The Rising Incidence of Type 1 Diabetes Is Accounted for by Cases With Lower-Risk Human Leukocyte Antigen Genotypes

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OBJECTIVE — The rising incidence of type 1 diabetes has been attributed to environment, implying a lesser role for genetic susceptibility. However, the rise could be accounted for by either more cases with classic high-risk genes or by cases with other risk genes. Separately, for any degree of genetic susceptibility, age at presentation may decrease in a permissive environment. To examine these possibilities, human leukocyte antigen (HLA) class II DRB1 genes known to confer risk for type 1 diabetes were analyzed in relation to year of birth and age at diagnosis over the last five decades.

RESEARCH DESIGN AND METHODS — Caucasoid subjects ($n = 462$) diagnosed with type 1 diabetes before age 18 between 1950 and 2005 were DRB1 genotyped.

RESULTS — Mean ± SD age at diagnosis, 8.5 ± 4.5 years, did not differ across decades. Recent diagnosis was associated with a lower proportion but unchanged incidence of the highest-risk DRB1 genotype DR3,4 (2000–2005, 28% vs. 1950–1969, 79%; $P < 0.0001$) and a higher proportion of lower-risk genotypes DR4,X and DR3,X (2000–2005, 48% vs. 1950–1969, 20%; $P = 0.0002$). The frequency of the DRX,X genotype was low (≤3%) across decades. Recent birth was associated with a lower age at diagnosis for lower risk DR3,3 and DR4,4 ($P < 0.0001$) and DR4,X ($P < 0.0001$) and DR3,X ($P = 0.015$) genotypes but not for DR3,4.

CONCLUSIONS — The rising incidence and decreasing age at diagnosis of type 1 diabetes is accounted for by the impact of environment on children with lower-risk HLA class II genes, who previously would not have developed type 1 diabetes in childhood.

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T ype 1 diabetes is an autoimmune disease that destroys the insulin-producing β-cells in the islets of the pancreas, resulting in hyperglycemia and associated complications. It is one of the most common chronic diseases from childhood, requiring continuous or multiple daily insulin injections and blood glucose monitoring at substantial personal and economic cost. Genetic susceptibility accounting for at least half the lifetime risk, together with environmental conditions, leads to the development of type 1 diabetes (1). Although type 1 diabetes is a polygenic disease, the human leukocyte antigen (HLA) genes, which code for molecules that bind and present peptide antigens to T-cells, account for approximately half of the genetic risk (2,3).

The incidence of childhood-onset type 1 diabetes has been increasing progressively over the last half century (4,5). Gillespie et al. (6) reported that the proportion of children with the highest-risk HLA genotype for type 1 diabetes (DR3,4

and DQ2,8) was significantly lower in the Bart's-Oxford cohort of children in the U.K. diagnosed between 1985 and 2002 compared with children in the Golden Years cohort diagnosed between 1920 and 1946. On the other hand, the proportion of children with lower-risk genotypes (DR4,X and DR3,X) was higher in the Bart's-Oxford cohort. Their findings were consistent with a Finnish study by Hermann et al. (7) in which children who developed type 1 diabetes between 1939 and 1965 carried a higher proportion of high-risk HLA genes compared with those diagnosed between 1990 and 2001. Gillespie et al. (6) concluded that "the rising incidence of type 1 diabetes in children has resulted from exposure of a genetically susceptible subgroup of the population to an environment that is increasingly conducive to diabetes development." Both Hermann et al. (7) and Gillespie et al. (6) compared contemporary subjects with cohorts diagnosed in previous eras when survival from type 1 diabetes was significantly less than it is today. Their analyses were based on the assumption that the HLA profile of long-term survivors was representative of that of the whole population of children with diabetes in the past, which is not necessarily the case. In fact, Gillespie et al. (6) acknowledge that only a minority of children who developed diabetes in the 1940s and 1950s are likely to have survived to the present. Many studies have documented improved survival rates, especially since the 1960s (8–10).

If the environment increasingly impacts on the expression of type 1 diabetes, then the contribution of genetic susceptibility in newly diagnosed cases may change over time, and, for the same degree of genetic susceptibility, age at diagnosis may decrease over time. We wanted to test these possibilities while avoiding selection bias in the reference population.

