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ABSTRACT: The HLA genotype DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 confers a 25-fold increase in the risk of type 1 diabetes. In persons with this genotype, DRB1*0405, *0402, and *0401 subtypes have been reported to further increase risk, whereas the *0403 and *0406 alleles confer a relative protection. We compared the frequencies of the DRB1*04 alleles in 193 type 1 diabetic patients with the HLA-DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype (140 non-Hispanic white [NHW] and 53 Hispanic) and 205 nondiabetic controls (142 NHW and 63 Hispanic). In addition, 87 NHW first-degree relatives of type 1 diabetes patients were studied: 33 positive and 54 negative for autoantibodies to insulin, GAD65, or IA-2. The HLA-DRB1 was typed using standard PCR SSOP methods. DRB1*0401 (OR, 2.19; 95% CI, 1.36–3.54) in NHW and *0405 (OR, 3.78; 95% CI, 1.43–10.0) in Hispanics were significantly associated with T1DM, whereas DRB1*0403 was protective (OR, 0.19; 95% CI, 0.04–0.89 in NHWs; OR, 0.10; 95% CI, 0.01–0.83 in Hispanics). Associations between the DRB1*04 alleles and prediabetic islet autoimmunity were generally in the same direction as those with diabetes. Among diabetic patients, the mean age of diagnosis appeared to be higher among those with the *0403 and *0407 allele compared with the others. In summary, on the DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotypes, the *0403 allele confers relative protection from type 1 diabetes and development of islet autoantibodies in both Hispanics and NHWs and is associated with older age at diabetes diagnosis. Although the associations between diabetes and *0401 and *0405 appear to differ somewhat between Hispanics and NHWs, overall there is no significant difference between these two ethnic groups.

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The cause of type 1 diabetes (T1DM) is multifactorial and includes the effects of genes at several loci.[1,2] The *IDDM1* locus, including the HLA-DR and DQ genes, is the only major genetic determinant, accounting for up to 50% of the familiar clustering of the disease.[3,4] Approximately 95% of all T1DM cases have either the DRB1*03,DQB1*0201 or the DRB1*04,DQB1*0302 haplotype. Although only 2% of the general population are DRB1*0301,DQB1*0201/DRB1*04,DQB1*0302 heterozygotes,[5] this genotype is present in 30–40% of T1DM patients[6] and in up to 52% of those who develop diabetes in the first 10 years of life.[7] Interestingly, minor differences within the DRB1*04 alleles modify the risk of diabetes.[6,8–18] Previous reports have shown that, on DQB1*0302 haplotypes, the DRB1*0405 (in Caucasoids, blacks, and Asians), *0402, and *0401 alleles (in Caucasoids) increase the risk, whereas the *0403 (in Caucasoids and Chinese) and *0406 (in Japanese) alleles confer relative protection. Other alleles appear to be neutral. Comparable data for the U.S. population are lacking.

The goal of this study was to (1) determine the population frequencies of the HLA-DRB1*04 alleles in children with diabetes and nondiabetic controls who have the HLA genotype DRB1*03,DQB1*0201/DRB1*04,DQB1*0302; (2) explore the effect of various DRB1*04 alleles on the age of diabetes diagnosis; (3) compare the frequencies of DRB1*04 alleles in nondiabetic relatives of diabetic children to explore if the same alleles are associated with diabetes and the preclinical phase of diabetes marked by the presence of islet autoantibodies; and (4) determine whether the frequencies of the DRB1*04 alleles and the association with diabetes differ between Colorado Hispanic and non-Hispanic white (NHW) children.

METHODS

Study Population

Only persons with the HLA-DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype that live in Colorado were eligible. Diabetic patients were identified from the Barbara Davis Center for Childhood Diabetes in Denver. Nondiabetic controls were children without T1DM among the first-degree relatives followed by the Diabetes Autoimmunity Study in the Young.[5] In addition, 95 nondiabetic first-degree relatives of people with type 1 diabetes were included. Of those, 35 had pre-diabetes (normal blood sugar, but persistent islet autoantibodies present), whereas 60 were negative for islet autoantibodies.

