HLA Class I and Genetic Susceptibility to Type 1 Diabetes

Results From the Type 1 Diabetes Genetics Consortium

Janelle A. Noble,[1] Ana Maria Valdes,[2] Michael D. Varney,[3] Joyce A. Carlson,[4] Priscilla Moonsamy,[5] Anna Lisa Fear,[1] Julie A. Lane,[1] Eva Lavant,[4] Rebecca Rappner,[4] Anthony Louey,[3] Patrick Concannon,[6] Josyf C. Mychaleckyj,[6] and Henry A. Erlich,[1,5] for the Type 1 Diabetes Genetics Consortium

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; $P = 4 \times 10^{-11}$) and B*3906 (10.31; $P = 4 \times 10^{-10}$). 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*3906, 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-*DQB1*/*DQA1* (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

From the [1]Children's Hospital Oakland Research Institute, Oakland, California; the [2]Department of Twin Research and Genetic Epidemiology, King's College London, London, U.K.; the [3]Victorian Transplantation and Immunogenetics Service, Australian Red Cross Blood Service, Melbourne, Australia; [4]Clinical Chemistry, University Hospital, Malmö, Sweden; [5]Roche Molecular Systems, Pleasanton, California; and the [6]Center for Public Health Genomics, University of Virginia, Charlottesville, Virginia.

Corresponding author: Janelle A. Noble, jnoble@chori.org.

Received 15 May 2010 and accepted 13 August 2010. Published ahead of print at http://diabetes.diabetesjournals.org on 26 August 2010. DOI: 10.2337/db10-0699.

TABLE 1
Descriptive statistics of sample studied

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.

to be associated with type 1 diabetes. Subsets of the T1DGC 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.T1DGC.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 tested 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 T1DGC 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.T1DGC.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 T1DGC HLA genotyping centers (Oakland and Alameda, CA; Melbourne, Australia; and Malmö, 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, because of the extreme polymorphism of the HLA genes, some alleles were indistinguishable from others in our genotyping assay. Alleles exhibiting type 1 diabetes association after adjustment of the data for LD with HLA class II DR-DQ–encoding haplotypes, or that modify the risk for one of the risk haplotypes in our "relative odds ratio" analysis, are listed here with the alternative alleles that are consistent with the probe binding pattern for the called allele: A*0101 (0104N, 0108, 0109, 0111N); A*0201 (0209, 0222, 0225, 0229, 0230, 0231, 0232N, 0233, 0242, 0243N, 0253N, 0259, 0260, 0264, 0266, 0267, 0268, 0270, 0271, 0274, 0275, 0277, 0282N, 0283N, 0285, 0286); A*1101 (1105, 1107, 1109, 1112, 1113, 1115, 1121N, 1122, 1123); A*2402 (2409N, 2411N, 2420, 2423, 2425, 2426, 2427, 2429, 2430, 2431, 2434, 2435, 2437, 2439, 2440N, 2443, 2447, 2448N, 2449, 2452, 2453); A*3201 (3205, 3208, 3209); A*6601 (no ambiguous alleles); B*0702 (0710, 0721, 0722, 0723, 0730, 0735); B*1801 (1803, 1805, 1808, 1815, 1817N, 1820); B*3502 (3504, 3509); B*3906 (no ambiguous alleles); B*4403 (4413, 4432, 4436, 4438); B*5701 (B*5706); C*0401 (0405, 0409N, 0412); C*0501 (0503, 0505, 0507N, 0509, 0510); C*0701 (0706, 0716, 0718, 0720, 0724, 0727); and C*1601 (no ambiguous alleles). In this article, we also refer to general serologic nomenclature, e.g., DR3, DR4, DR1, and DR8, to refer to haplotypes bearing the *DRB1**03xx, *DRB1**04xx, *DRB1**01xx, and *DRB1**08xx alleles, respectively. The term DR2 is used in this article to refer to the most common, and highly type 1 diabetes protective, Caucasian DR2 haplotype (*DRB1**1501-*DQA1**0102-*DQB1**0602).