RESEARCH DESIGN AND METHODS — Subjects with type 1 diabetes were from the Australian Type 1 Diabetes DNA Repository (11,12). They had been diagnosed by a specialist physician on the basis of hyperglycemia requir-

Table 1— *Proportion of HLA-DRB1 genotypes according to year of diagnosis of type 1 diabetes*

HLA-DRB1 genotype	Year of diagnosis					$P*$
	1950–1969	1970–1979	1980–1989	1990–1999	2000–2005	
DR3,4	15 (79)	35 (60)	42 (47)	98 (37)	8 (28)	<0.0001
DR4,4	0 (0)	5 (9)	4 (4)	31 (12)	4 (14)	0.039
DR3,3	0 (0)	7 (12)	7 (8)	19 (7)	2 (7)	0.800
DR4,X	2 (10)	8 (14)	28 (31)	68 (25)	10 (34)	0.034
DR3,X	2 (10)	1 (2)	7 (8)	42 (16)	4 (14)	0.009
DRX,X	0 (0)	2 (3)	1 (1)	9 (3)	1 (3)	0.950

Data are *n* (%). *P value is for trend across decades.

ing immediate and ongoing insulin therapy. All subjects were of European descent and lived in metropolitan Melbourne or the wider state of Victoria. They were recruited in an unselected manner from diabetes clinics, including the Royal Children's Hospital, which until recently followed up the majority of children and teenagers with type 1 diabetes in Victoria. Virtually all individuals with type 1 diabetes in Victoria attend diabetes clinics or are seen by specialists with attachments to these clinics. Subjects (*n* = 462) diagnosed before 18 years of age between 1950 and 2005 (91% after 1975) were typed for HLA-DRB1 alleles.

HLA typing

HLA-DRB1 genotyping was performed by PCR-based sequence-specific oligonucleotide hybridization, modified from the 11th International Histocompatibility Workshop protocol to accommodate subsequently described sequence polymorphisms (13). Briefly, PCR primers were designed to provide either generic or allele-specific amplification of exon 2 of the DRB1 allele. The PCR-amplified DNA was rendered single stranded by exposure to sodium hydroxide, immobilized onto nylon membranes, and hybridized with biotinylated oligonucleotide probes designed to detect sequence polymorphisms between DRB1 alleles. Stringent washing was performed in the presence of 3 mol/l tetramethylammonium chloride and the hybridized product detected using a streptavidin/alkaline phosphatase conjugate and chemiluminescent substrate CDP-star (Roche Diagnostics, Castle Hill, NSW, Australia). Allele assignments were made by comparing patterns of hybridization to those predicted from published sequences.

Statistics

Subjects were grouped by decade of birth or diagnosis (1950–1969, combined due to small numbers; 1970–1979; 1980–1989; 1990–1999; and 2000–2005) and from the highest (DR3,4) to intermediate (DR3,3 or DR4,4; DR4,X or DR3,X) to the lowest (DRX,X, where X denotes a non-3 or non-4 allele) HLA genotypes for type 1 diabetes risk. Genotypes between decade groups were compared by the χ^2 test for trend. Mean age at diagnosis across decades was analyzed by ANOVA. Statistics were performed with GraphPad Prism version 3.0 software (La Jolla, CA).

RESULTS — The mean age at diagnosis of the total cohort of type 1 diabetes subjects was (means ± SD) 8.5 ± 4.5 years, and this did not differ across decades of diagnosis (P = 0.54, ANOVA). DRB1 genotypes were first analyzed according to decade of diagnosis. The proportion of the highest-risk genotype, DR3,4, decreased significantly over time from 79% in 1950–1969 to 28% in 2000–2005 (P < 0.0001) (Table 1) (Fig. 1). On the other hand, the proportion of the heterozygous intermediate-risk genotypes, DR4,X and DR3,X, increased significantly over this period (Table 1); taken together, they increased from 20 to 48% (P = 0.0002) (Fig. 1). Homozygosity for DR4, but not DR3, increased over time, and the lowest-risk genotype, DRX,X, was consistently low (≤3%) (Table 1).

Matched for DRB1 genotype and decade of birth, subjects were analyzed for age at diagnosis. There was no change across time in age at diagnosis for the high-risk DR3,4 genotype, but for the intermediate risk genotypes (DR4,4; DR3,3; DR4,X; and DR3,X) age at diagnosis decreased over time (Fig. 2).

