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Race-Specific Type 1 Diabetes Risk of HLA-DR7 Haplotypes

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Abstract

Objective—To test the hypothesis that closely-related HLA haplotypes containing the DRB1*07:01 gene (“DR7” haplotypes) derived from European and African populations differ in their genetic susceptibility for type 1 diabetes (T1D) depending on the DQ- α molecule present.

Research Design and Methods—A combined total of ninety-eight African American T1D patients from the Type 1 Diabetes Genetics Consortium and from Children's Hospital and Research Center Oakland were genotyped for the HLA class II loci DRB1, DQA1, and DQB1. DNA samples extracted from newborn blood spot cards from African Americans born in California (n=947) were used as a population-based control group.

Results—Among African American cases, the European-derived DRB1*07:01-DQA1*02:01-DQB1*02:01g haplotype was protective for T1D risk (odds ratio (OR)=0.34; 95% CI 0.14 - 0.78; p<0.011), but the African-derived DRB1*07:01-DQA1*03:01-DQB1*02:01g haplotype increased T1D risk (OR=3.96; 95% CI 1.94 - 8.08; p<5.5E-05).

Conclusions—The effect of DRB1*07:01-DQB1*02:01g on T1D susceptibility depends on the DQA1 allele. DRB1*07:01-DQA1*02:01-DQB1*02:01g is protective for T1D however, the presence of DQA1*03:01 on the DRB1*07:01-DQB1*02:01g haplotype not only renders the DR7 haplotype not protective, it creates a haplotype with significant T1D risk. These data underscore the importance of assessing genetic effects within ethnic context.

Keywords

type 1 diabetes; genetic risk; HLA class II; HLA DR7; African American

INTRODUCTION

Type 1 diabetes is an autoimmune disease resulting in the immunological destruction of the insulin producing beta cells of the pancreas, which leads to dysregulation of glucose metabolism. The incidence of type 1 diabetes varies widely around the world, probably due, among several other factors, to ethnic differences in HLA allele and haplotype frequencies across populations, with T1D incidence and prevalence being much higher among individuals of European descent than in other ethnic groups (1).

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CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHOR CONTRIBUTIONS

J.A.N. served as principal investigator of the project, assisted with interpretation and compilation of data, and co-wrote the manuscript. J.J. generated HLA genotyping data. J.A.L. assisted with data analysis and reviewed the manuscript. A.M.V. performed all of the statistical analysis and co-wrote the manuscript.

Type 1 diabetes has a strong genetic component: approximately 40-50% of the familial clustering of type 1 diabetes can be attributed to allelic variation in the HLA region (2, 3). The direct involvement of the HLA class II DRB1, DQA1 and DQB1 genes in type 1 diabetes is well established, with a hierarchy ranging from very predisposing to very protective effects (4, 5). The most highly predisposing DRB1-DQB1 haplotypes are DRB1*03:01-DQA1*05:01-DQB1*02:01g and DRB1*04:01/2/4/5-DQA1*03:01-DQB1*03:02 with odds ratios > 3.0 and > 8.0, respectively (4). Other haplotypes are strongly protective, including DRB1*15:01-DQA1*01:02-DQB1*06:02 (OR=0.03) and the DR7 haplotypes DRB1*07:01-DQB1*02:01-DQB1*02:01g (OR=0.32) and DRB1*07:01-DQA1*02:01-DQB1*03:03 (OR=0.02) (4).

Susceptibility to type 1 diabetes is strongly associated with alleles at the DQA1 and DQB1 loci, which encode the alpha and beta chains of the DQ heterodimer. In general, heterodimers that are DQ α Arg52-positive and DQ β Asp57-negative represent high genetic risk for type 1 diabetes. DRB1 alleles, DP-encoding alleles, and HLA class I alleles also affect type 1 diabetes susceptibility (4, 6, 7).

HLA allele frequency distributions vary among populations as do haplotypic combinations of HLA alleles. Several haplotypes containing the allele DRB1*07:01, referred to as “DR7” haplotypes, are known. One particular DR7 haplotype, DRB1*07:01-DQA1*03:01-DQB1*02:01g, is seen only in African populations, while the closely-related DRB1*07:01-DQA1*02:01-DQB1*02:01g is common in European populations and has been shown in multiple studies to be protective for type 1 diabetes. DQA1*03:01 encodes a DQ α chain that is Arg52-positive, whereas DQA1*02:01 is Arg52-negative, leading to the hypothesis that the DQ molecule encoded by the African DR7 should be more predisposing than that encoded by the European DR7. This is consistent with previous observations that the “Negro” haplotype, described as DR7-DQA1*0301-DQB1*0201 is positively associated with type 1 diabetes in Afro-Caribbeans (8, 9).

