HLA DR-DQ Haplotypes and Genotypes and Type 1 Diabetes Risk

Analysis of the Type 1 Diabetes Genetics Consortium Families

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OBJECTIVE—The Type 1 Diabetes Genetics Consortium has collected type 1 diabetic families worldwide for genetic analysis. The major genetic determinants of type 1 diabetes are alleles at the HLA-DRB1 and DQB1 loci, with both susceptible and protective DR-DQ haplotypes present in all human populations. The aim of this study is to estimate the risk conferred by specific DR-DQ haplotypes and genotypes.

RESEARCH DESIGN AND METHODS:—Six hundred and seven Caucasian families and 38 Asian families were typed at high resolution for the DRB1, DQA1, and DQB1 loci. The association analysis was performed by comparing the frequency of DR-DQ haplotypes among the chromosomes transmitted to an affected child with the frequency of chromosomes not transmitted to any affected child.

RESULTS—A number of susceptible, neutral, and protective DR-DQ haplotypes have been identified, and a statistically significant hierarchy of type 1 diabetes risk has been established. The most susceptible haplotypes are the DRB1*0301-DQA1*0501-DQB1*0201 (odds ratio [OR] 3.64) and the DRB1*0405-DQA1*0301-DQB1*0302, DRB1*0401-DQA1*0301-DQB*0302, and DRB1*0402-DQA1*0301-DQB1*0302 haplotypes (ORs 11.37, 8.39, and 3.63), followed by the DRB1*0404-DQA1*0301-DQB1*0302 (OR 1.59) and the DRB1*0801-DQB1*0401-DQB1*0402 (OR 1.25) haplotypes. The most protective haplotypes are DRB1*1501-DQA1*0102-DQB1*0602 (OR 0.03), DRB1*1401-DQA1*0101-DQB1*0503 (OR 0.02), and DRB1*0701-DQA1*0201-DQB1*0303 (OR 0.02).

CONCLUSIONS—Specific combinations of alleles at the DRB1, DQA1, and DQB1 loci determine the extent of haplotypic risk. The comparison of closely related DR-DQ haplotype pairs with different type 1 diabetes risks allowed identification of specific amino acid positions critical in determining disease susceptibility. These data also indicate that the risk associated with specific HLA haplotypes can be influenced by the genotype context and that the *trans*-complementing heterodimer encoded by DQA1*0501 and DQB1*0302 confers very high risk. ***Diabetes* 57: 1084–1092, 2008**

Type 1 diabetes is a common autoimmune disorder resulting from the immunological destruction of the insulin-producing β-cells of the pancreas, leading to dysregulation of glucose metabolism. Type 1 diabetes clusters in families with an overall genetic risk ratio (λ-s) of ~15 (1). The concordance of type 1 diabetes among monozygotic and dizygotic twins argues for a strong genetic determinant of disease and a significant environmental factor required to elicit the disease in genetically predisposed individuals. Approximately 40–50% of the familial clustering of type 1 diabetes can be attributed to allelic variation in the HLA region, and a recent linkage analysis reported a logarithm of odds score of 116 (genome-wide P value $<1.0 \times 10^{-4}$) for this region (2). A large number of studies have demonstrated that specific alleles at the DRB1, DQA1, and DQB1 loci are strongly associated with type 1 diabetes (3–7). However, allelic variation at these loci cannot account fully for the pattern of HLA haplotype sharing among affected sibpairs (8). Moreover, the association analysis of other HLA loci (class I and DPB1) and other polymorphisms within the HLA region has revealed the presence of additional type 1 diabetes susceptibility loci in this region (9–19). To aid in the search for additional type 1 diabetes genes within and outside the HLA region, an international collaboration (the Type 1 Diabetes Genetics Consortium) has collected and is continuing to collect a large number of type 1 diabetic families (multiplex and simplex) from various populations (20). These samples were genotyped at high resolution for all classical HLA loci at three genotyping centers. The large sample size of this study allows stratification analysis for haplotypes and genotypes, allowing, in turn, the investigation of DR-DQ genotype context effects suggested by previous smaller studies (4,21,22). This sample size also allows statistically significant estimates of risk for individual DR-DQ haplotypes and the establishment of a risk hierarchy ranging from highly predisposing to highly pro-

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AFBAC, affected family-based control; SNP, single nucleotide polymorphism.

TABLE 1
Descriptive characteristics of the study subjects

Ethnicity	Origin	Families	Affected siblings per family	Sex (% women)	Age at onset
Caucasian	Australia New Zealand	14.6 (94)	2.03 ± 0.29	51.93	10.34 ± 8.08
	Europe	49.4 (318)	2.01 ± 0.08	61.54	12.28 ± 4.99
	North America	29.9 (193)	1.99 ± 0.20	45.18	10.66 ± 7.19
	Other	0.2 (1)	2.00 ± 0.00	44.35	8.33 ± 5.93
Non-Caucasian	East Asian	5.9 (38)	1.05 ± 0.23	0.00	10.50 ± 7.78

Data are % (n) or means ± SD.

tective. The availability of these haplotype type 1 diabetes risk estimates allows the analysis of closely related DR-DQ haplotype pairs that differ significantly in risk to identify specific amino acid residues that are critical in determining disease susceptibility.

Given the strong effect of the DR and DQ alleles on type 1 diabetes risk, and the strong linkage disequilibrium within the HLA region, the data presented here will provide the framework for the analysis of major histocompatibility complex single nucleotide polymorphism (SNP) and microsatellite markers and of the HLA class I and DP alleles. Such future analyses will require stratification and adjustment of the association data conditional on the HLA-DR and DQ alleles and genotypes.

RESEARCH DESIGN AND METHODS

The subjects included in this dataset (April 2006 data freeze) comprise newly collected samples and do not include previously collected families from the Human Biological Data Interchange. Thus, these data represent an independent cohort for evaluating associations seen in the Human Biological Data Interchange family collection (4). The descriptive characteristics of the study population are shown in Table 1 and in Supplementary Table 1, which is detailed in the online appendix (available at http://dx.doi.org/10.2337/db07-1331). The Caucasian families were recruited in Europe, North America, and Australia/New Zealand and consisted of two parents and at least two affected siblings. Asian families, recruited primarily from the Philippines, included both simplex and multiplex families. HLA DR-DQ haplotypes were determined by familial transmission.