The incidence of childhood-onset type 1 diabetes in Australia has doubled in the last 20 years, from 11.3 cases per 100,000 person-years in 1985 to 23.2 in 2002 (14). On the assumption that the DRB1 genotype frequencies were representative of the total population of individuals with type 1 diabetes, the contribution of DRB1 genotypes to the increasing incidence of type 1 diabetes was examined. The numbers of incident cases (rev. in 14) were related to specific DRB1 genotype proportions. As shown in Table 2, the population incidence of type 1 diabetes in subjects with the highest-risk
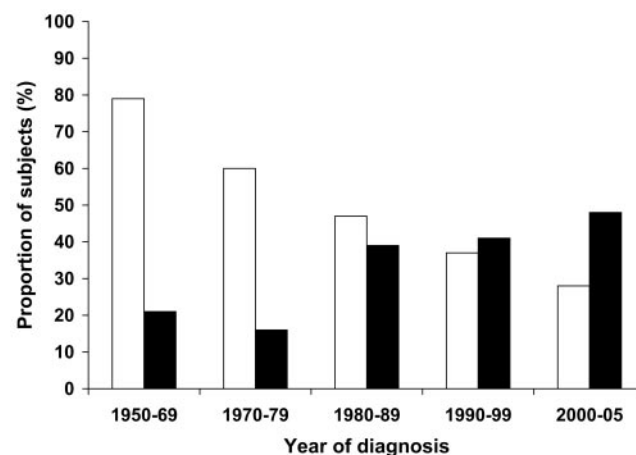

Figure 1—*The proportion of HLA-DRB1 genotypes in childhood-onset type 1 diabetes according to year of diagnosis (where X is a non-3 or non-4 DR allele).* □*, DR3,4;* ■*, DR4,X and DR3,X.*
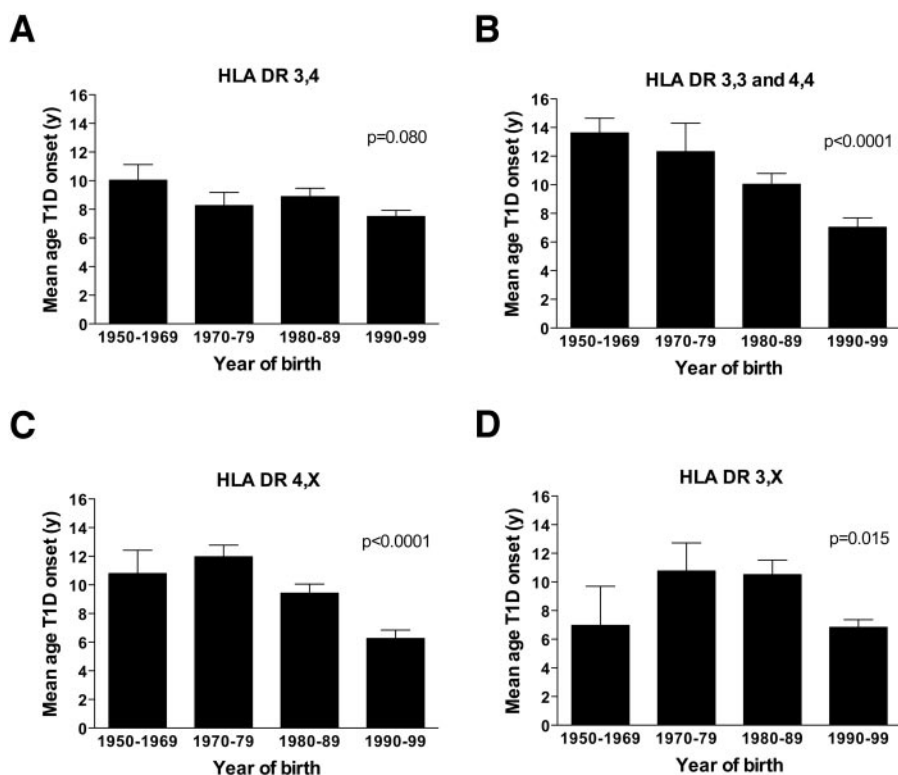
A

B

C

D

Figure 2—*Mean age at diagnosis of type 1 diabetes by decade of birth according to HLA-DRB1 genotypes. Error bars indicate the standard deviation.*

genotype (DR3,4) has remained unchanged. In contrast, the number of cases with intermediate-risk genotypes (DR3,3 or DR4,4 and DR3,X or DR4,X) has increased, accounting for the increase in disease incidence.

CONCLUSIONS — HLA class II profiling demonstrates that the genetic contribution in individuals diagnosed with type 1 diabetes has changed over the last five decades. The contemporary increase in disease incidence is accounted for by individuals with lower-risk HLA class II genotypes who, in previous eras, would not have developed diabetes in childhood. It would appear, therefore, that changing environmental conditions have increased the penetrance of these lower-risk genotypes. Our findings are similar to those reported in the northern hemisphere (6,7) but are more likely to be free of selection bias based on survival, as 91% of our subjects were diagnosed after 1975. Subjects were unselected and predominantly of European Anglo-Celtic origin, with no change over the decades to suggest dilution by immigrants of other ethnic backgrounds. Nevertheless, the possibility that they were not entirely representative of the total population of indi-

viduals with type 1 diabetes across the decades remains a potential limitation. With this caveat, environmental conditions operating in both hemispheres therefore appear to be permissive for the increasing incidence of type 1 diabetes among individuals without the classic high-risk DR3,4 genotype. Furthermore, the mean age at diagnosis has decreased in children with lower-risk genotypes, representing further evidence for the impact of environment.

