HLA Class I and Genetic Susceptibility to Type 1 Diabetes

Results From the Type 1 Diabetes Genetics Consortium

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OBJECTIVE—We report here genotyping data and type 1 diabetes association analyses for HLA class I loci (A, B, and C) on 1,753 multiplex pedigrees from the Type 1 Diabetes Genetics Consortium (T1DGC), a large international collaborative study.

RESEARCH DESIGN AND METHODS—Complete eight-locus HLA genotyping data were generated. Expected patient class I (HLA-A, B, and C) allele frequencies were calculated, based on linkage disequilibrium (LD) patterns with observed HLA class II DRB1-DQA1-DQB1 haplotype frequencies. Expected frequencies were compared to observed allele frequencies in patients.

RESULTS—Significant type 1 diabetes associations were observed at all class I HLA loci. After accounting for LD with HLA class II, the most significantly type 1 diabetes–associated alleles were B*5701 (odds ratio 0.19; P = 4 × 10−11) and B*3906 (10.31; P = 4 × 10−16). Other significantly type 1 diabetes–associated alleles included A*2402, A*0201, B*1801, and C*0501 (predisposing) and A*1101, A*3201, A*6601, B*0702, B*4403, B*3502, C*1601, and C*0401 (protective). Some alleles, notably B*5701, appear to modulate the risk of all DRB1-DQA1-DQB1 haplotypes on which they reside, suggesting a class I effect that is independent of class II. Other class I type 1 diabetes associations appear to be specific to individual class II haplotypes. Some apparent associations (e.g., C*1601) could be attributed to strong LD to another class I susceptibility locus (B*4403).

CONCLUSIONS—These data indicate that HLA class I alleles, in addition to and independently from HLA class II alleles, are associated with type 1 diabetes. Diabetes 59:2972–2979, 2010

Type 1 diabetes is an autoimmune disease characterized by progressive T-cell–mediated destruction of the pancreatic β-cells. Both genetic and environmental factors are involved in disease susceptibility; the major genetic susceptibility determinants are the highly polymorphic HLA loci on chromosome 6p21—more specifically the class II loci, HLA-DRB1, HLA-DQA1/DQB1 (see the study by Erlich et al. [1] and references therein), and, to a lesser extent, HLA-DPB1/DPA1 (2–6). These genes, however, cannot completely explain the association between type 1 diabetes and the HLA region. Several studies have shown that HLA class I genes (A, B, and C) are associated with type 1 diabetes (7–11). Products of the HLA class I genes bind and present peptide antigens. The HLA class I peptide antigen complexes function both in shaping the T-cell repertoire in the thymus and in initiating antigen-specific T-cell–mediated cytotoxicity, providing a plausible immunological rationale to explain the genetic association. The extremely high linkage disequilibrium (LD) within the HLA region, combined with the strong susceptibility effects of the HLA DR- and DQ-encoding loci, can confound association studies of any loci in the region. Thus, apparent susceptibility effects of HLA class I alleles may, in some cases, be attributable to their presence on highly protective or predisposing HLA DRB1-DQA1-DQB1 haplotypes.

Compared with the hundreds of studies of HLA class II association with type 1 diabetes, only a handful of reports focus on HLA class I and type 1 diabetes (7–12), and only a subset of these include molecular genotyping and consideration of LD with class II in association analyses. Some alleles have appeared consistently associated with type 1 diabetes both at the serologic and allele level, including A*24(02) and B*39(06), with and without conditioning on DR-DQ. HLA class I loci are extremely polymorphic, with a total of 2,893 alleles assigned for the three loci as of October 2009. Thus, large sample sizes are crucial to generate sufficient class I data for adequately powered disease association studies. The Type 1 Diabetes Genetics Consortium (T1DGC) is an international collaborative project that has ascertained the largest set of multiplex type 1 diabetes families in existence for the study of the genetic basis of type 1 diabetes susceptibility. All samples collected by the T1DGC are genotyped at all classical HLA loci (DRB1, DQA1, DQB1, DPA1, DPB1, A, B, and C) as well as for single nucleotide polymorphisms (SNPs) in the insulin and CTLA4 genes that have repeatedly been shown
to be associated with type 1 diabetes. Subsets of the TIDGC collection have been genotyped for candidate gene SNPs reported to be associated with type 1 diabetes (the “Rapid Response” project), genome-wide microsatellites, and genome-wide SNPs (www.TIDGC.org).

