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The Role of HLA Class II Genes in Insulin-Dependent Diabetes Mellitus: Molecular Analysis of 180 Caucasian, Multiplex Families

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Summary

We report here our analysis of HLA class II alleles in 180 Caucasian nuclear families with at least two children with insulin-dependent diabetes mellitus (IDDM). DRB1, DQA1, DQB1, and DPB1 genotypes were determined with PCR/sequence-specific oligonucleotide probe typing methods. The data allowed unambiguous determination of four-locus haplotypes in all but three of the families. Consistent with other studies, our data indicate an increase in DR3/DR4, DR3/DR3, and DR4/DR4 genotypes in patients compared to controls. In addition, we found an increase in DR1/DR4, DR1/DR3, and DR4/DR8 genotypes. While the frequency of DQB1*0302 on DR4 haplotypes is dramatically increased in DR3/DR4 patients, DR4 haplotypes in DR1/DR4 patients exhibit frequencies of DQB1*0302 and DQB1*0301 more closely resembling those in control populations. The protective effect of DR2 is evident in this data set and is limited to the common DRB1*1501-DQB1*0602 haplotype. Most $DR2^+$ patients carry the less common DR2 haplotype DRB1*1601-DQB1*0502, which is not decreased in patients relative to controls. DPB1 also appears to play a role in disease susceptibility. DPB1*0301 is increased in patients ($P < .001$) and may contribute to the disease risk of a number of different DR-DQ haplotypes. DPB1*0101, found almost exclusively on DR3 haplotypes in patients, is slightly increased, and maternal transmissions of DRB1*0301-DPB1*0101 haplotypes to affected children occur twice as frequently as do paternal transmissions. Transmissions of DR3 haplotypes carrying other DPB1 alleles occur at approximately equal maternal and paternal frequencies. The complex, multigenic nature of HLA class II–associated IDDM susceptibility is evident from these data.

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Introduction

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disorder characterized by destruction of insulin-producing beta cells in the pancreas. Progression to disease involves both genetic and environmental factors. The environmental component of IDDM susceptibility is not well understood, although viral infection has been suggested as a triggering event (Fohlman and Friman 1993; Trucco and LaPorte 1995). The genetic component of the disease is evident by familial clustering, ethnic variation in disease prevalence, and MZ versus DZ twin studies (see, e.g., Thomson 1988).

In humans, as well as in animal models, IDDM is a multigenic disease. The HLA component of IDDM genetic risk was discovered in the 1970s, with both association and affected-sib-pair data (Singal and Blajchman 1973; Cudworth and Woodrow 1974; Nerup et al. 1974). More recently, several candidate non-HLA loci have been investigated, with inconsistent results (Field 1991). One of these loci, the 5′ flanking polymorphism of the insulin gene, now referred to as *IDDM2,* was initially detected with case-control association data and confirmed with nuclear family–based association data (Bell et al. 1984; Spielman et al. 1989; Thomson et al. 1989). Several groups have mapped additional IDDM genes by using affected-sib-pair linkage analysis. In addition to the HLA region (IDDM1-6p) and the insulin gene region (IDDM2-11p), linkages reported in at least two independent data sets include IDDM3 (15q), IDDM4 (11q), IDDM5 (6q), IDDM7 (2q), and IDDM8 (6q) (Davies et al. 1994; Field et al. 1994; Hashimoto et al. 1994; Copeman et al. 1995; Luo et al. 1995; Owerbach and Gabbay 1995).

Linkage studies have demonstrated that the HLA region, termed *IDDM1,* is the major genetic determinant of IDDM susceptibility (see, e.g., Davies et al. 1994). From affected-sib-pair HLA haplotype sharing data, Risch (1987) estimated that the HLA component of IDDM susceptibility (λ_s for HLA) accounts for a 3.42-fold increased risk in siblings over the population prevalence, compared to an observed 15-fold increased risk in siblings due to all genetic factors (λ_s). Under a multi-

plicative model, Risch calculated that HLA contributes ~44% to the genetic risk for IDDM.

Initial association studies of serologically determined HLA alleles with IDDM showed increased frequencies of B8-DR3 and B15(w62)-DR4 in Caucasians (Svejgaard et al. 1980; Tiwari and Terasaki 1985). Both DR3 and DR4 are very strongly associated with IDDM: over 93% of IDDM patients have at least one of these alleles, compared to 43% of controls (Thomson et al. 1988), with a significant increase in risk for DR3/DR4 heterozygotes (Rotter et al. 1983; Thomson 1983; Louis and Thomson 1986). Predisposing effects have also been demonstrated for both DR1 and DR8, while DR2 is known to be highly protective (see, e.g., Thomson et al. 1988).

Previous DNA-based HLA typing studies suggested that the DQ molecule may be responsible for HLA associations with IDDM, for example, the DR4 haplotype DRB1*0401-DQA1*03-DQB1*0302 is strongly predisposing to disease, while DRB1*0401-DQA1*03-DQB1*0301 is not (Todd et al. 1987; Horn et al. 1988*a;* Nepom and Erlich 1991). Recent studies, however, indicate that DRB1 (Tait and Harrison 1991; Caillat-Zucman et al. 1992; Cucca et al. 1993; Erlich et al. 1993*b;* Luo et al. 1995) and DPB1 (Easteal et al. 1990; Erlich 1991; Tait and Harrison 1991; Erlich et al. 1996) may influence the disease process as well.

The Human Biological Data Interchange (HBDI) is a repository for cell lines derived from IDDM families. The HBDI collection was established in part for the purpose of mapping IDDM-associated, non-HLA genes by linkage analysis. Most of the HBDI families are nuclear families with unaffected parents and at least two affected siblings. Some of these HBDI samples were used in mapping of the recently reported IDDM genes.

In this study, we report our results from HLA class II typing of 180 multiplex IDDM families. PCR/SSOP typing was performed at the DRB1, DQA1, DQB1, and DPB1 loci. In addition to determining genotypes at each locus, the family-based samples allowed unambiguous determination of four-locus haplotypes in most cases, as well as distinction of maternal from paternal transmissions of haplotypes. The results of our analysis of these data not only confirm HLA associations with IDDM susceptibility that have been reported from other studies but also reveal previously unreported HLA effects.

Subjects, Material, and Methods

Subjects

Most of the family-based samples used in this study (166 families) were provided as purified genomic DNA from the HBDI. DNA samples derived from the remaining 14 families were provided by Dr. Donald Bowden (Bowman Gray School of Medicine, Wake Forest University).

Families analyzed in this study were multiplex (more than one affected child). Parents in all families were unaffected, with the exception of one family that had an affected father in whom the disease was not clearly categorized as type I or type II diabetes. The presence of a diseased parent in this family does not bias the results of our analyses, as described by Thomson (1995*a*). All the families were Caucasian, with the exception of one, which reported Native American ancestry. The HLA haplotypes in this family, however, were similar to those of Caucasian families and did not contain alleles known to be specific to Native American populations.

Age at diagnosis was known for 326 of the 360 affected individuals analyzed. The distribution of age at onset for these 326 patients was 71% diagnosed at $\leq$14 years of age, 20% diagnosed at 15–25 years of age, and 9% diagnosed at $\geq$26 years of age. HLA typing was performed on samples derived from both of the parents, affected children, and unaffected children in all families.

HLA Typing

HLA class II typing for DRB1, DQB1, and DPB1 was performed on all samples by using PCR/SSOP methods that have been described elsewhere (Bugawan et al. 1990, 1991; Bugawan and Erlich 1991; Erlich et al. 1991*a*, 1993*a;* Scharf et al. 1991). In brief, genomic DNA was amplified by PCR with primers specific for the second exon of the relevant locus. The resultant PCR product was then denatured and immobilized onto nylon membrane. Membranes were subsequently hybridized with horseradish peroxidase (HRP)-labeled or, in some cases, biotin-labeled, sequence-specific oligonucleotide probes. Following stringent washing, membranes hybridized to biotin-labeled probes were incubated with streptavidin-horseradish peroxidase. Bound probe was detected by means of a colorimetric detection system using the substrate tetramethylbenzidine. The pattern of probes hybridizing to a given sample indicated the identity of the alleles represented in the PCR product. DQA1 HLA typing was performed by a method similar to that described above. However, for DQA1, biotinylated PCR primers were used to generate biotinylated PCR products that were subsequently hybridized to a set of unlabeled oligonucleotide probes immobilized on a membrane. This method is referred to as "reverse dot-blot" (Saiki et al. 1989). In most families, DQA1 typing was performed only on samples from parents, and DQA1 genotypes of children were inferred. Haplotype assignments for DQA1 alleles were made using known patterns of linkage disequilibrium; any ambiguities in haplotype assignments were resolved by typing DNA from at least one child in the family.