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 sibs.

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 (H_0) is that class I allele frequencies will differ between patients and controls *1*) because of LD between the class I loci and *DRB1-DQB1* and *2*) due to chance (sampling), thus implying that class I loci are neutral relative to disease predisposition.

Under H_0, the expected allele frequencies at a given class I allele can be computed using the equation derived by Thomson (15)

$$q_{\exp\, classIj} = p_{classI_j} + \sum_{i}^{K} D_{ij} \frac{q_{classIIi}}{p_{classIIi}}$$

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 >0.05% 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 2
HLA class I allele frequencies in 3,506 chromosomes from 1,753 type 1 diabetic probands and 1,585 affected family-based control chromosomes

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*0235	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)	0.934
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*3402	0.2 (6)	0.1 (1)	0.336
A*3601	0 (1)	0.1 (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
B*1510	0.1 (4)	0 (0)	0.179
B*1515	0 (1)	0 (0)	0.501
B*1516	0 (1)	0.1 (1)	0.564
B*1517	0.4 (15)	0.3 (4)	0.343
B*1518	0.2 (6)	0.3 (5)	0.305
B*1524	0.1 (3)	0 (0)	0.244
B*1525	0 (0)	0.1 (1)	0.137
B*1537	0 (1)	0 (0)	0.501
B*1571	0 (0)	0.1 (1)	0.137
B*1573	0 (0)	0.1 (1)	0.137
B*1801	10.2 (357)	6.3 (100)	**2.E-05**
B*1812	0 (1)	0 (0)	0.501
B*2701	0 (0)	0.1 (1)	0.137
B*2702	0.3 (9)	0.4 (6)	0.458
B*2703	3.2 (113)	3.1 (49)	0.807
B*2707	0 (1)	0.1 (2)	0.184
B*3501	3.6 (126)	6.3 (100)	**2.E-05**
B*3502	0.3 (12)	1.5 (23)	**1.E-05**
B*3503	1.6 (56)	1.5 (23)	0.698
B*3508	0.3 (12)	0.5 (8)	0.392
B*3512	0 (0)	0.1 (1)	0.137
B*3701	0.7 (23)	1 (16)	0.182
B*3801	1.8 (63)	2.5 (40)	0.091
B*3901	1.2 (42)	1.2 (19)	0.998
B*3906	3.2 (112)	0.4 (7)	**3.E-09**
B*4001	5.6 (198)	4.4 (69)	**0.062**
B*4002	0.6 (20)	1.6 (25)	**4.E-04**
B*4006	0.1 (3)	0.2 (3)	0.318
B*4009	0 (1)	0 (0)	0.501
B*4014	0 (0)	0.1 (2)	**0.035**
B*4101	1 (34)	0.3 (4)	**0.006**
B*4102	0.1 (2)	0.4 (6)	**0.007**
B*4201	0 (1)	0 (0)	0.501
B*4202	0 (0)	0.1 (1)	0.137
B*4402	6 (210)	9 (143)	**1.E-04**
B*4403	2 (69)	5.7 (91)	**2.E-12**
B*4404	0 (0)	0.1 (1)	0.137
B*4405	0.2 (7)	0.1 (1)	0.255
B*4501	0.5 (17)	0.4 (7)	0.835
B*4701	0.3 (10)	0.4 (6)	0.582
B*4702	0 (1)	0 (0)	0.501
B*4801	0.1 (2)	0.1 (1)	0.934
B*4901	2.2 (77)	1.1 (17)	**0.006**
B*5001	2.1 (75)	1.2 (19)	**0.022**
B*5002	0.1 (2)	0.1 (1)	0.934
B*5101	3 (104)	4.2 (67)	**0.023**
B*5102	0 (0)	0.1 (1)	0.137
B*5105	0 (0)	0.1 (1)	0.137
B*5107	0 (1)	0.1 (1)	0.564
B*5108	0.1 (4)	0.1 (1)	0.591
B*5201	0.3 (12)	1.1 (18)	**0.001**
B*5301	0.1 (5)	0.5 (8)	**0.018**
B*5501	0.9 (30)	1.6 (26)	**0.013**
B*5601	0.6 (20)	0.6 (10)	0.795
B*5701	0.5 (19)	3.5 (56)	**4.E-16**
B*5703	0 (1)	0.3 (5)	**0.006**
B*5801	0.9 (31)	1.1 (18)	0.397
B*7301	0.3 (9)	0.1 (1)	0.149
B*8101	0 (1)	0 (0)	0.501
C*0102	2 (69)	3 (48)	**0.021**