**RESEARCH DESIGN AND METHODS**

The subjects included in this dataset were comprised of newly collected samples and samples from previously existing collections. The sample set included 1,753 Caucasian multiplex type 1 diabetes families compiled from four existing collections (DAN = Denmark; HBDI = Human Biological Data Interchange; JOS = Joslin Diabetes Center; SAR = Sardinia) and newly collected from four TIDGC networks (AP = Asia Pacific network; EUR = European network; NA = North American network; UK = United Kingdom network). The total number of affected offspring is 3,577. All samples were collected with appropriate informed consent, and all collections were done with the approval of the appropriate institutional review board at the collection sites (www.TIDGC.org). Table 1 includes the descriptive statistics for the collection.

**Genotyping methods.** All samples, including existing collections, were genotyped using standardized protocols, including inter- and intra-lab quality control procedures, at one of three TIDGC HLA genotyping centers (Oakland and Alameda, CA; Melbourne, Australia; and Malmo, Sweden) (1). High-resolution HLA genotyping was performed with a PCR-based sequence-specific oligonucleotide probe system. Briefly, a series of oligonucleotide probes, corresponding to known polymorphic sequence motifs in the HLA genes, was immobilized onto a backed nylon membrane to create a “linear array.” 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. The PCR product was denatured and subsequently hybridized to the appropriate linear array. After hybridization and wash, arrays were incubated with streptavidin-horseradish peroxidase, followed by the chromogenic substrate tetramethylbenzidine. Images were created by placing the arrays on a flatbed scanner, and probe intensities were measured as pixel values with a proprietary genotyping software called StripScan. Preliminary genotypes were determined with StripScan, and then data from StripScan were imported into Sequence Compilation and Rearrangement Evaluation (SCORE) software (13) for final genotyping calling and export of data to the coordinating center. Genotyping was completed for each sample for each locus (i.e., no failed typing). All primary probe-binding data were reported to the coordinating center; allele calls were reported at four-digit resolution. We note here that, due to, for example, only B*4403, we expect the frequencies of C*1601 alleles due to, for example, only B*4403, we expect the frequencies of C*1601 alleles introduced, only haplotypes at the susceptibility loci with an average frequency of ≥0.5% in combined cases and controls were used. Moreover, only class II–class I haplotypes where the expected frequency in controls in the absence of LD would be >0.5% were included in the analysis.

**Statistical analysis.** Control haplotypes were determined based on the affected family-based control (AFBAC) method (14). The transmission of overall haplotypes at all typed loci (DPA1, DPB1, DRB1, DQA1, DQB1, HLA-C, HLA-B, HLA-A) was used to determine AFBAC haplotypes; in this case, those parental haplotypes never transmitted to the affected sib-pair. Because only the proband from each family is used in the analyses, this approach does not introduce a bias because of the nonindependence between sib-pairs.

**Adjustment for LD with DRB1-DQB1 haplotypes.** The expected allele frequencies were computed, given known HLA DR-DQ primary associations with type 1 diabetes and their observed haplotype frequencies in both patients and controls. Briefly, the null hypothesis (H0) is that class I allele frequencies will differ between patients and controls 1) because of LD between the class I loci and DQB1 and 2) due to chance (sampling), thus implying that class I loci are neutral relative to disease predisposition. Under H0, the expected allele frequencies at a given class I allele can be computed using the equation derived by Thomson (15)

\[ q_{exp}^i = \frac{p_{classIj} \cdot \sum_{j=1}^{K} D_{ij} \cdot q_{haplotypej}}{p_{classIIi}} \]

where \( D_{ij} \) denotes the pair-wise LD coefficient between the \( i \)th DRB1-DQB1 haplotype and the \( j \)th class I allele in the control sample, \( q \) denotes the allele or haplotype frequency in patients, \( p \) denotes the frequency in the AFBAC, and \( q_{exp} \) denotes the expected frequency in patients under the assumption of no involvement of the class I allele in disease. This method relies on sampling estimates of pair-wise LD between a putative second disease locus and the DRB1-DQB1 haplotypes and on 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 class I alleles. 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 (7). Given the large number of classical loci haplotypes and the error that rare haplotypes could introduce, only haplotypes at the susceptibility loci with an average frequency of ≥0.5% in combined cases and controls were used. Moreover, only class II–class I haplotypes where the expected frequency in controls in the absence of LD would be >0.5% were included in the analysis.