Currently, the total number of HLA class II molecules encoded by reported allele sequences includes 123 at the DRB1 locus, 12 at the DQA1 locus, 22 at the DQB1 locus, and 60 at the DPB1 locus (Bodmer et al. 1995). Our PCR/SSOP typing system allows us to distinguish nearly all of these allele sequences. A few allele distinctions that cannot be made with our typing systems are noted here. The DRB1 allele 1603 (Rosenlicht et al. 1993) is indistinguishable from DRB1*1601. Such DRB1*1601/3 ambiguities are reported here as "DRB1*1601," although the relative frequencies of the two alleles are unknown. DPB1*0501 is not distinguished from DPB1*3801 in this analysis. Because DPB1*3801 is very rare, we have assigned all DPB1*0501/3801 ambiguities as "DPB1*0501." The alleles DQB1*0201 and DQB1*0202 differ at a residue which lies outside exon 2; therefore, the region is not present in the PCR product used for HLA typing analysis. These alleles are referred to as "DQB1*02." Similarly, with our current typing protocol, DQA1*0301 is indistinguishable from DQA1*0302, and samples with this type are termed "DQA1*03." Our DQA1 typing system does not distinguish DQA1*0104; these alleles are categorized as "DQA1*0101." In addition, DQA1*0502 and DQA1*0503 are not distinguished from DQA1*0501 in this system and are listed as "DQA1*0501."

Data Analysis

Data from family-based samples allowed unambiguous assignment of alleles to three-locus haplotypes (DRB1-DQA1-DQB1) in all families and to four-locus haplotypes (DRB1-DQA1-DQB1-DPB1) in 177 of the 180 families. Allele and haplotype frequencies were determined by the method of gene counting. Identity-by-descent (ibd) values of parental haplotypes in the affected sib pairs could be determined unambiguously in all except the 11 families in which one parent had indistinguishable haplotypes (i.e., was homozygous at all four loci tested). Siblings with identical haplotypes inherited from a homozygous parent were considered ibd.

Most families in the study (165) had only two children with IDDM. Of the 15 families with more than two affected children, 14 had three. In seven of these families, all three affected children were HLA-identical. In the other seven families with three affected children, as well as in the single family with five affected children, two of the affected children were chosen at random for all our analyses. The AFBAC (affected family–based controls) population of HLA haplotypes never transmitted to the affected sib pair was determined (Thomson 1995*b*). This control population provides an unbiased estimate of the overall population (control) HLA allele and haplotype frequencies, under the reasonable assumption of zero recombination between the marker (HLA) and disease, and random mating assumptions, i.e., no population stratification, admixture, or migration effects (Thomson 1995*b*).

Parental transmitted versus never-transmitted alleles and haplotypes were tested for differences using a χ^2 contingency table test for heterogeneity (Thomson 1995*b*). In cases where previous knowledge of association with IDDM exists, frequencies of transmitted alleles and haplotypes, reflecting the average of the two affected offspring, were compared to the AFBAC control frequencies. The Bonferoni correction for multiple comparisons was deemed overly conservative for these data and was not applied in any of our tests.

Tests for differences in predispositional and protective effects of HLA alleles, haplotypes, and genotypes were performed using the odds ratio method for haplotypes and genotypes (Woolf 1955) and the relative predispositional effects (RPE) method (Payami et al. 1989). For the RPE method, the χ^2 contingency table test for heterogeneity is performed at each round of analysis, rather than recalculation of expected values as in the original method. This prevents bias toward positive associations (G.T., unpublished results). Stratification analysis was used to detect the additional influence of HLA DR-DQ alleles or haplotypes in the presence or absence of particular HLA DPB1 alleles and vice versa (Klitz et al. 1994). The affected-sib-pair data were tested for evidence of linkage by deviation from random expectations of 1/4, 1/2, and 1/4 for the sharing of 2, 1, and 0 parental haplotypes ibd, respectively, by using the mean ibd value test (Blackwelder and Elston 1985, 1986).

Results

DRB1-DQA1-DQB1 Haplotype Associations with IDDM

Table 1 lists three-locus (DRB1-DQA1-DQB1) patient and AFBAC haplotypes from PCR/SSOP typing of 180 multiplex IDDM families. Control frequencies calculated from the CEPH family samples (Begovich et al. 1992) are also listed for comparison. The sample sizes for the AFBAC and the CEPH family haplotypes are nearly identical, and, for most haplotypes, AFBAC frequencies and CEPH family frequencies are quite similar. Notable differences include the haplotype DRB1*0404-DQA1*03-DQB1*0302, which is present at lower frequency in the AFBAC population than in CEPH ($P < .005$), and the haplotype DRB1*1104-DQA1*0501-DQB1*0301, which is more frequent in the AFBAC population than in CEPH ($P < .025$).

As expected, the data show a strong predisposing effect for DRB1*0301-DQA1*0501-DQB1*0201 and DRB1*0401-DQA1*03-DQB1*0302 haplotypes. In this data set, a strong predisposing effect for DRB1*0404-DQA1*03-DQB1*0302 haplotypes was

Table 1

Analysis of DR-DQ Haplotypes in Caucasian, Multiplex IDDM Families

Haplotype			Observed Frequency					
DRB1	DQA1	DQB1	Patient	AFBAC	CEPH[a]	OR	χ^2	*P*-value[b]
0101	0101	0501	.076	.061	.073[c]	1.27	.522	...
0101	0101	0503	.001	0	...	...	.386	...
0101	0102	0504	.003	.004	...	.77	.034	...
0102	0101	0501	.010	0	...[c]	...	2.703	...
0103	0101	0501	.010	.014	.008	.67	.291	...
0301	0501	0201	.319	.094	.088	4.55	36.227	.001
0401	0301	0301	.017	.043	.053	.38	3.904	.050
0401	0301	0302	.253	0.04	.042	8.21	44.603	.001
0401	0301	0305	0	.004	...	0	1.295	...
0402	0301	0302	.022	.018	.004	1.24	.138	...
0403	0301	0302	0	.004	.015	0	1.295	...
0404	0301	0302	.107	.018	.076	6.54	18.207	.001
0404	0301	0303	0	.004	...	0	1.295	...
0405	0301	0201	.007	0	...	...	1.931	...
0405	0301	0302	.018	.004	.004	5.09	2.790	.100
0405	0301	0401	0	.004	...	0	1.295	...
0407	0301	0301	.001	.025	.008	.05	7.553	.010
0408	0301	0301	0	.007	...	0	2.590	...
0408	0301	0304	.006	0	...	...	1.544	...
0411	0301	0302	0	.004	...	0	1.295	...
0413	0301	0302	.003	0	...	...	.772	...
0701	0201	0201	.018	.108	.08	.15	22.140	.001
0701	0201	0303	0	.036	.053	0	12.950	.001
0801	0401	0402	.028	.014	.031	1.96	1.282	...
0807	0401	0402	.001	0	...	...	.386	...
0901	0301	0201	.003	0	...	...	.772	...
0901	0301	0303	.006	.014	...	.38	1.301	...
1001	0101	0501	.004	0	.001	...	1.158	...
1101	0102	0602	0	.007	...	0	2.590	...
1101	0501	0301	.011	0.05	.073	.21	8.565	.005
1101	0501	0302	.003	0	...	...	.772	...
1103	0501	0301	0	.014	.015	0	5.180	.050
1104	0501	0301	.001	0.04	.008	.03	12.686	.001
1104	0103	0603	0	.004	...	0	1.295	...
1201	0501	0301	.004	.014	.011	.29	1.901	...
1301	0103	0501	0	.004	...	0	1.295	...
1301	0103	0603	.008	.065	.038	.12	15.168	.001
1302	0102	0604	.029	.043	.034	.67	.872	...
1302	0102	0606	0	.011	...	0	3.885	.05
1303	0501	0301	.003	.014	.019	.19	2.698	...
1401	0101	0503	0	0.04	.042	0	14.245	.001
1401	0101	0602	0	.004	...	0	1.295	...
1404	0101	0503	.001	0	.004		.386	...
1501	0102	0502	.001	.004	.004	.39	.325	...
1501	0102	0602	.003	.155	.156	.02	52.481	.001
1501	0102	0603	0	.004	...	0	1.295	...
1501	0401	0402	.003	0	...	...	.772	...
1502	0103	0601	0	.004	.008	0	1.295	...
1601	0102	0502	.019	.007	.011	2.74	1.669	...
	n		360	278	262			

[a] CEPH frequencies from table 3 of Begovich et al. (1992).
[b] χ^2 and *P*-values were calculated on the basis of patient and AFBAC frequencies.
[c] Frequency of 0101/02-0101-0501.