Genotyping methods. HLA genotyping was performed with a PCR-based sequence-specific oligonucleotide probe system. Oligonucleotide probes, corresponding to known polymorphic sequence motifs in the HLA genes, were immobilized onto a nylon membrane. Relevant polymorphic exons (exon 2 for HLA class II genes; exons 2 and 3 for HLA class I genes) were amplified with biotinylated PCR primers, denatured, and hybridized to the immobilized probe array. After hybridization and wash, arrays were incubated with streptavidin–horseradish peroxidase, followed by the chromogenic substrate tetramethylbenzidine. Images of results obtained by a colorimetric reaction were created with a flatbed scanner, and probe intensities were measured as pixel values with a proprietary genotyping software, StripScan. Preliminary genotypes were determined with StripScan, and then data from StripScan were imported into Sequence Compilation and Rearrangement Evaluation (SCORE) software for final genotype calling and export of data to the coordinating center.

HLA genotyping was performed at four sites in three geographic regions, including Oakland and Alameda, California; Melbourne, Australia; and Malmö, Sweden. Equipment and reagents were standardized for all laboratories, with initial certification and annual genotyping exercises conducted to assure concordance among laboratories. HLA genotyping data are presented at the four-digit level (i.e., DRB1*0101) so that synonymous polymorphisms, which are documented in the 5th and 6th digits, are not reported. After an initial training session, DNA from a panel of 40 selected cell lines with known HLA genotypes at all loci was used for certification. Within the limitations of the ambiguities (see below), there was 100% agreement with known HLA types. Selected samples from each network of the Type 1 Diabetes Genetics Consortium recruitment have also been reused as an ongoing blinded internal quality control procedure. The overall agreement between sites (total of four analyses per sample) for 372 samples (744 alleles) on 93 analysis plates was 100% for DQA1, 99.6% for DQB1, and 98.2% for DRB1 for a total of 99.3% overall. For simplicity, we have also used the general serologic nomenclature, for example, DR3 and DR4, to refer to haplotypes bearing the DRB1*03 and DRB1*04 alleles.

Because this typing system did not target all polymorphic sites, some alleles were not distinguished. For example, DQB1*0201, found on DR3 haplotypes, and DQB1*0202, found on DR7 haplotypes, have the same probe reactivity pattern in our typing system because the distinguishing polymorphism is located in the third exon. To address this issue, we have adopted the convention suggested by Cano et al. (23) of referring to the probe reactivity pattern consistent with these two common DQB1 alleles and with the very rare allele DQB1*0204 as DQB1*0201g, a group designation encompassing all DQB1 alleles with the same second exon sequence (for DQA1 and DQB1 designations, see Supplementary Table 2).

Statistical analysis. Data from these family-based samples allowed unambiguous assignment of alleles at three-locus haplotypes (DRB1-DQA1-DQB1) in all families. Control haplotypes were determined by the affected family-based control (AFBAC) method, based on haplotypes not transmitted to any affected child (24). This control population provides an unbiased estimate of the overall population (control) HLA allele and haplotype frequencies under the assumption, which is reasonable in this case, of zero recombination between the marker and the disease loci. The statistical significance of differences in allele/haplotype/genotype frequencies between type 1 diabetes cases (probands) and AFBACs or between subgroups of patients was assessed using a Pearson's χ^2 test.

RESULTS

Association of DR-DQ haplotypes. The distribution of DRB1-DQA1-DQB1 haplotypes among the probands and the AFBAC chromosomes (never transmitted to affected) is shown in Tables 2 (Caucasian) and 3 (East Asian). We have analyzed the disease associations at the level of these three-locus haplotypes rather than that of individual loci because it has been well established that the extent of type 1 diabetes risk is determined by specific combinations of DRB1, DQA1, and DQB1 alleles (3,6,7). For example, the DRB1*0401-DQA1*0301g-DQB1*0302 haplotype has an odds ratio (OR) of 8.39 while the DRB1*0401-DQA1*0301g-DQB1*0301 has an OR of 0.35 (Table 2), implicating the DQB1*0302 allele as a critical susceptibility allele. However, comparing the type 1 diabetes risk of DRB1*0401-DQA1*0301g-DQB1*0302 with the protective DRB1*0403-DQA1*0301-DQB1*0302 (OR 0.27) reveals the risk conferred by DRB1 alleles. These comparisons illustrate the importance of both DRB1 and DQB1 alleles in determining type 1 diabetes risk in an epistatic interaction. There is also considerable evidence that allelic variation at other HLA loci can modulate the risk conferred by specific DR-DQ haplotypes (9,11,13–16,18); the effects of other loci within the HLA region on type 1 diabetes risk in this dataset will be the subject of subsequent reports from the Type 1 Diabetes Genetics Consortium.

Although 44 different DR-DQ haplotypes ($n \geq 2$) were identified in this dataset (Table 2), highly significant P values for the association of many individual haplotypes and narrow CIs for the OR estimates were achieved. One of the striking patterns of HLA-DR-DQ type 1 diabetes associations, observed in this and in previously reported datasets, is the multiplicity of highly associated DR-DQ haplotypes and their risk hierarchy, ranging from highly predisposing to highly protective.