In addition, for specific HLA-B (B*4403) and HLA-C (C*1601) associations, we tested whether the association was due to the 4403-1601 haplotype or to only one of the loci. Under the null hypothesis that the association seen was due to, for example, only B*4403, we expect the frequencies of C*1601 alleles on these haplotypes to be the same in transmitted and nontransmitted haplotypes. This analysis can be extended to include additional predisposition effects from other classical HLA loci (in this case, DRB1*0701 HLA-B*4403 haplotypes). If the allele under study (C*1601) has no effect on type 1 diabetes risk, the transmission proportions of this allele should be the same, condi-

**TABLE 1**

| Cohort | Origin | Collection | Pedigrees | Type 1 diabetic sibs per pedigree | Type 1 diabetic offspring in cohort | Type 1 diabetic male | Type 1 diabetic female | Male (%) | Age of onset (years) | Age of onset (range) |
|--------|--------|------------|-----------|----------------------------------|-----------------------------------|---------------------|----------------------|---------|---------------------|---------------------|
| AP     | Asia Pacific | New       | 150       | 2.02 ± 0.24                      | 303                               | 153                 | 150                  | 50.50   | 10.06 ± 7.43        | 0–37                |
| DAN    | Denmark | Extant    | 94        | 2.05 ± 0.33                      | 194                               | 105                 | 90                   | 54.12   | 11.73 ± 8.30        | 0.8–49              |
| EUR    | Europe | New       | 470       | 2.01 ± 0.10                      | 945                               | 513                 | 432                  | 54.29   | 10.76 ± 7.26        | 1–35                |
| HBDI   | U.S.   | Extant    | 416       | 2.10 ± 0.42                      | 879                               | 471                 | 410                  | 53.58   | 11.90 ± 8.22        | 0.8–50              |
| JOS    | U.S.   | Extant    | 57        | 2.14 ± 0.58                      | 122                               | 60                  | 62                   | 49.18   | 11.22 ± 7.31        | 1–31                |
| NA     | U.S.   | New       | 407       | 2.01 ± 0.21                      | 819                               | 435                 | 384                  | 53.11   | 8.763 ± 6.45        | 0–34                |
| SAR    | Sardinia | Extant    | 52        | 1.98 ± 0.14                      | 101                               | 47                  | 54                   | 46.53   | 12.34 ± 7.90        | 0.7–34              |
| UK     | U.K.   | New       | 107       | 2.00 ± 0.13                      | 214                               | 97                  | 117                  | 45.33   | 7.65 ± 4.48         | 0–23                |
| Total  |        |           | 1,753     | 2.03 ± 0.28                      | 3,577                             | 1,881               | 1,699                | 52.59   | 10.44 ± 7.41        | 0–50                |