Ethnicity was self-reported (for minors reported by parents) using the U.S. Census classification. Informed consent to genetic typing was obtained from all study participants.

HLA Typing

Samples were first typed for the presence of the HLA-DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype at Roche Molecular Systems, Inc., in Alameda, California, or at the Barbara Davis Center for Childhood Diabetes in Denver. At the Roche Molecular Systems, whole blood (15–25 μL) was used in PCR amplification with 11 biotinylated primers to specifically coamplify the DRB1 and the DQB1 locus.[5] The amplification was conducted in the 9600 Thermal Cycler (Perkin-Elmer). Hybridization and detection were performed by adding the amplified DNA to strips containing 18 immobilized sequence specific oligonucleotide primers (SSOP) for DRB1 and DQB1. Each biotinylated DNA was denatured and hybridized to an individual strip for 30 min at 50°C in 4× SSPE/0.5% SDS. The wash step for 15 min at 50°C in 1× SSPE/0.1% SDS was followed by incubation with streptavidin-HRP and a chromogenic substrate. After color development, the strips were scanned and a simple computer program assigned alleles based on the probe reactivity pattern. At the Barbara Davis Center, DNA samples were typed for the HLA-DQB1 and DQA1 using standard kits (Dynal SSP; Dynal Biotech, Ltd., Merseyside, United Kingdom). For all samples, Dynal Classic SSP DRB1*04 kit (Dynal) was used to determine the DRB1*04 sequence.

Measurement of Autoantibodies

A group of 95 first-degree relatives (87 NHWs) with the HLA-DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype were tested for islet autoantibodies, and 35 (including 33 NHWs) were persistently positive. All measures of autoantibodies in blood were performed in the laboratory of George Eisenbarth of the Barbara Davis Center. We used radioassays for insulin, GAD_{65}, and IA-2 autoantibodies as previously described.[19,20] Persistent islet autoimmunity was defined as presence of at least one autoantibody (IAA, GAA, or IA-2) above the 99th percentile on two or more consecutive visits and at the most recent visit. This definition is highly predictive of type 1 diabetes. As of September 2002, 21 of the 35 islet autoantibody positive and initially nondiabetic relatives who met this definition had converted to diabetes.

Statistical Analysis

All analyses were conducted in SAS version 8.3 (SAS Institute, Cary, NC). Comparisons between frequencies of DRB1*04 subtypes among T1DM patients and control subjects were done by PROC FREQ, using χ^2 test or Fisher's exact test, when appropriate, with the acceptance p level of 0.05 and no correction for multiple comparisons. The univariate odds ratios (OR) and the 95% confidence intervals (CI) were calculated using the PROC LOGISTIC to evaluate potential association between the DRB1*04 alleles and diabetes or prediabetes.

PROC LOGISTIC was also used to formally test if the association between various DRB1*04 subtypes varied by ethnicity. Mean age at diabetes onset by the DRB1*04 allele status was compared using the Kruskal-Wallis test.

TABLE 1. Distribution of DRB1*04 alleles among Colorado T1DM patients and nondiabetic healthy controls with the DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype

	Non-Hispanic whites				Hispanics			
DRB1*04 allele	Diabetic patients (n = 140)	Controls (n = 142)	p	OR (95% CI)	Diabetic patients (n = 53)	Controls (n = 63)	p	OR (95% CI)
0401	90 (64%)	64 (45%)	**0.0013**	**2.19 (1.36–3.54)**	7 (13%)	16 (25%)	0.11	0.45 (0.17–1.19)
0402	10 (7%)	12 (9%)	0.83	0.83 (0.35–2.00)	6 (11%)	2 (3%)	0.14	3.89 (0.75–20.1)
0403	2 (1%)	10 (7%)	**0.034**	**0.19 (0.04–0.89)**	1 (2%)	10 (16%)	**0.011**	**0.10 (0.01–0.83)**
0404	35 (25%)	44 (31%)	0.29	0.72 (0.44–1.25)	18 (34%)	15 (24%)	0.30	1.65 (0.73–3.71)
0405	2 (1%)	8 (6%)	0.10	0.24 (0.05–1.16)	17 (32%)	7 (11%)	**0.010**	**3.78 (1.43–10.0)**
0406	–	1 (1%)	–	–	–	1 (2%)	–	–
0407	1 (1%)	2 (1%)	1.00	0.50 (0.05–5.62)	4 (8%)	10 (16%)	0.25	0.43 (0.13–1.47)
Other	–	1 (1%)	–	–	–	2 (3%)	–	–

