HLA Class II Genotyping of African American Type 1 Diabetic Patients Reveals Associations Unique to African Haplotypes

Janelle A. Noble,[1] Jameel Johnson,[1] Julie A. Lane,[1] and Ana M. Valdes[1,2]

HLA genotyping was performed in African American type 1 diabetic patients (n = 772) and controls (n = 1,641) in the largest study of African Americans and type 1 diabetes reported to date. Cases were from Children's Hospital and Research Center Oakland and from existing collections (Type 1 Diabetes Genetics Consortium [T1DGC], Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications [DCCT/EDIC], and Genetics of Kidneys in Diabetes [GoKinD]). Controls were from the T1DGC and from newborn bloodspot cards. The diversity of HLA DRB1-DQA1-DQB1 haplotypes and genotypes is far greater than that found in Europeans and European Americans. Association analyses replicated many type 1 diabetes risk effects of European-derived haplotypes but also revealed novel effects for African-derived haplotypes. Notably, the African-specific "DR3" haplotype DRB1*03:02-DQA1*04:01-DQB1*04:02 is protective for type 1 diabetes, in contrast to the common and highly-susceptible DR3 DRB1*03:01-DQA1*05:01-DQB1*02:01. Both DRB1*07:01 and DRB1*13:03 haplotypes are predisposing when they include DQA1*03:01-DQB1*02:01g but are protective with DQA1*02:01-DQB1*02:01g. The heterozygous DR4/DR9 genotype, containing the African-derived "DR9" haplotype DRB1*09:01-DQA1*03:01-DQB1*02:01g, exhibits extremely high risk (odds ratio = 30.88), approaching that for DR3/DR4 in European populations. Disease risk assessment for African Americans differs greatly from risk assessment in European populations. This has profound implications on risk screening programs and underscores the need for high-resolution genotyping of multiple populations for the rational design of screening programs with tests that will fairly represent the population being screened.
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Type 1 diabetes is an autoimmune disease characterized by destruction of insulin-producing β-cells. The incidence and prevalence of type 1 diabetes are much higher for populations of European descent than for other ethnic groups (1). In the United States, diabetes mellitus is more common among nonwhite populations, including African Americans, than among non-Hispanic white (European ancestry) populations (2). Because type 2 diabetes is far more prevalent than type 1 diabetes in African American adults, and because the incidence of type 2 diabetes is increasing in African American youth because of the obesity epidemic, the burden of type 1 diabetes in African American youth has been less emphasized in the literature than that of type 2 diabetes (3). However, type 1 diabetes presents a serious burden among African American youth younger than 10 years of age, and African American adolescents are impacted substantially by both type 1 and type 2 diabetes (3). In fact, although type 2 diabetes incidence is increasing, type 1 diabetes is still approximately three-fold more common than type 2 diabetes in the African American pediatric population at Children's Hospital and Research Center Oakland. Moreover, early disease onset lengthens disease duration and likely leads to complications at relatively young ages. African American individuals with diabetes are at higher risk for the chronic complications of diabetes than are non-Hispanic white (European) Americans, particularly for diabetic nephropathy (4).

The incidence of type 1 diabetes varies widely around the world, partly because of ethnic differences in HLA allele and haplotype frequencies across populations. Susceptibility to type 1 diabetes is strongly associated with alleles at the DRB1 locus and at the DQA1 and DQB1 loci, which encode the α-chain and β-chain of the DQ heterodimer. In general, heterodimers that are DQα Arg52–positive and DQβ Asp57–negative represent high genetic risk for type 1 diabetes (5). Because the heterodimeric DQ molecule is encoded by two polymorphic genes, DQA1 and DQB1, individuals heterozygous for DQA1-DQB1 haplotypes have the potential to express up to four different DQ molecules on the cell surface. Heterodimers encoded *in trans* are postulated to help explain the extremely high risk of the heterozygous "DR3/DR4" genotype, which confers the highest genetic risk known for type 1 diabetes. Data from many reports show that specific combinations of alleles in DRB1-DQA1-DQB1 haplotypes are associated with type 1 diabetes risk.

To date, few studies have been reported on HLA association with type 1 diabetes in African Americans, and some early reports may be confounded by the inclusion of type 2 diabetes patients. This study is the largest of its kind reported to date (772 cases, 1,641 controls) and was made possible by combining data from newly collected samples with data from existing collections.

RESEARCH DESIGN AND METHODS

Genotyping methods. For consistency, all study subjects, including newly collected samples, samples from blood cards, and samples received from the National Institute of Diabetes and Digestive and Kidney Diseases repository (https://www.niddkrepository.org) were genotyped in the laboratory at Children's Hospital and Research Center Oakland using PCR sequence-specific oligonucleotide probe methodology with "linear arrays" as previously described for the Type 1 Diabetes Genetics Consortium (T1DGC) (6,7). The DQB1 linear array assay does not query exon 3 of the DQB1 gene; thus, the alleles DQB1*02:01 and DQB1*02:02 cannot be distinguished. The designation DRB1*02:01g is used in this report to designate this ambiguity.

From the [1]Children's Hospital Oakland Research Institute, Oakland, California; and [2]Academic Rheumatology, University of Nottingham, City Hospital, Nottingham, U.K.
Corresponding author: Janelle A. Noble, jnoble@chori.org.
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Study subjects