Data are means ± SD unless otherwise indicated.
## Table 2

| Allele | Type 1 diabetes (n) | AFBAC (n) | P    |
|--------|---------------------|-----------|------|
| A*0101 | 17.7 (621)          | 16.1 (256)| 0.214|
| A*0102 | 0.1 (4)             | 0 (0)     | 0.179|
| A*0103 | 0 (0)               | 0.1 (2)   | 0.035|
| A*0201 | 30.6 (1,074)        | 27.6 (438)| 0.069|
| A*0202 | 0.2 (8)             | 0 (0)     | 0.057|
| A*0205 | 2.1 (73)            | 1.2 (19)  | 0.030|
| A*0206 | 0.3 (9)             | 0.2 (3)   | 0.646|
| A*0211 | 0 (1)               | 0 (0)     | 0.501|
| A*0220 | 0 (0)               | 0.1 (1)   | 0.137|
| A*0234 | 0 (0)               | 0.1 (1)   | 0.137|
| A*0301 | 11.9 (417)          | 13.1 (207)| 0.271|
| A*0302 | 0.5 (16)            | 0.3 (4)   | 0.282|
| A*0305 | 0 (0)               | 0.1 (1)   | 0.137|
| A*1101 | 3.4 (119)           | 6.9 (109) | 5.E-08|
| A*1105 | 0 (0)               | 0.1 (1)   | 0.137|
| A*2301 | 1.2 (43)            | 1.8 (29)  | 0.094|
| A*2401 | 0 (0)               | 0.1 (1)   | 0.137|
| A*2402 | 11.3 (396)          | 7.8 (123) | 2.E-04|
| A*2403 | 0.3 (10)            | 0.1 (1)   | 0.114|
| A*2501 | 2.3 (80)            | 2.6 (42)  | 0.432|
| A*2601 | 2.6 (90)            | 2.8 (44)  | 0.671|
| A*2607 | 0 (0)               | 0.1 (1)   | 0.137|
| A*2608 | 0.1 (2)             | 0 (0)     | 0.342|
| A*2901 | 0.3 (12)            | 0.4 (6)   | 0.840|
| A*2902 | 2.3 (80)            | 3.4 (54)  | 0.022|
| A*3001 | 0.7 (25)            | 1.1 (17)  | 0.191|
| A*3002 | 3.3 (117)           | 1.7 (27)  | 0.001|
| A*3004 | 0.1 (2)             | 0.1 (1)   | 0.034|
| A*3010 | 0 (1)               | 0 (0)     | 0.501|
| A*3101 | 2.1 (74)            | 1.9 (30)  | 0.614|
| A*3201 | 2.1 (73)            | 4.2 (67)  | 2.E-05|
| A*3204 | 0 (0)               | 0.1 (1)   | 0.137|
| A*3301 | 0.8 (27)            | 1 (16)    | 0.390|
| A*3303 | 0.4 (14)            | 0.3 (5)   | 0.650|
| A*3304 | 0.2 (6)             | 0.1 (1)   | 0.336|
| A*3601 | 0 (1)               | 0.2 (2)   | 0.184|
| A*6601 | 0.1 (3)             | 0.6 (10)  | 4.E-04|
| A*6602 | 0.1 (2)             | 0 (0)     | 0.342|
| A*6801 | 2.6 (91)            | 2.7 (43)  | 0.811|
| A*6802 | 0.4 (14)            | 1.3 (20)  | 5.E-04|
| A*6901 | 0 (0)               | 0.1 (1)   | 0.137|
| A*7401 | 0 (0)               | 0.1 (1)   | 0.137|
| A*8001 | 0 (1)               | 0 (0)     | 0.501|
| B*0702 | 7 (244)             | 14.7 (233)| 7.E-17|
| B*0704 | 0 (0)               | 0.1 (1)   | 0.137|
| B*0705 | 0.7 (26)            | 0.3 (4)   | 0.035|
| B*0707 | 0 (0)               | 0.1 (1)   | 0.137|
| B*0801 | 21 (735)            | 10.9 (173)| 4.E-15|
| B*0809 | 0 (0)               | 0.1 (1)   | 0.137|
| B*1302 | 1.8 (64)            | 2 (31)    | 0.752|
| B*1401 | 0.2 (8)             | 0.6 (9)   | 0.052|
| B*1402 | 1.7 (60)            | 2.6 (41)  | 0.040|
| B*1501 | 12.3 (432)          | 4.5 (71)  | 2.E-16|
| B*1502 | 0.1 (2)             | 0 (0)     | 0.342|
| B*1503 | 0.1 (5)             | 0.1 (1)   | 0.444|
| B*1507 | 0.1 (3)             | 0 (0)     | 0.244|
| B*1508 | 0 (1)               | 0 (0)     | 0.501|
| B*1509 | 0.1 (2)             | 0.1 (1)   | 0.934|

**Continued**
RESULTS

Frequency data for HLA-A, HLA-B, and HLA-C alleles in probands (n = 1,753) are shown in Table 2. Proband frequencies were compared with allele frequencies from AFBAC (15), which are alleles that are not transmitted from parent to any affected child. We previously reported HLA class I association data for families from the Human Biological Data Interchange (HBDI) (7,9). The HBDI families were re-genotyped with high-resolution reagents and are included as part of the TIDGC collection. We performed association analyses on the TIDGC families who were not from the HBDI and found that the results were not significantly different for the two groups. These data are shown in supplementary Table 1, available in on online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db10-0689/DC1.

