HLA DPA1, DPB1 Alleles and Haplotypes Contribute to the Risk Associated With Type 1 Diabetes

Analysis of the Type 1 Diabetes Genetics Consortium Families

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OBJECTIVE—To determine the relative risk associated with DPA1 and DPB1 alleles and haplotypes in type 1 diabetes.

RESEARCH DESIGN AND METHODS—The frequency of DPA1 and DPB1 alleles and haplotypes in type 1 diabetic patients was compared to the family based control frequency in 1,771 families directly and conditional on HLA (B)-DRB1-DQA1-DQB1 linkage disequilibrium. A relative predispositional analysis (RPA) was performed in the presence or absence of the primary HLA DR-DQ associations and the contribution of DP haplotype to individual DR-DQ haplotype risks examined.

RESULTS—Eight DPA1 and thirty-eight DPB1 alleles forming seventy-four DPA1-DPB1 haplotypes were observed; nineteen DPB1 alleles were associated with multiple DPA1 alleles. Following both analyses, type 1 diabetes susceptibility was significantly associated with DPB1*0301 (DPA1*0103-DPB1*0301) and protection with DPB1*0402 (DPA1*0103-DPB1*0402) and DPA1*0103-DPB1*0101 but not DPA1*0201-DPB1*0101. In addition, DPB1*0202 (DPA1*0103-DPB1*0202) and DPB1*0201 (DPA1*0103-DPB1*0201) were significantly associated with susceptibility in the presence of the high risk and protective DR-DQ haplotypes. Three associations (DPB1*0301, *0402, and *0202) remained statistically significant when only the extended HLA-A1-B8-DR3 haplotype was considered, suggesting that DPB1 alone may delineate the risk associated with this otherwise conserved haplotype.

CONCLUSIONS—HLA DP allelic and haplotypic diversity contributes significantly to the risk for type 1 diabetes; DPB1*0301 (DPA1*0103-DPB1*0301) is associated with susceptibility and DPB1*0402 (DPA1*0103-DPB1*0402) and DPA1*0103-DPB1*0101 with protection. Additional evidence is presented for the susceptibility association of DPB1*0202 (DPA1*0103-DPB1*0202) and for a contributory role of individual amino acids and DPA1 or a gene in linkage disequilibrium in DR3-DPB1*0101 positive haplotypes. ***Diabetes*** **59:2055–2062, 2010**

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Insulin-dependent autoimmune or type 1 diabetes is a common autoimmune disorder of unknown etiology which results in the destruction of the insulin-secreting pancreatic β-cells. The concordance rate in monozygotic twins is estimated to be 30 to 50% with an average risk to sibs of 6% (1) and an overall genetic risk ratio (λ-s) of about 15 (2). The increasing incidence of type 1 diabetes in a genetically homogeneous population, however, clearly indicates that environmental factors also play a key role (3,4).

Multiple association studies and genome linkage and association scans have confirmed that the greatest genetic risk is associated with variation within the HLA region located on chromosome six with evidence for modest associations at other regions in addition to HLA (5,6). In particular allelic, haplotypic or genotypic differences at the HLA class II DRB1, DQA1 or DQB1 loci have been shown in many studies, including a subset of this dataset (7), to have the greatest association.

The most plausible mechanism explaining the association with genes of the HLA class II region is their role in presentation of peptides derived from exogenous protein to CD4+ T-helper cells which, in the case of type 1 diabetes, may result in an inappropriate T cell immune response against self-antigens on the pancreatic β-cells. Allelic differences at the DRB1, DQA1 and DQB1 loci have been shown to influence the peptide binding and T cell stimulatory capacities of the individual HLA molecules (8), suggesting that such differences contribute to the association of individual or groups of alleles with autoimmune diseases. Genetic polymorphisms at other loci, both within and outside the HLA region may, in addition, contribute to and influence the magnitude of the immune response.

The HLA DPA1 and DPB1 genes are the third set of classical HLA class II loci which code for the DP antigen and have been associated with a lower immunostimulatory capacity and level of expression (9,10) although differences at individual DPB amino acids have been associated with an increased proliferative response in the mixed lymphocyte reaction (11,12). Similarly, a single DPB amino acid, glutamic acid at position 69, has been shown to contribute to graft versus host disease in otherwise HLA identical sibling bone marrow transplantation (13) and susceptibility to Beryllium disease (14).

Association studies of HLA-DPB1 and type 1 diabetes have shown multiple associations with conflicting re-

sults. The following have been reported as susceptibility alleles in populations of different ethnic backgrounds: DPB1*0201, *0202, *0301, *0401, *0402, *1701 and the following as protective alleles; DPB1*0101, *0202, *0401, *0402, *1701 (15–23). Other studies have reported weak or no association with HLA DPB1 alleles (24,25). The conflicting nature of these association studies may be a reflection of population specific differences, inconsistent typing approaches, differences in study design or inadequately powered studies.