NOTE: Significant differences (at $\alpha = 0.05$) are denoted by boldface type.

RESULTS

The frequencies of HLA-DRB1*04 subtypes in T1DM cases and controls with the HLA-DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype are shown, by ethnicity, in FIGURE 1. Formal comparisons of these frequencies are summarized in TABLE 1. The DRB1*0403 allele was protective from diabetes both in NHWs (OR, 0.19; 95% CI, 0.04–0.89) and in Hispanics (OR, 0.10; 95% CI, 0.01–0.83). Consistent with previous reports, the *0401 allele conferred increased risk in NHWs (OR, 2.19; 95% CI, 1.36–3.54), but this was not the case in Hispanics (OR, 0.45; 95% CI, 0.17–1.19). On the other hand, DRB1*0405 (OR, 3.78; 95% CI, 1.43–10.0) conferred increased risk in Hispanics, but not in NHWs. The *0404 allele appeared to be neutral in both ethnic groups, concordant with previous findings. The risk associated with other alleles was inconclusive because of low numbers. Overall, there was no evidence for a statistically significant difference between Hispanics and NHWs in the DRB1*04 subtype associations with diabetes ($p > 0.9$).

In addition, 95 NHW nondiabetic first-degree relatives of type 1 diabetes patients were studied. Of those, 87 were NHW and, among these, 33 were persistently positive and 54 negative for islet autoantibodies to insulin, GAD65, or ICA512. The direction and strength of the association between DRB1*04 alleles and either diabetes or prediabetes were remarkably consistent (FIG. 2). This confirms that the

DRB1*04 alleles affect both the early stages of the process, initiation of autoimmunity and its progression to overt diabetes.

Among the NHW diabetic patients, the mean age of diagnosis (FIG. 3) appeared to be higher among those with the *0403 allele (16.7 years) compared with those with the *0401 (10.0 years), *0402 (8.2 years), *0404 (9.8 years), and *0405 (3.8 years). Thus, generally, the more protective the allele, the older the age at diagnosis.

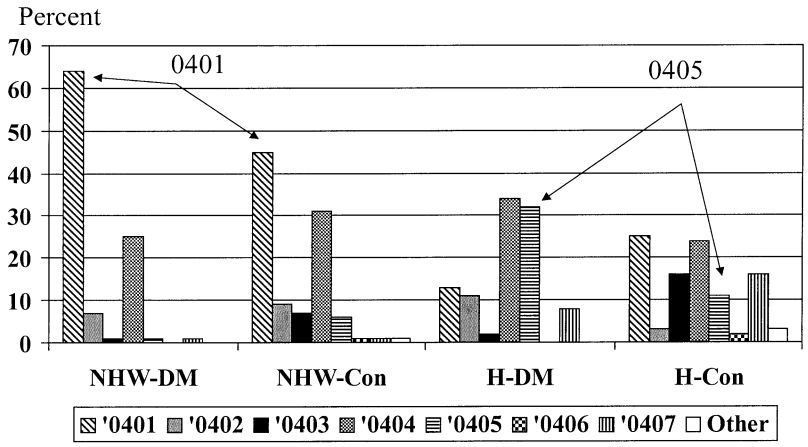

FIGURE 1. HLA-DRB1*04 subtypes in T1DM patients and controls with the DR3/4-DQ2/8 genotype (NHW, non-Hispanic white; H, Hispanic).