Linkage disequilibrium. The HLA region exhibits some of the strongest LD in the genome, and the strongest type 1 diabetes disease associations in the region have been well established to come from the HLA class II genes encoding the DR and DQ antigens. Thus, primary association data for HLA region markers must be adjusted for LD with strongly associated class II alleles. To adjust for LD to DR and DQ, expected allele frequencies among patients are adjusted mathematically to reflect the LD observed in the sample set (15) and compared with the observed allele frequencies among patients. Alleles that remain significantly associated with type 1 diabetes after adjustment for LD are shown in Table 3. Association data for all alleles after LD adjustment can be found in supplementary Table 2. After LD adjustment, the two most strongly type 1 diabetes–associated alleles are B*5701 (protective) and B*3906 (predisposing). For further confirmation, transmission disequilibrium testing (TDT) analysis stratified by DRB1-DQB1 haplotype was performed and showed significant type 1 diabetes protection for B*5701 on DRB1*0101-DQB1*0501 and DRB1*0301-DQB1*0201 haplotypes and significant type 1 diabetes risk for B*3906 on DRB1*0101-DQB1*0501, DRB1*0301-DQB1*0201, DRB1*0404-DQB1*0302, and DRB1*0801-DQB1*0402 haplotypes (data not shown). Stratified TDT analysis was also performed on A*0204 and showed significant type 1 diabetes risk on eight DRB1-DQB1 haplotypes, including DRB1*0101-DQB1*0501, DRB1*0101-DQB1*0504, DRB1*0301-DQB1*0201, DRB1*0401-DQB1*0302, DRB1*0404-DQB1*0302, DRB1*0701-DQB1*0303, DRB1*0801-DQB1*0402, and DRB1*1601-DQB1*0502 (data not shown). An overview of LD for selected type I diabetes–associated class I alleles is shown in Fig. 1. Type I diabetes–associated class II allele groups, including DR3, DR4, DR1, DR8 (susceptible), and DR2 (protective) are also included. As expected,
A*0101 and B*0801, which are part of the conserved, extended haplotype known as “A1-B8-DR3,” or simply “8.1,” are in strong LD with each other. However, even stronger LD was observed for the alleles B*4403 and C*1601. Frequencies for both of these alleles were decreased in patients compared with controls (Table 2), and this apparent protective effect remained even after adjustment of the data to account for LD with DR and DQ (Table 3). These two alleles are in strong LD (\(r^2 = 49\), Fig. 1); thus, most haplotypes that contain one of these two alleles contain the other as well, making distinction of effects of individual alleles difficult. The T1DGC has amassed such a large collection of families that haplotypes containing one or the other of these alleles were found. The data shown in Table 4 suggest that the transmission proportion for haplotypes carrying B*4403 does not differ significantly whether or not the haplotype carries C*1601 as well. On the other hand, for haplotypes carrying C*1601, the transmission proportion is lower when B*4403 is also included on the haplotype. This difference did not reach statistical significance; however, comparison of these two alleles on a single haplotype, DR7, was significant. The transmission proportion of DR7-B*4403-C*not1601 haplotypes is low (10.99%), whereas transmission proportion for DR7-

**FIG. 1. LD diagram for selected HLA alleles. The \(r^2 \times 100\) value of LD between pairs of alleles is shown.**

| DR      | HLA-B     | HLA-C     | Not trans | Trans | Trans proportion (%) | P      |
|---------|-----------|-----------|-----------|-------|----------------------|--------|
| All     | B*4403    | All       | 282       | 118   | 29.50                |        |
| All     | B*4403    | C*1601    | 156       | 66    | 29.73                |        |
| All     | B*4403    | not C*1601| 115       | 40    | 25.81                | NS     |
| All     | All       | C*1601    | 173       | 80    | 31.62                |        |
| All     | B*4403    | C*1601    | 156       | 66    | 29.73                |        |
| All     | not B*4403| C*1601    | 17        | 14    | 45.16                | <0.13  |
| DR7†    | B*4403    | not C*1601| 81        | 10    | 10.99                |        |
| DR7     | not B*4403| C*1601    | 3         | 4     | 57.14                | <0.0074|
| DR7     | B*4403    | C*1601    | 124       | 36    | 22.50                | <0.023 |
| DR7     | B*4403    | not C*1601| 81        | 10    | 10.99                |        |
| DR7     | B*4403    | C*1601    | 124       | 36    | 22.50                |        |
| DR7     | not B*4403| C*1601    | 3         | 4     | 57.14                | <0.036 |