The HLA DPA1 and DPB1 loci are highly polymorphic with 28 DPA1 and 136 DPB1 alleles defined as of October, 2009 (http://www.ebi.ac.uk/imgt/hla/). Association analyses in the HLA region are complicated by the occurrence of extensive linkage disequilibrium between loci such that the classical HLA loci, A, B, C, DR, DQ and DP, as well as other genes in this region, are often inherited as a "block". The DR/DQ recombination frequency per meiosis between DR-DQ and DP has been estimated to range between 1–3% (13,26). Estimates of the relative contribution of HLA DP to susceptibility or protection against type 1 diabetes must therefore consider the potential influence of co-inherited loci, some of which are strongly associated with type 1 diabetes.

The Type 1 Diabetes Genetic Consortium (T1DGC) is a large worldwide collaborative study of type 1 diabetes families that have been collected in a highly standardized fashion from various populations (27). High resolution HLA typing has been performed at eight loci at four genotyping centers using standardized typing protocols and reagents (28). The large sample size and addition of DPA1 typing permits, for the first time, an association analysis of the DPA1 locus in addition to DPB1 and DPA1-DPB1 haplotypes, which encode the antigen presenting alpha and beta chain heterodimer with type 1 diabetes.

RESEARCH DESIGN AND METHODS

The dataset used comprised 1,149 new Caucasian type 1 diabetes multiplex families combined with a number of existing type 1 diabetes DNA collections that are largely Caucasian, some of which have previously been HLA typed and analyzed (20,23), including the Danish (n = 95), Human Biological Data Interchange (HBDI) repository (Philadelphia, PA; n = 418), Sardinian (n = 52), and Joslin Diabetes Collections (n = 57). Where the proband was not indicated an affected sib was chosen at random.

Genotyping. The methods of HLA genotyping have previously been described (7). Briefly, four digit HLA typing was reported using oligonucleotide probes that were immobilized to a nylon membrane and hybridized with biotin-labeled locus specific PCR products. Genotyping was performed using the Stripscan and Score proprietary software prior to upload to the central coordinating committee. Regular quality assurance typing performed on blind samples and between laboratories indicate typing agreement to be greater than 98% for DPA1 and DPB1 (28).

Statistical analysis. Control haplotypes (n = 2,759) were determined based on the affected family-based control (AFBAC) method (29). The statistical significance of differences in allele frequencies between type 1 diabetes probands and AFBACs was assessed using a Pearson's χ^2 test. P values were adjusted for multiple alleles or haplotypes by applying the Bonferroni correction of multiplying the nominal P value by the number of alleles or haplotypes compared (P_{adj}). Only alleles or haplotypes for which f > 5% in either proband or AFBAC or where the nominal $P < 0.05$ and f > 1% were compared. We note that the Bonferroni correction is conservative for these data as the haplotypes are not independent.

Because only the proband from each family is used in the analysis and the AFBAC method uses at most two haplotypes from each pedigree, this approach does not introduce a bias due to the nonindependence between sibs.

Adjustment for linkage disequilibrium (LD) with DR-DQ (HLA-B) haplotypes. The expected allele frequencies were computed, given LD and relative haplotype penetrance. In this case the null hypothesis (H_0) is that DPA1, or DPB1 allele or DPA1-DPB1 haplotype frequencies will differ between patients and controls due to (1) linkage disequilibrium between DP and DRB1-DQB1 (and eventually also HLA-B) and (2) due to chance (sampling), thus implying that DP is neutral relative to disease predisposition.

Under H_0 the expected allele frequencies at DPB1 or DPA1 alleles or DPA1-DPB1 haplotypes can be computed using the equation derived by Thomson (30):

$$q_{\exp\,\mathrm{DP}_j} = p_{\mathrm{DP}_j} + \sum_{i}^{K} D_{ij}\frac{q_{\mathrm{DR\text{-}DQ\text{-}(DP)}_i}}{p_{\mathrm{DR\text{-}DQ\text{-}(DP)}_i}}$$

where D_{ij} denotes pairwise linkage disequilibrium between the ith DR-DQ (HLA-B) haplotype and the jth DPB1 or DPA1 allele or DPA1-DPB1 haplotype in the control sample, q denotes the allele or haplotype frequency in probands, p denotes frequency in AFBACs, and q_{exp} denotes the expected frequency in probands under the assumption of no involvement in disease. This method relies on sampling estimates of pairwise linkage disequilibrium between DP and DRB1-DQB1 and of the proband and control frequencies derived from the samples under study. Thus, there will be a sampling error associated with the computed value for expected DP allele or haplotype frequencies. The larger the control sample the smaller this error would be. This has been taken into account in the statistical tests carried out as previously described (31). Given the large number of DR-DQ-B haplotypes and the error that very rare haplotypes could introduce, only haplotypes at the susceptibility loci with an average frequency of 0.5% or higher in combined cases and controls were used. Moreover, only DP-DR-DQ (B) haplotypes where the expected frequency in controls in the absence of LD would be >0.05% were included in the analysis. Of the 75 observed DPA1-DPB1 haplotypes, a statistical test for association with type 1 diabetes conditional on DR-DQ or DR-DQ-HLA-B was carried out only on 22 haplotypes which had an expected frequency in type 1 diabetes of >0.5%.

