Ethnic Differences in Human Leukocyte Antigen Markers of Susceptibility to IDDM

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OBJECTIVE — To determine whether genetic differences explain the lower risk of developing insulin-dependent diabetes mellitus (IDDM) for Hispanic versus non-Hispanic white children in Colorado.

RESEARCH DESIGN AND METHODS — Hispanic (n = 62) and non-Hispanic white (n = 82) subjects with IDDM identified from the Colorado IDDM Registry and healthy, nondiabetic control subjects were recruited. Human leukocyte antigen (HLA) serologic typing and sequence-specific oligonucleotide typing of DQA1 and DQB1 alleles were performed.

RESULTS — HLA and allele associations with IDDM were similar in both ethnic groups. HLA-DR3 and HLA-DR4 were more common in IDDM subjects in both ethnic groups. Subjects with DQB1 alleles encoding aspartic acid (Asp) in position 57 were less likely to have IDDM, irrespective of ethnic background. HLA-DR3 was less common among Hispanic subjects than non-Hispanic white control subjects (4.4 vs. 17.5%, Hispanics vs. non-Hispanic whites, P = 0.04).

CONCLUSIONS — These data suggest that the lower prevalence of HLA-DR3 in the Hispanic population, a pattern consistent with the presence of Amerindian admixture, may explain the lower rate of IDDM in the Hispanic population.

Data from the Colorado Insulin-Dependent Diabetes Mellitus (IDDM) Registry have indicated that Hispanic children are about half as likely to develop IDDM as non-Hispanic white children (1). This ethnic difference is consistent with reports of other Hispanic groups and racial/geographic variation in incidence demonstrated by international comparisons of data from IDDM registries (2–4).

Although the reasons for these ethnic/racial variations are unclear, they may result from differences in exposure to environmental factors and/or the prevalences of genetically susceptible individuals (5–6). The prevalence of human leukocyte antigen DR3 (HLA-DR3) among Hispanics has been reported to be similar to (7–9), higher than (10), or lower than (J. Danilovs, unpublished observations) the prevalence among Caucasians. Because Colorado Hispanics are a genetically admixed group of Caucasian and Native American ancestry and IDDM is rare among Native Americans, genetic heritage may explain the lower risk of IDDM among Hispanics (11,12).

This case-control study was conducted to determine whether HLA-DR3, HLA-DR4, and DQB1 alleles encoding aspartic acid (Asp) at position 57 of the β-chain, markers highly associated with risk of IDDM in other Caucasian populations, are associated with risk of IDDM in both ethnic groups and examine ethnic differences in the prevalences of these risk markers among nondiabetic individuals.

RESEARCH DESIGN AND METHODS

— The Colorado IDDM Registry is a state-wide, population-based incidence registry that identified cases through a physician surveillance network and periodic hospital record reviews (13). Children eligible for inclusion were residents of the state, <18 years of age at the diagnosis of IDDM between 1 January 1978 and 31 December 1988, and placed on insulin therapy within two weeks of the diagnosis date. Cases with diabetes secondary to other disorders were excluded. All registered people who identified themselves as Hispanic (n = 118) and a similar size random sample of those who reported

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IDDM, insulin-dependent diabetes mellitus; HLA, human leukocyte antigen; Asp, aspartic acid; BMI, body mass index; CI, confidence interval; OR, odds ratio.

Table 1—*Characteristics of full participants by IDDM status and ethnicity*

	Hispanic				Non-Hispanic			
	n	IDDM	*n*	No IDDM	*n*	IDDM	*n*	No IDDM
Age at visit (years)	62	15.8 ± 5.4	56	17.0 ± 6.2	82	15.7 ± 6.1	75	16.9 ± 6.9
Age at diagnosis (years)	62	9.4 ± 4.3			82	9.4 ± 4.3		
Duration of IDDM at visit (years)	62	5.8 ± 3.3			82	5.8 ± 3.0		
Male (% [*n*])	62	41.9 (26)	56	48.2 (27)	82	43.9 (36)	75	58.7 (44)
GHb (%)	54	11.6 ± 2.8			67	11.1 ± 2.0		
Insulin dose (U/kg)	46	0.88 ± 0.31			66	0.92 ± 0.24		
BMI (kg/m^2)	56	22.2 ± 4.4	56	21.9 ± 4.2	71	21.3 ± 3.8	74	21.3 ± 4.7

Data are means ± SD.

themselves as non-Hispanic white (*n* = 122) were eligible for this study (14).