T1DGC cases and controls. The T1DGC was a large, worldwide, collaborative study aimed at collecting and genotyping new type 1 diabetes families in a highly standardized fashion, from multiple populations, to aid in the search for additional type 1 diabetes genes within and outside the HLA region (8). An individual was designated as affected if he or she had documented type 1 diabetes with onset at younger than 35 years of age, had used insulin within 6 months of diagnosis, and had no concomitant disease or disorder associated with diabetes. To avoid confounding of the data from population substructure, HLA frequency distributions from different groups of African American samples in the T1DGC were compared before sample selection. No significant differences were seen among groups collected in different geographic areas, with the exception of the samples collected in Canada, which differed significantly from the other groups ($P < 0.05$ overall; $P < 0.01$ at 4 of the 10 most common haplotypes; data not shown). The Canadian samples were not included in this study. For individuals of European origin, collections were limited to families; however, for African American and Hispanic individuals, cases and controls were collected as well. Thus, some subjects have family information and others do not. For families with more than one affected child, only data from the proband were used for this study to maintain independence of the samples. High-resolution HLA genotyping was performed at eight classical major histocompatibility complex loci by four genotyping centers using standardized typing protocols, reagents, and quality control procedures (7). In total, 664 African American type 1 diabetic cases from this collection were included in the current study. This included 31 Children's Hospital Oakland (CHO) study patients. Subjects were recruited with the approval of local ethics committees or institutional review boards. Additional details can be found on the T1DGC website (www.t1dgc.org). African American control subjects (n = 649), recruited and genotyped as part of the T1DGC, were included in the analyses reported here. For cases with family information available, haplotypes were determined using identity by descent. For nonfamily-based cases and controls, haplotypes were predicted based on established patterns of linkage disequilibrium from published data and from the extensive family-based data from the T1DGC (6,9).

CHO study cases. The CHO study is an ongoing study of pediatric diabetes mellitus patients recruited from the patient population of Children's Hospital and Research Center Oakland, formerly called CHO. Ethnic distribution of the type 1 diabetes patients recruited for the CHO study collection is 38% European American, 27% African American, 25% Hispanic, and the remaining 10% is of other or mixed ethnicity. This distribution reflects both the diverse patient population at Children's Hospital and Research Center Oakland and the emphasis on recruitment of non-European patients for the study. Of 67 African American type 1 diabetic patients enrolled in the CHO study, 36 were not enrolled in the T1DGC. Samples from these 36 subjects were genotyped at the DRB1, DQA1, and DQB1 loci and included in the present report. The CHO study patients received diagnoses from Children's Hospital and Research Center Oakland pediatric endocrinologists using available clinical data including, but not limited to, autoantibodies, C-peptide measurements, age, and BMI. Diagnoses for African American patients were reviewed by a second pediatric endocrinologist. Subjects were included in the study if the diagnosis of type 1 diabetes was unequivocal to the endocrinologists. Subjects with known or suspected type 2 diabetes, maturity-onset diabetes of youth, or other forms of diabetes were excluded. Ethnicity was determined by self-report and confirmed by clinician interview. The CHO study patients are a mixture of individuals and family-based samples. Haplotypes were determined using identity-by-descent when possible and were predicted using known patterns of linkage disequilibrium, as for the T1DGC cases and controls, when family data were not available. The CHO study was approved by the Children's Hospital and Research Center Oakland Institutional Review Board.

Cases from existing collections. Samples were obtained from the National Institute of Diabetes and Digestive and Kidney Diseases repository for African American patients collected as part of two large studies, the Diabetes Complications Control Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) and the Genetics of Kidney Disease in Diabetes (GoKinD) (10). Both studies are primarily comprised of individuals of European descent; however, a small proportion of the subjects are African American. Genotypes from 28 patients from the DCCT/EDIC collection and 44 patients from the GoKinD collection were generated with the same method used for genotyping the T1DGC collection, and haplotypes were predicted as for the other samples in this report.

Population-based controls. Extracted DNA samples from 992 dried blood cards (Guthrie cards) from newborns in California were used as a population-based control sample. These newborns were reported by the mothers to be of African American ethnicity. No other information about these subjects is available to the research team. Although the possibility exists that the collection may contain future patients, the low prevalence of type 1 diabetes in African Americans suggests that the number of future type 1 diabetic patients in this sample set should be no more than one (3). Haplotypes were predicted using known patterns of linkage disequilibrium for HLA class II alleles from published literature and from the family-based T1DGC data (6,9). The samples were obtained from and used with permission from the State of California Department of Public Health Genetic Disease Screening Program. Genotyping was performed with the approval of the Children's Hospital and Research Center Oakland Institutional Review Board. To address the issue of whether population substructure might confound these results, the HLA frequency distribution of the combined blood card and T1DGC control set was compared with the distribution in controls from a smaller recent study of HLA and type 1 diabetes in African Americans (11). The two data sets did not differ significantly (P = 0.16; data not shown).

Genotype groups. To facilitate analysis of genotype risk, haplotypes were grouped into the following categories: DR3 Cau: DRB1*03:01-DQA1*05:01-DQB1*02:01g; DR4: DRB1*04:01/02/04/05/08-DQA1*03:01-DQB1*03:02/02:01g; DR3 Af: DRB1*03:02-DQA1-*04:01-DQB1*04:02; DR7 Af: DRB1*07:01-DQA1*03:01-DQB1*02:01g; DR7 Cau: DRB1*07:01-DQA1*02:01-DQB1*02:01g; DR9 Af: DRB1*09:01-DQA1*03:01-DQB1*02:01g; DR8 Af: any DRB1*08 except DRB1*08:01-DQA1*04:01-DQB1 04:02; DR11-12-14: any DRB1*11, *12, or *14 haplotype; DR13: any DRB1*13 haplotype; DR15-DQ6: DRB1*15:01/03-DQB1*0602; and X: any other haplotype.

Statistical analysis. Haplotype frequencies between cases and controls were compared using a Pearson χ^2 test. Odds ratios (ORs) and 95% CIs were computed.

RESULTS

HLA diversity of African Americans. The diversity of HLA haplotypes is far higher in African Americans than in European-derived populations. From the total of 772 cases and 1,641 controls analyzed for this report, 257 different DRB1-DQA1-DQB1 haplotypes and 958 different genotypes were observed. Table 1 lists 59 individual haplotypes that were seen at least seven times total for cases and controls. For comparison, the previous report of European and European American data from 607 patients (1,214 haplotypes) and 898 control haplotypes from the T1DGC showed 44 haplotypes that appeared at least twice (6); this African American sample set had 144 haplotypes that appeared at least twice. The full set of 251 observed African American haplotypes is provided in Supplementary Table 1.

