect linkage disequilibrium with a susceptible DR-DQ haplotype in the general population or, alternatively, this allele could confer increased risk independently. Although virtually all (12 of 14) of the DPB1*0301 patients also carried DRB1*04 (Table 4), this association was not statistically significant because most of the non-DPB1*0301 patients were also DRB1*04. Additionally, family analysis revealed that many (8 of 18 haplotypes in IDDM patients) of the DRB1 alleles coupled to and segregating with DPB1*0301 were not DRB1*04 (Table 5).

The presence of these eight non-DR4 haplotypes suggests that the observed disease association with DPB1*0301 cannot be attributed simply to linkage with DRB1*04 in these families. One-third (6 of 18) of these DPB1*0301 haplotypes, however, are DRB1*0405, the highest risk DR4 haplotype in this population (9), compared with a random expectation of 9.5% based on DRB1 allele frequencies in patients (9). However, DPB1*0301 is still increased even among DRB1*0405-negative patients; when genotypes in patients and control subjects (the randomly selected nonfamily control subjects) are compared for DPB1*0301 proportions in the absence of individuals bearing DRB1*0405, DPB1*0301 is more frequent in the patient population (RR = 8.8, $P = 0.005$) (Table 6). This observation suggests that a DPB1*0301 diabetogenic effect exists even in the absence of DRB1*0405.

The role of disequilibrium between DR4 and DPB1*0301–and more specifically between DRB1*0405 and DPB1*0301–can be addressed by examining disequilibrium in the total control sample. The list of all haplotypes having significant positive disequilibrium values among the Mexican-American control haplotypes (Table 1) includes several DR4 alleles, but DRB1*0405 is absent. In the total control sample, DRB1*0405 is present at a frequency of only 0.027, and DPB1*0301 has a frequency of 0.044. Of the three individuals

TABLE 4
Association of HLA-DPB1*0301 and DR4 among IDDM patients

	$DR4^+$ patients	Not $DR4^+$ patients
DPB1*0301	12	2
Not *0301	20	8

NS, Fisher's exact test.

TABLE 5
Linkage of DRB1 alleles with DPB1*0301 in IDDM patients

Patient no.	DPB1	Linked DRB1
1	0301	0405 (0101)
2	0301	0102 (0402)
3	0301/0301	0405, 0802
4	0301	0405 (0401)
5	0301	1302 (1602)
6	0301/0301	0410, 1302
7	0301	0405 (1602)
8	0301/0301	0701, 0405
9	0301/0301	0301/0301
10	0301	0802 (0401)
11	0301	0102 (0407)
12	0301	0404 (1001)
13	0301	0411 (0301)
14	0301	0405 (0301)

The DRB1 allele present on the non-DPB1*0301 haplotype is in parentheses.

in the sample of unrelated control subjects with DPB1*0301, two carry DRB1*0405; in one, the DRB1*0405 haplotype has DQB1*0301 and in the other, DQB1*0302. The haplotype estimation procedure gives a 0.5 probability for the haplotype DRB1*0405-DPB1*0301 for each of the individuals. The family-derived control subjects included six individuals with DPB1*0301, none of whom carried DRB1*0405. We conclude that, although the evidence is limited, it is possible that there is haplotypic association between these two relatively rare alleles in this population.

Mode of inheritance of class II susceptibility to IDDM. The analysis of the DR-DQ types previously obtained for these patients, their families, and the unrelated control subjects by the antigen/genotype frequency method (30) rejects both the recessive and the dominant modes of inheritance primarily because of the excess of DR3/DR4 heterozygotes (9). As previously discussed for this data set (9), susceptible DR4 haplotypes appear to confer a dominant risk for IDDM, whereas susceptible DR3 haplotypes confer increased, risk only in conjunction with another DR3 haplotype or a DR4 haplotype. For the susceptible DPB1*0301 allele, a single copy seems to be sufficient to increase IDDM risk. Of 14 DPB1*0301 patients, 3 were homozygous; given that the random expectation is 2.4, there is no evidence of a significant excess of DPB1*0301 homozygotes among patients.