TABLE 2
Frequency distribution of DRB1-DQB1 haplotypes in Caucasian probands and in AFBAC

DRB1	DQA1	DQB1	AFBAC	Proband	*Trans*	OR (95% CI)	χ^2	*P* value	Effect on T1D*
0101	0101	0501	9.0	6.6	44.6	0.71 (0.52–0.98)	3.96	0.047	
0101	0102	0504	0.2	0.1	50.0	0.37 (0.03–4.08)	0.70	0.40	
0102	0101	0501	1.0	0.7	32.5	0.66 (0.25–1.71)	0.74	0.39	
0103	0101	0501	0.6	0.1	25.0	0.15 (0.02–1.26)	3.99	0.046	
0301	0501	0201	12.5	34.1	70.9	3.64 (2.89–4.58)	95.33	2×10^{-22}	S3
0401	0301	0201	0.1	0.2	57.1	1.48 (0.13–16.37)	0.10	0.75	
0401	0301	0301	3.9	1.4	33.6	0.35 (0.20–0.63)	12.78	4×10^{-4}	
0401	0301	0302	4.5	28.1	84.5	8.39 (5.97–11.80)	156.73	6×10^{-36}	S2
0402	0301	0302	1.0	3.5	73.3	3.63 (1.76–7.49)	13.28	3×10^{-4}	S4
0403	0301	0302	1.2	0.3	13.3	0.27 (0.08–0.84)	5.69	0.017	
0404	0301	0302	3.2	5.0	57.1	1.59 (1.01–2.49)	3.88	0.049	S5
0404	0301	0402	0.1	0.2	50.0	1.48 (0.13–16.37)	0.10	0.75	
0405	0301	0201	0.3	0.5	42.1	1.48 (0.37–5.95)	0.31	0.58	
0405	0301	0302	0.2	2.5	87.3	11.37 (2.71–47.68)	16.88	4×10^{-5}	S1
0407	0301	0301	1.4	0.2	8.0	0.11 (0.03–0.50)	11.68	6×10^{-4}	
0408	0301	0301	0.4	0.1	20.0	0.18 (0.02–1.65)	2.81	0.094	
0408	0301	0304	0.3	0.7	56.5	1.98 (0.52–7.49)	1.03	0.31	
0701	0201	0201	10.1	3.5	28.2	0.32 (0.22–0.46)	35.67	2×10^{-9}	
0701	0201	0303	4.3	0.1	3.6	0.02 (0.00–0.13)	48.33	4×10^{-12}	P1
0801	0301	0302	0.0	0.2	71.4		2.17	0.14	
0801	0401	0402	2.4	3.1	50.0	1.25 (0.73–2.14)	0.68	0.41	
0803	0601	0301	0.3	0.0	0.0	0.00		3.96	0.047
0804	0401	0402	0.2	0.2	50.0	0.74 (0.10–5.26)	0.09	0.76	
0901	0301	0201	0.0	0.2	100.0		1.45	0.23	
0901	0301	0303	1.6	0.8	45.2	0.53 (0.23–1.19)	2.40	0.12	
1001	0101	0501	0.7	0.3	26.1	0.49 (0.14–1.75)	1.22	0.27	
1101	0501	0301	6.5	1.2	17.9	0.18 (0.10–0.32)	39.80	3×10^{-10}	
1102	0501	0301	0.4	0.2	23.1	0.37 (0.07–2.02)	1.40	0.24	
1103	0501	0301	1.0	0.2	25.0	0.25 (0.07–0.91)	5.06	0.024	
1104	0103	0603	0.3	0.1	12.5	0.25 (0.03–2.37)	1.69	0.19	
1104	0501	0301	2.3	0.2	6.3	0.07 (0.02–0.30)	21.88	3×10^{-6}	P4
1201	0501	0301	1.1	0.3	30.3	0.29 (0.09–0.94)	4.68	0.031	
1301	0103	0603	5.9	0.8	14.8	0.13 (0.07–0.26)	43.59	4×10^{-11}	
1302	0102	0604	2.6	2.2	46.1	0.87 (0.49–1.52)	0.26	0.61	
1302	0102	0609	0.3	0.0	22.2	0.00	3.96	0.047	
1303	0501	0301	1.0	0.1	10.0	0.08 (0.01–0.64)	9.01	0.003	P5
1401	0101	0503	2.1	0.0	0.0	0.02 (0.00–0.32)	23.51	1×10^{-6}	P2
1404	0101	0503	0.1	0.1	50.0	0.74 (0.05–11.85)	0.05	0.83	
1501	0102	0501	0.2	0.0	0.0	0.00	2.64	0.10	
1501	0102	0602	12.0	0.4	3.9	0.03 (0.01–0.07)	127.14	2×10^{-29}	P3
1501	0102	0603	0.3	0.0	0.0	0.00	3.96	0.047	
1502	0103	0601	0.7	0.2	13.3	0.25 (0.05–1.22)	3.37	0.066	
1601	0102	0502	2.0	1.0	35.7	0.49 (0.23–1.02)	3.67	0.055	
1602	0102	0502	0.1	0.1	50.0	0.74 (0.05–11.85)	0.05	0.83	
Total			898	1,214			721.72	5×10^{-124}	

Data are %. *For haplotypes with a frequency >1% in either probands or control subjects, the five most susceptible (S) and the five most protective (P) haplotypes are indicated. T1D, type 1 diabetes.

Risk hierarchy of DR-DQ haplotypes. The most susceptible haplotypes in this dataset are the DRB1*0301-DQA1*0501g-DQB1*0201g haplotype (OR 3.64; $P = 2 \times 10^{-22}$) and the DR4 haplotypes DRB1*0405-DQA1*0301g-DQB1*0302 (11.37; $P = 4 \times 10^{-05}$), DRB1*0401-DQA1*0301g-DQB1*0302 (8.39; $P = 6 \times 10^{-36}$), and DRB1*0402-DQA1*0301g-DQB1*0302 (3.63; $P = 3 \times 10^{-4}$). The other common DR4 haplotype, DRB1*0404-DQA1*0301g-DQB1*0302, is only moderately predisposing (1.59; $P = 0.049$), and the DRB1*0401-DQA1*0301g-DQB1*0301 haplotype appears neutral or moderately protective (see below).

There is also a hierarchy of risk among the relatively neutral DR-DQ haplotypes. Table 4 shows the estimated ORs for seven selected DR-DQ haplotypes, with ORs ranging from 0.3 to 1.25 in Table 2, after removal of the high-risk DR3 and DR4 and the protective haplotypes, an analysis termed relative predispositional effect (25). Clearly, the DRB1*0801-DQA1*0401g-DQB1*0402 haplotype is the next most predisposing after the DR3 and DR4 haplotypes, followed by DRB1*1302-DQA1*0102-DQB1*0604 and DRB1*0101-DQA1*0101g-DQB1*0501. The other four haplotypes (DRB1*0901-DQA1*0301g-DQB1*0303, DRB1*1601-DQA1*0102g-DQB1*0502, DRB1*0401-DQA1*0301g-DQB1*0301, and DRB1*0701-DQA1*0201-DQB1*0201g) appear to be neutral or moderately protective in this dataset with respect to type 1 diabetes risk. The risk hierarchy (ranking) of these seven DR-DQ haplotypes is the same with or without the removal of the high-risk and the protective DR-DQ haplotypes.