Table 2

Analysis of DR2 Haplotypes in IDDM Families

A. DR2 Allele Frequencies

Patients	AFBAC	CEPH
.03	.17	.18

B. Distribution of DR2$^+$ Haplotypes in IDDM Patients

		Proportion of DR2$^+$ Haplotypes (%)		
DRB1	DQB1	Patient (n = 19)	AFBAC (n = 48)	CEPH (n = 47)
1601	0502	74	4	6
1501	0602	11	90	87
1501	0502	5	2	2
1501	0603	0	2	0
1501	0402	11	0	0
1502	0601	0	2	4

also observed, although other studies have shown this haplotype to be mildly predisposing, neutral, or even somewhat protective (Sheehy et al. 1989; Erlich et al. 1993*b*; Cucca et al. 1995; Harfouch et al. 1995; Reijonen et al. 1995). The fact that our data set contains fewer control DRB1*0404-DQA1*03-DQB1*0302 haplotypes than observed in CEPH families might reflect sampling variation and partially explain this observation. Other DR4-DQB1*0302 high-risk haplotypes, including DRB1*0405-DQB1*0302 and DRB1*0402-DQB1*0302 (Caillat-Zucman et al. 1992; Erlich et al. 1993*b*; Cucca et al. 1995), were too rare in this study for statistical evaluation. By using the RPE method, after the strongly predisposing DR3 and DR4 haplotypes given above are removed from the analysis, the predisposing effects of haplotypes DR8 ($P < .001$) and DR1 ($P < .001$) become evident.

Several haplotypes appear to have a protective effect. The well-known protective effect of DR2 appears to be limited to the common Caucasian haplotype DRB1*1501-DQA1*0102-DQB1*0602, which is seen in only 2 of the 19 DR2$^+$ patients analyzed here (table 2). Three patients have DRB1*1501 on a haplotype with a DQB1 allele other than DQB1*0602. Most (74%) DR2$^+$ patients, however, have the haplotype DRB1*1601-DQA1*0102-DQB1*0502, which is rare among DR2$^+$ controls and may be slightly predisposing, although the numbers are too small for significance testing. Other protective haplotypes in these data include DR7, in combination with either DQB*0201 or DQB1*0303, and DR11 with DQA1*0501-DQB1*0301. DR11 haplotypes containing other DQB1 alleles are too rare to evaluate (table 1).

Genotype Effects

Table 3 lists genotypes present in affected children, with patient numbers and frequencies based on the average of the two affected children per family. Those genotypes observed in <3 patients (average <1.5) are not included. Most patient genotypes listed in table 3 are increased in the patient population over the estimated numbers based on Hardy-Weinberg equilibrium calculations using AFBAC frequencies. Some DR3/DR4 and DR1/DR4 genotypes (DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0401-DQA1*03-DQB1*0302, DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0404-DQA1*03-DQB1*0302, and DRB1*0101-DQA1*0101-DQB1*0501/DRB1*0401-DQA1*03-DQB1*0301) appear to exhibit synergy with respect to disease susceptibility, i.e., those genotypes are seen significantly more often than expected based on recessive expectations calculated from patient haplotype frequencies (Louis and Thomson 1986).

When genotypes are stratified by parent of origin, the only genotype with a significant increase ($P < .005$) in observed patient frequency over expected patient frequency (based on patient haplotype frequencies) is for the genotype DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0401-DQA1*03-DQB1*0302, where the DR3 haplotype in inherited from the mother and the DR4 haplotype is inherited from the father (data not shown). DR4/DR4 and DR3/DR3 genotypes are present in patients in excess over the expected population genotype frequencies; however, these two genotypes appear less frequently than expected for patients under a recessive model because of the large excess of DR3/DR4 heterozygotes. DR1/DR4 genotypes differ from DR3/DR4 genotypes with respect to DQB1 alleles and DRB1 subtypes (see Haplotype Sharing in Affected Sibs).

Haplotype Sharing in Affected Sibs

HLA haplotype sharing in 180 IDDM-affected sib pairs is illustrated in table 4*A*, which shows the percentages of sib pairs sharing two, one, and zero haplotypes and the deviation from random expectations of 25%, 50%, and 25%, respectively ($P < .00001$). Under the assumption that HLA effects (as opposed to non-HLA or environmental effects) would be expected to be more pronounced in HLA-identical, affected siblings than in unrelated patients, we examined the distribution of genotypes of the HLA-identical sib pairs in this data set (table 4*B*). In the 180 families we typed, we found 109 pairs of siblings ibd at the DRB1, DQA1, and DQB1 loci. As expected, DR3/DR4 genotypes were found at highest frequency (45%), followed by DR4/DR4. DR3/DR3, DR1/DR4, DR1/DR3, and DR4/DR8 genotypes were also seen at frequencies higher than those expected in controls.

The weakest HLA effect on IDDM susceptibility might be expected in the affected sib pairs with no HLA

Table 3

Patient Genotypes

Genotype		Number					
DRB1-DQA1-DQB1	DRB1-DQA1-DQB1	Observed	Expected[a]	χ^2	*P*	OR[b]	*P*[c]
0301-0501-0201	0401-0301-0302	44.0	29.13	7.591	.01	41.88	<.001
0301-0501-0201	0404-0301-0302	21.5	12.36	6.759	.01	37.43	.001
0301-0501-0201	0301-0501-0201	10.0	18.33	3.786	.1	6.69	.025
0101-0101-0501	0401-0301-0302	9.0	6.95	.605	...	10.59	.01
0101-0101-0501	0301-0501-0201	8.0	8.79	.071	...	4.04	.1
0401-0301-0302	0801-0401-0402	5.5	2.53	3.487	.1	28.34	.05
0401-0301-0302	0401-0301-0302	5.5	11.48	3.115	.1	25.76	.05
0401-0301-0302	0404-0301-0302	4.5	9.68	2.772	.1	32.94	.05
0101-0101-0501	0401-0301-0301	3.5	.46	20.090	.001	3.82	...
0301-0501-0201	1601-0102-0502	3.5	2.24	.709	...	14.85	.1
0301-0501-0201	0402-0301-0302	3.5	2.57	.337	...	5.93	...
0301-0501-0201	1302-0102-0604	3.5	3.26	.018	...	2.34	...
0101-0101-0501	1302-0102-0604	2.5	.81	3.526	.1	2.57	...
0401-0301-0302	1601-0102-0502	2.5	1.77	.301	...	25.34	...
0301-0501-0201	0901-0301-0303	2.0	.61	3.167	...	4.03	...
0401-0301-0302	1301-0103-0603	2.0	.74	2.145	...	1.81	...
0401-0301-0302	1101-0501-0301	2.0	1.01	.970	...	2.49	...
0404-0301-0302	0404-0301-0302	2.0	2.03	0	...	...	...
0301-0501-0201	0405-0301-0302	2.0	2.06	.002	...	15.55	...
0101-0101-0501	0701-0201-0201	1.5	.5	2	...	.62	...
0404-0301-0302	0801-0401-0402	1.5	1.07	.173	...	16.8	
0401-0301-0302	0701-0201-0201	1.5	1.65	.014	...	1.07	

[a] Expected numbers of patient genotypes were calculated on the basis of patient haplotype frequencies and recessive expectations under Hardy-Weinberg equilibrium. Comparison of expected patient genotypes to observed patient genotypes was used to generate the χ^2 and *P*-values listed.

[b] OR for patient genotypes compares patient genotype frequencies to expected control genotype frequencies (on the basis of AFBAC haplotype frequencies, using Hardy-Weinberg expectations).

[c] The significance level associated to the χ^2 derived from comparing the expected number of control genotypes, on the assumption of a sample size of 180, and the observed number of patient genotypes.

class II haplotypes in common. However, examination of the 11 "share-0" affected sib pairs in this data set revealed that 9 (41%) of the 22 individuals had a DR3/DR4 genotype. This illustrates the strength of the HLA effect, especially the DR3/DR4 synergistic effect, on genetic susceptibility to IDDM even in the zero haplotype–sharing class. Statistical comparison of the "share-2" group with the combined "share-1" and "share-0" groups revealed no heterogeneity with respect to genotype distribution.

DR4-Associated Susceptibility

The DRB1*0302 allele on DR4 haplotypes is a well-known marker for IDDM susceptibility (Nepom and Erlich 1991); however, the risk conferred by this allele appears to be influenced not only by the DRB1*04 allele on the haplotype (Cucca et al. 1995) but also by the DR genotype. The importance of genotypic context in assigning risk to an allele is shown in table 5. Consistent with other reports (Horn et al. 1988*a*; Tait et al. 1988; Owerbach et al. 1989; Erlich et al. 1990), our data suggest that the predisposing effect of DQB1*0302 is less strong in DR1/DR4 heterozygotes than in DR3/DR4 heterozygotes. Table 5*A* shows a comparison of DQB1 alleles on DR4 haplotypes in DR3/DR4 and DR1/DR4 genotypes. In DR3/DR4 genotypes, DR4 haplotypes carrying the DQB1*0302 allele (Ala 57) were significantly overrepresented (97%), while DQB1*0301 (Asp 57) was rarely seen (1.4%). In addition, the 15 patients with DR4/DR8 genotypes in this study all carried DQB1*0302 on their DR4 haplotype, and most DR4/DR4 patients were homozygous for DQB1*0302 (data not shown), consistent with a predisposing effect for DQB1*0302 (Ala 57). However, this strong bias for DQB1*0302 is not apparent in DR1/DR4 heterozygous patients, where DRB1*0302 and DRB1*0301 on the DR4 haplotypes are seen in proportions resembling those of Caucasian control populations. This suggests that the contribution of the DQ molecule to overall disease susceptibility is genotype dependent.