In addition, the contribution of DP was considered on individual DRB1-DQA1-DQB1 and extended HLA haplotypes by a direct comparison between the allele and haplotype distribution between proband and AFBAC for individual haplotypes.

RESULTS

Frequency distribution of DPA1 and DPB1 alleles and haplotypes. Eight DPA1 and thirty-eight DPB1 alleles were observed which formed seventy-four different DPA1-DPB1 haplotypes; nineteen DPB1 alleles were associated with multiple DPA1 alleles. The most frequent DPB1 allele, *0401, was observed in association with five different DPA1 alleles. While the DPA1-DPB1 haplotypic diversity is much greater than that previously observed, only fourteen DPA1-DPB1 haplotypes were observed at a frequency of greater than one percent in either the probands or AFBACs. The inclusion of DPA1 in HLA-DP associations with type 1 diabetes permits, for the first time, an analysis of the contribution of this locus and an analysis of DPA1-DPB1 haplotypes.

A direct comparison of the DPA1, DPB1, and DPA1-DPB1 frequency distribution, unadjusted for linkage disequilibrium, reveals a significant association with susceptibility to type 1 diabetes with DPB1*0101, 0202, 0301 and the DPA1-DPB1 haplotypes; 0201-0101, 0103-0202, 0103-0301. Protection against type 1 diabetes was significantly associated with DPA1*0202, DPB1*0402, 1001, 1101 and the DPA1-DPB1 haplotypes; 0103-0402, 0201-1101 (Table 1). DPB1*0101 was the only DPB1 allele observed with multiple DPA1 alleles that each had a frequency greater than 1% allowing analysis of the role of DPA1 in type 1 diabetes risk.

Association analyses of HLA-DP with type 1 diabetes are complicated by the presence of extensive linkage disequilibrium between HLA loci that include the established primary susceptibility and protective DRB1, DQA1, and DQB1 loci. To address the issue of linkage disequilibrium, association analyses were performed conditional on DRB1-DQA1-DQB1 linkage disequilibrium (Table 2) and HLA-B-DRB1-DQA1-DQB1 linkage disequilibrium (Fig. 1)

TABLE 1
Proband and affected family-based control (AFBAC) HLA DPA1, DPB1 allele, and DPA1-DPB1 haplotype frequency distribution in 1,771 type 1 diabetes families

	AFBAC (%) (n = 2,759)	Proband (%) (n = 3,542)	P_{adj}	Odds Ratio	95% CI		AFBAC (%) (n = 2,759)	Proband (%) (n = 3,542)	P_{adj}	Odds Ratio	95% CI
DPA1*											
0103	82.4	83.6		1.1							
0104	0.5	1.1		2.0	1.1–3.6						
0201	13.7	13.5		1.0	0.8–1.2						
0202	3.2	1.6	2E-4	0.5	0.4–0.7						
DPB1*						DPA1*-DPB1*					
0101	5.6	7.4	0.05	1.4	1.1–1.7	0103-0101	1.3	0.6		0.5	0.3–0.8
						0201-0101	3.4	6.4	2E-6	1.9	1.5–2.5
						0202-0101	0.9	0.4		0.5	0.2–0.9
0201	13.5	15.2		1.1	1.0–1.4	0103-0201	12.6	14.9		1.2	1.0–1.4
0202	0.7	2.8	7E-9	4.4	2.6–7.3	0103-0202	0.5	2.8	5E-10	5.3	3.0–9.1
0301	10.4	15.9	5E-8	1.6	1.4–1.9	0103-0301	10.1	15.6	2E-8	1.7	1.4–2.0
0401	42.0	40.2		0.9		0103-0401	40.8	39.3		0.9	
0402	11.5	5.3	9E-17	0.4	0.4–0.5	0103-0402	11.2	5.1	1E-16	0.4	0.3–0.5
1001	1.8	1.0	0.04	0.5	0.3–0.8	0103-0601	1.5	2.2		1.5	1.0–2.2
1101	2.1	0.9	9E-4	0.4	0.3–0.7	0201-1001	1.5	0.9		0.6	0.4–0.9
1401	1.3	0.8		0.6	0.4–1.0	0201-1101	1.7	0.8	0.02	0.5	0.3–0.8
1501	0.7	1.5		2.1	1.2–3.5	0104-1501	0.5	1.0		2.0	1.0–3.7
1701	1.7	1.0		0.6	0.4–0.9	0201-1701	1.6	1.0		0.6	0.4–1.0