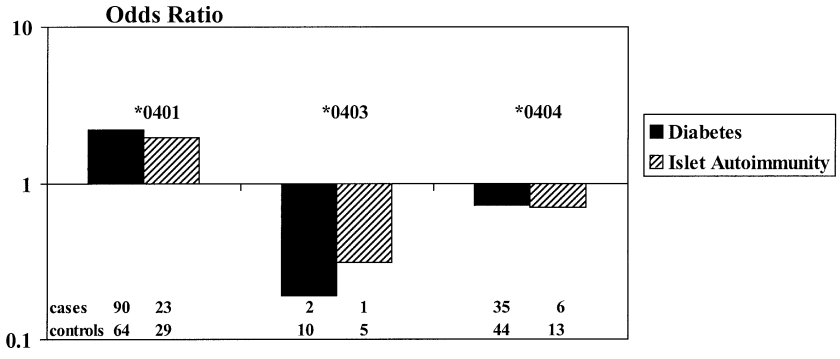

FIGURE 2. Diabetes and islet autoimmunity (persistent insulin, GAD, or IA-2 autoantibodies) are generally associated with the same DRB1*04 alleles.

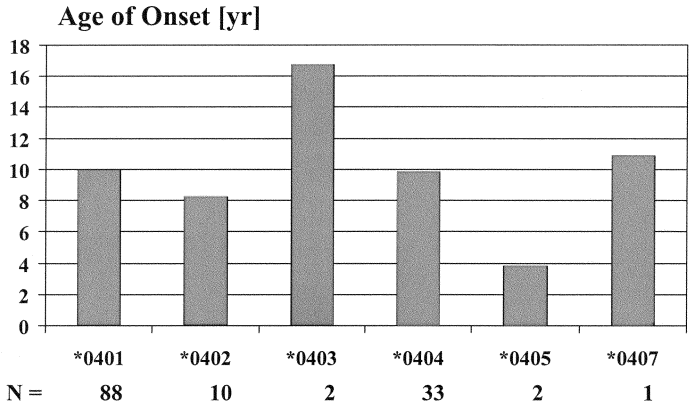

FIGURE 3. Effect of the DQB1*04 allele on the mean age of T1DM onset among children with the HLA-DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype (NHW children, $n = 136$).

DISCUSSION

The DRB1*04 alleles are fairly frequent in the general population (20% of Colorado residents carry at least one DRB1*04 allele) and even more frequent among diabetic children (65% of those carry at least one DRB1*04 allele). We selected individuals with the highest-risk HLA genotype of Hispanic and NHW ethnicity to evaluate previously reported associations between the DRB1*04 subtypes and T1DM. Only 2% in the general population and 30–40% of T1DM patients carry this high-risk DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype.

In NHWs, associations between DRB1*04 alleles and diabetes were similar to those previously reported (*0401 conferred risk, whereas *0403 was protective). The 0402 allele, previously shown to be a high-risk DRB1*04 allele, was unexpectedly not associated with diabetes in this data set. In Hispanics, *0405 allele conferred risk and *0403 was protective. Generally, the same DRB1*04 alleles were associated with development of islet autoimmunity and progression to diabetes. This is most clearly seen in the *0403 allele, which conferred protection to diabetes in both ethnic groups and was associated with an older age at diabetes diagnosis.

This is the first report on the associations between childhood diabetes and the HLA-DRB1*04 alleles in the U.S. population, outside of the multiplex family collection of the Human Biological Data Interchange.[4] In addition, there is a paucity of data on DRB1*04 allele frequencies in Hispanic populations,[21] and our preliminary data for the Hispanic population suggest intriguing similarities and differences compared with NHWs. For instance, the DRB1*0405 allele was a major risk allele in Hispanics (present in 32% of T1DM patients and 11% of controls), whereas it was decreased in frequency in NHWs (both in patients [1%] and controls [6%]; $p < 0.01$). Finally, to our knowledge, this is the first report documenting that the protective

TABLE 2. Prevalence of DRB1*04 alleles in diabetic children and nondiabetic controls with the HLA-DQB1*0201,DQA1*0501,DRB1*03/DQB1*0302,DQA1*0301,DRB1*04 genotype in different populations