Type 1 diabetes association of DR-DQ haplotypes. Twenty-nine of the 59 haplotypes shown in Table 1 show statistically significant association (positive or negative) with type 1 diabetes. Even if a highly conservative Bonferroni correction for multiple testing were applied, 18 of these haplotypes would remain significantly associated. Some of these are haplotypes that are well-established to contribute to type 1 diabetes risk in European-derived populations, including the predisposing DRB1*03:01-DQA1*05:01-DQB1*02:01g and DRB1*04:01/02/04/05-DQA1*03:01-DQB1*03:02 and the protective DRB1*15:01-DQA1*01:02-DQB1*06:02 and DRB1*14:01-DQA1*01:01-DQB1*05:03 haplotypes. Other strongly associated haplotypes were revealed in these African American data that were not seen in studies of European-derived populations. Notable examples of protective haplotypes revealed in this data set include DRB1*03:02-DQA1*04:01-DQB1*04:02, DRB1*15:03-DQA1*01:02-DQB1*06:02, DRB1*08:04-DQA1*04:01-DQB1*03:01, and DRB1*13:03-DQA1*02:01-DQB1*02:01g. Predisposing haplotypes not seen in European-derived populations include DRB1*16:01-DQA1*01:02-DQB1*05:02, DRB1*13:03-DQA1*03:01-DQB1*02:01g, DRB1*09:01-DQA1*03:01-DQB1*02:01g, and DRB1*07:01-DQA1*03:01-DQB1*02:01g.

DQA1*03:01-DQB1*02:01g haplotype. The DQ-encoding haplotype DQA1*03:01-DQB1*02:01g appears coupled to six different DRB1 alleles observed more than seven times in these data and is significantly predisposing for type 1 diabetes when coupled with DRB1*04:05, 07:01, 09:01, or 13:03 (Table 1). The remaining DRB1 alleles observed in

TABLE 1
African American haplotype associations with type 1 diabetes

DRB1	DQA1	DQB1	Control	%	T1D	%	*P**	OR	(95% CI)	African American†
01:01	01:01	05:01	77	2.30	42	2.70	0.44	1.16	(0.8–1.70)	
01:02	01:01	05:01	114	3.50	39	2.50	0.08	0.72	(0.50–1.04)	
01:03	01:01	05:01	10	0.30	2	0.13	0.26	0.42	(0.09–1.94)	
03:01	05:01	02:01g	224	6.80	398	25.80	**1.33E-65**	4.74	(3.97–5.66)	
03:02	04:01	04:02	175	5.30	14	0.90	**4.29E-13**	0.16	(0.09–0.28)	+
03:02	05:01	02:03	7	0.21	2	0.13	0.53	0.61	(0.13–2.92)	
04:01	03:01	03:01	28	0.90	16	1.00	0.53	1.22	(0.66–2.26)	
04:01	03:01	03:02	31	0.90	130	8.40	**3.86E-40**	9.64	(6.48–14.34)	
04:02	03:01	03:02	4	0.10	13	0.80	**8.44E-05**	6.96	(2.27–21.38)	
04:03	03:01	03:02	8	0.24	3	0.19	0.74	0.80	(0.21–3.01)	
04:04	03:01	03:02	25	0.80	50	3.20	**1.21E-10**	4.36	(2.69–7.07)	
04:05	03:01	02:01g	3	0.10	20	1.30	**1.59E-08**	14.34	(4.26–48.34)	
04:05	03:01	03:02	28	0.90	125	8.10	**1.13E-39**	10.24	(6.76–15.50)	
04:07	03:01	03:01	9	0.27	0	0.03	0.08	0.12	(0.01–2.03)	
04:07	03:01	03:02	13	0.40	5	0.30	0.7	0.82	(0.29–2.30)	
07:01	02:01	02:01g	273	8.30	83	5.40	**4.47E-04**	0.63	(0.49–0.81)	
07:01	02:01	03:03	17	0.50	8	0.50	1	1.00	(0.43–2.32)	
07:01	03:01	02:01g	43	1.30	67	4.30	**7.94E-11**	3.42	(2.32–5.04)	+
08:01	04:01	04:02	17	0.50	13	0.80	0.18	1.63	(0.79–3.37)	
08:02	04:01	04:02	8	0.24	1	0.06	0.18	0.27	(0.03–2.12)	
08:04	04:01	03:01	104	3.20	11	0.70	**2.52E-07**	0.22	(0.12–0.41)	+
08:04	04:01	04:02	18	0.50	7	0.50	0.67	0.83	(0.34–1.98)	
08:04	05:01	03:01	35	1.10	8	0.50	0.06	0.48	(0.22–1.04)	
08:06	01:02	06:02	13	0.40	0	0.00	**2.59E-02**	0.08	(0.00–1.38)	+
09:01	03:01	02:01g	91	2.80	128	8.30	**4.74E-17**	3.17	(2.41–4.18)	+
09:01	03:01	03:03	17	0.50	13	0.80	0.18	1.63	(0.79–3.37)	
10:01	01:01	05:01	64	2.00	11	0.70	**1.30E-03**	0.36	(0.19–0.69)	
11:01	01:02	05:01	10	0.30	2	0.13	0.26	0.42	(0.09–1.94)	
11:01	01:02	05:02	10	0.30	6	0.40	0.64	1.28	(0.46–3.52)	
11:01	01:02	06:02	107	3.30	1	0.10	**4.46E-12**	0.02	(0.00–0.14)	
11:01	03:01	02:01	12	0.40	11	0.70	0.1	1.96	(0.86–4.44)	
11:01	05:01	03:01	77	2.30	12	0.80	**1.81E-04**	0.33	(0.18–0.60)	
11:01	05:02	03:01	4	0.12	4	0.26	0.27	2.13	(0.53–8.52)	
11:01	05:02	03:01	4	0.12	4	0.26	0.27	2.13	(0.53–8.52)	
11:02	05:01	03:01	123	3.70	15	1.00	**1.04E-07**	0.25	(0.15–0.43)	
11:04	05:01	03:01	17	0.50	2	0.10	**0.045**	0.25	(0.06–1.08)	
12:01	01:01	05:01	91	2.80	19	1.20	**9.33E-04**	0.44	(0.27–0.72)	
12:01	05:01	03:01	18	0.50	9	0.60	0.88	1.06	(0.48–2.37)	
13:01	01:02	05:01	31	0.90	5	0.30	**1.99E-02**	0.34	(0.13–0.88)	
13:01	01:03	05:01	6	0.18	1	0.06	0.32	0.35	(0.04–2.94)	
13:01	01:03	06:03	79	2.40	18	1.20	**4.55E-03**	0.48	(0.29–0.80)	
13:01	01:03	06:04	11	0.34	3	0.19	0.40	0.58	(0.65–0.16)	
13:01	01:03	06:08	13	0.40	2	0.13	0.12	0.33	(0.76–0.07)	
13:01	01:03	06:09	13	0.40	2	0.13	0.12	0.33	(0.76–0.07)	
13:01	03:01	03:03	19	0.60	10	0.60	0.77	1.12	(0.52–2.41)	
13:02	01:02	05:01	58	1.80	13	0.80	**1.34E-02**	0.47	(0.26–0.86)	
13:02	01:02	05:02	11	0.30	3	0.20	0.40	0.58	(0.16–2.08)	
13:02	01:02	06:04	66	2.00	44	2.80	0.07	1.43	(0.97–2.10)	
13:02	01:02	06:09	72	2.20	26	1.70	0.25	0.76	(0.49–1.20)	
13:03	02:01	02:01g	43	1.30	4	0.30	**5.58E-04**	0.20	(0.07–0.55)	+
13:03	03:01	02:01g	1	0.03	6	0.39	**0.002**	12.80	(1.54–106.41)	
13:03	05:01	03:01	45	1.40	10	0.60	**0.028**	0.47	(0.24–0.93)	+
13:04	05:01	03:01	40	1.20	10	0.60	0.07	0.53	(0.26–1.06)	
14:01	01:01	05:03	52	1.60	1	0.10	**2.62E-06**	0.04	(0.01–0.29)	
15:01	01:02	06:02	96	2.90	3	0.20	**6.49E-10**	0.06	(0.02–0.20)	
15:03	01:02	06:02	353	10.80	20	1.30	**2.84E-28**	0.11	(0.07–0.17)	
15:03	03:01	02:01g	5	0.15	3	0.19	0.74	1.28	(0.30–5.35)	
16:01	01:02	05:02	2	0.10	7	0.50	**0.003**	7.47	(1.55–36)	+
16:02	01:02	05:02	38	1.20	11	0.70	0.15	0.61	(0.31–1.20)	
16:02	05:01	03:01	6	0.18	1	0.06	0.32	0.35	(0.04–2.94)	
16:02	05:01	03:01	6	0.18	1	0.06	0.32	0.35	(0.04–2.94)	
Other haplotypes	299		46							
Total			3,282		1,544					