To test this hypothesis, high-resolution HLA genotyping was performed in a collection of African American type 1 diabetes cases and population based controls. Case data were combined with HLA genotyping data for African American probands in the Type 1 Diabetes Genetics Consortium (T1DGC). Data from DR7-positive subjects of both European and African descent from the existing T1DGC collection were utilized for the analyses presented here.

SUBJECTS AND METHODS

Study subjects

T1DGC patients—The T1DGC is 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 (10). An individual was designated as affected if he or she had documented type 1 diabetes with onset at <35 years of age, had used insulin within 6 months of diagnosis, and had no concomitant disease or disorder associated with diabetes. High resolution HLA genotyping has been performed at eight classical MHC loci by four genotyping centers using standardized typing protocols, reagents, and quality control procedures (11). In total, 74 African American type 1 diabetes probands from this collection were included in the current analysis. In addition, 168 type 1 diabetes cases and 96 unaffected siblings of European descent from T1DGC families, who were carriers of at least one DRB1*07:01 allele, were included for analysis. T1DGC samples were family-based; thus, haplotypes were determined using identity-by-descent. Subjects were recruited with

the approval of local ethics committees and/or Institutional Review Boards. Additional details can be found on the T1DGC website (www.t1dgc.org).

CHO study patients—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 (CHRCO), formerly called Children’s Hospital Oakland (CHO). Ethnicity distribution of the type 1 diabetes patients recruited for the CHO study collection is 38% Caucasian, 27% African American, 25% Hispanic, with the remaining 10% of other or mixed ethnicity. This distribution reflects both the diverse patient population and the emphasis on recruitment of non-Caucasian patients for the study. Twenty-four African American type 1 diabetes patients from the CHO study were genotyped at the DRB1, DQA1, and DQB1 loci and included in the present report. The patients collected from Children’s Hospital and Research Center Oakland (CHRCO) were diagnosed by the 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 T1D was unequivocal to the endocrinologists. Subjects with known or suspected type 2 diabetes (T2D), maturity-onset diabetes of youth (MODY), 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 where possible and deduced using known patterns of linkage disequilibrium when family data were not available. The CHO study is approved by the CHRCO Institutional Review Board.

Population based controls—Extracted DNA samples from 947 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 is available to the research team from these individuals. 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 diabetes patients in this sample set should be fewer than one. Haplotypes were determined by hand using known patterns of linkage disequilibrium for HLA class II alleles. 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 CHRCO Institutional Review Board.

HLA genotyping: The methods of HLA genotyping have previously been described (4). Both the T1DGC samples and the CHO study samples were genotyped with the same technology. Briefly, HLA genotyping at four-digit resolution was achieved using oligonucleotide probes that were immobilized to a nylon membrane and hybridized with biotin-labeled, locus-specific PCR amplification products. Genotyping was performed using the Stripscreen and SCORE proprietary software prior to upload, for the T1DGC samples, to the coordinating center. Regular quality assurance genotyping performed on blinded replicate samples and among laboratories indicated both inter- and intra-laboratory concordance to be greater than 99% (11). For T1DGC samples, family-based data allowed the assignment of three locus haplotypes (*DRB1-DQA1-DQB1*) for statistical analyses. Phased genotypes for HLA classical loci were generated either by hand or using in-house built macros and double-checked manually by two independent individuals. The designation “DQB1*02:01g” is used throughout the manuscript to refer to the fact that the genotyping system did not distinguish DQB1*02:01 from DQB1*02:02 in these samples. The difference between these two alleles is encoded in exon 3 of the DQB1 gene, and the amino acid

residue encoded by the polymorphism does not contribute to the peptide-binding groove of the HLA molecule.

Haplotype phase assignment: Samples from the T1DGC were family-based; thus, haplotypes were unambiguously determined. Some patients from CHRCHO had family information available for direct determination of haplotypes. For patients without corresponding family members, and for controls, haplotypes were constructed by hand using established patterns of linkage disequilibrium for DRB1, DQA1, and DQB1 alleles.

Statistical analysis: Haplotype frequencies between cases and controls were compared using a Pearson's chi-squared test. Odds ratios and 95% confidence intervals were computed.

RESULTS

Consistent with the hypothesis, the data showed that in African American patients, the African-derived, predisposing DR7 (DRB1*07:01-DQA1*03:01-DQB1*02:01g) is present twice as frequently than the common, European protective DR7 (DRB1*07:01-DQA1*02:01-DQB1*02:01g). In fact, the DQA1*02:01 carrying haplotype is significantly protective in African Americans, just as it is in Caucasians, but the DQA1*03:01 carrying haplotype is significantly predisposing (Table 1). The effects of these two haplotypes are significantly different ($p < 3 \times 10^{-6}$).