Table 5*B* compares DRB1*04 alleles in DR3/DR4 versus DR1/DR4 genotypes. When the DR4 haplo-

Table 4

Affected Sib Pairs

A. Haplotype Sharing

	SHARE (%)		
	2	1	0
Expected	25.0	50.0	25.0
Observed	60.6	33.3	6.1

B. Distribution of Genotype Frequencies in Sib Pairs

	SHARE				
Genotype	2	1	0	AFBAC	CEPH
DR3/DR4	.450	.333	.409	.033	.037
DR4/DR4	.110	.100	.000	.030	.044
DR1/DR4	.083	.158	.091	.027	.034
DR3/DR3	.073	.033	.000	.009	.008
DR1/DR3	.046	.058	.045	.015	.014
DR8/DR4	.046	.042	.000	.005	.015
Other	.193	.275	.455	.262	.245
n	109	60	11		

types are categorized according to DRB1 subtype, we see that in DR3/DR4 genotypes, as in controls, DRB1*0401 is the most frequent subtype (63%), followed by DRB1*0404 (29%). In DR1/DR4 genotypes, DRB1*0401 frequency is slightly higher (74%) than in DR3/DR4 genotypes, while the proportion of DRB1*0404 is reduced markedly (5%). Comparison of DRB1*0401 to DRB1*0404 in these two genotypes reveals significant heterogeneity ($P < .005$). The highly predisposing DRB1*0405, which is rare in Caucasians, is infrequent in DR3/DR4 patients but is significantly increased in the DR1/DR4 patients ($P < .025$). This suggests that the subtype of the DR4 molecule may exert a bigger influence on disease susceptibility in a DR1/DR4 heterozygote than it does in a DR3/DR4 heterozygote.

DPB1 Analysis

All samples were typed for DPB1, and DPB1 alleles were assigned to haplotypes in 177 of the 180 families tested. Five samples in which a recombination event occurred between the DQB1 and DPB1 loci were identified. The entire data set contained a total of 1,051 informative meiotic events, indicating a DQ-DP recombination rate of .0048, similar to that (.008 ± .004) found in an analysis of the CEPH families (Begovich et al. 1992).

Analysis of the transmitted versus never-transmitted DPB1 alleles (table 6) indicates an association between DPB1*0301 and IDDM ($P < .001$). This effect has been observed previously in a study of Mexican-American IDDM families (Erlich et al. 1996). Even though the DPB1*0301 allele is present most often on patient DR4 and DR3 haplotypes, the fact that DPB1*0301 appears on several different transmitted haplotypes argues against disease association based wholly on a simple linkage-disequilibrium effect (table 7). In addition, examination of the AFBAC haplotypes containing DPB1*0301 does not indicate strong linkage disequilibrium with any single DRB1-containing haplotype.

Stratification analysis of all DPB1 alleles found on haplotypes containing DRB1*0301, DRB1*0401, or DRB1*0404 suggests that transmission of DR4 alleles carrying DPB1*0301 is more frequent than expected (data not shown). A similar increase of DPB1*0301 is not seen on DRB1*0301 haplotypes, suggesting that the putative predisposing effect of DPB1*0301 may be dependent on its DR context.

DPB1*0202, seen infrequently in Caucasian populations, shows an association with IDDM in these data, consistent with a recent report (Balducci-Silano et al. 1995) of an analysis of 42 Venezuelan IDDM families. In both the Venezuelan and Caucasian data, most of the DPB1*0202 alleles are found on haplotypes that also carry DRB1*0301 (12 of 14, in this study; data not shown); therefore, the observed effect may simply reflect linkage disequilibrium. DPB1*0202 has been reported

Table 5

Comparison of DR1/DR4 and DR3/DR4 Genotypes

Allele	DR1/DR4	DR3/DR4	*P*-value	AFBAC DR4	CEPH DR4
	A. Distribution of DQB1 Alleles on DR4 Haplotypes (%)				
0302	71.79	96.60	...	50.00	70.91
0301	17.95	1.36	.001	43.75	29.09
0304	5.13	1.36	...	0	...
0201	5.13	0.68	.1	0	...
0305	...	...	...	2.08	...
0303	...	...	...	2.08	...
0401	...	...	...	2.08	...
n	39	147	0.001	48	55
	B. Distribution of DRB1 Subtypes on DR4 Haplotypes (%)				
0401	74.36	61.22	...	50.00	45.45
0402	2.56	4.76	...	10.42	1.82
0403	...	...	...	2.08	7.27
0404	5.13	29.25	.01	12.50	36.36
0405	12.82	3.40	.025	4.17	1.82
0407	...	...	...	14.58	7.27
0408	5.13	1.36	...	4.17	...
0411	...	...	...	2.08	...
n	39	147	0.005	48	55

Table 6

DPB1 Frequencies in Caucasian, Multiplex Families

	Frequency				
DPB1	Patient	AFBAC	OR	χ^2	*P*-value
0101	.088	.047	1.94	3.70	.1
0201	.126	.170	.70	1.96	...
0202	.019	.000	...	5.35	.025
0301	.182	.080	2.57	11.97	.001
0401	.381	.438	.79	1.18	...
0402	.046	.098	.44	6.00	.025
0501	.015	.033	.46	2.00	...
0601	.036	.007	5.13	5.52	.025
0901	.007	.007	.96	0	...
1001	.018	.014	1.25	.13	...
1040	.000	.004	0	1.28	...
1101	.006	.025	.21	4.24	.05
1160	.000	.004	0	1.28	...
1301	.008	.018	.46	1.15	...
1401	.008	.011	.76	.10	...
1501	.018	.007	2.52	1.37	...
1601	.008	0	...	2.29	...
1701	.006	.025	.21	4.24	.05
1901	.013	0	...	3.44	.1
2001	.008	.004	2.31	.56	...
2301	.003	.007	0.38	.64	...
2601	.001	0	...	.38	...
5901	.003	0	...	.76	...
n	360	276			

to be in strong linkage disequilibrium with the extended haplotype B18, DR3 (Begovich et al. 1992).

DPB1*0601 also shows a positive association with disease (table 5). DPB1*0601 differs from DPB1*0301 at only two amino acid residues (positions 69 and 76). The appearance of DPB1*0601 on several haplotypes in patients, combined with its similarity to DPB1*0301, suggests that this allele may warrant further analysis as a risk factor.

Weak association with IDDM was seen for DPB1*0101, which is known to be in linkage disequilibrium with B8, DR3 haplotypes (Begovich et al. 1992). In our data, DPB1*0101 is found almost exclusively on DR3 haplotypes in patients, whereas, in controls, DPB1*0101 was found with approximately equal frequency on DR3 and on DR2 (DRB1*1501) haplotypes (fig. 1*A*). The strong linkage disequilibrium of DPB1*0101 with DR3 suggests that this allele may not be an independent risk factor. Our analysis of DR3-DPB*0101 haplotypes did, however, lead to an interesting observation. DRB1*0301-DPB1*0101 haplotypes were found to be transmitted to affected children approximately twice as often from mothers as from fathers, while DR3 haplotypes carrying DPB1 alleles other than 0101 show no transmission bias ($P < .02$) (fig. 1*B*). The effect of DPB1*0101 on maternal transmission of DR3 haplotypes may be partially responsible for conflicting reports of increased maternal transmission of DR3 haplotypes in IDDM patients (see Discussion).

The DPB1*0402 allele shows significant negative association with disease, in these data (table 5). This effect was previously observed in analysis of Mexican-American diabetic families (Erlich et al. 1993*b*). Stratification analysis of DPB1 molecules on DRB1*0301, DRB1*0401, or DRB1*0404 haplotypes shows that DRB1*0301-DPB1*0402 is significantly less predisposing than other DRB1*0301 haplotypes ($P < .005$) (data not shown), suggesting that DPB1*0402 may mitigate the predisposing effect of DRB1*0301 to some extent.

Discussion

HLA typing analysis of family material has several advantages over typing of individual samples. The typing data produced are generally more accurate, individual alleles can be assigned unambiguously to haplotypes, and analysis of parental transmission patterns is possible. The large number of families in this study has allowed a detailed analysis of the genetic factors in the HLA region that may contribute to IDDM susceptibility in Caucasians.

The multiplex nature of the families in this analysis allows us to use analytical methods developed for the study of affected sib pairs. One such method (Risch 1987) looked at affected sib pairs sharing zero parental HLA haplotypes to estimate that HLA represents 44% of the genetic risk for IDDM. Application of this method to our data results in an estimate of the HLA component of IDDM susceptibility of ~53%. These figures illus-

Table 7

Distribution of DRB1 Haplotypes Carrying the DPB1*0301 Allele

DRB1-DPB1	Patient (n = 20)	AFBAC (n = 13)	OR	*P*-value
0101-0301	5.5	1	4.30	...
0103-0301	1	0	...	
0301-0301	14	3	3.71	.05
0401-0301	13.5	1	10.80	.01
0402-0301	.5	0	...	...
0403-0301	0	1	0	...
0404-0301	9.5	0	...	.025
0405-0301	3.5	1	2.72	...
0407-0301	.5	1	.39	...
0701-0301	.5	1	.39	...
0801-0301	5.5	3	1.42	...
1101-0301	1.5	0	...	...
1301-0301	.5	1	.39	...
1302-0301	5.5	4	1.06	...
1401-0301	0	2	0	...
1501-0301	0	1	0	...