Only alleles or haplotypes with a frequency >5% in an individual category or >1% where the nominal P value was <0.05 were compared. The nominal P value was adjusted (P_{adj}) for multiple alleles or haplotypes by applying the Bonferroni correction of multiplying the nominal P value by the number of alleles or haplotypes compared.

to accommodate the more recent large association analyses which indicate an independent association of HLA-B (32,33). In addition, a relative predispositional analysis (RPA) (34) in which the primary susceptibility and protective DRB1-DQA1-DQB1 haplotypes are removed was performed to detect secondary associations not detected in the presence of the primary associations. Furthermore, an association analysis was performed comparing the frequency of DPA1-DPB1 haplotypes present on the more frequent individual DRB1-DQA1-DQB1 haplotypes.

Conditional analysis

DPA1. Following the correction for multiple allele testing, only DPA1*0201 ($P_{adj} = 5 \times 10^{-4}$, OR = 0.7) was significantly associated with protection to type 1 diabetes. DPA1*0201 was also protective when in haplotypic association with four DPB1 alleles; 0101, 0401, 1001 and 1101.

TABLE 2
Expected and observed proband DPA1, DPB1, and DPA1-DPB1 frequencies conditioned on HLA DRB1-DQA1-DQB1 linkage disequilibrium in 1,771 type 1 diabetes families

	Proband F_{exp} (%)	Proband F_{obs} (%)	P_{adj}	Odds Ratio (F_{obs}/F_{exp})	95% CI		Proband F_{exp} (%)	Proband F_{obs} (%)	P_{adj}	Odds Ratio (F_{obs}/F_{exp})	95% CI
DPA1*						DPA1-DPB1					
0103	80.3	82.8		1.2							
0104	0.5	1.0		2.0	1.1–3.7						
0201	17.2	13.4	5E-4	0.7	0.6–0.9						
0202	2.0	2.8		1.4	1.0–2.0						
DPB1*											
0101	11.4	8.0	2E-4	0.7	0.6–0.8	0103-0101	2.8	0.7	2E-10	0.2	0.1–0.4
						0201-0101	7.5	6.3		0.8	0.7–1.0
0201	13.0	15.2		1.2	1.0–1.4	0103-0201	12.1	14.7		1.3	1.1–1.5
0202	1.4	2.8	2E-3	2.0	1.4–3.0	0103-0202	1.4	2.8	2E-3	2.1	1.4–3.0
0301	10.1	15.6	4E-8	1.6	1.4–2.0	0103-0301	10.0	15.3	5E-8	1.6	1.4–1.9
0401	40.8	39.9		1.0		0103-0401	38.9	38.7		1.0	0.9–1.1
						0201-0401	1.2	0.7		0.6	0.3–1.0
0402	10.9	5.3	2E-14	0.5	0.4–0.6	0103-0402	10.7	5.1	7E-15	0.5	0.4–0.5
0501	1.1	1.7		1.6	1.0–2.5						
0601	1.5	2.2		1.5	1.0–2.3	0103-0601	1.4	2.2		1.6	1.1–2.3
1001	1.7	1.0		0.6	0.4–0.9	0201-1001	1.7	0.9	0.05	0.5	0.3–0.8
1101	1.6	0.9		0.5	0.3–0.9	0201-1101	1.6	0.8	0.03	0.5	0.3–0.8
1501	0.5	1.5	2E-3	3.0	1.6–5.4	0104-1501	0.5	1.0		2	1.1–3.7

Only alleles or haplotypes with a frequency >5% in an individual category or >1% where the nominal P value was <0.05 were compared. The nominal P value was adjusted (P_{adj}) for multiple alleles or haplotypes by applying the Bonferroni correction of multiplying the nominal P value by the number of alleles or haplotypes compared.

TABLE 3
Relative predispositional analysis; DPA1, DPB1 allele, and DPA1-DPB1 haplotype frequency distribution in 1,771 type 1 diabetes families in the presence or absence of the high-risk and protective DRB1-DQA1-DQB1 haplotypes