A random sample of households, stratified by Spanish surname, was selected from households with licensed drivers in 1987 who were born between 1 January 1937 and 31 December 1971 and who were residents of the Front Range (a 10-county region east of the Rocky Mountains) (15). A household enumeration was conducted to identify healthy, nondiabetic individuals 5–30 years of age. One eligible person was randomly selected from the household. Control subjects were frequency-matched to cases based on sex, age-group, and ethnicity. Of 1,137 households contacted, 23.0% (*n* = 262) had at least one eligible member, of whom 108 (41.2%) agreed to fully participate and 24 (9.2%) agreed to complete the questionnaires only. Additional control subjects were recruited through announcements at the University of Colorado Health Sciences Center and outpatient clinics (*n* = 23; 5 Hispanic and 18 non-Hispanic white). No differences were found in the age or years of maternal education between these groups of control subjects.

The examination included anthropometric skin reflectance (a measure of genetic admixture) and blood pressure measurements; a blood draw; self-administered questionnaires about early childhood factors, family history, medical history, and diet; and a physical activity interview. GHb was measured in IDDM subjects (Glyc-Affin GHb, Isolab, Akron, OH). HLA serologic typing was performed using commercially available trays (One Lambda, Los Angeles, CA) and standard techniques (16). DQA1 and DQB1 alleles were identified by use of polymerase chain reaction (17) and dot-blot analysis with labeled oligonucleotides as described previously (18–24). The numbers of individuals with HLA marker information varied because of technical problems, including insufficient numbers of cells available for both serology and sequence-specific oligonucleotides typing. A child from each of the two families with more than one child with IDDM was excluded from analyses.

Statistical analysis

The Statistical Analysis System was used to compute odds ratios (ORs) and confidence intervals (CIs) using Wolf's Method and Haldane's correction for small samples, tests of heterogeneity, and differences in proportions (25). For secondary analyses of HLA antigens and alleles with no specific a priori hypotheses, *P* values were corrected for the number of comparisons (*n* = 39) using the Bonferroni method (26).

RESULTS — Overall, 75 (63.5%) of the eligible Hispanic diabetic subjects (*n* = 118) and 99 (81.1%) of the non-Hispanic white diabetic subjects (*n* = 122) participated in the examination and/or questionnaires. Hispanic subjects with IDDM were less likely to participate than non-Hispanic white diabetic subjects (*P* = 0.004). No significant differences were found in age, duration of IDDM, age at diagnosis of diabetes, or sex for IDDM subjects by participation status in either ethnic group (data not shown). Genetic marker data were available on 89.6% of the full participants (*n* = 62 Hispanics and *n* = 82 non-Hispanic whites).

Age and sex did not differ by diabetes status or ethnicity. Among those with IDDM, no ethnic differences were observed in age at visit, age at diagnosis, duration of IDDM, sex, GHb, insulin dose, or body mass index (BMI) (Table 1).

As shown in Table 2 (primary analysis), HLA-DR3, HLA-DR4, and Asp were associated with IDDM in both ethnic groups. No significant ethnic differences were found in the ORs for these markers. The frequencies of HLA-DR3, HLA-DR4, and Asp were then examined by ethnicity among nondiabetic control subjects. The prevalence of HLA-DR3 was 75% lower among Hispanic control subjects than non-Hispanic white control subjects (Table 2, primary analysis) (*P* = 0.04). No ethnic differences were observed in the prevalences of HLA-DR4 or Asp between ethnic groups.