TABLE 3
Frequency distribution of DRB1-DQB1 haplotypes in East-Asian families and in AFBAC

DRB1	DQA1	DQB1	AFBAC	Proband	OR (95% CI)	χ^2	*P* value
0301	0501	0201	2.8	22.4	10.08 (2.24–45.45)	11.05	0.0009
0402	0301	0302	0.0	1.3	3.83 (0.05–310.02)	0.41	0.5216
0403	0301	0302	5.6	2.6	0.46 (0.08–2.59)	0.78	0.3772
0405	0103	0503	4.2	1.3	0.31 (0.03–3.02)	1.11	0.2917
0405	0301	0401	2.8	6.6	2.46 (0.46–13.13)	1.13	0.2879
0405	0301	0402	0.0	5.3	15.94 (0.28–918.70)	3.11	0.0777
0406	0301	0302	1.4	1.3	0.95 (0.06–15.42)	0.00	0.9695
0408	0301	0304	0.0	1.3	3.83 (0.05–310.02)	0.41	0.5216
0410	0301	0302	0.0	1.3	3.83 (0.05–310.02)	0.41	0.5216
0410	0301	0402	1.4	0.0	0.23 (0.00–18.98)	0.49	0.4831
0441	0301	0302	1.4	0.0	0.23 (0.00–18.98)	0.49	0.4831
0701	0201	0201	2.8	1.3	0.47 (0.04–5.26)	0.39	0.5324
0701	0201	0303	2.8	0.0	0.12 (0.00–7.48)	1.46	0.2272
0803	0103	0601	1.4	0.0	0.23 (0.00–18.98)	0.49	0.4831
0901	0301	0303	5.6	11.8	2.28 (0.67–7.78)	1.66	0.1971
1101	0103	0601	2.8	0.0	0.12 (0.00–7.48)	1.46	0.2272
1101	0501	0301	5.6	1.3	0.23 (0.02–2.08)	1.97	0.1607
1201	0501	0301	1.4	0.0	0.23 (0.00–18.98)	0.49	0.4831
1202	0102	0502	0.0	1.3	3.83 (0.05–310.02)	0.41	0.5216
1202	0601	0301	18.1	3.9	0.19 (0.05 0.69)	6.81	0.0091
1302	0102	0609	0.0	1.3	3.83 (0.05–310.02)	0.41	0.5216
1404	0101	0503	1.4	3.9	2.92 (0.30–28.72)	0.90	0.3440
1501	0102	0502	1.4	2.6	1.92 (0.17–21.63)	0.28	0.5956
1501	0102	0602	1.4	0.0	0.23 (0.00–18.98)	0.49	0.4831
1502	0101	0501	13.9	1.3	0.08 (0.01–0.66)	7.86	0.0050
1502	0102	0502	22.2	27.6	1.34 (0.63–2.83)	0.43	0.5106
Total			72	76		44.92	0.0085

Data are %.

These observations are generally consistent with previous reports in Caucasians (rev. in 22,26,27). In studies of Asian populations, in which the DRB1*0901-DQA1*0301g-DQB1*0303 haplotype is much more frequent, this haplotype is associated with type 1 diabetes in many studies (Table 3) (28,5,29) but not all (30).

The three most protective haplotypes are DRB1*1501-DQA1*0102-DQB1*0602 (OR 0.03; $P = 2 \times 10^{-29}$), DRB1*1401-DQA1*0101-DQB*0503 (0.02; $P = 1 \times 10^{-6}$), and DRB1*0701-DQA1*0201-DQB1*0303 (0.02; $P = 4 \times 10^{-12}$). Among the moderately protective haplotypes, the most common are DRB1*0701-DQA1*0201-DQB1*0201g (0.32; $P = 2 \times 10^{-9}$), DRB1*1301-DQA1*0103-DQB1*0603 (0.13; $P = 1 \times 10^{-6}$), DRB1*1101-DQA1*0501-DQB1*0301 (0.18; $P = 3 \times 10^{-10}$), and the closely related DRB1*1104-DQA1*0501-DQB1*0301 (0.07; $P = 3 \times 10^{-6}$). All of the DR-DQ haplotypes (DR11, -12, and -13) containing the DQA1*0501-DQB1*0301 alleles appear to be protective in this dataset. The protective association of DRB1*1202-DQA1*0601 (closely related to *0501) -DQB1*0301 reaches nominal statistical significance ($P = 0.0091$), even in this small Asian dataset (Table 3). DRB1*0403-DQA1*0301-DQB1*0302, which is rare among Caucasians but common among Asians, is protective in both groups, as noted above. The protective DRB1*0406-DQA1*0301-DQB1*0302 haplotype is restricted to Asian populations (28) and appears too infrequently in this Asian dataset to assess type 1 diabetes risk.