Only haplotypes seen at least seven times total in patients and controls are included. +, positive. T1D, type 1 diabetes. *Given the number of tests performed, a $P = 0.00071$ is required for statistical significance after a Bonferroni correction for multiple tests. †Haplotypes marked in the African American column include type 1 diabetes associated haplotypes observed in these African American subjects but not observed in European American type 1 diabetes association studies. Significant *P* values are indicated in boldface.

TABLE 2
HLA class II selected genotype associations with type 1 diabetes in African Americans

DRB1	DQA1	DQB1	*DRB1*	*DQA1*	*DQB1*	Control	%	T1D	%	OR	(95% CI)	*P*
01:01	01:01	05:01	03:01	05:01	02:01g	6	0.4	12	1.6	4.31	(1.61–11.52)	**1.59E-03**
01:02	01:01	05:01	03:01	05:01	02:01g	11	0.7	5	0.6	0.97	(0.33–2.79)	0.95
01:02	01:01	05:01	07:01	03:01	02:01g	1	0.1	6	0.8	12.86	(1.54–107.02)	**2.29E-03**
03:01	05:01	02:01g	03:01	05:01	02:01g	6	0.4	69	8.9	26.78	(11.50–61.92)	**7.25E-29**
03:01	05:01	02:01g	10:01	01:01	05:01	6	0.4	2	0.3	0.71	(0.14–3.51)	0.67
03:01	05:01	02:01g	11:01	01:02	06:02	8	0.5	0	0.0	0.13	(0.00–2.30)	0.09
03:01	05:01	02:01g	11:02	05:01	03:01	8	0.5	0	0.0	0.13	(0.00–2.30)	0.09
03:01	05:01	02:01g	12:01	01:01	05:01	6	0.4	2	0.3	0.71	(0.14–3.51)	0.67
03:01	05:01	02:01g	13:02	01:02	06:04	4	0.2	7	0.9	3.75	(1.09–12.85)	**2.43E-02**
03:01	05:01	02:01g	13:02	01:02	06:09	5	0.3	7	0.9	3.00	(0.94–9.47)	0.05
03:01	05:01	02:01g	13:02	01:02	05:01	6	0.4	2	0.3	0.71	(0.14–3.51)	0.67
01:02	01:01	05:01	04:01	03:01	03:02	1	0.1	6	0.8	12.86	(1.54–107.03)	**2.29E-03**
03:01	05:01	02:01g	04:01	03:01	03:02	1	0.1	32	4.1	71.01	(9.68–520.70)	**1.17E-15**
03:01	05:01	02:01g	04:05	03:01	02:01g	1	0.1	6	0.8	12.86	(1.54–107.03)	**2.29E-03**
04:01	03:01	03:02	13:02	01:02	06:04	0	0.0	6	0.8	25.73	(1.43–461.33)	**5.82E-04**
03:01	05:01	02:01g	04:04	03:01	03:02	0	0.0	18	2.3	78.43	(4.71–1,304.57)	**9.97E-10**
03:01	05:01	02:01g	04:05	03:01	03:02	0	0.0	35	4.5	156.03	(9.55–2,547.74)	**1.00E-17**
03:02	04:01	04:02	04:05	03:01	03:02	3	0.2	5	0.6	3.56	(0.84–14.95)	0.06
13:02	01:02	06:04	04:05	03:01	03:02	0	0.0	8	1.0	34.40	(1.97–599.74)	**6.09E-05**
01:02	01:01	05:01	07:01	02:01	02:01g	11	0.7	2	0.3	0.39	(0.08–1.74)	0.20
03:01	05:01	02:01g	07:01	03:01	02:01g	2	0.1	21	2.7	22.95	(5.36–98.12)	**1.04E-09**
03:01	05:01	02:01g	07:01	02:01	02:01g	20	1.2	19	2.5	2.05	(1.08–3.85)	**2.49E-02**
03:02	04:01	04:02	07:01	02:01	02:01g	10	0.6	1	0.1	0.21	(0.02–1.65)	0.10
04:01	03:01	03:02	07:01	02:01	02:01g	1	0.1	9	1.2	19.37	(2.44–153.17)	**8.31E-05**
04:01	03:01	03:02	07:01	03:01	02:01g	2	0.1	7	0.9	7.51	(1.55–36.23)	**3.21E-03**
04:04	03:01	03:02	07:01	02:01	02:01g	1	0.1	7	0.9	15.03	(1.84–122.35)	**7.56E-04**
04:05	03:01	03:02	07:01	02:01	02:01g	2	0.1	12	1.6	12.96	(2.89–58.00)	**1.61E-05**