The prevalence of Asp genotypes

Table 2—*Frequency of HLA antigens and alleles by IDDM and ethnicity*

	Hispanic			Non-Hispanic		
	IDDM (%)	No IDDM (%)	OR (95% CI)	IDDM (%)	No IDDM (%)	OR (95% CI)
Primary analyses						
HLA-DR3	44.4	4.4	17.60* (3.87, 80.09)	52.0	17.5	5.12* (2.32, 11.31)
HLA-DR4	61.1	37.0	2.68* (1.19, 60.03)	61.3	34.9	2.96* (1.47, 5.93)
Asp	28.9	60.0	0.27* (0.12, 0.63)	25.4	63.5	0.20* (0.09, 0.42)
Secondary analyses						
HLA-A						
1	18.6	16.4	1.17 (0.44, 3.09)	35.1	26.1	1.53 (0.75, 3.12)
2	50.9	50.9	1.00 (0.48, 2.08)	54.6	55.1	0.98 (0.51, 1.89)
3	10.2	12.7	0.78 (0.24, 2.47)	24.7	33.3	0.66 (0.32, 1.35)
11	8.5	7.3	1.18 (0.30, 4.64)	7.8	14.5	0.50 (0.17, 1.45)
24	33.9	27.3	1.37 (0.61, 3.05)	11.7	7.3	1.69 (0.54, 5.33)
25	3.4	5.5	0.61 (0.10, 3.79)	7.8	5.8	1.37 (0.37, 5.09)
26	10.2	7.3	1.44 (0.39, 5.42)	3.9	4.4	0.89 (0.17, 4.57)
28	11.9	12.7	0.92 (0.30, 2.83)	6.5	5.8	1.13 (0.29, 4.38)
29	3.4	10.9	0.29 (0.06, 1.49)	7.8	10.1	0.75 (0.24, 2.35)
31	13.6	12.7	1.08 (0.36, 3.19)	9.1	10.1	0.89 (0.29, 2.67)
HLA-B						
7	13.6	10.9	1.28 (0.41, 3.96)	15.6	27.5	0.49 (0.22, 1.09)
8	22.0	3.6	7.49* (1.61, 34.94)	39.0	23.2	2.11* (1.03, 4.36)
13	3.4	7.3	0.45 (0.08, 2.55)	6.5	5.8	1.13 (0.29, 4.38)
14	8.5	7.3	1.18 (0.30, 4.64)	5.2	7.3	0.70 (0.18, 2.72)
17	0.0	1.8	0.31 (0.01, 7.65)	1.3	0.0	2.73 (0.11, 68.01)
18	6.8	7.3	0.93 (0.22, 3.90)	10.4	10.1	1.03 (0.35, 3.00)
27	10.2	5.5	1.96 (0.47, 8.26)	6.5	7.3	0.89 (0.25, 3.21)
35	13.6	29.1	0.38* (0.15, 0.98)	11.7	10.1	1.17 (0.41, 3.34)
38	6.8	9.1	0.73 (0.19, 2.86)	0.0	2.9	0.17 (0.01, 3.69)
44	15.3	25.5	0.53 (0.21, 1.34)	16.9	27.5	0.54 (0.24, 1.19)
50	13.6	0.0	18.32* (1.03, 325.5)	5.2	1.5	3.73 (0.41, 34.17)
51	10.2	5.5	1.96 (0.47, 8.26)	3.9	5.8	0.66 (0.14, 3.05)
60	6.8	3.6	1.93 (0.34, 10.97)	9.1	10.1	0.89 (0.29, 2.67)
62	1.7	7.3	0.22 (0.02, 2.03)	22.1	10.1	2.51 (0.97, 6.48)
HLA-DR						
1	14.8	13.0	1.16 (0.37, 3.63)	10.7	7.9	1.39 (0.43, 4.47)
2	3.7	10.9	0.32 (0.06, 1.71)	2.7	14.3	0.16* (0.03, 0.79)
5	5.6	34.8	0.11† (0.03, 0.41)	2.7	15.9	0.15* (0.03, 0.69)
7	13.0	26.1	0.42 (0.15, 1.18)	21.3	20.6	1.04 (0.46, 2.38)
DQA1						
0101,0102,0103	17.3	44.4	0.26* (0.10, 0.66)	32.8	58.1	0.35* (0.17, 0.73)
0201	11.5	24.4	0.40 (0.14, 1.20)	18.8	25.8	0.66 (0.28, 1.55)
0301	80.8	40.0	6.30*† (2.53, 15.68)	68.8	40.3	3.26*† (1.57, 6.78)
0401,0501,0601	57.7	57.8	1.00 (0.44, 2.24)	60.9	50.0	1.56 (0.77, 3.16)