Continued

TABLE 2
Continued

Continued on facing page

TABLE 2
Continued

Allele	Type 1 diabetes (*n*)	AFBAC (*n*)	*P*
C*0202	3.9 (136)	4.4 (69)	0.435
C*0206	0 (1)	0.1 (1)	0.564
C*0302	0.3 (12)	0.4 (7)	0.591
C*0303	5.5 (194)	3.9 (62)	**0.017**
C*0304	13.5 (475)	6.6 (105)	**1.E-11**
C*0305	0.1 (3)	0 (0)	0.244
C*0314	0 (1)	0 (0)	0.501
C*0401	6 (212)	11.6 (184)	**4.E-11**
C*0403	0 (0)	0.1 (1)	0.137
C*0501	11.3 (397)	9.5 (150)	**0.061**
C*0602	5.9 (208)	8.4 (133)	**0.002**
C*0606	0 (1)	0 (0)	0.501
C*0701	25.7 (900)	15 (238)	**1.E-13**
C*0702	10.8 (378)	15.6 (247)	**6.E-06**
C*0704	1 (34)	1.9 (30)	**0.007**
C*0801	0.1 (5)	0.1 (2)	0.884
C*0802	1.9 (66)	3.2 (50)	**0.005**
C*1202	0.3 (12)	1.1 (17)	**0.001**
C*1203	5.7 (200)	6.4 (102)	0.322
C*1402	0.7 (25)	1.2 (19)	0.084
C*1502	1.4 (50)	2 (32)	0.123
C*1504	0.2 (8)	0.1 (2)	0.447
C*1505	0.8 (27)	0.3 (5)	**0.058**
C*1601	1.2 (43)	3.8 (60)	**3.E-09**
C*1602	0.5 (17)	0.2 (3)	0.119
C*1604	0.1 (4)	0.4 (6)	**0.049**
C*1701	0.8 (27)	0.7 (11)	0.771
C*1801	0 (1)	0.1 (1)	0.564

P values lower than 0.1 are shown in boldface type.

tioned on the *DRB1-HLA-B* haplotype or *HLA-B* allele. Both affected sibs from each family were used. Deviations from the random expectation were assessed using a χ^2 test where the total sum in the contingency table was ≥200, or a Fisher's exact test otherwise. This methodological approach has been described and discussed in detail elsewhere (16).

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 T1DGC collection. We performed association analyses on the T1DGC 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/db10-0699/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*2402 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 1 diabetes–associated class I alleles is shown in Fig. 1. Type 1 diabetes–associated class II allele groups, including DR3, DR4, DR1, DR8 (susceptible), and DR2 (protective) are also included. As expected,

TABLE 3
HLA class I alleles significantly different from expected values when accounting for LD with *DRB1-DQB1*