†DRB1*0701 DQB1*0201.
B*not4403-C*1601 haplotypes (57.14%) showed no apparent protective effect (P value for difference 0.0074). The number of DR7 haplotypes carrying C*1601 without B*4403 is small (n = 7 total, three transmitted and four not transmitted), so these data should be interpreted with caution. Other haplotype combinations were examined, including B*1501-C*0303, which suggested that, on DR3 haplotypes, the predisposing effect of B*1501-C*0303 may be attributable to the C*0303 or something in LD with it, rather than to the B*1501 allele; however, the result did not reach statistical significance (P = 0.069, data not shown). Data were too sparse for meaningful analysis of any other class I allele combinations. This result underscores both the complexity of statistical analysis of individual HLA alleles in this region of strong LD and the need for very large datasets to distinguish true susceptibility effects from apparent effects attributable to LD of a given allele with a second allele with strong type 1 diabetes susceptibility.

**Disease susceptibility effects in multiple DR-DQ haplotypes.** Even when an HLA allele exhibits an apparent strong effect on type 1 diabetes susceptibility, the abundance of both classic HLA loci and other immunologically relevant loci, such as TNF, in the region can limit the confidence that an apparent type 1 diabetes association is direct to the locus under investigation. One criterion that can help evaluate whether a particular HLA allele itself is affecting type 1 diabetes susceptibility is to see if the effect of the allele is consistent across multiple DR-DQ haplotypes. DR5 haplotypes (containing DRB1*0301 and DR4 haplotypes (with DRB1*0404, except for 0403 and 0406, and carrying DQB1*0302) are established as the most highly type 1 diabetes–predisposing haplotypes in Caucasians, and the common DR2 haplotype DRB1*1501-DQB1*0602, is the most strongly protective. We examined the effects of the HLA-A, HLA-B, and HLA-C alleles in the context of these specific haplotypes, as well as on the more moderately predisposing DR1 and DR8 haplotypes, to look for susceptibility effects that were seen in more than one haplotypic context and that, when significantly associated, always had the same effect qualitatively (i.e., always protective, or always predisposing). For each of the five DR haplotypes being tested (DR3, DR4, DR1, DR8, and DR2), we compared haplotypes carrying a given class I allele to that haplotype carrying any allele at the locus in question. To determine the value referred to here as the “relative odds ratio,” we arbitrarily set the odds ratio (OR) for each tested DR-DQ haplotype, regardless of class I (e.g., “DR3-any,” “DR4-any,” etc.), to 1.0 and compared individual haplotypes with given class I alleles to the “any” baseline to generate a relative OR. In other words, the total of any of the tested haplotypes (e.g., DR4, DR1, etc.) provides the baseline, set at a value of 1.0, to which haplotypes of each category with particular class I alleles (e.g., DR4-B*3906, DR1*0401) can be compared. Thus, although the absolute OR for DR3-A*0101 is 1.69, the relative OR for DR3-A*0101, compared with the entire set of DR3 haplotypes (DR3-any), is 0.69. This means that a DR3 haplotype carrying A*0101 is less predisposing than the average of all DR3 haplotypes, even though DR3-A*0101 is still predisposing overall. A summary of alleles with significant relative OR in multiple DR haplotypes is shown in Table 5. Values are included for any allele that showed a significant relative OR on more than one of our selected type 1 diabetes–associated haplotypes. Also, the direction of the effect, i.e., protective (OR < 1) or susceptible (OR > 1), was compared for consistency among haplotypes. The most striking example of an effect that was seen consistently in multiple haplotypic contexts is that of B*3906, which significantly increases the risk of four of five tested haplotypes and shows a nonsignificant trend toward increased risk in the remaining one (DR3). DR2-B*3906 haplotypes, while still type 1 diabetes protective overall, are significantly less type 1 diabetes protective than DR2 haplotypes with an unspecified HLA-B allele. This difference argues that the type 1 diabetes predisposing effect is coming from the HLA-A allele itself, rather than from an unidentified locus in LD with B*3906 and is consistent with other studies (10,11). We note here that the allele B*5701, which exhibited the strongest type 1 diabetes association in the LD-adjusted data, did not significantly affect any of the DR haplotypes tested; however, for all predisposing DR haplotypes tested, all relative ORs for B*5701 on predisposing haplotypes were < 1. These relative ORs, including DR3-B*5701 (OR 0.43, 95% CI 0.17 – 1.05), DR4-B*5701 (0.57, 0.22 – 1.42), DR8-B*5701 (0.22, 0.00 – 4.94), and DR1-B*5701 (0.16, 0.02 – 1.27), while individually not reaching statistical significance, were all suggestive of a protective effect of the B*5701 allele. A larger sample size may be required to demonstrate significant haplotype-specific effects for this allele.