	Presence of high-risk and protective DR-DQ haplotypes					Absence of high-risk and protective DR-DQ haplotypes				
	% AFBACs (n = 1,102)	% Probands (n = 2,626)	P_{adj}	Odds Ratio	95% CI	% AFBACs (n = 1,656)	% Probands (n = 916)	P_{adj}	Odds Ratio	95% CI
DPA1*										
0103	83.7	83.7		1.0		81.5	83.4		1.1	1.0–1.2
0104						0.7	2.2	0.002	3.3	1.6–7.0
0201	14.2	14.4		1.0	0.8–1.3	13.3	10.9		0.8	0.6–1.0
DPB1*										
0101	9.5	9.3		1.0	0.8–1.3					
0201	9.6	15.3	1E-04	1.7	1.3–2.2	16.1	15.0		0.9	0.7–1.2
0202	1.3	3.7	8E-04	2.9	1.7–5.2					
0301	8.7	14.9	1E-05	1.8	1.4–2.4	11.5	18.6	3E-05	1.8	1.4–2.2
0401	50.5	40.6	2E-04	0.7	0.5–0.8					
0402	6.4	4.8		0.7	0.5–1.0	14.9	6.8	7E-08	0.4	0.3–0.6
1501						0.8	2.8	3E-04	3.7	1.9–7.2
2301	1.3	0.2	5E-04	0.1	0.04–0.4					
DPA1-DPB1										
0103-0101	2.5	0.8	7E-04	0.3	0.2–0.6					
0201-0101	7.0	8.2		1.2	0.9–1.6					
0103-0201	9.3	14.9	1E-04	1.7	1.4–2.2	14.9	14.7		1.0	0.8–1.3
0103-0202	1.1	3.7	2E-04	3.4	1.9–6.3					
0103-0301	8.4	14.6	1–05	1.9	1.5–2.4	11.2	18.6	7E-06	1.8	1.4–2.3
0103-0401	49.5	39.7	2E-04	0.7	0.5–0.8	35.1	38.1		1.1	1.0–1.4
0103-0402	6.4	4.6		0.7	0.5–1.0	14.4	6.4	6E-08	0.4	0.3–0.6
0103-2301	1.3	0.2	5E-05	0.1	0.04–0.4					
0104-1501				0.3	0.2–0.6	0.6	2.2	2E-03	3.7	1.7–7.9

DPA1, DPB1, and DPA1-DPB1 alleles and haplotypes with a frequency >5% in an individual category or >1% where the nominal P value was <0.05 were compared in the presence or absence of the following high-risk and protective DRB1-DQA1-DQB1 haplotype: 0301-0501-0201, 0401-0301-0302, 0402-0301-0302, 0404-0301-0302, 0405-0301-0302, 0701-0201-0303, 1401-0101-0503 and 1501-0102-0602. The nominal P value was adjusted (P_{adj}) for multiple alleles or haplotypes by applying the Bonferroni correction of multiplying the nominal P value by the number of comparisons.

The DPA1 allele in association with DPB1*0101 appears to significantly contribute to the risk associated with DPB1*0101 (described below).

DPB1. DPB1*0301 was associated with susceptibility following the DRB1-DQA1-DQB1 conditional analysis ($P_{adj} = 4 \times 10^{-8}$, OR = 1.6) and in haplotypic association with DPA1*0103 ($P_{adj} = 5 \times 10^{-8}$, OR = 1.6). DPB1*0402 was associated with protection following the DRB1-DQA1-DQB1 conditional analysis ($P_{adj} = 2 \times 10^{-14}$, OR = 0.5) and in haplotypic association with DPA1*0103 ($P_{adj} = 7 \times$

TABLE 4
DPA1-DPB1 haplotype frequency odds ratios for individual DRB1-DQA1-DQB1 haplotypes

			DPA1-DPB1									
	Proband	AFBAC	0103-0101		0201-0101	0103-0201	0103-0202		0103-0301		0103-0402	
DRB1-DQA1-DQB1	(n)	(n)	OR	P_{adj}	OR	OR	OR	P_{adj}	OR	P	OR	P_{adj}
04XX-0301-0302	1,393	239				1.4			1.6		0.7	
0401-0301-0302	909	114	0.2	0.04		1.6			2.0		0.7	
0402-0301-0302	135	36				2.2			1.9		0.5	
0404-0301-0302	223	90				1.2			1.6		0.9	
0301-0501-0201	1,224	342	0.2	5.E-06	0.8	1.5	2.5	0.04	1.7		0.4	5.E–03
A1-B8-DR3	436	146	0.3		0.8	1.9	3.5	0.03	1.2	0.03	0.4	0.04
A30-B18-DR3	99	26				0.4	3.0		0.8		0.9	
DR3 other	691	170	0.1	5.E-06	0.8	1.5	2.2		1.3		0.5	
0101-0101-0501	195	236				0.8			2.2		0.3	1.E–03
1302-0103-0604	93	80				0.7			1.9		0.1	0.02
0701-0201-0201	94	270				1.0			1.1		0.3	
1101-0501-0301	32	162				2.1			1.8		0.2	
0401-0301-0301	63	111				2.2					0.4	

The odds ratio (OR) was calculated for DPA–DPB1 haplotypes with a frequency >5% in an individual category or >1% where the nominal P value was <0.05. The nominal P value was adjusted (P_{adj}) for multiple alleles or haplotypes by applying the Bonferroni correction of multiplying the nominal P value by the number of comparisons. DR3 (DRB1*0301-DQA1*0501-DQB1*0201) positive haplotypes were subdivided into the presence or absence of the extended A*0101-B*0801-C*0701-DRB1*0301-DQA1*0501-DQB1*0201 and A*3002-B*1801-C*0501-DRB1*0301-DQA1*0501-DQB1*0201 positive haplotypes.