Allele	Type 1 diabetes observed (%)	Type 1 diabetes expected (%)†	OR (95% CI)	*P*
A*0101	17.7	21.9	0.77 (0.66–0.88)	0.002
A*0201	30.6	24.7	1.35 (1.17–1.54)	3.E-04
A*1101	3.4	6.2	0.53 (0.40–0.70)	8.E-06
A*2402	11.4	7.0	1.71 (1.37–2.12)	5.E-06
A*3201	2.1	3.8	0.54 (0.37–0.75)	4.E-04
A*6601	0.1	0.6	0.16 (0.04–0.57)	9.E-04
A*6802	0.4	1.2	0.34 (0.17–0.69)	0.002
B*0702	7.0	11.4	0.58 (0.47–0.70)	3.E-07
B*0705	0.7	0.2	3.42 (1.11–10.5)	0.023
B*1501	12.3	9.4	1.36 (1.11–1.65)	0.004
B*1801	10.2	5.2	2.05 (1.59–2.61)	3.E-08
B*3501	3.6	4.9	0.72 (0.53–0.96)	0.028
B*3502	0.3	1.2	0.29 (0.14–0.60)	5.E-04
B*3906	3.2	0.3	10.31 (4.21–25.1)	4.E-10
B*4002	0.5	1.2	0.45 (0.23–0.84)	0.012
B*4101	1.0	0.2	4.49 (1.48–13.6)	0.004
B*4102	0.1	0.3	0.19 (0.03–0.96)	0.020
B*4403	1.9	4.5	0.42 (0.30–0.59)	4.E-07
B*4901	2.2	0.9	2.6 (1.45–4.63)	0.001
B*5001	2.1	1.0	2.27 (1.30–3.94)	0.003
B*5201	0.3	0.9	0.37 (0.17–0.80)	0.009
B*5301	0.1	0.4	0.33 (0.10–1.06)	0.055
B*5701	0.5	2.8	0.19 (0.11–0.32)	4.E-11
B*5703	0.0	0.3	0.11 (0.01–0.96)	0.013
C*0303	5.5	3.8	1.48 (1.10–1.99)	0.010
C*0401	6.0	9.3	0.63 (0.50–0.78)	6.E-05
C*0501	11.3	7.6	1.56 (1.26–1.93)	9.E-05
C*1202	0.3	0.9	0.39 (0.17–0.83)	0.014
C*1402	0.7	1.4	0.52 (0.29–0.93)	0.027
C*1505	0.8	0.3	2.78 (1.01–7.62)	0.039
C*1601	1.2	3.0	0.39 (0.25–0.58)	5.E-06

†Expected values calculated based on LD of class I alleles with DR-DQ–encoding loci in this population.