04:05	03:01	03:02	07:01	03:01	02:01g	2	0.1	7	0.9	7.51	(1.55–36.23)	**3.21E-03**
07:01	02:01	02:01g	07:01	02:01	02:01g	14	0.9	0	0.0	0.08	(0.00–1.26)	**1.76E-02**
10:01	01:01	05:01	07:01	02:01	02:01g	13	0.8	0	0.0	0.08	(0.00–1.37)	**2.30E-02**
11:01	05:01	03:01	07:01	02:01	02:01g	9	0.5	0	0.0	0.12	(0.00–2.03)	0.07
11:01	01:02	06:02	07:01	02:01	02:01g	13	0.8	0	0.0	0.08	(0.00–1.37)	**2.30E-02**
11:02	05:01	03:01	07:01	02:01	02:01g	10	0.6	3	0.4	0.64	(0.17–2.32)	0.49
12:01	01:01	05:01	07:01	02:01	02:01g	9	0.5	0	0.0	0.12	(0.00–2.03)	0.07
13:01	01:03	06:03	07:01	02:01	02:01g	11	0.7	1	0.1	0.19	(0.02–1.49)	0.08
13:02	01:02	06:04	07:01	02:01	02:01g	5	0.3	3	0.4	1.28	(0.30–5.36)	0.74
13:02	01:02	06:09	07:01	02:01	02:01g	7	0.4	1	0.1	0.30	(0.03–2.46)	0.24
03:01	05:01	02:01g	08:04	04:01	03:01	9	0.5	3	0.4	0.71	(0.19–2.62)	0.60
01:01	01:01	05:01	09:01	03:01	02:01g	4	0.2	5	0.6	2.67	(0.71–9.97)	0.13
01:02	01:01	05:01	09:01	03:01	02:01g	1	0.1	5	0.6	10.70	(1.24–91.79)	**6.97E-03**
03:01	05:01	02:01g	09:01	03:01	03:03	1	0.1	5	0.6	10.70	(1.24–91.79)	**6.97E-03**
03:01	05:01	02:01g	09:01	03:01	02:01g	7	0.4	23	3.0	7.18	(3.06–16.80)	**1.52E-07**
04:01	03:01	03:02	09:01	03:01	02:01g	0	0.0	8	1.0	34.40	(1.97–599.74)	**6.09E-05**
04:05	03:01	03:02	09:01	03:01	02:01g	0	0.0	15	1.9	65.10	(3.88–1,091.06)	**2.65E-08**
07:01	02:01	02:01g	09:01	03:01	02:01g	7	0.4	6	0.8	1.83	(0.61–5.46)	0.27
09:01	03:01	02:01g	09:01	03:01	02:01g	1	0.1	9	1.2	19.37	(2.44–153.17)	**8.31E-05**
09:01	03:01	02:01g	12:01	01:01	05:01	5	0.3	4	0.5	1.71	(0.45–6.37)	0.42
01:02	01:01	05:01	11:02	05:01	03:01	7	0.4	1	0.1	0.30	(0.03–2.46)	0.24
03:01	05:01	02:01g	15:01	01:02	06:02	8	0.5	0	0.0	0.13	(0.00–2.30)	0.09
01:01	01:01	05:01	15:03	01:02	06:02	12	0.7	0	0.0	0.09	(0.00–1.49)	**3.01E-02**
01:02	01:01	05:01	15:03	01:02	06:02	9	0.5	1	0.1	0.24	(0.02–1.86)	0.14
03:01	05:01	02:01g	15:03	01:02	06:02	27	1.6	3	0.4	0.23	(0.07–0.77)	**9.88E-03**
03:02	04:01	04:02	15:03	01:02	06:02	27	1.6	1	0.1	0.08	(0.01–0.57)	**1.27E-03**
07:01	02:01	02:01g	15:03	01:02	06:02	34	2.1	1	0.1	0.06	(0.00–0.44)	**2.22E-04**
08:04	04:01	03:01	15:03	01:02	06:02	15	0.9	2	0.3	0.28	(0.06–1.23)	0.07
09:01	03:01	02:01g	15:03	01:02	06:02	9	0.5	0	0.0	0.12	(0.00–2.03)	0.07
11:01	01:02	06:02	15:03	01:02	06:02	11	0.7	0	0.0	0.10	(0.00–1.63)	**3.95E-02**
11:02	05:01	03:01	15:03	01:02	06:02	18	1.1	0	0.0	0.06	(0.00–0.97)	**6.17E-03**
12:01	01:02	05:01	15:03	01:01	06:02	8	0.5	0	0.0	0.13	(0.00–2.30)	0.09
13:02	01:02	06:04	15:03	01:02	06:02	10	0.6	1	0.1	0.21	(0.02–1.65)	0.10
13:02	01:02	05:01	15:03	01:02	06:02	8	0.5	0	0.0	0.13	(0.00–2.30)	0.09
13:02	01:02	06:09	15:03	01:02	06:02	8	0.5	0	0.0	0.13	(0.00–2.30)	0.09
15:01	01:02	06:02	15:03	01:02	06:02	10	0.6	0	0.0	0.11	(0.00–1.81)	0.05
15:03	01:02	06:02	15:03	01:02	06:02	24	1.5	1	0.1	0.09	(0.01–0.64)	**2.71E-03**
Other						1,146	69.8	357	46.2			**1.09E-09**