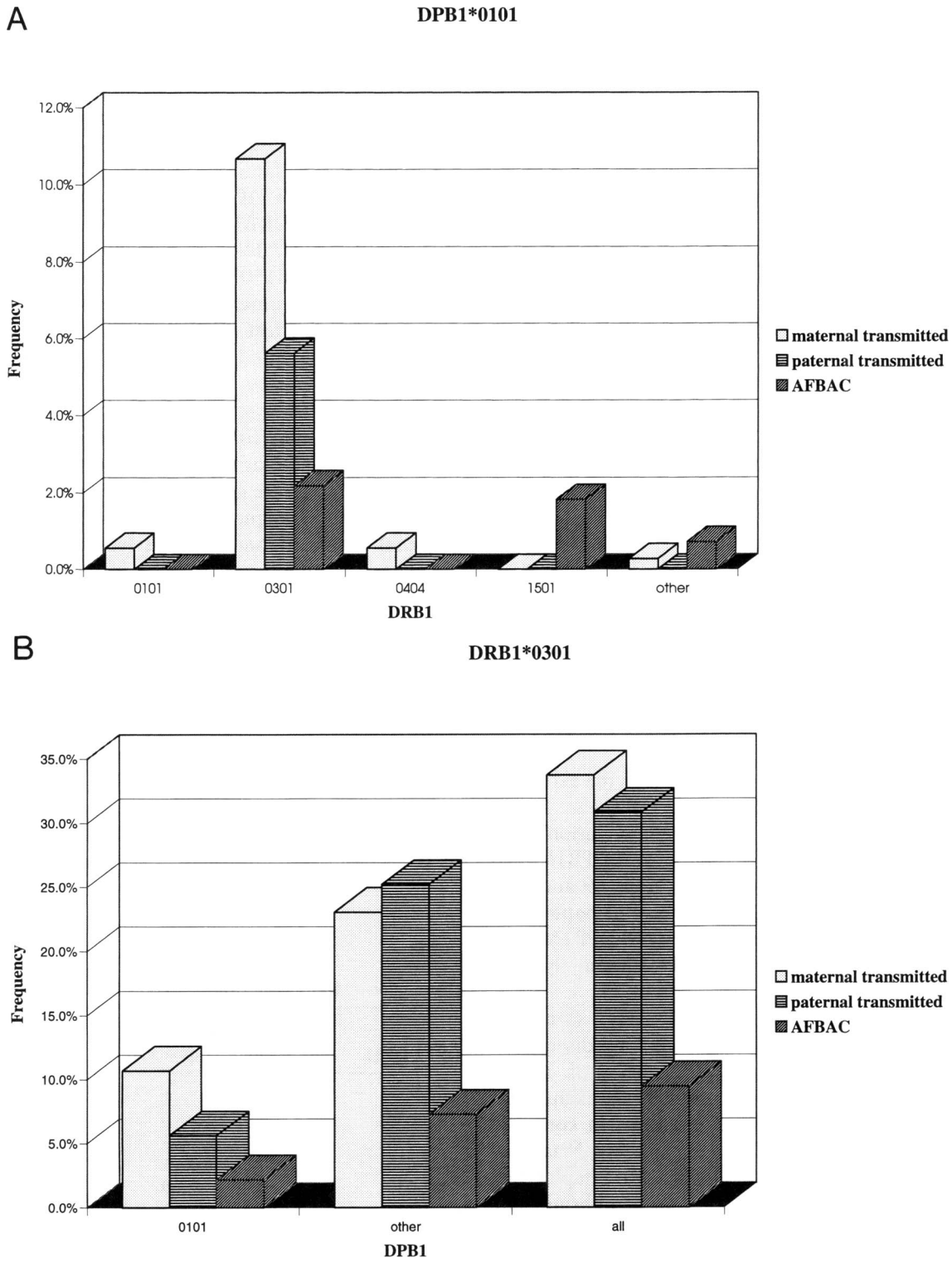

Figure 1 Excess maternal transmission of DRB1*0301-DPB1*0101 haplotypes. *A*, Examination of DPB1*0101 haplotypes. DPB1*0101 is nearly always found on DRB1*0301 haplotypes in patients. In controls, however, DPB1*0101 is found on both DRB1*0301 and DRB1*1501 haplotypes in approximately equal frequencies. *B*, Analysis of DRB1*0301 haplotypes. Examination of all DRB1*0301 haplotypes shows a slight, but not statistically significant, increase in maternal transmissions. Separation of DRB1*0301 haplotypes carrying the DPB1*0101 allele from DRB1*0301 haplotypes carrying all other DPB1 alleles indicates that the excess in maternal transmission of DRB1*0301 haplotypes can be accounted for by the excess maternal transmission of the DRB1*0301-DPB1*0101 haplotype. The difference between maternal versus paternal transmission of DRB1*0301-DPB1*0101 haplotypes and maternal versus paternal transmission of DRB1*0301-DPB1*not 0101 haplotypes is significant ($P < .03$).

trate the magnitude of the HLA contribution to disease susceptibility and emphasize the importance of determining HLA context in studies of other genetic IDDM risk factors.

IDDM Risk Assessment

Our data confirm results from many previous studies that indicate the prominent role of DR3 and DR4 haplotypes in disease susceptibility and the pronounced effect of DR3/DR4 heterozygosity. The data also indicate, as previously reported, that DR3/DR3 and DR4/DR4 genotypes confer significant risk. DR1/DR4, DR1/DR3, and DR4/DR8 genotypes are also overrepresented. DR8, which is relatively rare in Caucasians, is increased only in DR4/DR8 genotypes. In assessing HLA-based IDDM risk, these three genotypes are frequently included in "3/*X*" or "4/*X*" categories ($X \neq$ DR3, DR4, or DR2). Our data suggest that the risk associated with these genotypes may be significant enough to warrant their analysis separately from these categories.

The protective effect of DR2 is well established; consequently, IDDM patients whose genotypes include a DR2 allele are rarely observed. Because of the large size of our data set, we were able to type 21 $DR2^+$ patients from 15 of the 180 families in the analysis. Nineteen of these patients were used in the statistical analyses; two were excluded by random selection of two affected children from families containing three or more affected children. Only two $DR2^+$ patients had the haplotype DRB1*1501-DQB1*0602, which is present in ~90% of control DR2 haplotypes (see Results). Instead, most of the $DR2^+$ patients contained the relatively rare DRB1*1601-DQB1*0502 haplotype. This observation indicates that IDDM risk assessment will benefit from a level of typing specificity greater than that available with serological typing. Because the frequency of the DRB1*1601-DQB1*0502 haplotype is low in some Caucasian populations, only a small number of individuals serologically typed as $DR2^+$ would be mistakenly categorized as protected. However, serology-based categorization of all $DR2^+$ individuals as protected could have a more serious effect in population studies or screening programs of groups with higher frequencies of nonprotective DR2 haplotypes.

Molecular Basis of IDDM Susceptibility

The DQ molecule is well known to contribute to HLA association with IDDM; the identity of the amino acid residue at position 57 (aspartic acid vs. alanine, valine, or serine) of the DQβ chain is thought to play a key role in determining disease protection or susceptibility (Todd et al. 1987; Horn et al. 1988*a*). In this data set, as in many others, the DQB1*0302 allele (Ala 57) on DR4 haplotypes is strongly predisposing to disease, while the DQB1*0602 allele (Asp 57) on DRB1*1501 haplotypes is strongly protective. However, DQβ position 57 alone is insufficient to fully explain the effect of HLA on disease.

The extent of protection from disease afforded by the DQB1 Asp 57 residue appears to vary not only with the DQβ chain on which it is found, but also on the HLA context. For example, although the DQB1*0602 allele (Asp 57) on DRB1*1501 haplotypes is strongly protective, DQB1*0402 (Asp 57) on DRB1*1501 haplotypes is not protective (Erlich et al. 1991*b*). DQB1*0301 encodes an aspartic acid residue at position 57, and haplotypes containing this allele are decreased in patients. The apparent protective effect of DQB1*0301, however, is not as pronounced as that of DQB1*0602. In addition, DQB1*0301 appears more protective on DR11 haplotypes than it does on DR4 haplotypes (see table 1), suggesting that, at least in some cases, protection from disease may involve not only the DQβ polypeptide chain but also the DR molecule or the DQα chain.