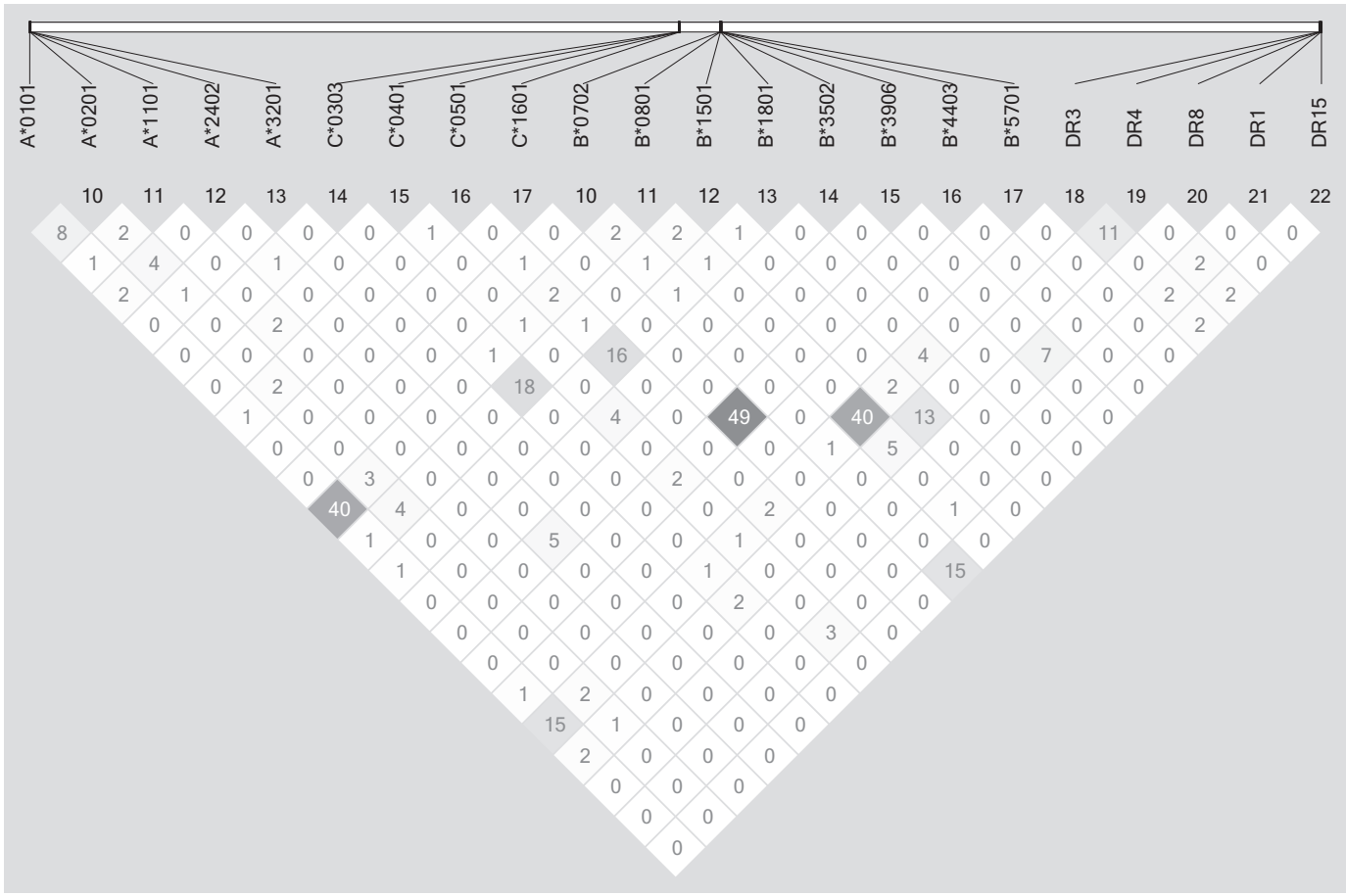

FIG. 1. LD diagram for selected HLA alleles. The $r^2 \times 100$ value of LD between pairs of alleles is shown.

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-

TABLE 4
Protective effect of C*1601 appears to be caused by LD with B*4403

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	
DR7	B*4403	not C*1601	81	10	10.99	<0.023
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 directly due 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. DR3 haplotypes (containing *DRB1**0301) and DR4 haplotypes (with *DRB1**04 alleles, 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*B*5701) 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-B* 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*4403, and C*0501) had opposite effects on different haplotypes, suggesting that the observed effects represent either haplotype-specific effects or type 1 error. In addition to B*3906, six additional alleles had risk effects of the same type on more than one haplotype. These include the predisposing alleles A*2402, B*1801, 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 T1DGC 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

