Trends in High-Risk HLA Susceptibility Genes Among Colorado Youth With Type 1 Diabetes

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OBJECTIVE — Type 1 diabetes is associated with a wide spectrum of susceptibility and protective genotypes within the HLA class II system. It has been reported that adults diagnosed with youth-onset type 1 diabetes more recently have been found to have fewer classical high-risk HLA class II genotypes than those diagnosed several decades ago. We hypothesized that such temporal trends in the distribution of HLA-DR, DQ genotypes would be evident, and perhaps even stronger, among 5- to 17-year-old Hispanic and non-Hispanic white (NHW) youth diagnosed with type 1 diabetes in Colorado between 1978 and 2004.

RESEARCH DESIGN AND METHODS — HLA-DR, DQ was typed using PCR and sequence-specific oligonucleotide hybridization in 100 youth diagnosed during the period of 1978–1988 and 264 diagnosed during 2002–2004. Logistic regression was used to adjust for confounders and assess temporal trends.

RESULTS — The frequency of the highest-risk genotype (DRB1*03-DQB1*02/DRB1*04-DQB1*03) was higher (39%) in children diagnosed during the period 1978–1988 than in those diagnosed during 2002–2004 (28%). A similar pattern was observed in NHWs and Hispanics.

CONCLUSIONS — We found that high-risk HLA genotypes are becoming less frequent over time in youth with type 1 diabetes of NHW and Hispanic origin. This temporal trend may suggest that increasing environmental exposure is now able to trigger type 1 diabetes in subjects who are less genetically susceptible.

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The incidence of type 1 diabetes in youth under 20 years of age is increasing worldwide (1). Recently, we reported an annual average increase of 2.3% in youth 0–17 years of age in Colorado, with the greatest increase reported in children under 5 years of age (2). Trends of such magnitude and consistency can only be explained by changes in environmental risk factors (3). Recent hypotheses argue that an increased environmental pressure associated with type 1 diabetes risk may reduce the need for a strong background of genetic susceptibility in order for type 1 diabetes to develop.

Type 1 diabetes has a genetic component that is neither sufficient nor necessary, in terms of current known determinants. HLA class II region polymorphisms alone explain 40–50% of familial clustering in type 1 diabetes risk (4). There is an approximate 5-fold increase in risk of type 1 diabetes for the homozygous expression of either DRB1*03 or DRB1*04 and a 14-fold increased risk for those with heterozygous expression (DRB1*03/DRB1*04) (5). Although the DRB1 and the DQA1 and DQB1 genes are important in determining susceptibility to type 1 diabetes, examining the penetrance of these genes has provided a better understanding of the involvement of environmental risk factors. Four studies (6–9) have explored potential temporal changes in the frequency and/or distribution of HLA genotypes associated with type 1 diabetes susceptibility. All have suggested a decreasing frequency of high-risk HLA genotypes over time in adults diagnosed with childhood-onset type 1 diabetes. A study conducted in the U.K. reported a 12% decrease in the frequency of the high-risk HLA genotype (DRB1 03-DQA1*0501-DQB1*0201/DRB1 04-DQA1*0301-DQB1*0302) between individuals diagnosed recently compared with 50 years ago (8). A similar decrease in the frequency of the high-risk HLA genotype (7.1% over a 62-year time period) was noted in Finland (9). These studies, however, were all performed among surviving adults with childhood-onset type 1 diabetes and thus may have been biased by changes in mortality rates among people with type 1 diabetes over the last 50 years, especially if mortality was associated with specific HLA genotypes. Further studies, which are not limited to adult survivors and capture younger individuals close to the time of diagnosis, are needed to address the question of changes in type 1 diabetes–related HLA genotypes over time.

The purpose of this report was to determine potential temporal trends in the frequency distribution of HLA susceptibility genes (HLA-DR and HLA-DQ genotypes) among 5- to 17-year-old Hispanic and non-Hispanic white (NHW) youth with type 1 diabetes from Colorado, newly diagnosed with diabetes between 1978 and 2004.