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did not differ by ethnicity among cases ($P = 0.72$) or control subjects ($P = 0.33$) (Table 3). Of the nondiabetic Hispanics, 38.6% were in the highest risk group (non-Asp) compared with 35.5% of non-Hispanic white, nondiabetic control subjects. Khalil et al. (27) have suggested that the combination of a DQA1 chain encoding arginine at position 52 and a DQB1 chain without Asp in position 57 is diabetogenic. In this study, this combination was associated with a similar increase in risk of diabetes in both ethnic groups. Among nondiabetic control subjects, the prevalence of this sus-

Table 2—*Continued*

	Hispanic			Non-Hispanic		
	IDDM (%)	No IDDM (%)	OR (95% CI)	IDDM (%)	No IDDM (%)	OR (95% CI)
DQB1						
0201	63.5	33.3	3.47* (1.50, 8.03)	63.5	46.0	2.04* (1.00, 4.16)
0401,0402	3.9	4.4	0.86 (0.12, 6.37)	6.4	4.8	1.36 (0.29, 6.32)
0501,0502,0604	11.5	24.4	0.40 (0.14, 1.20)	28.6	19.1	1.70 (0.74, 3.91)
0601,0603	1.9	8.9	0.20 (0.02, 1.87)	1.6	31.8	0.04*† (0.004, 0.27)
0301	17.3	51.1	0.20*† (0.08, 0.51)	17.5	36.5	0.37* (0.16, 0.84)
0302	69.2	26.7	6.19*† (2.55, 14.99)	49.2	25.4	2.85* (1.34, 6.04)
0303	5.9	0.0	6.57 (0.33, 130.7)	0.0	1.6	0.32 (0.01, 7.95)

Asp, persons with DQB1*0402, 0401, DQB1*0602, 0603, DQB1*0301, or DQB1*0303; alleles encoding Asp in position 57. Markers present in <5% of participants were not included. Sample sizes were 54 Hispanics with IDDM, 46 Hispanic nondiabetic control subjects, 75 non-Hispanic whites with IDDM, 63 non-Hispanic white control subjects for comparison of HLA-DR antigens; 52 Hispanics with IDDM, 45 Hispanic control subjects, 64 non-Hispanic whites with IDDM and 62 non-Hispanic white control subjects for comparisons of DQA1 and DQB1 alleles and Asp; 59 Hispanics with IDDM, 55 Hispanic control subjects, 77 non-Hispanic whites with IDDM, and 69 non-Hispanic white control subjects for comparisons of HLA-A and HLA-B antigens.

* $P < 0.05$, uncorrected.

† $P < 0.05$, corrected for the number of comparisons.

ceptibility marker did not differ by ethnicity (19.7% Hispanics vs. 11.1% non-Hispanic whites, $P = 0.24$).

The attributable risk for HLA-DR3 among non-Hispanic whites was 0.42, suggesting that 6.5 of 15.4 cases (per 100,000/year) that occurred in Colorado were accounted for by HLA-DR3 (28). If the prevalence of this antigen among non-Hispanic whites was lowered to that of the Hispanic population (i.e., 75% lower), only 1.6 of these cases would have occurred, and the overall incidence of IDDM among non-Hispanic whites would be similar to the rate observed in the Hispanic population (10.5/100,000/year vs. 9.7/100,000/year, predicted versus observed).