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

Allele	DR3	DR4	DR8	DR1	DR2	Number	Consistent effect
A*0101	**0.69 (0.59–0.78)**	**0.75 (0.57–0.98)**				**2**	**Yes**
A*0201			0.58 (0.38–0.89)				
A*0301		1.47 (1.14–1.88)					
A*1101			X	0.60 (0.38–0.91)			
A*2402	**1.69 (1.25–2.28)**	**1.52 (1.15–2.01)**	**3.31 (1.90–5.74)**	**2.18 (1.51–3.14)**		**4**	**Yes**
A*2601		0.65 (0.46–0.89)			X		
A*2902	2.22 (1.03–4.78)						
A*3101		0.57 (0.41–0.79)					
A*3201		**0.57 (0.36–0.88)**	**0.19 (0.04–0.89)**		X	**2**	**Yes**
A*6601	X	0.10 (0.01–0.98)					
A*6801	0.62 (0.38–0.99)			3.32 (1.14–9.64)	4.83 (1.02–22.6)	3	No
B*0801	0.68 (0.62–0.74)						
B*1801	**1.58 (1.31–1.90)**	**1.74 (1.19–2.53)**				**2**	**Yes**
B*3501	X			4.79 (1.07–21.4)	4.79 (1.07–21.4)	2	No
B*3503	X			X	14.54 (2.66–79.3)		
B*3801	X	0.70 (0.49–0.97)	X				
B*3901	X		X	2.44 (1.01–5.88)			
B*3906	**11.56 (0.68–195.0)†**	**2.70 (1.16–6.29)**	**14.03 (5.55–35.4)**	**7.15 (3.89–13.1)**	**14.54 (2.66–79.3)**	**4**	**Yes**
B*4001		0.71 (0.56–0.88)					
B*4402			0.34 (0.13–0.84)				
B*4403	4.83 (1.12–20.6)	0.5 (0.32–0.77)	X	X		2	No
B*4901	X	1.61 (1.01–2.53)	X	X	X		
B*5001	1.95 (1.22–3.11)		X	X	X		
B*5101			0.36 (0.13–0.96)				
B*5301	X	X	X	X	28.11 (1.75–449.0)		
B*5601	0.10 (0.01–0.81)	X	X	X	X		
C*0102	0.30 (0.11–0.77)						
C*0401					4.82 (1.87–12.3)		
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				
C*0701	**0.70 (0.60–0.80)**		**0.26 (0.08–0.80)**	**0.51 (0.28–0.92)**		**3**	**Yes**
C*0702		**1.31 (1.00–1.70)**	**3.65 (2.42–5.48)**	**1.94 (1.41–2.65)**		**3**	**Yes**
C*1202	X	0.15 (0.03–0.61)	X	X	X		
C*1402	X		0.33 (0.11–0.92)		X		
C*1502		0.57 (0.37–0.87)		X			
C*1604	X	0.38 (0.15–0.90)					

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.

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

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. Thorough 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.

ACKNOWLEDGMENTS

This research uses resources provided by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Human Genome Research Institute (NHGRI), National Institute of Child Health and Human Development (NICHD), and Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418. This work was also supported by R01 DK61722 (J.A.N.).

No potential conflicts of interest relevant to this article were reported.

J.A.N. generated data/wrote the manuscript. A.M.V. performed all statistical analyses/wrote the manuscript. M.D.V., J.A.C., P.M., A.L.F., J.A.L., E.L., R.R., and A.L. generated data. P.C. contributed to discussion/reviewed the manuscript. J.C.M. contributed to discussion. H.A.E. generated data/reviewed and edited the manuscript.