The temporal change in the genetic contribution to type 1 diabetes raises several points for discussion. First, studies of type 1 diabetes may need to consider stratification by year of birth and diagno-

sis to dissect the relative influences of genes and environment. This concept is also more broadly relevant to other human diseases in which polygenetic susceptibility and environment interact. Second, the HLA profile of classic, childhood-onset type 1 diabetes has shifted and is now similar to that of adults with autoimmune diabetes, who are characterized as having lower-risk HLA genes (15,16). However, adults diagnosed today with autoimmune diabetes were born >30 years ago when the contribution of environment was presumably much less. Born today, would they reach adulthood before developing type 1 diabetes? Third, it appears that the shift in the distribution of HLA genotypes began in the 1980s. A variety of environmental factors including infectious exposure in early life, the quantity and composition of food intake, and the amount of exercise and sleep (with concomitant obesity) have been implicated in promoting the rising incidence of type 1 diabetes. Since the 1980s, the incidence of type 1 diabetes in Australia has more then doubled (14). A change in individuals diagnosed in the 1980s could reflect the impact of environmental factors acting in early life in the 1970s. Two candidate factors may be worthy of comment. First, up until the mid-1970s newborns in Victorian hospitals were housed together in nurseries for up to a week, but this practice ceased when the high prevalence of diarrhea associated with rotavirus infection was found to be significantly reduced by "rooming-in" with the mother (17). The possibility that rotavirus infection in the neonate could protect against type 1 diabetes is supported by findings in the nonobese diabetic (NOD) mouse model of the disease (18); interestingly, though, rotavirus infection postweaning in the NOD mouse did not protect but rather increased disease incidence (18). The latter result is consistent with our

Table 2—*Incident cases of type 1 diabetes by HLA DRB1 genotype*

	1985		2002	
	Incidence 11.3/100,000 person-years*		Incidence 23.2/100,000 person-years	
DRB1 genotype	Proportion	Cases†	Proportion	Cases
DR3,4	47%	5.3	28%	6.5
DR3,3 and DR4,4	12%	1.4	21%	4.9
DR3,X and DR4,X‡	39%	4.4	48%	11.1
DRX,X	1%	0.1	3%	0.7

*Based on ref. 14. †Calculated as incidence times proportion DRB1 genotype. ‡Where X is non-3 or non-4 DRB1 allele.

finding in children followed longitudinally in whom rotavirus infection was positively associated with evidence of islet autoimmunity (19). Second, the prevalence of obesity, and presumably insulin resistance, in Australian children began to accelerate in the 1970s (20). We have shown that insulin resistance is an independent risk factor for development of type 1 diabetes in children with islet autoimmunity (21). Therefore, insulin resistance promoted by changing environmental conditions may contribute to the pathogenesis and rising incidence of type 1, as well as type 2, diabetes.

The stable incidence of DR3,4 across time suggests that this genotype is resistant to the influence of environment. One interpretation is that adaptive T-cell–mediated immunity responsible for β-cell destruction is optimal with DR3,4 but not with lower-risk genotypes. However, the latter can be complemented by innate immunity promoted (together with insulin resistance) by a proinflammatory environment (22). Our findings illustrate that the contribution of HLA genes to type 1 diabetes has changed but not lessened over time. Indeed, genetic susceptibility to type 1 diabetes is no less relevant as more individuals fall under the shadow of a "diabetogenic" environment.