Several studies have suggested that predisposing DQ molecules may be encoded either *in cis* or *in trans* (Nepom et al. 1987; Ronningen et al. 1991; Hu et al. 1993). *Trans*-encoded DQ molecules have been proposed as a basis for the strong disease susceptibility observed in DR3/DR4 heterozygotes. DR4 haplotypes include genes encoding the alleles DQA1*03 and DQB1*0302 (other DQB1 alleles are found on DR4 haplotypes but are rarely seen in Caucasian DR3/DR4 patients). In Caucasians, DR3 haplotypes include the alleles DQA1*0501 and DQB1*0201. Consequently, a DR3/DR4 genotype could produce DQ heterodimers consisting of the gene products of DQA1*03 and DQB1*0201 or of DQA1*0501 and DQB1*0302. The DQ heterodimer formed by the products of the DQA1*03 and DQB1*0201 alleles is encoded *in cis* in certain haplotypes, e.g., coupled to DRB1*0405, DRB1*0701, or DRB1*0901 (in African populations), that appear to be IDDM-associated (Todd et al. 1989). The same molecule is encoded *in trans* by the DR3/DR9 genotype, associated with IDDM in Chinese (Hu et al. 1993; Huang et al. 1995), and the DR3/DR9 genotype is slightly increased in this data set.

Although the *trans*-encoded DQ heterodimers represent an attractive explanation for genotype-specific predisposition to IDDM, the story is certainly more complex than this simple model. Neither of the DQ heterodimers described above appears to be sufficient to confer susceptibility. For example, DR4/DR7 heterozygotes should generate a DQA1*03-DQB1*0201 *trans*-encoded heterodimer; however, DR4/DR7 genotypes are not associated with IDDM risk. Similarly, a DR4/DR11 heterozygote (on which the DR4 haplotype includes a DQB1*0302 allele) should produce the *trans*-encoded DQ molecule encoded by DQA1*0501 and DQB1*0302, but DR4/DR11 genotypes are not increased in patient populations. Therefore,

the likelihood exists that the presence of both *trans*-encoded DQ heterodimers, perhaps in combination with the *cis*-encoded DQ molecules and in the absence of protective DR or DQ molecules is required to produce the full magnitude of DR3/DR4-associated susceptibility.

Another example in which *trans*-encoded DQ molecules might help to explain increased susceptibility is in the DR4/DR8 genotype. A DR4/DR8 heterozygote could produce a DQ molecule derived from DQA1*03 and DQB1*0402 alleles (Ronningen et al. 1991). This molecule would be nearly identical to the DQ molecule encoded on the haplotype DRB1*0405-DQA1*03-DQB1*0401, a haplotype associated with IDDM risk in the Japanese population (Ronningen et al. 1991; Awata et al. 1992*a*, 1992*b*). DQB1*0401 and DQB1*0402 differ at only one residue. We suggest also that the alternate *trans*-encoded heterodimer in a DR4/DR8 heterozygote, namely, that encoded by DQA1*0401 and DQB1*0302, may be involved. DQA1*0401 (found on DR8) is very similar to the DQA1*0501 allele seen on DR3; consequently, the *trans*-encoded heterodimer derived from DQA1*0401 on DR8 and DQB1*0302 on DR4 may function in a similar manner to the DQA1*0501, DQB1*0302–encoded heterodimer that could be formed from a DR3/DR4 heterozygous genotype. The fact that 15 of 15 DR4/DR8 heterozygous patients in this study carry DQB1*0302 on their DR4 haplotype is consistent with this model. Thus, either of these potentially high-risk, *trans*-encoded DQ molecules may explain, at least in part, why DR4/DR8 heterozygotes are increased in patients but DR3/DR8 heterozygotes are not.

While the identity of the DQ molecule has proved to be a very significant risk factor for IDDM susceptibility, a growing body of evidence suggests that DQ is not the only molecule involved in conferring risk (Horn et al. 1988*b*; Sheehy et al. 1989; Caillat-Zucman et al. 1992; Erlich et al. 1993*b*). On DR4 haplotypes, the subtype of the DRB1 molecule has been shown to have a dramatic effect on disease susceptibility when the DQ molecule is constant (DQA1*03-DQB1*0302) (Erlich 1991; Erlich et al. 1993*b*; Cucca et al. 1995; Harfouch et al. 1995). In a case-control study of Sardinian patients, DRB1*0405-DQA1*03-DQB1*0302 was shown to be highly predisposing, while DRB1*0403-DQA1*03-DQB1*0302 showed significant protection (Cucca et al. 1995). DRB1*0401-DQA1*03-DQB1*0302 and DRB1*0404-DQA1*03-DQB1*0302 were present in the Sardinian study in numbers too small to be significant. Another study suggested that DRB1*0404-DQA1*03-DQB1*0302 exhibits a protective effect (Reijonen et al. 1995). In our study, DRB1*0401-DQA1*03-DQB1*0302 and DRB1*0404-DQA1*03-DQB1*0302 were both shown to be significantly predisposing, although DRB1*0401 appears to confer a greater risk than does DRB1*0404 in DR3/DR4 and, particularly, in DR1/DR4 patients. These two haplotypes are among the most-common DR4 haplotypes found in Caucasian populations. Taken together, data from these studies not only indicate that the DRB1 molecule can play a role in IDDM susceptibility but also stress the importance of considering population prevalence in estimating risk for specific HLA alleles and haplotypes.

In general, the risk or protection conferred by an individual HLA molecule can be mitigated or augmented depending on the HLA context in which it is found. For example, the Sardinian data described above (Cucca et al. 1995) show that the predisposing effect of DQB1*0302 is not evident when that allele is found on a haplotype including DRB1*0403. Data presented here suggest that the predisposing effect of DQB1*0302 is also lessened or absent in the DR1/DR4 genotype where the DRB1 effect seems to predominate. The data presented here illustrate the importance of considering not only the haplotype but also the identity of the HLA alleles on the other chromosome in estimating of individual IDDM risk.

The DQB1*0301 allele represents an illustrative example. DR11 haplotypes in this data set, DR12 haplotypes in a Chinese population (Huang et al. 1995), and DRB1*1402 haplotypes in a Mexican-American population (Erlich et al. 1993*b*), all of which carry the DQA1*0501 allele and the DQB1*0301 allele, appear to confer significant protection. In addition, in our Caucasian data, examination of the DQB1*0301 allele on DRB1*0401 haplotypes, which carry the DQA1*03 allele, reveals a decrease in patient populations. Finally, when DR3/DR4 heterozygotes, which comprise nearly half of our patient population, are examined, nearly all DQB1 alleles on DR4 haplotypes are DQB1*0302; DQB1*0301 is almost never seen (see Results). Taken together, these data point to a protective role for DQB1*0301.

Other lines of evidence, however, exist that suggest that DQB1*0301, by itself, does not confer protection. Examination of DQB1 alleles in DR1/DR4 heterozygous patients shows both DQB1*0302 and DQB1*0301 represented in proportions approximating those seen in control populations. The strong bias toward DQB1*0302 and away from DQB1*0301, observed in DR3/DR4 heterozygotes, is not present in DR1/DR4 patients. Also, the haplotype DRB1*1602-DQA1*0501-DQB1*0301, found in Hispanic and Native American populations, does not confer IDDM protection (Erlich et al. 1993*b*). In conclusion, DQB1*0301 does not appear to confer protection by itself but may be protective when combined with certain DR molecules.

Contribution of the DP Molecule to Disease Susceptibility

The availability of molecular typing systems for DP alleles (Bugawan et al. 1988, 1990) has prompted the

study of disease associations with DP. In some cases, DP may be increased in patient populations simply due to linkage disequilibrium of certain DP alleles with DR-DQ haplotypes known to confer risk. In other cases, such as for DPB1*0301 presented here, the DP molecule appears to contribute to IDDM risk. Association of DPB1*0301 with IDDM has been observed previously in a study of Mexican-American IDDM families (Erlich et al. 1996). While the increased frequency of DPB1*0301 in patients may be due in part to linkage disequilibrium, its presence on several different patient haplotypes suggests that DPB1*0301 may contribute to the overall IDDM risk of these haplotypes. This conclusion is supported by the excess of DPB1*0301 seen on DR8 and DR1 haplotypes, although the number of patients with these haplotypes is too small to achieve statistical significance. The appearance of this allele as a risk factor in independent studies of two ethnically different populations argues strongly in favor of DP involvement in IDDM susceptibility.

The mechanism of DP involvement in disease susceptibility is unclear. The cell-surface DP molecule may be actively presenting peptides that influence disease susceptibility. On the other hand, DP could play a more passive role. Peptides from the DP molecule itself, presented by the DR molecule, might influence the T-cell repertoire in ontogeny or activate diabetogenic or regulatory T cells in the periphery. Presentation of HLA class II–derived peptides by other HLA class II molecules has been demonstrated (de Koster et al. 1992; Schroeijers et al. 1992). Clearly, many more data are required to address this question.