T1D, type 1 diabetes. Significant *P* values are indicated in boldface.

combination with this DQ haplotype, DRB1*15:03 and DRB1*11:01, are generally considered protective. However, even these protective alleles are seen more frequently in cases than in controls when coupled with the DQA1*03:01-DQB1*02:01g haplotype, although the effect does not reach statistical significance (Table 1). These data are consistent with the hypothesis that DQA1*03:01-DQB1*02:01g is highly predisposing. As previously reported for African Americans, the effect of DRB1*07:01 is predisposing when in haplotype with DQA1*03:01-DQB1*02:01g but is protective when combined with the European-derived DQ haplotype DQA1*02:01-DQB1*02:01g (12). The exception to this rule is that DRB1*07:01-DQA1*02:01-DQB1*02:01g, which is generally protective, is highly predisposing when in genotype combination with DRB1*04 alleles, in which case the DQA1*03:01 and DQB1*02:01g gene products are encoded *in trans* (Table 2). The only other haplotype combination in which the European DR7 appears modestly predisposing is when the other haplotype is DRB1*03:01-DQB1*05:01-DQB1*02:01g (OR = 2.05, Table 2).

A similar result is seen in these data with the African allele DRB1*13:03. DRB1*13:03-DQA1*03:01-DQB1*02:01g is significantly predisposing (OR = 12.82; $P = 0.002$), whereas DRB1*13:03-DQA1*02:01-DQB1*02:01g is significantly protective (OR = 0.2; $P = 5.58 \times 10^{-4}$; Table 1). DQA1*02:01 and DQA1*03:01 differ at seven amino acid positions, including Arg 52, which is thought to be important in type 1 diabetes risk (Fig. 1) (5). This result is notable for its consistency with the DR7 observation, although given the small number of observed haplotypes, replication in a separate data set of similar or larger size to the one used in this report would be useful if such a data set were available.

Type 1 diabetes risk specific to African American genotypes. Table 2 shows 64 genotypes observed at least six times from >900 genotypes observed in this data set. Of these, 35 show significant association, either positive or negative, with type 1 diabetes. As expected, heterozygous genotypes containing DRB1*03:01-DQA1*05:01-DQB1*02:01g and DRB1*04:01/04/05-DQA1*03:01-DQB1*03:02 (DR3/DR4) show the highest positive association with disease (OR = 156.03 for the DR3/DR4 genotype with DRB1*04:05, 78.43 with DRB1*04:04; OR = 71.01 with DRB1*04:01). These three genotypes combined represent 10.9% of the total patient population and only 0.1% of the controls.

Notably, the haplotype DRB1*09:01-DQA1*03:01-DQB1*02:01g (OR = 3.17; Table 1) also exhibits very high risk when in genotype combination with DR4 (OR = 65.10 with DRB1*04:05; OR = 34.40 with DRB1*04:01; Table 2).

DRB1-DQA1-DQB1 haplotypes were placed into 11 categories based on their effect on type 1 diabetes susceptibility, as described in the Research Design and Methods section. Genotype association analyses were then performed; the results are provided in Table 3. In general, genotypes containing DR4, non-African DR3, and the African-derived DR7 and DR9 haplotypes are predisposing, whereas DR15 and the African-derived DR3 (DRB1*03:02-DQA1*04:01-DQB1*04:02) are the most highly protective.

DISCUSSION

The HLA contribution to the total genetic susceptibility for type 1 diabetes has been estimated at ~50% (13,14). The association of HLA with type 1 diabetes was first observed nearly 40 years ago (15). In addition to its role in transplantation and infectious disease, HLA is also well-known to contribute to the genetic susceptibility to other autoimmune diseases (16). The genetics of susceptibility to type 1 diabetes has been extensively studied since the initial observation, most notably including the T1DGC, which was created in an attempt to find all of the other genetic determinants of type 1 diabetes susceptibility (8). The overall