RESEARCH DESIGN AND METHODS

— Subjects included in this study were identified through two different diabetes registry studies conducted 13 years apart: the Colorado Type 1 Diabetes Registry and the Colorado Site of the SEARCH for Diabetes in Youth Study. The Colorado Type 1 Diabetes Registry was a statewide registry developed to ascertain all new cases of type 1 diabetes in 0- to 17-year-olds in Colorado

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Table 1—*Characteristics of the study population by time period (1978–1988 and 2002–2004) and participation status in the HLA typing*

	NHW			Hispanic		
	Nonparticipants	Participants	*P*	Nonparticipants	Participants	*P*
1978–1988						
n	58	59	122	75	41	120
Sex (%male/%female)	54/46	45/55	0.17	37/63	41/59	0.66
Family history (%yes/%no)	54/46	44/56	0.15	55/45	69/31	0.16
Age-group at diagnosis (years)						
5–9	38 (22)	41 (24)		28 (21)	32 (13)	
10–14	48 (28)	42 (25)	0.64	52 (39)	57 (23)	0.42
15–17	14 (8)	17 (10)		20 (15)	11 (5)	
Mean age at diagnosis (years)	11.2 ± 3.3	11.1 ± 3.4	0.73	11.8 ± 3.1	11.2 ± 3.0	0.27
2002–2004						
n	287	225	512	48	39	87
Sex (%male/%female)	53/47	53/47	0.95	56/44	49/51	0.48
Family history (%yes/%no)	48/52	58/42	0.04	59/41	69/31	0.32
Age-group at diagnosis (years)						
5–9	38 (109)	38 (86)		40 (19)	36 (14)	
10–14	46 (132)	48 (108)	0.76	52 (25)	49 (19)	0.59
15–17	16 (46)	15 (31)		8 (4)	13 (6)	
Mean age at diagnosis (years)	11.2 ± 3.4	10.5 ± 3.4	0.39	10.8 ± 3.0	11.5 ± 4.4	0.32

Data are % (*n*) and means ± SD unless otherwise indicated.

from 1 January 1978 to 31 December 1988 (10,11). SEARCH is a multicenter, population-based observational registry that ascertained new cases of physician-diagnosed diabetes in youth 0–19 years of age from 1 January 2002 forward (12). A detailed description of this population (2), the Colorado Type 1 Diabetes Registry (10,11), and SEARCH have been published (12). Type 1 diabetes was defined as use of insulin within 2 weeks from diagnosis. Completeness of case ascertainment was assessed by the capture-recapture method (13,14) and estimated to be 96–97% over time (2).

Youth aged 5–17 years identified with incident type 1 diabetes during the period of 1978–1988 and 2002–2004 were eligible to participate in this analysis. In 1978–1988, all Hispanic youth ($n = 120$) and a similar size random sample of NHW ($n = 122$) youth identified by the Colorado Type 1 Diabetes Registry were eligible for genetic typing, and 41 Hispanic (34%) and 59 NHW youth (48%) had HLA measured (15). In 2002–2004, all youth over the age of 4 years of Hispanic ($n = 87$) or NHW ($n = 512$) origin were eligible for genetic typing. A total of 39 Hispanic (45%) and 225 NHW (44%) youth participated. Age at diagnosis, sex, and family history of diabetes were assessed to determine whether the youth with HLA measured were representative of the overall registered population in each period and racial/ethnic group.

Date of diagnosis and date of birth were obtained from medical records. Family history of diabetes was obtained from medical record abstraction (71% in 1978–1988 and 85% in 2002–2004) or from self-administered questionnaires completed within 5 years from the date of diagnosis (17% in 1978–1988 and 3% in 2002–2004). Children who had a sibling, parent, or grandparent with any type of diabetes were defined as having a positive family history.

To assess whether the sample of youth with available HLA typing was representative of the entire patient population of youth with type 1 diabetes diagnosed in these two time periods, demographic and clinical characteristics of study participants were compared with those of nonparticipants. Table 1 shows these characteristics according to time period and ethnicity. In 1978–1988, there were no significant differences for either NHW or Hispanic youth. In 2002–2004, the only significant difference between those with and without HLA typing was a higher frequency of a positive family history of diabetes in NHW participants with typing available. However, no significant relationship was noted between having the highest-risk HLA genotype (DRB1*03-DQB1*02/DRB1*04-DQB1*03) and having a positive family history of diabetes in either time period ($P = 0.96$). These data suggest that our samples of NHW and Hispanic youth with measured HLA are likely representative of the larger eligible population with type 1 diabetes in both periods, or that they differ in characteristics not strongly related with the frequency of high-risk HLA genotypes. Therefore, we believe that any observed trends or lack thereof in the frequency and distribution of HLA genotypes over time in our population are not likely to be due to selection bias. All participants provided informed consent and both the Colorado Type 1 Diabetes Registry and SEARCH were approved by relevant institutional review boards.