In our “relative OR” analysis for specific haplotypes, ten other alleles exhibited a significant risk modulation for at least two of the five haplotypes tested. Four of these (A*6801, B*3501, B*4402, and B*4403) had opposite effects from apparent effects attributable to LD to another class I allele. For example, a study of Filipino type 1 diabetic patients and control subjects showed that A*2407 appears protective (A*2402, B*3501, and C*0702 and the protective alleles A*0101, A*3201, and C*0701.

**DISCUSSION**

Unraveling the effects of individual HLA class I alleles on type 1 diabetes susceptibility requires taking into account both the allele itself and its context (LD pattern). The results presented here show apparent class I associations that are specific to a single haplotype and associations that can be accounted for by LD to another class I allele. However, these data also show associations that are consistent across multiple DR-DQ haplotypes, suggesting that they represent true independent disease-associated alleles.

One method of decreasing the complexity of HLA class I disease association data are to bin alleles into two-digit resolution, based on serologic reactivity, which can increase statistical power by decreasing sparseness. However, not all alleles within a serologic category necessarily have the same effect on type 1 diabetes susceptibility. For example, a study of Filipino type 1 diabetic patients and control subjects showed that A*2407 appears protective for type 1 diabetes, whereas the closely related A*2402 allele is highly predisposing (12). Two HLA-B alleles, B*4402 and B*4403, that differ at only a single encoded amino acid residue have been shown to stimulate strong allogeneic responses in hematopoietic transplant (17). In the TIDGC dataset presented here, B*4402 appears neutral for type 1 diabetes risk, whereas B*4403 is highly protective (supplemental Table 2). Thus, analysis of individual alleles at the four-digit level is preferable and can
only be done with large datasets. The T1DGC collection represents, to our knowledge, the largest existing dataset of its kind and includes high-resolution genotyping results reported at four-digit resolution.

Even when genotyping resolution is at the allele level, the effects of any given locus in the HLA region cannot be interpreted in isolation. Some extended HLA haplotypes are conserved over several megabases. “A1-B8-DR3” (also called “8.1”) has almost no variation over nearly 4 Mb of DNA (18). Consequently, an association study of any genetic locus within this haplotype must necessarily take into account the effects of all of the other loci on the conserved haplotype. Stratifying by other HLA loci has shown that A*0101, seen more frequently in patients than in control subjects, is actually protective for type 1 diabetes when LD of A*0101 with the type 1 diabetes–predisposing haplotype DRB1*0301-DQA1*0501-DQB1*0201 is taken into account (7).

In some cases, effects of alleles that appear type 1 diabetes associated, even after adjustment of the data for LD with DR-DQ haplotypes can still be misleading. C*1601 appears protective for disease, and the protective effect persists even after adjustment of the data for LD with DR-DQ. Closer examination of LD patterns in the data reveal that C*1601 is in strong LD with B*4403, which also appears strongly type 1 diabetes protective. Examination of this large dataset allowed the observation that the apparent type 1 diabetes effect for C*1601 can be explained by its LD to B*4403, but the B*4403 allele appears protective in the absence of C*1601. Given the function of HLA class II antigens, the B*4403 allele itself seems a likely candidate for a causative allele, although the formal possibility remains that the disease protection may come from an unidentified allele at a locus in LD with B*4403. Unraveling susceptibility for other HLA class I allele combinations will require even larger datasets than the current one.