To determine whether other ethnic differences existed in the relationships of HLA antigens and alleles to IDDM, we examined the associations of HLA-A and HLA-B antigens, additional HLA-DR antigens (other than HLA-DR3 and HLA-DR4), and specific DQA1 and DQB1 alleles with IDDM in both ethnic groups (Table 2, secondary analysis). After adjusting for the number of comparisons, DQA1*0301 remained significantly associated with IDDM in both ethnic groups, whereas DQB1*0602, 0603 was associated with IDDM among non-Hispanic whites only, and HLA-DR5, DQB1*0301, and DQB1*0302 were associated with IDDM among Hispanics only. No statistically significant ethnic differences were detected among nondiabetic control subjects in the prevalences of any of these antigens and alleles after correcting for the number of comparisons.

CONCLUSIONS— The genetic markers HLA-DR3, HLA-DR4, and Asp conferred similar risk of IDDM in both ethnic groups, consistent with previous reports (9–12,19,21,22,29–34). The prevalences of Asp alleles in both Hispanics and non-Hispanic whites were similar to those reported in other Caucasian populations (29,30,34). These data suggest that the markers of genetic susceptibility to IDDM and the degree of risk they confer do not differ between Hispanics and non-Hispanic whites.

We did find that HLA-DR3 was significantly more common in non-Hispanic whites than in Hispanics, a pattern consistent with the incidence differential. Because HLA-DR3 is less common among Native American than Caucasian populations (35,36), these results suggest that Amerindian admixture may explain the decreased risk of IDDM seen among Hispanics.

The prevalence of Asp alleles was not different by ethnicity in nondiabetic

Table 3—*Prevalence of Asp genotypes in nondiabetic control subjects by ethnicity*

	Hispanic		Non-Hispanic	
	IDDM *n* (%)	No IDDM *n* (%)	IDDM *n* (%)	No IDDM *n* (%)
Asp	5 (8.2)	15 (34.1)	3 (5.9)	15 (24.2)
Asp/non-Asp	11 (18.0)	12 (27.3)	12 (23.5)	25 (40.3)
Non-Asp	45 (73.8)	17 (38.6)	36 (70.6)	22 (35.5)

Asp includes those Asp/Asp and Asp/blank. Non-Asp includes those non-Asp/non-Asp and non-Asp/blank.

subjects in contrast with a report from other registries (34). Also, no ethnic difference was found in the prevalence of the high-risk DQA1 and DQB1 heterodimer (27). Our data suggest that the prevalence of genetically susceptible people is lower among Hispanics, but that DQ markers may not reliably differentiate populations.

HLA-DR5, DQB1*0301, and DQB1*0302 were associated with IDDM in Hispanics only, whereas DQB1*0602, 0603 was associated with IDDM among non-Hispanic whites only. These data suggest the existence of some differences in markers of IDDM by ethnicity, but these results need to be confirmed by additional studies.

Among diabetic subjects, our participation rate was low but no significant differences were observed between participants and nonparticipants, suggesting little probable bias. The control subjects were slightly more likely to be from higher socioeconomic strata because they were identified through a household that contained at least one licensed driver and a telephone, which may have led to an overestimate of the prevalence of Caucasian genes in the Hispanic population and an underestimate of the magnitude of the HLA-DR3 prevalence difference between ethnic groups (38,39).

Although our data clearly suggest that the lower prevalence of HLA-DR3 may explain the incidence differential between ethnic groups, it also may be a surrogate for cultural differences in lifestyles and environmental exposures. In San Antonio, Texas, socioeconomic status (38,39) and acculturation correlated with Amerindian admixture (H.P. Hazuda, unpublished observations). Thus, studies of potential environmental agents should be conducted in genetically typed populations to determine the relative importance of genetic and environmental factors in explaining incidence differentials observed between populations.

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