DR-DQ diplotype risk. The genotypic risk for DR-DQ diplotypes consisting of the high-risk DRB1*0301 and *04 haplotypes and the four DR-DQ haplotypes from Table 4 with ORs >1.0 is shown in Supplementary Table 3. The ORs are estimated by comparing the observed frequency of diplotypes among probands with the estimated frequency

TABLE 4
Relative predispositional effects of seven DR-DQ haplotypes

Haplotype	AFBAC	Type 1 diabetes	Association in all samples			Association excluding DR3, DR4, and protective haplotypes*		
			OR (95% CI)	*P* value	Rank	OR (95% CI)	*P* value	Rank
DRB1*0801 DQA1*0401 DQB1*0402	2.45	3.05	1.25 (0.73–2.14)	0.41	1	2.58 (1.48–4.48)	0.0013	1
DRB1*1302 DQA1*0102 DQB1*0604	2.56	2.23	0.87 (0.49–1.52)	0.61	2	1.72 (0.96–3.07)	0.076	2
DRB1*0101 DQA1*0101 DQB1*0501	9.02	6.60	0.71 (0.52–0.98)	0.047	3	1.55 (1.08–2.22)	0.029	3
DRB1*0901 DQA1*0301 DQB1*0303	1.56	0.82	0.53 (0.23–1.19)	0.12	4	1.00 (0.44–2.28)	0.73	4
DRB1*1601 DQA1*0102 DQB1*0502	2.00	0.99	0.49 (0.23–1.02)	0.055	5	0.93 (0.44–1.96)	0.66	5
DRB1*0401 DQA1*0301 DQB1*0301	3.90	1.40	0.35 (0.20–0.63)	4×10^{-4}	6	0.65 (0.36–1.20)	0.17	6
DRB1*0701 DQA1*0201 DQB1*0201	10.10	3.50	0.32 (0.22–0.46)	2×10^{-9}	7	0.58 (0.38–0.87)	0.015	7

Data are %. *The protective haplotypes excluded are DRB1*150x DQB1*060x; all DRB1*11, -*12, -*13, and -*14 haplotypes with DQB1*0301; DRB1*140x DQB1*050x; and DRB1*0701 DQB1*0303 haplotypes.

TABLE 5
Amino acid differences between closely related DR-DQ haplotypes that differ in type 1 diabetes risk

DRB1*1301-DQA1–0103-DQB1*0603 (OR 0.13)	vs.	DRB1*1302-DQA1*0102-DQB1*0604 (OR 0.87)
DRB1 Val-86		Gly-86
DQA1 Arg-41		Lys-41
DQB1 Asp-57, Gly-70, Ala-86		Val-57, Arg-70, Gly-86
DRB1*0401-DQA1*0301g-DQB1*0302 (OR 8.39)	vs.	DQB1*0404-DQA1*0301g-DQB1*0302 (OR 1.59)
DRB1 Lys-71, Gly-86		Arg-71, Val-86
DRB1*1502-DQA1*0101g-DQB1*0501 (OR 0.08)	vs.	DRB1*1502-DQA1*0102-DQB1*0502 (OR 1.34)
DQA1 Glu-34		Gln-34
DQB1 Val-57		Ser-57

of diplotypes, based on observed AFBAC haplotype frequencies and Hardy-Weinberg equilibrium assumptions. Because the estimated "control" frequencies are low, the CIs are broad, and a statistically significant hierarchy of risk for all diplotypes cannot be established from these data, but clearly, the DR3/4, the DR4/4, and DR4/8 diplotypes (excluding DRB1*0404) have the highest risk. The estimated risk for the DR3/3 diplotype is somewhat lower than in other studies (31); this difference may reflect the well-known risk heterogeneity of the DR3 haplotype (32,33), which implicates loci other than DRB1, DQA1, and DQB1 in determining the extent of risk on DR3 haplotypes.

Type 1 diabetes risk in closely related DR-DQ haplotypes. As noted above, analysis of the type 1 diabetes association of various DR-DQ haplotypes has clearly established that type 1 diabetes risk is determined by specific combinations of DRB1, DQA1, and DQB1 alleles. In some cases, the risk for type 1 diabetes differs significantly between two closely related DR-DQ haplotypes, implicating specific alleles and polymorphisms in disease susceptibility. Comparing the sequences of such haplotype pairs can be instructive in that potentially important polymorphic amino acid residues that distinguish the two haplotypes can be identified.

Among the protective DR-DQ haplotypes, the DR7 haplotype bearing DQB1*0303 is significantly more protective than the one bearing DQB1*0201g (OR 0.02 [95% CI 0.00–0.13] vs. 0.32 [0.22–0.46]). The DRB1 and DQA1 alleles are identical, and the DQB1 alleles differ at 11 amino acid positions encoded in the second exon, including Ala-57 for *0201g vs. Asp-57 for *0303, consistent with the correlation between the presence of Asp-57 and protection, noted by Todd et al. (34) and Horn et al. (35).

The type 1 diabetes risk for the protective DRB1*1301-DQA1*0103-DQB1*0603 (OR 0.13 [95% CI 0.07–0.26]) and the moderately predisposing/neutral DRB1*1302-DQA1*0102-DQB1*0604 haplotype (0.87 [0.49–1.52]) is significantly different. These haplotypes differ at all three loci, but the alleles differ by only one to three amino acid residues (Table 5).

In this dataset, the two most common DR4 haplotypes have ORs with nonoverlapping CIs. The DRB1*0401-DQA1*0301g-DQB1*0302 haplotype (OR 8.39 [95% CI 5.97–11.80]) and the DRB1*0404-DQA1*0301g-DQB1*0302 (1.59 [1.01–2.49]) differ only at amino acid positions 71 and 86 (Lys-Gly vs. Arg-Val) of DRB1 (Table 5). Position 86 contributes to pocket 1 while position 71 contributes to pockets 4 and 7 of the peptide-binding groove.

Among the Asian haplotypes (Table 3), the DRB1*1502-DQA1*0101-DQB1*0501 is protective (OR 0.08 [95% CI 0.01–0.66]), whereas the DRB1*1502-DQA1*0102-DQB1*0502 appears neutral or slightly predisposing (1.34 [0.63–2.83]). Although the Asian population sample in this dataset is relatively small, a 2×2 table analysis indicates that these two common DR2 haplotypes, which differ only at DQA1 position 34 (Glu vs. Gln) and DQB1 position 57 (Val vs. Ser) (Table 5), confer different risk for type 1 diabetes ($P = 0.015$), consistent with the results of an earlier case-control study of Filipino type 1 diabetes (5), which showed that DRB1*1502-DQB1*0501 was significantly protective but that DRB1*1502-DQB1*0502 was not. This Asian protective haplotype with DQB1 Val-57 represents an exception to the "general rule" that protective haplotypes encode DQB1 Asp-57. Another exception to this pattern are the susceptible Asian haplotypes DRB1*0405-DQA1*0301-DQB1*0401 and -*0402 haplotypes (Table 3), which also encode Asp-57. We note that these two DQB1 alleles are also exceptional in that they are the only alleles encoding a Leu, rather than a Pro, at position 56.