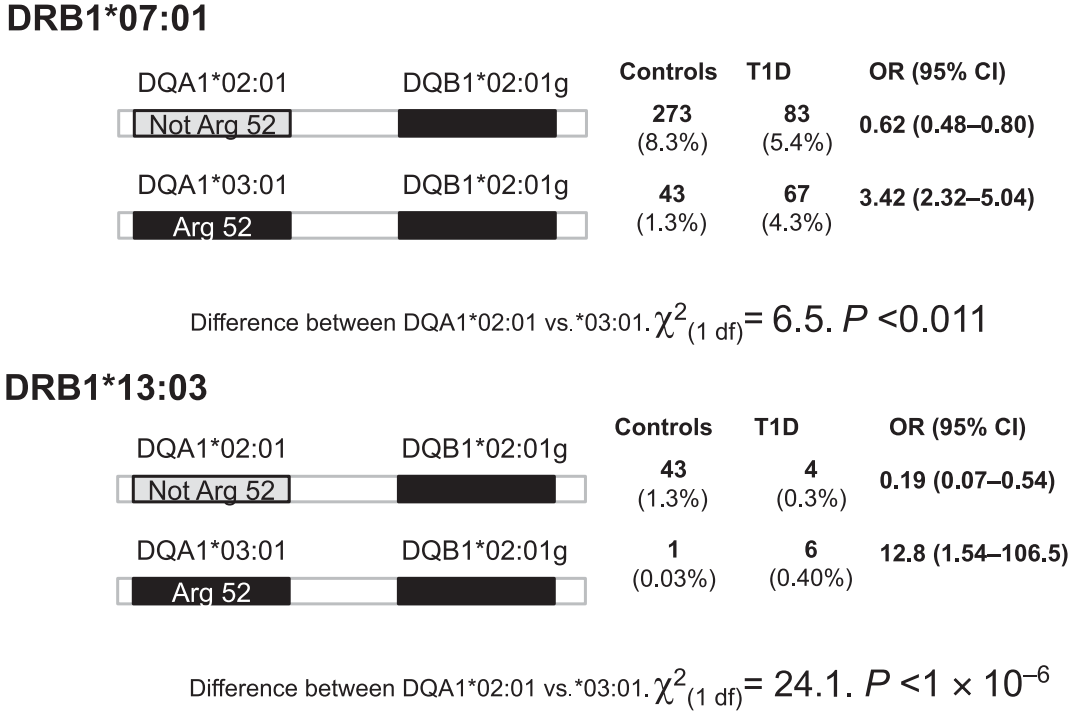

FIG. 1. Illustration of the increase in risk for DQA1*03:01-DQB1*02:01g compared with DQA1*03:01-DQB1*02:01g on DRB1 haplotypes carrying DRB1*07:01 and DRB1*13:03. T1D, type 1 diabetes.

TABLE 3
HLA class II genotype categories associated with type 1 diabetes in African Americans

Genotype*		Control	%	T1D	%	OR	95% CI	*P*
DR3Cau	DR4	5	0.3	96	12.4	46.47	18.8–114.69	**4.9E-42**
DR4	DR9Af	2	0.1	28	3.6	30.84	7.32–129.81	**5.9E-13**
DR3Cau	DR3Cau	6	0.4	69	8.9	26.75	11.5–61.89	**7.9E-29**
DR3Cau	DR7Af	2	0.1	21	2.7	22.92	5.35–97.98	**1.1E-09**
DR9Af	DR9Af	1	0.1	9	1.2	19.34	2.44–152.97	**8.4E-05**
DR4	DR4	2	0.1	17	2.2	18.45	4.25–80.07	**7.8E-08**
DR4	DR7-DQ2	5	0.3	29	3.8	12.77	4.92–33.12	**2.7E-11**
DR4	DR8Af	1	0.06	5	0.65	10.70	1.24–91.79	**0.007**
DR4	DR13	13	0.8	52	6.7	9.04	4.89–16.78	**1.1E-16**
DR4	DR7Af	4	0.2	16	2.1	8.66	2.88–25.90	**4.2E-06**
DR3Cau	DR9Af	7	0.4	23	3.0	7.17	3.06–16.78	**1.6E-07**
DR4	X	24	1.5	55	7.1	5.17	3.17–8.41	**7.5E-13**
DR7Af	X	9	0.5	14	1.8	3.35	1.44–7.77	**3.0E-03**
DR9Af	X	18	1.1	25	3.2	3.02	1.63–5.56	**2.4E-04**
DR3Cau	X	42	2.6	54	7.0	2.86	1.89–4.32	**3.5E-07**
DR9Af	DR13	13	0.8	15	1.9	2.48	1.17–5.24	**0.014**
DR3Cau	DR7-DQ2	20	1.2	19	2.5	2.05	1.08–3.85	**0.025**
DR4	DR11-12-14	20	1.2	15	1.9	1.61	0.81–3.15	0.17
DR3Cau	DR13	43	2.6	30	3.9	1.50	0.93–2.41	0.10
DR3Cau	DR8Af	13	0.8	8	1.0	1.31	0.54–3.17	0.55
X	X	58	3.5	18	2.3	0.65	0.38–1.11	0.12
DR13	DR13	51	3.1	13	1.7	0.53	0.28–0.98	0.045
DR7-DQ2	DR13	52	3.2	11	1.4	0.44	0.22–0.85	0.013
DR13	X	92	5.6	18	2.3	0.40	0.24–0.67	**4.4E-04**
DR3Cau	DR11-12-14	39	2.4	6	0.8	0.32	0.13–0.76	**0.007**
DR7-DQ2	X	52	3.2	7	0.9	0.28	0.12–0.61	**9.2E-04**
DR15-DQ6	DR8Af	25	1.5	3	0.4	0.25	0.07–0.83	**0.016**
DR11-12-14	DR11-12-14	50	3.0	5	0.6	0.21	0.08–0.52	**2.7E-04**
DR11-12-14	DR13	75	4.6	7	0.9	0.19	0.08–0.41	**5.3E-06**
DR11-12-14	DR7-DQ2	34	2.1	3	0.4	0.18	0.05–0.60	**1.8E-03**
DR3Cau	DR15-DQ6	35	2.1	3	0.4	0.18	0.05–0.58	**1.4E-03**
DR11-12-14	X	100	6.1	8	1.0	0.16	0.07–0.33	**4.3E-08**
DR3Af	DR11-12-14	29	1.8	2	0.3	0.14	0.03–0.60	**2.3E-03**
DR3Af	DR13	31	1.9	2	0.3	0.13	0.03–0.56	**1.4E-03**
DR11-12-14	DR8Af	34	2.1	2	0.3	0.12	0.02–0.51	**6.7E-04**
DR15-DQ6	X	69	4.2	4	0.5	0.12	0.04–0.32	**1.2E-06**
DR7-DQ2	DR11-12-14	18	1.1	1	0.1	0.12	0.01–0.87	**0.012**
X	DR8Af	44	2.7	2	0.3	0.09	0.02–0.38	**5.8E-05**
DR15-DQ6	DR13	78	4.8	3	0.4	0.08	0.02–0.24	**4.8E-08**
DR15-DQ6	DR11-12-14	31	1.9	1	0.1	0.07	0.00–0.49	**4.6E-04**
DR15-DQ6	DR9Af	16	1.0	0.5	0.1	0.07	0.00–1.10	**0.012**
DR3Af	DR15-DQ6	32	2.0	1	0.1	0.07	0.00–0.47	**3.6E-04**
DR15-DQ6	DR15-DQ6	34	2.1	1	0.1	0.06	0.00–0.44	**2.2E-04**
DR15-DQ6	DR7-DQ2	39	2.4	1	0.1	0.05	0.00–0.38	**6.4E-05**
DR13	DR8Af	40	2.4	1	0.1	0.05	0.00–0.37	**5.0E-05**
DR11-12-14	DR15-DQ6	48	2.9	1	0.1	0.04	0.00–0.31	**7.0E-06**
DR3Af	X	38	2.3	0.5	0.1	0.03	0.00–0.44	**4.4E-05**
Other		148	9.0	53	6.9			
Total		1,641		772				