		Frequency of specific DR*04 alleles						
		U.S.[4]	Norwegian[13]		Belgian[9]		French[11]	
	T1DM risk	Diabetics ($n = 180$)	Diabetics ($n = 191$)	Controls ($n = 235$)	Diabetics ($n = 174$)	Controls ($n = 73$)	Diabetics ($n = 92$)	Controls ($n = 9$)
0401	Increased	62%	76%	50%	70%	64%	38%	22%
0402	Increased	5%	2%	1%	9%	3%	20%	11%
0403	Lowest	–	1%	3%	–	8%	–	11%
0404	Reduced	30%	19%	44%	11%	14%	21%	56%
0405	Highest	3%	3%	1%	8%	3%	17%	–
0406	Lowest	–	–	–	–	–	–	–
0407	Reduced	–	–	–	–	7%	–	–
0408		–	–	1%	1%	1%	–	–
0410		–	–	–	1%	–	–	–

NOTE: Reported hierarchy of diabetes risk in previous studies: *0405 > *0402 > *0401 > *0404 > *0403 > *0406. The Human Biological Data Interchange data set (U.S.[4]) included diabetic patients only from multiplex families, that is, with more than one affected child.

effect of the *0403 allele extends into the earliest stages of the autoimmune process. Understanding of the structural reasons for this protective effect may yield new clues concerning design of a "diabetes vaccine".

Minor polymorphic substitutions in the peptide-binding groove of class II HLA molecules can lead to drastic variation in peptide-binding selectivity and interactions with specific T cell receptors. Several groups have attempted to develop a model based on characteristics of relevant pockets of HLA-DR and HLA-DQ molecules to explain variability in associations with T1DM.[13,22,23] Undlien *et al.*[13] presented a plausible hypothesis that the DRB1*0404, 0403, 0406, and perhaps 0407 confer an increasing degree of protection, possibly by binding a common protective peptide.

Note that most studies of the effect of DRB1*04 subtypes have focused on DR4/4 or DR4/X individuals and reported that the DRB1*04 subtype effect was harder to demonstrate in DR3/4 genotypes[21] (TABLE 2). Here, we report that the effect of DRB*04 subtypes can be seen even in the high-risk DR3/4 genotype in two different populations. The previously reported hierarchy of diabetes risk associated with DRB1*04 subtypes, 0405 > 0402 > 0401 > 0404 > 0403 > 0406, generally was confirmed in our study.

This research would have been greatly aided by the inclusion of more Hispanic children as well as other U.S. ethnic groups. U.S. Hispanics are a very heterogeneous group, even within a relatively small state like Colorado. Although ethnicity was defined based on self-reports using the U.S. Census definitions, some degree of misclassification is possible because of variable perception of affiliation with this ethnic group.

In conclusion, the HLA-DRB1*0403 allele confers protection from islet autoimmunity and progression to diabetes. The protective association of the *0403 allele merits further study of the differences in the structure of the peptide-binding domain and deducted structure of peptides that are bound. Hispanics, compared with NHWs with the DRB1*03,DQB1*0201/DRB1*04,DQB1*0302 genotype, appeared to differ only slightly in the associations between different DRB1*04 alleles and diabetes. Further studies of a larger group of Hispanics from Colorado and other areas are needed to validate this observation.