HLA typing

DRB1 and DQB1 typing was performed using OneLambda PCR assays that were standard for the time period in which they were used (1978–1988 and 2002–2004) (16). The main difference between assays was the number of DQB1 alleles identified that had sequence-specific oligonucleotides (SSOs) available for typing. There were seven DQB1 SSOs identified for typing in 1978–1988, and there were 100 DQB1 SSOs identified for typing in 2002–2004. Due to the difference in the number of DQB1 alleles identified and the availability of SSOs for typing these alleles over the two periods, only the gene locus was used in the analysis to compare genotypes (e.g., DQB1*02 vs. DQB1*0201). Additionally, the current PCR sequence–specific oligonucleotide probes used in 2002–2004 allowed for a larger number

Table 2—*Distribution of HLA class II genotypes (DRB1-DQB1) and odds ratios (ORs) for the association between genotype and period (1978–1988 vs. 2002–2004), according to participants' ethnicities*

	NHW		Hispanic		All	
	n (%)	OR (95% CI)*	*n* (%)	OR (95% CI)*	*n* (%)	OR (95% CI)*
DRB1*03-DQB1*02/DRB1*04-DQB1*03						
1978–1988	24 (41)		15 (37)		39 (39)	
2002–2004	63 (28)	**0.6 (0.3–0.99)**	12 (31)	0.8 (0.3–1.9)	75 (28)	**0.6 (0.4–0.99)**
DRB1*04-DQB1*03/DRB1*04-DQB1*03, DRB1*04-DQB1*03/X†, DRB1*04-DQB1*03/unknown						
1978–1988	18 (31)		12 (29)		30 (30)	
2002–2004	96 (43)	1.7 (0.9–3.0)	15 (38)	1.5 (0.6–3.8)	111 (42)	**1.7 (1.1–2.8)**
DRB1*03-DQB1*02/DRB1*03-DQB1*02, DRB1*03-DQB1*02/X†, DRB1*03-DQB1*02/unknown						
1978–1988	12 (20)		8 (19)		20 (20)	
2002–2004	50 (22)	1.1 (0.6–2.3)	5 (13)	0.6 (0.2–2.0)	55 (21)	1.1 (0.6–1.9)
X/X, X/unknown						
1978–1988	5 (8)		6 (15)		11 (11)	
2002–2004	16 (7)	0.8 (0.3–2.4)	7 (18)	1.3 (0.4–4.2)	23 (8.7)	0.8 (0.4–1.6)

*OR for the association between period 2002–2004 versus 1978–1988 and carrying the high-risk genotype compared with all other genotypes. †DRB1*03-DQB1*02/DRB1*04-DQB1*03 not included. Data in bold are statistically significant.

of samples to be typed at one time compared with the prior assay (17,18). Previous validation studies based on OneLambda Luminex SSO typing yielded similar results when compared with the earlier SSO assay (19).

Genotypes assessed in both time periods were categorized as high risk (DRB1*03-DQB1*02/DRB1*04-DQB1*03); moderate to low risk (DRB1*04-DQB1*03/DRB1*04-DQB1*03, DRB1*04-DQB1*03/X, where X is not DRB1*03-DQB1*02, and DRB1*04-DQB1*03/unknown); low risk (DRB1*03-DQB1*02/DRB1*03-DQB1*02, DRB1*03-DQB1*02/X, where X is not DRB1*04-DQB1*03, and DRB1*03-DQB1*02/unknown); and neutral risk (X/X or X/unknown). The X haplotype denotes all other haplotypes not defined above. The unknown haplotype denotes a haplotype that was unable to be typed.

Statistical analyses

Descriptive univariate analysis was used to determine the frequency of the genotypes for each period. χ^2 statistics were used to compare frequencies of HLA haplotypes by race and period and genotypes by high-risk (DRB1*03-DQB1*02/DRB1*04-DQB1*03) versus all other genotypes. Multivariate logistic regression was used to adjust for potential confounders (onset age, sex, family history of diabetes, and race/ethnicity) and assess temporal trends. High-risk was compared with all other genotypes using all other genotypes as the referent group. SAS version 9.1 (20) was used for all analyses.

RESULTS — A total of 364 youth (100 in 1978–1988, and 264 in 2002–2004) with new-onset type 1 diabetes at age 5–17 years had HLA genes measured as described above.