Perhaps the most striking observation from the DPB1 data in this analysis is the apparent increase of maternal transmission of DRB1*0301-DPB1*0101 haplotypes. Analysis of DPB1*0101 alone revealed an increase in patients versus controls. In control haplotypes, DPB1*0101 was usually coupled to the predisposing DRB1*0301 or to the protective DRB1*1501. DPB*0101 was found almost exclusively on DR3 haplotypes in patients, suggesting that its increase in patients may simply be due to linkage disequilibrium. Closer examination of the patient DR3-DPB1*0101 haplotypes, however, revealed a notable difference in the number of maternal transmissions versus paternal transmissions, although DR3 haplotypes without DPB1*0101 showed no excess of maternal transmission. Thus, DPB1*0101 does not appear to represent an independent IDDM risk factor; however, this allele (or something linked to it) may have some effect on parental transmission patterns for the DR3 haplotypes on which it is carried.

Increased maternal transmission of DR3 haplotypes is a controversial issue. Several studies have suggested that heterozygous DR3/DR4 IDDM patients are more likely to result from maternal transmission of DR3 and paternal transmission of DR4 than vice versa (Vadheim et al. 1986; Deschamps et al. 1990; Clerget-Darpoux et al. 1991; Margaritte-Jeannin et al. 1995). In contrast, other studies report no parent-of-origin effect (Bain et al. 1994; Undlien et al. 1995). The different conclusions reached by different researchers may be due to factors other than just the DRB1 and DQ alleles carried on these haplotypes.

Because analysis of DP polymorphisms has only recently become possible, most studies of HLA class II association with IDDM have focused on DR and DQ. In addition, the linkage disequilibrium between DQ and DP is generally much weaker than that between DR and DQ; therefore, haplotype assignment of DPB1 alleles requires the use of family material. Perhaps some of the reported differences concerning maternal transmission of DR3 might be explained by DR-DP haplotype analysis. For example, on the basis of the results of this study, a difference in maternal versus paternal DR3 transmissions might be expected in a population with a very high frequency of the DPB1*0101 allele. DR3 haplotype analysis in populations with low DPB1*0101 frequency would not be expected to reveal an excess of maternal transmission of DR3-DPB1*0101 haplotypes; thus, maternal and paternal transmissions of DR3 would not be expected to differ in such a population.

A thorough understanding of the genetic basis of IDDM susceptibility will require analysis of HLA alleles in the context of extended haplotypes as well as in genotypes. These analyses dictate the use of very large sets of family-based samples of varied ethnic backgrounds. Combining the results of large-scale genetic studies, such as this one, with data generated from other kinds of diabetes research should lead to a more complete comprehension of the disease process. This information can, in turn, lead to more accurate prediction and even prevention or delay of onset of the disease.

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References

Awata T, Kuzuya T, Matsuda A, Iwamoto Y, Kanazawa Y (1992*a*) Genetic analysis of HLA class II alleles and susceptibility to type 1 (insulin-dependent) diabetes mellitus in Japanese subjects. Diabetologia 35:419–424

——— (1992*b*) Genetic analysis of HLA class II alleles and susceptibility to type 1 (insulin-dependent) diabetes mellitus in Japanese subjects: erratum. Diabetologia 35:906

Bain SC, Rowe BR, Barnett AH, Todd JA (1994) Parental origin of diabetes-associated HLA types in sibling pairs with type I diabetes. Diabetes 43:1462–1468

Balducci-Silano PL, Layrisse ZE, Dominguez E, Gunzcler P, Lanes R, Amaro R, Zaro R (1995) HLA class II genes and susceptibility to IDDM in an ethnically mixed population. Autoimmunity 21:13

Begovich AB, McClure GR, Suraj VC, Helmuth RC, Fildes N, Bugawan TL, Erlich HA, et al (1992) Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. J Immunol 148:249–258

Bell GI, Horita S, Karam JH (1984) A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. Diabetes 33:176–183

Blackwelder WC, Elston RC (1985) A comparison of sib-pair linkage tests for disease susceptibility loci. Genet Epidemiol 2:85–97

——— (1986) A comparison of sib-pair linkage tests for disease susceptibility loci: erratum. Genet Epidemiol 3:379

Bodmer JG, Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Charron D, Dupont B, et al (1995) Nomenclature for factors of the HLA system, 1995. Tissue Antigens 46:1–18

Bugawan TL, Begovich AB, Erlich HA (1990) Rapid HLA-DPB typing using enzymatically amplified DNA and nonradioactive sequence-specific oligonucleotide probes. Immunogenetics 32:231–241

——— (1991) Rapid HLA-DPB typing using enzymatically amplified DNA and nonradioactive sequence-specific oligonucleotide probes: erratum. Immunogenetics 34:413

Bugawan TL, Erlich HA (1991) Rapid typing of HLA-DQB1 DNA polymorphism using nonradioactive oligonucleotide probes and amplified DNA. Immunogenetics 33:163–170

Bugawan TL, Horn GT, Long CM, Mickelson E, Hansen JA, Ferrara GB, Angelini G, et al (1988) Analysis of HLA-DP allelic sequence polymorphism using the in vitro enzymatic DNA amplification of DP-alpha and DP-beta loci. J Immunol 141:4024–4030

Caillat-Zucman S, Garchon HJ, Timsit J, Assan R, Boitard C, Djilali-Saiah I, Bougneres P, et al (1992) Age-dependent HLA genetic heterogeneity of type 1 insulin-dependent diabetes mellitus. J Clin Invest 90:2242–2250

Clerget-Darpoux F, Babron MC, Deschamps I, Hors J (1991) Complementation and maternal effect in insulin-dependent diabetes. Am J Hum Genet 49:42–48

Copeman JB, Cucca F, Hearne CM, Cornall RJ, Reed PW, Ronningen KS, Undlien DE, et al (1995) Linkage disequilibrium mapping of a type 1 diabetes susceptibility gene (IDDM7) to chromosome 2q31-q33. Nat Genet 9:80–85

Cucca F, Lampis R, Frau F, Macis D, Angius E, Masile P, Chessa M, et al (1995) The distribution of DR4 haplotypes in Sardinia suggests a primary association of type I diabetes with DRB1 and DQB1 loci. Hum Immunol 43:301–308

Cucca F, Muntoni F, Lampis R, Frau F, Argiolas L, Silvetti M, Angius E, et al (1993) Combinations of specific DRB1, DQA1, DQB1 haplotypes are associated with insulin-dependent diabetes mellitus in Sardinia. Hum Immunol 37:85–94

Cudworth AG, Woodrow JC (1974) Letter: HL-A antigens and diabetes mellitus. Lancet 2:1153

Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, et al (1994) A genome-wide search for human type 1 diabetes susceptibility genes. Nature 371:130–136

de Koster HS, van Rood JJ, Termijtelen A (1992) HLA-DR peptide-induced alloreactive T cell lines reveal an HLA-DR sequence that can be both "dominant" and "cryptic": evidence for allele-specific processing. Eur J Immunol 22:1531–1539

Deschamps I, Hors J, Clerget-Darpoux F, Gardais E, Robert JJ, Marcelli-Berge A, Lestradet H, et al (1990) Excess of maternal HLA-DR3 antigens in HLA DR3,4 positive type 1 (insulin-dependent) diabetic patients. Diabetologia 33:425–430

Easteal S, Kohonen-Corish MR, Zimmet P, Serjeantson SW (1990) HLA-DP variation as additional risk factor in IDDM. Diabetes 39:855–857

Erlich HA (1991) HLA class II sequences and genetic susceptibility to insulin dependent diabetes mellitus. Baillieres Clin Endocrinol Metab 5:395–411

Erlich H, Bugawan T, Begovich A, Scharf S (1993*a*) Analysis of HLA class II polymorphism using polymerase chain reaction. Arch Pathol Lab Med 117:482–485

Erlich H, Bugawan T, Begovich AB, Scharf S, Griffith R, Saiki R, Higuchi R, et al (1991*a*) HLA-DR, DQ and DP typing using PCR amplification and immobilized probes. Eur J Immunogen 18:33–55

Erlich HA, Bugawan TL, Scharf S, Nepom GT, Tait B, Griffith RL (1990) HLA-DQ beta sequence polymorphism and genetic susceptibility to IDDM. Diabetes 39:96–103

Erlich HA, Griffith RL, Bugawan TL, Ziegler R, Alper C, Eisenbarth G (1991*b*) Implication of specific DQB1 alleles in genetic susceptibility and resistance by identification of IDDM siblings with novel HLA-DQB1 allele and unusual DR2 and DR1 haplotypes. Diabetes 40:478–481

Erlich HA, Rotter JI, Chang JD, Shaw SD, Raffel LJ, Klitz W, Bugawan TL, et al (1996) Association of HLA-DPB1*0301 with insulin dependent diabetes mellitus in Mexican-Americans. Diabetes 45:610-614

Erlich HA, Zeidler A, Chang J, Shaw S, Raffel LJ, Klitz W, Beshkov Y, et al (1993*b*) HLA class II alleles and susceptibility and resistance to insulin dependent diabetes mellitus in Mexican-American families. Nat Genet 3:358–364

Field LL (1991) Non-HLA region genes in insulin dependent diabetes mellitus. Baillieres Clin Endocrinol Metab 5:413–438