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REFERENCES

1. TODD, J.A. 1995. Genetic analysis of type 1 diabetes using whole genome approaches. Proc. Natl. Acad. Sci. USA **92:** 8560–8565.
2. COX, N.J. *et al*. 2001. Seven regions of the genome show evidence of linkage to type 1 diabetes in a consensus analysis of 767 multiplex families. Am. J. Hum. Genet. **69:** 820–830.
3. DAVIES, J.L. *et al*. 1994. A genome-wide search for human type 1 diabetes susceptibility genes. Nature **371:** 130–136.
4. NOBLE, J.A. *et al*. 1996. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. Am. J. Hum. Genet. **59:** 1134–1148.
5. REWERS, M. *et al*. 1996. Newborn screening for HLA markers associated with IDDM: Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia **39:** 807–812.
6. RONNINGEN, K.S. *et al*. 1992. HLA class II associations in insulin-dependent diabetes mellitus among blacks, Caucasoids, and Japanese. *In* HLA 1991: Proceedings of the 11th International Histocompatibility Workshop and Conference (Yokohama, Japan), pp. 713–722. Oxford University Press. London/New York.
7. VAN DER AUWERA, B. *et al*. 1995. Genetic susceptibility for insulin-dependent diabetes mellitus in Caucasians revisited: the importance of diabetes registries in disclosing interactions between HLA-DQ– and insulin gene–linked risk—Belgian Diabetes Registry. J. Clin. Endocrinol. Metab. **80:** 2567–2573.
8. ERLICH, H.A. *et al*. 1990. HLA-DQb sequence polymorphism and genetic susceptibility to IDDM. Diabetes **39:** 96–103.
9. VAN DER AUWERA, B. *et al*. 1995. DRB1*0403 protects against IDDM in Caucasians with the high-risk heterozygous DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype: Belgian Diabetes Registry. Diabetes **44:** 527–530.
10. CUCCA, F. *et al*. 1995. The distribution of DR4 haplotypes in Sardinia suggests a primary association of type I diabetes with DRB1 and DQB1 loci. Hum. Immunol. **43:** 301–308.
11. HARFOUCH-HAMMOUD, E. *et al*. 1996. Contribution of DRB1*04 variants to predisposition to or protection from insulin dependent diabetes mellitus is independent of dq. J. Autoimmun. **9:** 411–414.
12. YASUNAGA, S. *et al*. 1996. Different contribution of HLA-DR and -DQ genes in susceptibility and resistance to insulin-dependent diabetes mellitus (IDDM). Tissue Antigens **47:** 37–48.
13. UNDLIEN, D.E. *et al*. 1997. HLA-encoded genetic predisposition in IDDM: DR4 subtypes may be associated with different degrees of protection. Diabetes **46:** 143–149.

14. NEJENTSEV, S. *et al*. 1997. The effect of HLA-B allele on the IDDM risk defined by DRB1*04 subtypes and DQB1*0302. Diabetes **46:** 1888–1892.
15. ROEP, B.O. *et al*. 1999. HLA-DRB1*0403 is associated with dominant protection against IDDM in the general Dutch population and subjects with high-risk DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype. Tissue Antigens **54:** 88–90.
16. QIU, C. *et al*. 1997. Study on the association of HLA-DR4 with IDDM susceptibility in a Chinese population. Zhongguo Yi Xue Ke Xue Yuan Xue Bao **19:** 54–59.
17. BUGAWAN, T.L. *et al*. 2002. The association of specific HLA class I and II alleles with type 1 diabetes among Filipinos. Tissue Antigens **59:** 452–469.
18. ABID, K.H. *et al*. 2002. HLA polymorphism in type 1 diabetes Tunisians. Ann. Genet. **45:** 45–50.
19. YU, L. *et al*. 2000. Early expression of anti-insulin autoantibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes. Proc. Natl. Acad. Sci. USA **97:** 1701–1706.
20. YU, L. *et al*. 2001. Expression of GAD65 or ICA512(IA-2) autoantibodies amongst cytoplasmic ICA positive relatives is associated with eligibility for DPT-1. Diabetes **50:** 1735–1740.
21. ERLICH, H.A. *et al*. 1993. HLA class II alleles and susceptibility and resistance to insulin dependent diabetes mellitus in Mexican-American families. Nat. Genet. **3:** 358–364.
22. DJOULAH, S. *et al*. 1999. A new predictive model for insulin-dependent diabetes mellitus susceptibility based on combinations of molecular HLA-DRB1 and HLA-DQB1 pockets. Tissue Antigens **54:** 341–348.
23. SANJEEVI, C.B. *et al*. 2002. The combination of several polymorphic amino acid residues in the DQα and DQβ chains forms a domain structure pattern and is associated with insulin-dependent diabetes mellitus. Ann. N.Y. Acad. Sci. **958:** 362–375.