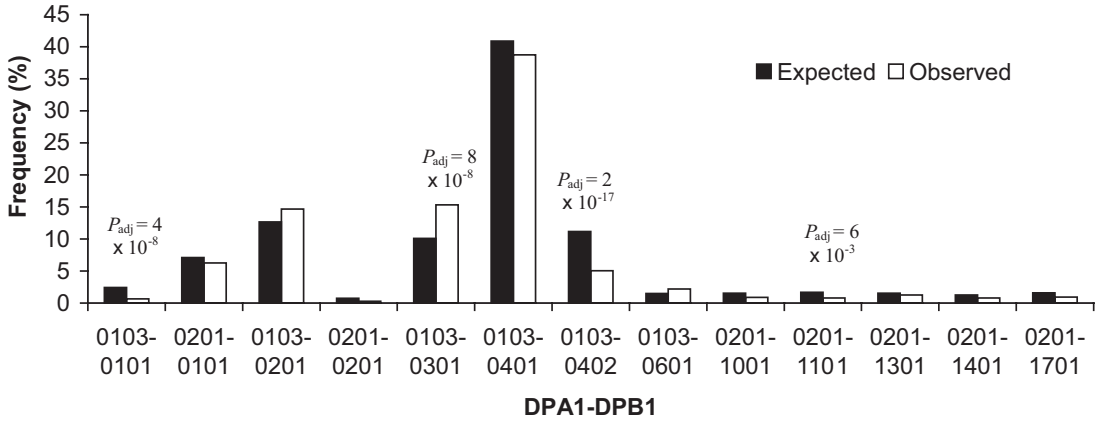

FIG. 1. Expected and observed proband DPA1-DPB1 haplotype frequencies conditioned on HLA-B-DRB1-DQA1-DQB1 linkage disequilibrium. Alleles or haplotypes with a frequency of less than 5% for an individual category with a non significant comparison or less than 1% where the nominal $P < 0.05$ are not shown. Nominal P value was adjusted for the number of haplotypes compared (P_{adj}).

10^{-15}, RR = 0.5). DPB1*0101 was associated with protection following the DRB1-DQA1-DQB1 conditional analysis ($P_{adj} = 2 \times 10^{-4}$, OR = 0.7) and in haplotypic association with DPA1*0103 ($P_{adj} = 2 \times 10^{-10}$, OR = 0.2). However, no association was observed with the more common DPA1*0201-DPB1*0101 haplotype; the nonoverlapping 95% confidence intervals for the Odds Ratios between the two DPB1*0101 haplotypes suggests a contributory role of DPA1 (Table 2). These observations were confirmed in the HLA-B-DRB1-DQA1-DQB1 conditional analysis (Fig. 1) and are the most widely reported and now replicated DP associations with type 1 diabetes.

The low frequency DPB1*0202 allele was associated with susceptibility following the DRB1-DQA1-DQB1 conditional analysis ($P_{adj} = 4 \times 10^{-3}$, OR = 2.0) and in haplotypic association with DPA1*0103 ($P_{adj} = 7 \times 10^{-3}$, OR = 2.1). This association was confirmed following the HLA-B-DRB1-DQA1-DQB1 conditional analysis where the expected frequency of DPA1*0103-DPB1*0202 was zero whereas the observed frequency in type 1 diabetic patients was 2.8% (data not shown).

Relative predispositional analysis. The distinct hierarchy of risk and protection established for individual DRB1-DQA1-DQB1 haplotypes among the T1DGC families has been reported (7). The role of HLA DP was therefore considered both in the presence or absence of the DRB1*0301-DQA1*0501-DQB1*0201, DRB1*0401-DQA1*0301-DQB1*0302, DRB1*0402-DQA1*0301-DQB1*0302, DRB1*0404-DQA1*0301-DQB1*0302, DRB1*0405-DQA1*0301-DQB1*0302 high risk DR-DQ haplotypes and the DRB1*0701-DQA1*0201-DQB1*0303, DRB1*1401-DQA1*0101-DQB1*0503 and DRB1*1501-DQA1*0102-DQB1*0602 protective haplotypes (Table 3).

DPB1*0301 was significantly associated with susceptibility both in the presence or absence of the high risk and protective DR-DQ haplotypes either individually or in association with DPA1*0103 with an odds ratio of between 1.8 and 1.9. DPB1*0402, either individually or in association with DPA1*0103, was observed to be significantly associated with protection only in the absence of the high risk and protective DR-DQ haplotypes with an odds ratio of 0.4.

The protective association of DPA1*0103-DPB1*0101 and susceptibility association of DPB1*0202 (DPA1*0103-DPB1*0202) only observed in the high risk and protective DR-DQ haplotypes is consistent with the presence of DR3 positive haplotypes in this group. The low frequency susceptibility association with DPB1*1501 (or DPA1*0104-DPB1*1501) observed in the absence of high risk and protective DR-DQ haplotypes and in the conditional analysis (Table 2) may represent an additional secondary association but requires confirmation.