Genotype effects and *trans*-complementing DQ heterodimers. The specific combination of DR-DQ haplotypes (i.e., diplotypes) is also known to affect type 1 diabetes risk. In many different studies of type 1 diabetes among Europeans, the risk of DR3/DR4 heterozygotes is higher than that of DR3/3 and DR4/4 homozygotes (22,36–39). The DR3/DR4 heterozygous genotype can produce two DQ heterodimers encoded *in cis* and two encoded *in trans* (the product of DQA1*0301 from the DR4 haplotype combined with the product of DQB1*0201g from the DR3 haplotype, as well as the product of DQA1*0501 from the DR3 haplotype paired with the product of DQB1*0302 from the DR4 haplotype). One explanation for the extremely high risk of the DR3/DR4 haplotype is that one or both of the DQ molecules encoded *in trans* confers greater type 1 diabetes risk than either of the DQ molecules encoded *in cis*.

Comparison of DR3/4s in European ancestry and Asian populations supports the idea that the *trans*-encoded molecule produced by DQA1*0501 and DQB1*0302 may confer the highest risk. The frequency of DR3/4s in all European ancestry patients ($n = 1,220$) is 38.1%; virtually all (99.4%) of these patients carry a DQB1*0302 allele on the DR4 haplotype. The proportion of patients with the DR3/4-DQB1*0302 genotype is similar in all three European ancestry populations (36.7% in the U.S., 36.5% in Europe, and 45.0% in Australia, among probands), but this genotype is found in only 2.5% in the general European ancestry population (40). However, as observed in previous analyses of Filipino type 1 diabetic patients, in whom the risk of the DR3/4 genotype was less than that of DR3/3 and DR3/9 genotypes (5), the frequency of the DR3/4 genotype is not dramatically increased among Asian patients in this dataset (6 of 41 or 14.6%). The small Asian sample size (Table 3) means that the risk estimate for DR3/4 genotypes, based on estimating control frequencies from the AFBAC haplotype frequencies, although approx-

TABLE 6
DQB1 alleles on DR4 haplotypes in DR4$^+$ patient genotypes

Genotype	DQB1*0201	DQB1*0301	DQB1*0302	DQB1*0304	DQB1*0400	Total†
DR1/DR4	0.0	13.1	81.8	5.1	0.0	99
DR3/DR4	0.4	0.7	98.5	0.4	0.0	453
DR4/DR4	1.6	5.6	89.3	1.6	2.0	252
DR4/DR8	0.0	2.5	97.5	0.0	0.0	40

Data are %. †Data are *n*.

imately twofold lower than in Caucasians, has wide CIs. Among Asian patients, as in the analysis of Caucasian patients (see below; Table 6), the distribution of DQB1*0302 alleles among DR4$^+$ patients is instructive. Only two of these six DR3/4 patients carried the DQB1*0302 allele, but these were the only two susceptible DRB1*04-DQB1*0302 haplotypes in this dataset. In the Filipino type 1 diabetes case-control study (5), 3 of 8 DR3/4 patients carried a susceptible DRB1*04-DQB1*0302 haplotype, whereas among the 3 DR4/4 and 20 DR4/X patients, only 3 carried the DQB1*0302 allele. Thus, although the proportion of Asian DR3/4 patients carrying the DQB1*0302 allele is much lower than that of Caucasian DR3/4 patients (Table 6), the Asian DR3/4 patients are enriched for the DQB1*0302 allele relative to the Asian DR4/4 and DR4/X patients.

We attribute the absence of the high-risk DR3/4 effect among this and other Asian populations (5) to the very low frequency of DR4 haplotypes (other than the protective DRB1*0403 and *0406 haplotypes) carrying the DQB1*0302 allele. The common high-risk Asian DR4 haplotypes are DRB1*0405-DQA1*0301-DQB1*0401 and DRB1*0405-DQA1*0301-DQB1*0402 (Table 3) (5,28,30).

This interpretation argues that the very high risk associated with the DR3/4 genotype in Caucasian populations reflects the critical importance of the *trans*-complementing α-β heterodimer encoded by the DQA1*0501 allele from the DR3 haplotype and the DQB1*0302 allele on the DR4 haplotype and that this risk is greater than that conferred by the other *trans*-complementing DQ α-β heterodimer encoded by the DQA1*0301 allele from the DR4 haplotype and the DQB1*0201g allele from the DR3 haplotype. This DQ heterodimer, like the one encoded *in cis* by DQA1*0301 and DQB1*0302, may confer type 1 diabetes risk as well (41) but to a lesser degree (22) (Table 2). The DQA1*0301 and DQB1*0201g alleles are present *in cis* on some type 1 diabetes–associated DRB1*0405, DRB1*0701, and DRB1*0901 haplotypes.

Moreover, this DQ heterodimer (DQA1*0301-DQB1*0201g) is encoded *in trans* in all of the Asian DR3/4 heterozygotes; the lower type 1 diabetes risk associated with these Asian DR3/4 heterozygotes compared with the European ancestry DR3/4s with DQB1*0302 argues that the high-risk DR3/4 effect reflects primarily the high risk conferred by the DQA1*0501-DQB1*0302 heterodimer. We note that in these DR3/4 heterozygotes, four different DQ heterodimers encoded *in cis* and *in trans* may contribute to high type 1 diabetes risk, but as discussed above, we propose that DQA1*0501-DQB1*0302 confers the greatest risk.