Field LL, Tobias R, Magnus T (1994) A locus on chromosome 15q26 (IDDM3) produces susceptibility to insulin-dependent diabetes mellitus. Nat Genet 8:189–194

Fohlman J, Friman G (1993) Is juvenile diabetes a viral disease? Ann Med 25:569–574

Harfouch E, Timsit J, Boitard C, Bach JF, Caillat-Zucman S (1995) Contribution of DR4 alleles to IDDM predisposition or protection is independent from DQ. Autoimmunity 21:14

Hashimoto L, Habita C, Beressi JP, Delepine M, Besse C, Cambon-Thomsen A, Deschamps I, et al (1994) Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. Nature 371:161–164

Horn GT, Bugawan TL, Long CM, Erlich HA (1988*a*) Allelic

sequence variation of the HLA-DQ loci: relationship to serology and to insulin-dependent diabetes susceptibility. Proc Natl Acad Sci USA 85:6012–6016

Horn GT, Bugawan TL, Long CM, Manos MM, Erlich HA (1988*b*) Sequence analysis of HLA class II genes from insulin-dependent diabetic individuals. Hum Immunol 21:249–263

Hu CY, Allen M, Chuang LM, Lin BJ, Gyllensten U (1993) Association of insulin-dependent diabetes mellitus in Taiwan with HLA class II DQB1 and DRB1 alleles. Hum Immunol 38:105–114

Huang H-S, Peng J-T, She JY, Zhang L-P, Chao CCK, Liu K-H, She J-X (1995) HLA-encoded susceptibility to Insulin-dependent diabetes mellitus is determined by DR and DQ genes as well as their linkage disequilibria in a Chinese population. Hum Immunol 44:210–219

Klitz W, Aldrich CL, Fildes N, Horning SJ, Begovich AB (1994) Localization of predisposition to Hodgkin disease in the HLA class II region. Am J Hum Genet 54:497–505

Louis EJ, Thomson G (1986) Three-allele synergistic mixed model for insulin-dependent diabetes mellitus. Diabetes 35: 958-963

Luo D-F, Bui MM, Muir A, Maclaren NK, Thomson G, She J-X (1995) Affected-sib-pair mapping of a novel susceptibility gene to insulin-dependent diabetes mellitus (*IDDM8*) on chromosome 6q25-q27. Am J Hum Genet 57:911–919

Margaritte-Jeannin P, Clerget-Darpoux F, Hors J, Deschamps I (1995) Testing parental imprinting in insulin-dependent diabetes mellitus by the marker-association-segregation-χ^2 method. Am J Hum Genet 56:1080–1087

Nepom BS, Schwarz D, Palmer JP, Nepom GT (1987) Transcomplementation of HLA genes in IDDM. HLA-DQ alpha- and beta-chains produce hybrid molecules in DR3/4 heterozygotes. Diabetes 36:114–117

Nepom GT, Erlich H (1991) MHC class-II molecules and autoimmunity. Annu Rev Immunol 9:493–525

Nerup J, Platz P, Andersen OO, Christy M, Lyngsoe J, Poulsen JE, Ryder LP, et al (1974) HL-A antigens and diabetes mellitus. Lancet 2:864–866

Owerbach D, Gabbay KH (1995) The HOXD8 locus (2q31) is linked to type I diabetes. Interaction with chromosome 6 and 11 disease susceptibility genes. Diabetes 44:132–136

Owerbach D, Gunn S, Gabbay KH (1989) Primary association of HLA-DQw8 with type I diabetes in DR4 patients. Diabetes 38:942–945

Payami H, Joe S, Farid NR, Stenszky V, Chan SH, Yeo PPB, Cheah JS, et al (1989) Relative predispositional effects (RPEs) of marker alleles with disease: HLA-DR alleles and Graves disease. Am J Hum Genet 45:541–546

Reijonen H, Tuokko J, Ilonen J, Akerblom HK, Childhood Diabetes in Finland (DiMe) Study Group (1995) The effect of HLA-DR4 subtype on the risk of IDDM carried by DQB1*0302. Paper presented at the 9th International Congress of Immunology, San Francisco, July 23–29

Risch N (1987) Assessing the role of HLA-linked and unlinked determinants of disease. Am J Hum Genet 40:1–14

Ronningen KS, Gjertsen HA, Iwe T, Spurkland A, Hansen T, Thorsby E (1991) Particular HLA-DQ alpha beta heterodimer associated with IDDM susceptibility in both DR4-DQw4 Japanese and DR4-DQw8/DRw8-DQw4 whites. Diabetes 40:759–763

Rosenlicht JW, Hartung K, Deicher H, Frey J (1993) A novel HLA-DRB1-DR2 allele associated with HLA mistyping. Immunogenetics 37:479

Rotter JI, Anderson CE, Rubin R, Congleton JE, Terasaki PI, Rimoin DL (1983) HLA genotypic study of insulin-dependent diabetes the excess of DR3/DR4 heterozygotes allows rejection of the recessive hypothesis. Diabetes 32:169–174

Saiki RK, Walsh PS, Levenson CH, Erlich HA (1989) Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. Proc Natl Acad Sci USA 86: 6230–6234

Scharf SJ, Griffith RL, Erlich HA (1991) Rapid typing of DNA sequence polymorphism at the HLA-DRB1 locus using the polymerase chain reaction and nonradioactive oligonucleotide probes. Hum Immunol 30:190–201

Schroeijers WE, de Koster HS, Verduyn W, Schreuder GM, Termijtelen A (1992) T-cell recognition of class II products that result from the combined presence of two different HLA haplotypes. Hum Immunol 33:229–234

Sheehy MJ, Scharf SJ, Rowe JR, Neme de Gimenez MH, Meske LM, Erlich HA, Nepom BS (1989) A diabetes-susceptible HLA haplotype is best defined by a combination of HLA-DR and -DQ alleles. J Clin Invest 83:830–835

Singal DP, Blajchman MA (1973) Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes 22:429–432

Spielman RS, Baur MP, Clerget-Darpoux F (1989) Genetic analysis of IDDM: summary of GAW5 IDDM results. Genet Epidemiol 6:43–58

Svejgaard A, Platz P, Ryder LP (1980) Insulin dependent diabetes mellitus. In: Terasaki P (ed) Histocompatibility tesing 1980. University of California Press, Los Angeles and Berkeley, pp 638–656

Tait BD, Harrison LC (1991) Overview: the major histocompatibility complex and insulin dependent diabetes mellitus. Baillieres Clin Endocrinol Metab 5:211–228

Tait BD, Mraz G, Harrison LC (1988) Association of HLA-DQw3 (TA10-) with type I diabetes occurs with DR3/4 but not DR1/4 patients. Diabetes 37:926–929

Thomson G (1983) Investigation of the mode of inheritance of the HLA associated diseases by the method of antigen genotype frequencies among diseased individuals. Tissue Antigens 21:81–104

——— (1988) HLA disease associations: models for insulin dependent diabetes mellitus and the study of complex human genetic disorders. Annu Rev Genet 22:31–50

——— (1995*a*) Analysis of complex human genetic traits: an ordered-notation method and new tests for mode of inheritance. Am J Hum Genet 57:474–486

——— (1995*b*) HLA disease associations: models for the study of complex human genetic disorders. Crit Rev Clin Lab Sci 32:183–219

Thomson G, Robinson WP, Kuhner MK, Joe S, Klitz W (1989) HLA and insulin gene associations with IDDM. Genet Epidemiol 6:155–160

Thomson G, Robinson WP, Kuhner MK, Joe S, MacDonald MJ, Gottschall JL, Barbosa J, et al (1988) Genetic heterogeneity, modes of inheritance, and risk estimates for a joint

study of Caucasians with insulin-dependent diabetes mellitus. Am J Hum Genet 43:799–816

Todd JA, Bell JI, McDevitt HO (1987) HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 329:599–604

Todd JA, Mijovic C, Fletcher J, Jenkins D, Bradwell AR, Barnett AH (1989) Identification of susceptibility loci for insulin-dependent diabetes mellitus by trans-racial gene mapping. Nature 338:587–589

Trucco M, LaPorte R (1995) Exposure to superantigens as an immunogenetic explanation of type I diabetes mini-epidemics. J Pediatr Endocrinol Metab 8:3–10

Tiwari JL, Terasaki PI (1985) HLA and disease. Springer, New York

Undlien DE, Akselsen HE, Joner G, Dahl-Jorgensen K, Aagenæs Ø, Søvik O, Thorsby E, et al (1995) No difference in the parental origin of susceptibility HLA class II haplotypes among Norwegian patients with insulin-dependent diabetes mellitus. Am J Hum Genet 57:1511–1514

Vadheim CM, Rotter JI, Maclaren NK, Riley WJ, Anderson CE (1986) Preferential transmission of diabetic alleles within the HLA gene complex. N Engl J Med 315:1314–1318

Woolf B (1955) On estimating the relation between blood group and diseases. Ann Eugen 19:251–253