DPA1-DPB1 haplotype distribution in individual DRB1-DQA1-DQB1 haplotypes. A comparison of the DPA1-DPB1 odds ratios in the most frequent DRB1-DQA1-DQB1 haplotypes suggests that DPA1*0103-DPB1*0301 and DPA1*0103-DPB1*0201 are associated with susceptibility and DPA1*0103-DPB1*0402 with protection across most DR-DQ haplotypes (Table 4). Only a single DPA1-DPB1 association with either the combined or individual DR4-DQB1*0302 positive haplotypes reached statistical significance; susceptibility associated with DRB1*0401-DQA1*0301-DQB1*0302 was significantly reduced by the presence of DPA1*0103-DPB1*0101 ($P_{adj} = 0.04$, OR = 0.2). In contrast, the risk associated with DR3 (DRB1*0301-DQA1*0501-DQB1*0201) positive haplotypes was significantly reduced by the presence of DPA1*0103-DPB1*0402 ($P_{adj} = 5 \times 10^{-3}$, OR = 0.4) and the infrequent DPA1*0103-DPB1*0101 ($P_{adj} = 5 \times 10^{-6}$, OR = 0.2) and increased by the presence of DPA1*0103-DPB1*0202 ($P_{adj} = 0.04$, OR = 2.5).

The effect of DP haplotypes on DR3 haplotypes was further divided into three categories based on the presence of the extended haplotypes A1-B8-DR3 (A*0101-B*0801-C*0701-DRB1*0301-DQA1*0501-DQB1*0201) and A30-B18-DR3 (A*3002-B*1801-C*0501-DRB1*0301-DQA1*0501-DQB1*0201) and "other" DR3 haplotypes. The susceptibility associated with the A1-B8-DR3 haplotype was significantly reduced by the presence of DPA1*0103-DPB1*0402 ($P_{adj} = 0.04$, OR = 0.4) and increased by the presence of DPA1*0103-DPB1*0301 ($P_{adj} = 0.03$, OR = 1.2) and the low frequency DPA1*0103-DPB1*0202 ($P_{adj} = 0.03$, OR = 3.5). DPA1*0103-DPB1*0202 was associated with susceptibility on the A30-B18-DR3 extended haplotype as well as on "other" DR3 haplotypes but did not reach statistical significance in these haplotype comparisons.

The presence of DPA1*0103-DPB1*0101 ($P_{adj} = 5 \times 10^{-6}$, OR = 0.1) but not DPA1*0201-DPB1*0101 significantly reduced the risk associated with "other" DR3 positive haplotypes with a nonoverlapping 95% CI (data not shown) and nonsignificantly reduced the risk associated

with the A1-B8-DR3 haplotype. On the extended A1-B8-DR3 haplotype the only difference between those carrying DPA1*0101-DPB1*0101 and DPA1*0201-DPB1*0101 is likely to be the DPA1 allele or a gene in linkage disequilibrium.

DISCUSSION

Previous association studies of HLA DP polymorphism with type 1 diabetes using a variety of typing approaches in different populations have indicated a susceptibility association with DPB1*0301 and *0202 and a protective association with DPB1*0402 as well as a number of conflicting associations. In this publication, the Type 1 Diabetes Genetic Consortium provides a large scale association analysis of DPA1, DPB1 allele and DPA1-DPB1 haplotypes in a new type 1 diabetes Caucasian family collection combined with existing collections that were re-typed for HLA using the same platform. Seventy four different DPA1-DPB1 haplotypes were observed; 19 DPB1 alleles were associated with multiple DPA1 alleles which permits, for the first time, a more precise type 1 diabetes association analysis for DP and an assessment of the role of DPA1.

A direct comparison of proband and AFBAC frequency distributions showed that the frequency of one DPA1, four DPB1 alleles and four DPA1-DPB1 haplotypes differed significantly when probands and AFBACs were compared; the most significant association was protection with DPA1*0103-DPB1*0402.

HLA-DP association analyses in type 1 diabetes are complicated by the extensive linkage disequilibrium across the HLA region, in particular with the HLA-DR-DQ region, which confers high risk for type 1 diabetes. In order to adjust for these factors, analyses were performed conditional on HLA-DRB1-DQA1-DQB1 and HLA-B-DRB1-DQA1-DQB1 linkage disequilibrium and in the presence or absence of the primary susceptibility and protective haplotypes (relative predispositional analysis).