*Haplotype categories included in genotypes include: DR3 Cau = DRB1*03:01-DQA1*05:01-DQB1*02:01g; DR4 = DRB1 04:01/02/04/05/08-DQA1 03:01-DQB1 03:02/02:01g; DR3 Af = DRB1*03:02-DQA1*04:01-DQB1*04:02; DR7Af = DRB1*07:01-DQA1*03:01-DQB1-02:01g; DR7 Cau = DRB1*07:01-DQA1*02:01-DQB1 02:01g; DR9 Af = DRB1*09:01-DQA1*03:01-DQB1 02:01g; DR8 Af = any DRB1*08:00 haplotype except DRB1*08:01-DQA1*04:01-DQB1*04:02; DR11-12-14 = any DRB1*11:00,*12:00, or *14:00 haplotype; DR13 = any DRB1*13:00 haplotype; DR15-DQ6 = DRB1*15:01/03 with DQB1*0:602; X = any other haplotype. T1D, type 1 diabetes. Significant *P* values are indicated in boldface.

conclusion that can be drawn from the type 1 diabetes genetics literature is that HLA is, by far, the largest contributor to type 1 diabetes.

The genes encoding the classical HLA proteins are the most polymorphic in the human genome. A total of 9,154 alleles are reported to date for the genes encoding the six classical HLA proteins, which includes 1,418, 50, and 323 alleles for DRB1, DQA1, and DQB1, respectively (www.anthonynolan.org/HIG/). However, many of the reported alleles are rare. In a given population, only a subset of available alleles are seen; distributions of HLA alleles and haplotype combinations differ widely among populations (17). This can confound the interpretation of HLA association data generated from different populations. African populations tend to have the greatest diversity of HLA alleles observed among populations studied for HLA. Given admixture with European Americans, African Americans are quite diverse, containing HLA alleles and haplotypic

combinations not seen in European Americans. African Americans represent a good population in which to study effects of non-European alleles and haplotypes. Assessing the disease association of a given allele or haplotype is not possible unless the allele or haplotype is present in sufficient frequency to analyze in the population being studied. An allele may have a very strong effect on a disease; however, this effect is unlikely to be seen if the allele is rare in the population.

Type 1 diabetes risk appears to be different in African Americans compared with Europeans in that only ~12% of cases have DR3/DR4 genotypes. In studies of Europeans, the proportion of type 1 diabetic patients who are DR3/DR4 heterozygotes can be as high as 40% (14). The DR7 and DR9 haplotypes containing DQA1*03:01-DQB1*02:01g observed in the current study have not been reported in studies of Europeans (6), although DRB1*09:01 is consistently reported to be predisposing to type 1 diabetes in Asian populations (18,19), and DRB1*07 has been reported to be associated with levels of insulin antigen-2 autoantibodies (20). Either DRB1*07:01-DQA1*03:01-DQB1*02:01g or DRB1*09:01-DQA1*03:01-DQB1*02:01g was observed in eight of the 17 significantly predisposing genotypes shown in Table 3. These eight genotypes account for 19.5% of type 1 diabetes cases. The DR3 haplotype DRB1*03:02-DQA1*04:01-DQB1*04:02 is highly protective for type 1 diabetes; this haplotype is not seen at all in European populations. The presence of unique alleles and haplotypes in some populations but not others underscores the importance of studying HLA with type 1 diabetes in varied populations.

These data illustrate the importance of high-resolution HLA genotyping to assess type 1 diabetes risk. Using a limited resolution screen, an individual with a DR3 haplotype likely would be considered at high risk for disease. If, however, that individual were of African descent and carried the African-specific DR3 haplotype, they would actually be at low risk. Similarly, a low-resolution genotyping result showing DR7 might be considered protective for type 1 diabetes when, in fact, the individual might carry the high-risk African DR7 haplotype DRB1*07:01-DQA1*03:01-DQB1*02:01g.

Given HLA differences among groups and differences in type 1 diabetes risk between similar alleles and haplotypes specific to given populations, genetic screening programs for type 1 diabetes should be developed to be not only high-resolution but also specific to the ethnic group of an individual. Additional studies of HLA and type 1 diabetes in worldwide populations are needed to provide data for development of screening assays and to use cross-ethnic comparisons to aid in understanding the mechanism of HLA association with type 1 diabetes susceptibility.

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J.A.N. led the project and wrote the manuscript. J.J. researched data. J.A.L. researched data. A.M.V. performed all of the statistical analyses and wrote the manuscript. J.A.N. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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