Although the formal possibility that the susceptibility difference observed between the two DR7 haplotypes is due to a locus other than DQ, the simplest, and perhaps most plausible, explanation is that differences in peptide repertoire binding and or affinity for the two encoded DQ heterodimers affect type 1 diabetes susceptibility. A heterozygous individual can produce two different DQ α and two different DQ β chains. If the susceptibility encoded in the African DR7 haplotype is due to the DQA1*03:01-DQB1*02:01g-encoded heterodimer, then individuals with these two alleles encoded on different chromosomes would be expected to have increased type 1 diabetes risk as well. Thus, Caucasian DR7-positive type 1 diabetes patients (with DRB1*07:01-DQA1*02:01-DQB1*02:01g) might be expected to have a disproportionately high frequency of DQA1*03:01 on the second chromosome. To test this hypothesis, we examined the T1DGC class II Caucasian data and compared the frequency of DQA1*03:01 in the DR7+ patients to the frequency in DR7+ unaffected siblings. We found that Caucasian DR7+ patients are significantly more likely to carry DQA1*03:01 on their second chromosome than are unaffected DR7+ siblings. (49% vs 35%; $p < 0.035$; data not shown). We note, however, that the vast majority (89%) of haplotypes with DQA1*03:01 on the non-DR7 chromosome are DRB1*04-DQA1*03:01-DQB1*03:02, which conveys, in itself, very high T1D risk.

DISCUSSION

In this study we report that the effect of the HLA DRB1*07:01-DQB1*02:01g haplotype on type 1 diabetes susceptibility depends on the DQA1 allele present. DQA1*03:01 (DQ α Arg52-positive) results in increased risk and DQA1*02:01 (DQ α Arg52-negative) in decreased risk. In addition to DRB1*07:01, the DQA1*03:01-DQB1*02:01g haplotype was observed coupled to two other DRB1 alleles, DRB1*04:05 and DRB1*09:01, in the African American data set. Notably, both of these haplotypes were also significantly predisposing for type 1 diabetes (data not shown). This is consistent with previous reports of T1D association for DR9-DQA1*03:01-DQB1*02:01 in Afro-Caribbeans (8, 9). DQA1*02:01 and DQB1*02:01g were found *in cis* on only one non-DR7 haplotype in the African American data, DRB1*13:03-DQA1*02:01-DQB1*02:01g, which was seen only in controls

and not observed in patients. This effect, while consistent with the DR7 data, did not reach statistical significance. Our data are also in agreement with the previously published observation that no significant difference was observed between black and white patients with T1D in Allegheny County, PA in the frequency of non-Asp57 homozygosity at DQB1 (12). The analysis in Caucasian samples analysing a predisposing effect for DQA1*03:01-DQB1*02:01g encoded *in trans* supports our hypothesis, but, because most of the DQA1*03:01 containing non-DR7 haplotypes in this analysis also carry a DRB1*04:xx allele, we cannot rule out the possibility that the T1D susceptibility comes from the DRB1*04 haplotype, rather than the *trans*-encoded DQ heterodimer from the DR4/DR7 genotype.

On the other hand, the African American data presented here for DR7 haplotypes strongly suggest a predisposing effect for the combination of DQA1*03:01 and DQB1*02:01g when encoded on the same chromosome. This fits well with the hypothesis that the extremely high type 1 diabetes susceptibility observed for individuals heterozygous for DR3 and DR4 haplotypes is partly due to the DQ heterodimers that are encoded *in trans* on DR3/DR4 individuals, one of which is DQA1*03:01 from the DR4 haplotype combined with DQB1*02:01g from the DR3 haplotype. In other words, having antigen presentation by a DQ molecule that carries an α chain encoded by DQA1*03:01 and a β chain encoded by DQB1*02:01 appears to increase risk of type 1 diabetes, regardless of how the chains are genetically encoded.

In conclusion, our data show that the African DR7 haplotype is predisposing, rather than protective, for type 1 diabetes. This effect is likely due to the DQ molecule encoded by the DQA1*03:01 and DQB1*02:01g alleles, and increased risk can be seen when these two alleles are encoded either *in cis* (African DR7) or *in trans* (Caucasian DR3/DR4). This result illustrates the value of HLA disease association studies that compare data from multiple ethnic groups.

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Abbreviations

HLA	human leukocyte antigen
DR7	DRB1*07:01-DQA1*02:01-DQB1*02:01g or DRB1*07:01-DQA1*03:01-DQB1*02:01g
OR	Odds Ratio
CI	Confidence Interval
T1D	type 1 diabetes

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Table 1

Comparison of the effects of two DRB1*07:01-DQB1*02:01g haplotypes, one of African origin, the other of European origin in African American type 1 diabetes cases vs. population-based controls.

DRB1	DQA1	DQB1	controls	TYPE 1 DIABETES	p<	OR (95% CI)
07:01	02:01	02:01g	160 (8.45%)	6 (3.06%)	0.011	0.34 (0.14 - 0.78)
07:01	03:01	02:01g	28 (1.48%)	11 (5.61%)	5.5E-05	3.96 (1.94 - 8.08)
Any	other		1706 (90.07%)	179 (91.33%)		