REFERENCES

1. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, Mychaleckyj JC, Todd JA, Bonella P, Fear AL, Lavant E, Louey A, Moonsamy P, Type 1 Diabetes Genetics Consortium. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the Type 1 Diabetes Genetics Consortium families. Diabetes 2008;57:1084–1092
2. Noble JA, Valdes AM, Thomson G, Erlich HA. The HLA class II locus *DPB1* can influence susceptibility to type 1 diabetes. Diabetes 2000;49:121–125
3. Cucca F, Dudbridge F, Loddo M, Mulargia AP, Lampis R, Angius E, De Virgiliis S, Koeleman BP, Bain SC, Barnett AH, Gilchrist F, Cordell H, Welsh K, Todd JA. The HLA-*DPB1*–associated component of the IDDM1 and its relationship to the major loci HLA-*DQB1*, *-DQA1*, and *-DRB1*. Diabetes 2001;50:1200–1205
4. Valdes AM, Noble JA, Génin E, Clerget-Darpoux F, Erlich HA, Thomson G. Modeling of HLA class II susceptibility to type I diabetes reveals an effect associated with *DPB1*. Genet Epidemiol 2001;21:212–223
5. Cruz TD, Valdes AM, Santiago A, Frazer de Llado T, Raffel LJ, Zeidler A, Rotter JI, Erlich HA, Rewers M, Bugawan T, Noble JA. *DPB1* alleles are associated with type 1 diabetes susceptibility in multiple ethnic groups. Diabetes 2004;53:2158–2163
6. Stuchlikova M, Kantarova D, Michalkova D, Barak L, Buc M. Association of HLA-*DPB1* alleles with type I diabetes mellitus in Slovak population. Bratisl Lek Listy 2006;107:73–75
7. Noble JA, Valdes AM, Bugawan TL, Apple RJ, Thomson G, Erlich HA. The HLA class I A locus affects susceptibility to type 1 diabetes. Hum Immunol 2002;63:657–664
8. Tait BD, Colman PG, Morahan G, Marchinovska L, Dore E, Gellert S, Honeyman MC, Stephen K, Loth A. HLA genes associated with autoimmunity and progression to disease in type 1 diabetes. Tissue Antigens 2003;61:146–153
9. Valdes AM, Erlich HA, Noble JA. Human leukocyte antigen class I B and C loci contribute to type 1 diabetes (T1D) susceptibility and age at T1D onset. Hum Immunol 2005;66:301–313
10. Nejentsev S, Howson JM, Walker NM, Szeszko J, Field SF, Stevens HE, Reynolds P, Hardy M, King E, Masters J, Hulme J, Maier LM, Smyth D, Bailey R, Cooper JD, Ribas G, Campbell RD, Clayton DG, Todd JA, Wellcome Trust Case Control Consortium. Localization of type 1 diabetes susceptibility to the MHC class I genes *HLA-B* and *HLA-A*. Nature 2007;450:887–892
11. Howson JM, Walker NM, Clayton D, Todd JA. Confirmation of HLA class II independent type 1 diabetes associations in the major histocompatibility complex including *HLA-B* and *HLA-A*. Diabetes Obes Metab 2009; 11(Suppl. 1):31–45
12. Bugawan TL, Klitz W, Alejandrino M, Ching J, Panelo A, Solfelix CM, Petrone A, Buzzetti R, Pozzilli P, Erlich HA. The association of specific HLA class I and II alleles with type 1 diabetes among Filipinos. Tissue Antigens 2002;59:452–469
13. Helmberg W, Lanzer G, Zahn R, Weinmayr B, Wagner T, Albert E. Virtual DNA analysis: a new tool for combination and standardised evaluation of SSO, SSP and sequencing-based typing results. Tissue Antigens 1998;51: 587–592
14. Thomson G. Mapping disease genes: family-based association studies. Am J Hum Genet 1995;57:487–498
15. Thomson G. HLA DR antigens and susceptibility to insulin-dependent diabetes mellitus. Am J Hum Genet 1984;36:1309–1317
16. Valdes AM, Thomson G, Barcellos LF. Genetic variation within the HLA class III influences T1D susceptibility conferred by high-risk HLA haplotypes. Genes Immun 2010;11:209–218
17. Macdonald WA, Purcell AW, Mifsud NA, Ely LK, Williams DS, Chang L, Gorman JJ, Clements CS, Kjer-Nielsen L, Koelle DM, Burrows SR, Tait BD, Holdsworth R, Brooks AG, Lovrecz GO, Lu L, Rossjohn J, McCluskey J. A naturally selected dimorphism within the HLA-B44 supertype alters class I structure, peptide repertoire, and T cell recognition. J Exp Med 2003;198: 679–691
18. Aly TA, Eller E, Ide A, Gowan K, Babu SR, Erlich HA, Rewers MJ, Eisenbarth GS, Fain PR. Multi-SNP analysis of MHC region: remarkable conservation of HLA-A1–B8-DR3 haplotype. Diabetes 2006;55:1265–1269
19. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, Hallahan CW, Selig SM, Schwartz D, Sullivan J, Connors M. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc Natl Acad Sci U S A 2000;97:2709–2714
20. Trachtenberg E, Bhattacharya T, Ladner M, Phair J, Erlich H, Wolinsky S. The *HLA-B*/-C haplotype block contains major determinants for host control of HIV. Genes Immun 2009;10:673–677