Table 2 shows the frequency of HLA class II DRB1-DQB1 genotypes by time period and ethnicity. More children (both NHW and Hispanic) carried the high-risk genotype (DRB1*03-DQB1*02/DR04-DQB1*03) in 1978–1988 than in 2002–2004 ($P = 0.05$). Conversely, more children in 2002–2004 carried the moderate to low risk DRB1*04-DQB1*03 (homo- or heterozygous) genotypes than in 1978–1988 ($P = 0.04$). There was no significant difference by time period or ethnicity in frequency of the neutral risk, DRB1*03-DQB1*02 (homo- or heterozygous), or X/X genotypes. These patterns were similar for NHW and Hispanic youth with type 1 diabetes.

Using multiple logistic regression analysis (Table 2), we assessed the association between having the high-risk genotype (versus all other genotypes) and time period, controlling for age, sex, race/ethnicity, and family history of diabetes. There was a 40% lower odds for carrying the high-risk genotype in 2002–2004 versus 1978–1988 (odds ratio 0.60 [95% CI 0.36–0.99], $P = 0.05$). A decreasing trend over the two time periods was noted for both NHWs (13%) and Hispanics (6%). Conversely, there was a 70% greater chance of carrying the moderate- to low-risk genotypes in 2002–2004 versus 1978–1988 (1.7 [1.1–2.8], $P = 0.03$). No significant interactions between ethnicity and time period on the odds of having the highest risk genotype were noted. When stratified according to age-group (Table 3), the most dramatic decreasing trend in the prevalence of the high-risk genotype occurred in those aged 5–9 years (51% [1978–1988] vs. 32% [2002–2004], $P = 0.05$) with a coincident increasing trend in the proportion with the DRB1*04-DQB1*03 (homo- or heterozygous) genotype in the same age-group (19% [1978–1988] to 40% [2002–2004], $P = 0.03$). No significant differences over time were noted in the other age-groups.

CONCLUSIONS — We found that the distribution of HLA genotype changed in youth with type 1 diabetes diagnosed 27 years apart. Having type 1 diabetes at age 5–17 years in 2002–2004 was associated with an 11% difference or 0.6-fold lower odds for carrying the high-risk HLA genotype (DRB1*03-DQB1*02/DRB1*04-DQB1*03) compared with having type 1 diabetes in 1978–1988. The largest proportional difference (19%) over time in the high-risk genotype was

Table 3—*Distribution of HLA class II genotypes (DRB1-DQB1) and odds ratios (ORs) for the association between genotype and period (1978–1988 vs. 2002–2004) by age-group at diagnosis*

	5–9 years of age		10–14 years of age		15–17 years of age	
	n (%)	OR (95% CI)*	*n* (%)	OR (95% CI)*	*n* (%)	OR (95% CI)*
DRB1*03-DQB1*02/DRB1*04-DQB1*03						
1978–1988	19 (51)		14 (29)		6 (40)	
2002–2004	32 (32)	**0.45 (0.2–0.9)**	34 (27)	0.9 (0.4–1.8)	9 (24)	0.5 (0.1–1.7)
DRB1*04-DQB1*03/DRB1*04-DQB1*03, DRB1*04-DQB1*03/X†, DRB1*04-DQB1*03/unknown						
1978–1988	7 (19)		20 (42)		3 (20)	
2002–2004	40 (40)	**2.9 (1.2–7.3)**	55 (43)	1.1 (0.5–2.1)	16 (43)	3.0 (0.7–12.6)
DRB1*03-DQB1*02/DRB1*03-DQB1*02, DRB1*03-DQB1*02/X†, DRB1*03-DQB1*02/unknown						
1978–1988	7 (19)		9 (19)		4 (27)	
2002–2004	20 (20)	1.1 (0.4–2.8)	28 (22)	1.2 (0.5–2.8)	7 (19)	0.6 (0.6–2.6)
X/X, X/unknown						
1978–1988	4 (11)		5 (10)		2 (13)	
2002–2004	7 (8)	0.6 (0.2–2.3)	11 (8)	0.8 (0.3–2.5)	5 (14)	1.0 (0.2–5.9)

*OR for the association between period 2002–2004 versus 1978–1988 and carrying the high-risk genotype compared with all other genotypes. †DRB1*03-DQB1*02/DRB1*04-DQB1*03 not included. Data in bold are statistically significant.

found in 5- to 9-year-olds, suggesting that environmental pressures may have a greater impact on type 1 diabetes risk at earlier ages. This is the first documentation of such a trend in a representative biracial sample of youth with type 1 diabetes in the U.S.