Additional evidence supporting the high-risk DQA1*0501-DQB1*0302 heterodimer model is provided by the comparison of the proportion of DR4 haplotypes that carry DQB1*0302 in the DR3/4 patients (carrying DQA1*0501) with the proportion of DR4$^+$ patients who do not carry DQA1*0501 or the closely related DQA1*0401 (see below). Table 6 shows the distribution of DQB1*0302 alleles among various DR4$^+$ patient genotypes. The dramatic increase in the frequency of the DQB1*0302 allele among DR4$^+$ type 1 diabetic patients is observed primarily among DR3/4 and DR4/8 heterozygotes but not among other DR4$^+$ patients, such as DR1/4 heterozygotes, consistent with our previous reports (4,21). The proportion of DR4 haplotypes bearing DQB1*0302 is significantly higher among DR3/4 patients than among DR1/4 patients (98.5 vs. 81.8%; $P = 4.7 \times 10^{-8}$) and also higher than among DR4/4 patients (89.3%; $P = 4.7 \times 10^{-8}$). The frequency of DQB1*0302 among the AFBAC DR4 haplotypes in this dataset is 55.4%. Correspondingly, the proportion of DR4 DQB1*0301 haplotypes is significantly lower among DR3/4 patients than among DR1/4 patients (0.7 vs. 13.1%; $P = 2 \times 10^{-10}$) and among DR4/4 patients (5.6%; $P = 0.00015$). This pattern is consistent with a hierarchy of risk among DQ-α-β heterodimers with the *trans*-complementing DQA1*0501-DQB1*0302 conferring greater risk than the *cis*-encoded DQA1*0301-DQB1*0302 molecule or other DQ molecules. We note that, in the DR1/4 heterozygotes, the potential *trans*-heterodimer encoded by DQA1*0101 and DQB1*0302 is not formed (42,43) so that, according to this model, this genotype encodes only one high-risk DQ molecule *in cis*.

The pattern of linkage disequilibrium between DQA1 and DQB1 loci among all populations is characterized by an absence of certain DQA1 and DQB1 alleles *in cis* (i.e., DQA1*01 alleles; DQB1*02, -*03, and -*04 alleles; DQA1*02, -*03, -*04, -*05, and -*06 alleles; and DQB1*05 and -*06 alleles), presumably due to their failure to form heterodimers. However, no haplotypes encoding DQA1*0501-DQB1*0302 have been observed, despite the fact that these α- and β-chains can form a stable heterodimer when encoded *in trans* (44).

The proportion of DR4 DQB1*0302 haplotypes among DR4/8 patients (97.5%) is almost as high as that among DR3/4 patients (98.5%) and significantly higher than the proportion among DR1/4 patients (81.8%; $P = 0.03$). This observation suggests that the DQ heterodimer encoded by the DQA1*0401 (from the DR8 haplotype) and DQB1*0302 alleles also confers very high risk for type 1 diabetes. The protein encoded by the DQA1*0401 allele differs from the more common DQA1*0501 only by an Ile (*0401) to Ser (*0501) change at position 75. Another possible explanation (45) for the high risk associated with the DR4/8 genotype (22,27,40) (Supplementary Table 3) is based on the other *trans*-complementing DQ heterodimer, DQA1*0301-DQB1*0402—the same DQ molecule encoded *in cis* by Asian DRB1*0405 haplotypes—but this explanation seems less likely because this Asian DRB1*0405 haplotype confers only modest risk.

Although, as noted above, allelic variation at the DRB1 locus among DR4 haplotypes influences type 1 diabetes risk, the role of DRB1 DR4 subtypes in disease susceptibility appears to be influenced by genotype. In this dataset

TABLE 7
DRB1*04 subtypes (alleles) in DQB1*0302^{+} patient genotypes

As a percentage of genotype	*0401	*0402	*0403	*0404	*0405	*0406	*0407	*0408	Total†
DR1/DR4	74.4	5.8	0.0	8.1	5.8	0.0	0.0	5.8	86
DR3/DR4	66.4	11.8	0.2	13.1	7.6	0.0	0.0	0.9	450
DR4/DR13	73.3	16.0	0.0	1.3	9.3	0.0	0.0	0.0	75
DR4/DR4	77.3	5.0	0.4	10.3	5.4	0.0	0.0	1.7	242
DR4/DR7	64.9	5.4	0.0	10.8	13.5	0.0	0.0	5.4	37
DR4/DR8	79.5	5.1	0.0	10.3	0.0	0.0	5.1	0.0	39
DR4/DR9	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8
DR4/DRX	54.3	3.7	0.0	25.9	7.4	1.2	0.0	7.4	81

Data are %. †Data are *n*.

and in previous smaller studies (21), the DR1/4 patients show not only a lower proportion of DQB1*0302 alleles than observed in DR3/4 patients (see above) but, among the common DRB1*04-DQB1*0302 haplotypes, the distribution of DRB1*04 alleles is significantly different between these two genotypes (Table *7)*. The proportion of the high-risk DRB1*0401 and the lower-risk DRB1*0404 alleles is 74.4 and 8.1%, respectively, among DR1/4 patients compared with 66.6 and 13.1% among DR3/4 patients (P = 0.0087). We infer from these data that the difference in risk associated with the various DRB1*04 alleles is harder to discern in the presence of the high-risk DQ molecule encoded by DQA1*0501 and DQB1*0302 in DR3/4 heterozygotes.

DISCUSSION

The Type 1 Diabetes Genetics Consortium dataset provides a unique resource for genetic analysis because of the large sample size, the high-resolution HLA typing, and the quality control procedures for the genotype results. The association analyses presented here show a statistically significant risk hierarchy among the many associated DRB1-DQA1-DQB1 haplotypes, ranging from highly predisposing to highly protective, consistent with the results of previous studies (4,22,27) and with a recent meta-analysis of published studies (26). Based on the narrow CI for the risk estimates for individual DR-DQ haplotypes reported in Tables 2 and 3, comparisons of closely related haplotypes with significantly differing type 1 diabetes risks could be carried out, implicating specific alleles and amino acid polymorphisms in type 1 diabetes susceptibility (Table 5). One striking example of this analysis are the two Asian DRB1*1502 haplotypes that differ in type 1 diabetes risk (P = 0.015) but are distinguished only at DQA1 position 34 (Glu vs. Gln) and DQB1 position 57 (Val vs. Ser), consistent with the findings of Bugawan et al. (5). Some comparisons implicate critical polymorphisms in DQB1, some in DRB1, and some, like the comparison of DRB1*0701-DQA1*0201-DQB1*0201g (moderately protective) and the African DRB1*0701-DQA1*0301-DQB1*0201g (susceptible) (26), polymorphisms in DQA1. It should be noted, however, that non–HLA-DR and DQ loci in the major histocompatibility complex, which have been detected but remain to be localized, could modify the risks of these DR-DQ haplotypes. This caveat awaits future investigation, requiring complete genotyping of the class I loci, HLA-DPA1 and DPB1; the nonclassical but polymorphic *MICA* and *MICB* loci; and dense SNP/microsatellite maps.