The conditional analyses confirmed the previously described HLA-DP associations, now reported as DPA1-DPB1 haplotypes; susceptibility with DPB1*0301 (DPA1*0103-DPB1*0301), DPB1*0202 (DPA1*0103-DPB1*0202) and protection with DPB1*0402 (DPA1*0103-DPB1*0402). A novel haplotype association observed here, reflecting the potential role of DPA1, is the protective effect of DPB1*0101 in association with DPA1*0103 but not when linked to DPA1*0201. These alleles and haplotypes may either encode secondary class II HLA molecules involved in the presentation of a putative "diabetogenic peptide" or are in linkage disequilibrium with another putative causal gene.

The relative predispositional analysis, performed in the presence or absence of the high risk susceptibility and protective DR-DQ haplotypes, confirmed the association of susceptibility with DPA1*0103-DPB1*0301 across both groups, whereas protection with DPA1*0103-DPB1*0402 only reached statistical significance in the absence of the high risk susceptibility and protective DR-DQ haplotypes. The susceptibility association with DPA1*0103-DPB1*0202 and protective association with DPA1*0103-DPB1*0101 were observed only in the presence of the high risk susceptibility and protective DR-DQ haplotypes. This is consistent with the presence of DR3 positive haplotypes in this group.

The contribution of DP to DR3 positive haplotypes was further examined by comparing the risk associated with the presence or absence of the extended A1-B8-DR3 and A30-B18-DR3 haplotypes. The A1-B8-DR3 haplotype has previously been shown to be associated with a lower risk and the A30-B18-DR3 haplotype with a high risk to type 1 diabetes (35). The risk associated with the A1-B8-DR3 haplotype, which shows remarkable conservation of sequence between unrelated individuals (36), was significantly increased by the presence of DPA1*0103-DPB1*0301 and DPA1*0103-DPB1*0202 and reduced by the presence of DPA1*0103-DPB1*0402. DPB1 allele differences therefore appear to stratify the susceptibility risk on an otherwise conserved HLA haplotype. In contrast only a single DP haplotypic association with DR4-DQB1*0302 positive haplotypes was observed; the DRB1*0401-DQA1*0301-DQB1*0302 haplotype was significantly reduced by the presence of DPA1*0103-DPB1*0101. The DP haplotype *0103-*0202 is also increased among patient A30-B18-DR3 haplotypes, but the difference does not reach statistical significance, perhaps due to a power issue as DPA1*0103-DPB1*0202 has previously been associated with increased susceptibility when not in association with A30-B18-DR3 in Filipino type 1 diabetic patients (37). The risk associated with the remaining DR3 positive haplotypes was significantly decreased by the presence of DPA1*0103-DPB1*0101 but not by DPA1*0201-DPB1*0101 with nonoverlapping confidence intervals. It is plausible that HLA-DPA1 or another gene in linkage disequilibrium may contribute to the significant differences in associated risk. DPA1 is the closest expressed gene centromeric to HLA-DOA, which has recently been significantly associated with type 1 diabetes risk in a study that initially utilized the conservation of the A30-B18-DR3 haplotype to examine other DR3 positive haplotypes (38).

A comparison of the amino acid sequences of DPA1*0103 and DPA1*0201 shows they differ by two substitutions at positions 31 (M versus Q) and 50 (Q versus R); the latter substitution results in a charge difference with a limited effect on peptide binding (39). However, the same DPA1 allele difference in association with DPB1*0301 has been observed to significantly alter T cell recognition (40) and monoclonal antibody recognition (41), suggesting that these DPA1 differences do contribute to functional and antigenic differences. Evidence for the potential role of individual amino acids to type 1 diabetes susceptibility is also highlighted by the protective association of DPA1*0103-DPB1*0402 and the susceptibility association of DPA1*0103-DPB1*0201. A comparison between the two haplotype sequences reveals a single K to E difference at position 69 of DPB1. It is of note that a second susceptibility association, DPA1*0103-DPB1*0202, is also E positive at position 69, suggesting a critical role for this residue. This substitution has previously been implicated in influencing T cell recognition (12,42,43) and changing peptide binding properties, resulting in an increase in the affinity of pocket four for positively charged aromatic and polar residues (44). The corresponding position in the DR beta chain, amino acid residue 71, has been shown to strongly influence peptide binding in autoimmune related DRB1 alleles (45) and pocket four to be important in determining susceptibility of DRB1*04 alleles in type 1 diabetes (46).

The observations reported here confirm and further delineate the contributory role of DPA1 and DPB1 allelic and haplotypic diversity with the risk associated to type 1 diabetes. Susceptibility is associated with DPB1*0301

(DPA1*0103-DPB1*0301) and DPB1*0202 (DPA1*0103-DPB1*0202) and protection with DPB1*0402 (DPA1*0103-DPB1*0402) and DPA1*0103-DPB1*0101 but not DPA1**0201*-DPB1*0101. The pattern of DP association on several different DR-DQ haplotypes is consistent with the notion that specific DPA1 and DPB1 alleles play a potential causal role in type 1 diabetes susceptibility rather than as haplotype markers. Additional evidence is presented to suggest that individual amino acid substitutions may influence type 1 diabetes risk.

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