Our study supports previous findings from Finland (9) and the U.K (8), both including Caucasian adults with childhood-onset type 1 diabetes diagnosed up to 80 years apart. The U.K. study showed that the frequency of the high-risk genotype was 12% lower in adults diagnosed up to 15 years of age in 1985–2002 compared with those diagnosed between 1922–1946 and 21% lower in those diagnosed under 5 years of age (8). The Finnish study showed that the frequency of the high-risk genotype was 7.1% lower and that of protective genotypes was 7.2% higher in adults with childhood-onset type 1 diabetes diagnosed after 1990 compared with those diagnosed before 1965 (9). Two older studies conducted in Finland in the 1980s reported a decrease in HLA-DR3 by 25% for those diagnosed with type 1 diabetes in the 1980s compared with those diagnosed in the 1960s (6,7). However, the major limitation of these studies was the potential for selection bias. Neither of these studies provided information on the size or representativeness of the referent population. More importantly, two studies (8,9) measured HLA in adults who were diagnosed with type 1 diabetes as children in the first half of the 20th century, when early mortality associated with type 1 diabetes was high. Therefore, the frequency and distribution of HLA genotypes in this sample may be significantly influenced by factors associated with survival of type 1 diabetes. An underestimate of the proportional distribution of high-risk HLA genotypes in earlier time periods (due to a potential association of high-risk HLA genes with increased mortality) may have resulted in underestimating the true temporal trends in the proportion of type 1 diabetes cases with high-risk HLA genotypes in these earlier studies. In contrast, our study genotyped youth with type 1 diabetes relatively close to their disease onset and thus is not affected by survivor bias. In addition, we were able to provide evidence that the sample included in the analysis was representative of the Colorado population of youth with type 1 diabetes in both time periods.

Nevertheless, our study has several limitations. Importantly, although the same method was used for typing, due to the specific time periods in which they were performed, two slightly different assays were used, namely, OneLambda LabType Luminex SSO in 2002–2004 and OneLambda SSO in 1978–1988. We were unable to validate the results obtained with the earlier SSO assay due to the lack of stored DNA samples and inability to recontact participants. However, previous studies (19,21) suggest comparable agreement in serologic specificity with an 85% allele agreement. Due to this and the difference in the number of allele SSOs available for typing over time, only the gene locus was used in this analysis. By not using expanded allele information, we potentially introduced some misclassification, likely increasing the proportion with the high-risk genotype. However, misclassification would be similar for both time periods and therefore unlikely to influence our analysis of trends. Because for 2002–2004 we did have allele information on 100 DQB1 SSOs, we estimated the magnitude of the potential misclassification, and only four (1.5%) cases were misclassified as having the highest-risk genotype in 2002–2004 using the expanded allele information only. In addition, there were 12 youth from 1978–1988 that did not have complete genotypes. These youth were included in the study and classified as haplotype unknown. This categorization could have led to some misclassification. Specifically, if the unknown haplotype was DRB1*03-DQB1*02 or DRB1*04-DQB1*03, youth would have been classified as high risk based on the typed haplotype or as heterozygous for the above haplotypes. For example, in the extreme situation where all youth with the unknown haplotype in the first time period would have been in fact at "high-risk," the proportion of youth with the

highest risk genotype in 1978–1988 would have been 51% (instead of 39%). This would have made our trend estimates even more substantial (i.e., a 23% decrease in the proportion with high-risk genotype). Consequently, our estimated change of 11% is conservative.

There are several hypotheses proposed to explain the increase in type 1 diabetes incidence in youth and the earlier age at onset. The "accelerator" and "overload" hypotheses suggest that an increase in body mass in genetically susceptible individuals accelerates β-cell decline or stresses (overloads) the β-cells, leading to an early age at type 1 diabetes diagnosis (22,23). The "hygiene hypothesis" postulates that the decreasing early life exposure to infectious agents in Westernized societies has led to an impairment in the maturation of the immune system, thus permitting an increased occurrence of immune-mediated disorders, such as asthma and type 1 diabetes (24). Further testing is required for all of these hypotheses.

Given that there is an increasing incidence of type 1 diabetes in Colorado youth (2), our findings support the hypothesis that environmental pressures may be increasing the disease penetrance despite a shift in the distribution of HLA genotypes from high to moderate-low risk. Although this and previous studies (6–9) evaluating the distribution and frequency of HLA susceptibility genes over time have provided evidence in support of this hypothesis, the nature of environmental factors responsible for the increasing incidence of type 1 diabetes remains unknown.

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