References

1. Eisenbarth GS: Update in type 1 diabetes. *J Clin Endocrinol Metab* 92:2403–2407, 2007
2. Eisenbarth GS: *Type 1 Diabetes: Molecular, Cellular and Clinical Immunology*. 2.5 ed. Eisenbarth GS, Ed. Denver, CO, The Barbara Davis Center for Childhood Diabetes, 2007
3. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Bailey R, Nejentsev S, Field SF, Payne F, Lowe CE, Szeszko JS, Hafler JP, Zeitels L, Yang JH, Vella A, Nutland S, Stevens HE, Schuilenburg H, Coleman G, Maisuria M, Meadows W, Smink LJ, Healy B, Burren OS, Lam AA, Ovington NR, Allen J, Adlem E, Leung HT, Wallace C, Howson JM, Guja C, Ionescu-Tîrgoviste C, Genetics of Type 1 Diabetes in Finland, Simmonds MJ, Heward JM, Gough SC, Dunger DB, Wicker LS, Clayton DG, the Wellcome Trust Case Control Consortium: Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet* 39:857–864, 2007
4. Gale EA: The rise of childhood type 1 diabetes in the 20th century. *Diabetes* 51:3353–3361, 2002
5. The DIAMOND Project Group: Incidence and trends of childhood type 1 diabetes worldwide 1990–1999. *Diabet Med* 23:857–866, 2006
6. Gillespie KM, Bain SC, Barnett AH, Bingley PJ, Christie MR, Gill GV, Gale EA: The rising incidence of childhood type 1 diabetes and reduced contribution of high-risk HLA haplotypes. *Lancet* 364:1699–1700, 2004
7. Hermann R, Knip M, Veijola R, Simell O, Laine AP, Akerblom HK, Groop PH, Forsblom C, Pettersson-Fernholm K, Ilonen J: Temporal changes in the frequencies of HLA genotypes in patients with type 1 diabetes: indication of an increased environmental pressure? *Diabetologia* 46:420–425, 2003
8. Marks HH: Longevity and mortality of diabetics. *Am J Public Health* 55:416–423, 1965
9. McNally PG, Raymond NT, Burden ML, Burton PR, Botha JL, Swift PG, Burden AC, Hearnshaw JR: Trends in mortality of childhood-onset insulin-dependent diabetes mellitus in Leicestershire: 1940–1991. *Diabet Med* 12:961–966, 1995
10. Nishimura R, LaPorte RE, Dorman JS, Tajima N, Becker D, Orchard TJ: Mortality trends in type 1 diabetes: the Allegheny County (Pennsylvania) Registry 1965–1999. *Diabetes Care* 24:823–827, 2001
11. Morahan G, Huang D, Ymer SI, Cancilla MR, Stephen K, Dabadghao P, Werther G, Tait BD, Harrison LC, Colman PG: Linkage disequilibrium of a type 1 diabetes susceptibility locus with a regulatory IL12B allele. *Nat Genet* 27:218–221, 2001
12. Tait BD, Colman PG, Morahan G, Marchinovska L, Dore E, Gellert S, Honeyman MC, Stephen K, Loth A: HLA genes associated with autoimmunity and progression to disease in type 1 diabetes. *Tissue Antigens* 61:146–153, 2003
13. Kimura A, Sasazuki T: *Eleventh International Histocompatibility Workshop Reference Protocol for the HLA DNA-Typing Technique*. Oxford, Oxford University Press, 1992
14. Haynes A, Bower C, Bulsara MK, Jones TW, Davis EA: Continued increase in the incidence of childhood type 1 diabetes in a population-based Australian sample (1985–2002). *Diabetologia* 47:866–870, 2004
15. Tuomi T, Carlsson A, Li H, Isomaa B, Miettinen A, Nilsson A, Nissen M, Ehrnstrom BO, Forsen B, Snickars B, Lahti K, Forsblom C, Saloranta C, Taskinen MR, Groop LC: Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. *Diabetes* 48:150–157, 1999
16. Fourlanos S, Dotta F, Greenbaum CJ, Palmer JP, Rolandsson O, Colman PG, Harrison LC: Latent autoimmune diabetes in adults (LADA) should be less latent. *Diabetologia* 48:2206–2212, 2005
17. Bishop RF, Cameron DJ, Veenstra AA, Barnes GL: Diarrhoea and rotavirus infection associated with differing regimens for postnatal care of newborn infants. *J Clin Microbiol* 9:525–529, 1979
18. Harrison LC, Honeyman MC: Mucosa, not moocosa! In *Commentaries on Perspectives in Diabetes: 1988–1992*. Vol. 3. Robertson RP, Ed. American Diabetes Association, Alexandria, VA, 2006, p. 158
19. Honeyman MC Coulson BS, Stone NL, Gellert SA, Goldwater PN, Steele CE, Couper JJ, Tait BD, Colman PG, Harrison LC: Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. *Diabetes* 49:1319–1324, 2000
20. Norton K, Dollman J, Martin M, Harten N: Descriptive epidemiology of childhood overweight and obesity in Australia: 1901–2003. *Int J Pediatr* 1:232–238, 2006
21. Fourlanos S, Narendran P, Byrnes GB, Colman PG, Harrison LC: Insulin resistance is a risk factor for progression to type 1 diabetes. *Diabetologia* 47:1661–1667, 2004
22. Pickup JC: Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care* 27:813–823, 2004