We have discussed elsewhere that the protective DRB1*0403-DQA1*0301-DQB1*0302 differs from the susceptible DRB1*0407-DQA1*0301-DQB1*0302 (7) only at one position, amino acid residue 86 (Val vs. Gly), and has a significantly different risk for type 1 diabetes (25). Even in this large dataset, however, the frequency of the DRB1*0407 haplotype was too low for a risk estimate. This haplotype is much more common among Native Americans and their descendants (7,46) than among Europeans. Given the observed differences in haplotype frequencies among different populations, the risk comparison of closely related DR-DQ haplotype pairs is more robust when both haplotypes are present in the same population.

The comparison of type 1 diabetes risk and the distribution of DQB1*0302 alleles in DR3/4 and DR1/4 patients and control subjects illustrate the importance of genotype context. We infer from these data and from the high risk for DR3/4 heterozygotes among Europeans relative to Asians that the *trans*-complementing DQ heterodimer encoded by the DQA1*0501 allele on DR3 and the DQB1*0302 allele on some DR4 haplotypes confers very high risk. This inference is supported by the high risk conferred by the DR4/8 genotype (Supplementary Table 3) in which the closely related DQA1*0401 allele is on the DR8 haplotype. The other *trans*-complementing DQ heterodimer encoded by DQA1*0301 and DQB1*0201g in Caucasian DR3/4 heterozygotes also confers type 1 diabetes risk, as does the DQ heterodimer encoded *in cis* by DQA1*0301 and DQB1*0302 but to a lesser degree. We note that the rare African DRB1*0405 haplotype carrying the DQA1*0301-DQB1*0201g alleles *in cis* confers lower risk than those carrying DQA1*0301-DQB1*0302 (Table 2) (22), so it seems unlikely that this DQ heterodimer (α*0301-β*0201) encoded *in trans* would account for the elevated risk of DR3/4 heterozygotes among Europeans.

The increased risk of DR3/4-DQB1*0302 heterozygotes relative to DR3/3 and DR4/4 genotypes has led to the hypothesis that the *trans*-complementing DQ heterodimers are more effective in presenting diabetogenic epitopes to T-cells (44,47). Our proposal to explain the observed patterns of genotype risk described above is that the DQ heterodimer encoded by DQA1*0501 and DQB1*0302 confers the highest risk. Experimental studies have demonstrated that the peptide binding (47) and, more recently, that T-cell recognition of peptides bound to *trans*-encoded DQ heterodimers can differ significantly from that of *cis*-encoded DQ molecules (48).

At the genotype level, protective molecules encoded by one haplotype can affect the risk conferred by susceptible haplotypes so that a genotype with one protective haplotype, such as DRB1*1101-DQA1*0501-DQB1*0301/DRB1*0401-DQA1*0301-DQB1*0302, does not confer high

risk even though it encodes the putative high-risk DQ heterodimer *in trans*. Alternative explanations that do not invoke this DQ heterodimer for the synergistic effect of DR3 and DR4 haplotypes propose a type 1 diabetes–prone combination of different autoimmune pathways mediated by these two haplotypes (36,37).

The pattern of linkage disequilibrium between DQA1 and DQB1 loci among all populations is characterized by an absence of certain DQA1 and DQB1 alleles *in cis* (i.e., DQA1*01 alleles; DQB1*02, -*03, and -*04 alleles; DQA1*02, -*03, -*04, -*05, and -*06 alleles; DQB1*05 and -*06 alleles, presumably because of their failure to form heterodimers. No haplotypes encoding DQA1*0501-DQB1*0302 have been observed, although these α- and β-chains can form a stable heterodimer when encoded *in trans* (44). Perhaps a haplotype with these specific alleles *in cis*, because of the high risk for type 1 diabetes—a disease whose onset often predates reproduction—might have been subject to negative selection and/or failed to be maintained in populations because of the absence of positive or balancing selection of this particular haplotypic combination of the *DRB1* and *DQB1* alleles.

The comparison of DRB1 and DQB1 alleles in DR1/4 and DR3/4 patients and control subjects demonstrates that for the DR1/4 patients, allelic variation at the DRB1 locus (DRB1*0401 vs. *0404) appears to be the critical element on the DR4 haplotype whereas for the DR3/4 patients, allelic variation at the DQB1 locus (DQB1*0302 vs. *0301) seems critical in determining the extent of type 1 diabetes risk. Conceivably, a putative pathogenic autoantigen peptide might be presented by a DR molecule in the DR1/4 patients and by a DQ molecule in the DR3/4 patients. Whether the type 1 diabetes HLA class II associations reflect thymic-positive selection of autoreactive T-cells (susceptible HLA) or the deletion (negative selection) of autoreactive T-cells (protective HLA), preferential presentation of diabetogenic peptides, or alternative immunological mechanisms remains uncertain. At any rate, the data presented here on DRB1 and DQB1 allele distributions and the importance of genotype context support the genetic associations observed in previous smaller studies (4,21).

Given the critical role of DRB1 and DQB1 alleles in type 1 diabetes susceptibility, the identification of additional disease susceptibility loci within the HLA region will require adjusting the results of association analysis for linkage disequilibrium to DR-DQ haplotypes and will require stratification on the relevant DR-DQ genotypes. In general, the search for secondary type 1 diabetes genes is facilitated by a thorough characterization of the genetic risk heterogeneity of the primary disease genes, in this case, the alleles at the DRB1, DQA1, and DQB1 loci. The Type 1 Diabetes Genetics Consortium dataset includes genotypes for the other HLA loci (DPA1, DPB1, and HLA-A, -B, and -C) and for SNP and microsatellite genotypes. The DR-DQ data and analyses presented here provide the means for such adjustments and stratifications and a critical framework in the search for additional type 1 diabetes genes within the HLA region.

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