DISCUSSION

Confirmation that the major genetic determinant for IDDM is in the MHC has come from a recent genome-wide search for genes involved in IDDM susceptibility using microsatellite markers (15). A number of different MHC loci may play a role in IDDM susceptibility (6), as demonstrated by our previous

TABLE 6
Association of HLA-DPB1*0301 and IDDM excluding DRB1*0405 patients and control subjects

	Patients	Control subjects
DPB1*0301	8	2
Not *0301	25	52
n	33	54

RR = 8.3; 95% CI = 2.2–30.0; $P = 0.006$, Fisher's exact test.

studies of these 41 Mexican-American IDDM families, which indicated that both DRB1 and DQB1 alleles can contribute to susceptibility and resistance to IDDM (9). Here we report that in addition to these DR and DQ effects, polymorphism at DPB1 may also play a role in susceptibility. In particular, there is an increase (OR = 6.6, P = 0.0012) of DPB1*0301 among IDDM patients (Table 3). In a previous report using RFLP typing, an increased frequency of an RFLP pattern denoted DP3/6 was observed in Australian IDDM patients (31). However, some studies using PCR/SSO typing have failed to observe an increase in DPB1*0301 among patients in Taiwan (32).

Associations with a specific DPB1 allele could reflect linkage disequilibrium with a susceptible DR-DQ haplotype, or the associated allele could confer increased risk independently. Although these are often considered mutually exclusive explanations, our data suggest that both mechanisms may be involved. In this population, DPB1*0301 may be in linkage disequilibrium with the high-risk haplotype DRB1*0405,DQB1*0302, although both DPB1*0301 and DRB1*0405 are sufficiently rare in the general population that the evidence for linkage disequilibrium is limited and not statistically significant. This DR-DQ-DP haplotype is not among those showing significant linkage disequilibrium in the general population (Table 1). Moreover, DPB1*0301 is still increased among IDDM patients (RR = 8.8, P = 0.005) even when DRB1*0405-positive individuals are removed from the analysis (Table 6). Thus, it appears that the association of IDDM with DPB1*0301 cannot be attributed simply to linkage disequilibrium and that this DPB1 allele may be an additional risk factor for IDDM. Another potential explanation for disease association could be population admixture, given that our previous work in this population indicated that two specific European, rather than Native American, DR-DQ haplotypes were increased among IDDM patients. The decrease of DPB1*0402, a common Native American DPB1 allele found in linkage disequilibrium with DRB1*0407 and DRB1*0802 (Table 1), among IDDM patients (Table 2) could be accounted for in part by this explanation. However, DPB1*0301 is found in both European and Native American populations. Moreover, other DPB1 alleles found more frequently among Europeans (e.g., DPB1*0101) are not significantly increased among IDDM patients (25,26,29,33). Thus, the increase in DPB1*0301 in Mexican-American IDDM patients does not appear to be attributable to population stratification and may reflect the independent role of DPB1. A definitive demonstration of the independent effect of DPB1*0301 in IDDM will require additional studies. In our recent study of 117 white multiplex IDDM families, transmission analysis indicated that DPB1*0301 is also associated with IDDM in the population (J. Noble, A.M. Valdes, W.K., G. Thomson, and H.A.E., unpublished observations); DPB1*0301 does not appear to be in strong linkage disequilibrium with DR3 or DR4 alleles in whites (29).

Although DPB1*0301 has a RR of about 6 in this population, it appears to increase the IDDM risk primarily in individuals with high-risk DR, DQ genotypes. As noted above, 12 of 14 DPB1*0301 IDDM patients were also $DR4^+$, but the P-value for this observation was not significant. A larger number of patients will have to be studied to determine whether the DPB1*0301 effect is, as suggested, confined to $DR4^+$ patients. Since the DPB1*0301-associated risk, however, cannot be attributed completely to linkage disequilibrium with high-risk DR-DQ haplotypes, these observations suggest that the DPB1*0301 allele may interact in some way with the DR-DQ haplotypes (e.g., DR4) associated with disease to increase the overall risk for IDDM.

We have previously proposed that both DRB1 and DQB1 alleles can contribute to IDDM susceptibility as well as resistance (9); now it appears that DPB1*0301 can also increase the risk for IDDM. A variety of immunological mechanisms could account for the apparent contribution of several different class II molecules to IDDM risk. Conceivably, an autoantigen peptide can be bound and presented to pathogenic T-cells by the DPB1*0301 molecule as well as, for example, by DRB1*0405 and DQB1*0302 molecules. The likelihood of such a binding/presentation event is, by this hypothesis, increased in the presence of three molecules with this capacity. It is also possible that different autoantigen peptides could be presented by different class II molecules encoded by IDDM-associated alleles. Conceivably, a peptide derived from one disease-associated class II molecule could be presented by another class II molecule, influencing the T-cell receptor repertoire. In general, some of the disease-associated alleles could affect IDDM susceptibility by affecting the expressed T-cell receptor repertoire, whereas others could mediate their effect by antigen binding and presentation.

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