In the data reported here, the additional predisposing effect of the B*3906 allele was apparent on all predisposing haplotypes examined (DR3, DR4, DR1, DR8), strongly suggesting that the predisposing effect may be due to

### TABLE 5
Summary of statistically significant relative ORs on specific haplotypes for class I alleles

| Allele   | DR3          | DR4          | DR8          | DR1          | DR2          | Number |
|----------|--------------|--------------|--------------|--------------|--------------|--------|
| A*0101   | 0.69 (0.59–0.78) | 0.75 (0.57–0.98) | 0.58 (0.38–0.89) | 2 Yes        |
| A*0201   | 1.47 (1.14–1.88) | X            | 0.60 (0.38–0.91) | 4 Yes        |
| A*1101   | 1.52 (1.15–2.01) | 3.31 (1.90–5.74) | 2.18 (1.51–3.14) | 4 Yes        |
| A*2601   | 2.22 (1.03–4.78) | 0.57 (0.41–0.79) | 0.19 (0.04–0.89) | 2 Yes        |
| A*3101   | 0.57 (0.36–0.88) | X            | 0.10 (0.01–0.98) | 3 No         |
| A*3201   | 0.62 (0.38–0.99) | X            | 3.32 (1.14–9.64) | 4 Yes        |
| A*6601   | 0.68 (0.62–0.74) | X            | 4.83 (1.02–22.6) | 3 No         |
| B*1801   | 1.58 (1.31–1.90) | 1.74 (1.19–2.53) | 2 Yes        |
| B*3501   | 4.79 (1.07–21.4) | 4.79 (1.07–21.4) | 2 No         |
| B*3503   | 0.70 (0.49–0.97) | X            | 14.54 (2.66–79.3) | 3 No         |
| B*3901   | 2.44 (1.01–5.88) | X            | 28.11 (1.75–449.0) | 4 Yes        |
| B*3906   | 2.70 (1.16–6.29) | 14.03 (5.55–35.4) | 7.15 (3.89–13.1) | 4 Yes        |
| C*0102   | 0.30 (0.11–0.77) | X            | 4.82 (1.87–12.3) | 2 No         |
| C*0501   | 1.48 (1.21–1.81) | 0.29 (0.12–0.68) | 2 No         |
| C*0602   | 1.56 (1.09–2.21) | X            | 0.57 (0.37–0.87) | 3 Yes        |
| C*0701   | 0.70 (0.60–0.80) | 0.26 (0.08–0.80) | 3 Yes        |
| C*1202   | 0.15 (0.03–0.61) | X            | 1.94 (1.41–2.65) | 3 Yes        |
| C*1402   | 0.33 (0.11–0.92) | X            | 1.61 (1.01–2.53) | 2 No         |
| C*1502   | 0.38 (0.15–0.90) | X            | 0.38 (0.15–0.90) | 4 Yes        |

For each allele, the relative OR value represents a comparison of the DR haplotype carrying the allele to that DR haplotype carrying any class I allele. X = total n in contingency table ≤12. †Although the confidence interval overlaps 1, this result, with relative OR of 11.56, has been included for comparison. Data for seven alleles that exhibit a consistent disease association on more than one haplotype are indicated in boldface type.
B*3906 itself. The alternative explanation, that a putative causative allele at another locus in LD with B*3906 must be present on all haplotypes tested, is possible but seems unlikely. Other alleles had inconsistent effects across haplotypes, for example, A*6801 is protective in the context of DR3 haplotypes but predisposing in the context of DR1 and DR2 haplotypes, suggesting that these effects are not due to A*6801 but to alleles at other loci on the haplotypes.

The two strongest type 1 diabetes susceptibility effects observed in these data are the predisposing effect of B*3906 and the protective effect of B*5701. Both have been noted in other class I type 1 diabetes association studies. B*5701 has also been reported to be associated with restriction of virus replication in long-term progressors (19,20). B*5701 is strongly associated with adverse drug reaction (allergic hypersensitivity) to the nucleoside reverse transcriptase inhibitor abacavir, used to treat HIV-positive patients, leading to implementation of genetic screening programs to reduce the frequency of allergic hypersensitive responses.

In summary, the extreme polymorphism of the HLA class I alleles and the strong LD in the HLA region make association analysis difficult for individual alleles. The large size of the T1DGC dataset and the consistent genotyping resolution of the samples from the collection allow meaningful analyses to assess the contribution of classical HLA alleles to type 1 diabetes susceptibility. The data presented here argue that HLA class I alleles (minimally B*3906 and B*5701) should be considered for inclusion in type 1 diabetes genetic screening panels. Though understanding of the risk of individual HLA alleles will provide clues to biological mechanism of type 1 diabetes pathogenesis and, eventually, could lead to intervention or prevention targets. The results presented here represent a step toward this goal.

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