Kathleen M. Gillespie, Rachel J. Aitken, Isabel Wilson, Alistair J.K. Williams, and Polly J. Bingley

Early Onset of Diabetes in the Proband Is the Major Determinant of Risk in HLA DR3-DQ2/DR4-DQ8 Siblings

Islet autoimmunity is initiated in infancy, and primary prevention trials require children at high genetic risk to be identified before autoantibodies appear. To inform screening strategies, we evaluated risks of autoimmunity and diabetes associated with HLA DR3-DQ2/DR4-DQ8 in U.K. families. Extended HLA haplotypes were determined in 2,134 siblings from the Bart's-Oxford Study followed to a median age of 22 years. Risks of diabetes and islet autoimmunity (more than two antibodies) were estimated by survival analysis. Of 138 informative DR3-DQ2/DR4-DQ8 siblings, 63% shared both haplotypes with their diabetic proband, 29% shared one, and 8% shared neither. In HLA-identical DR3-DQ2/DR4-DQ8 siblings, the cumulative risk of diabetes by age 15 was 17% (vs. 6% in those sharing one haplotype or none; P = 0.095). Risk varied, however, with the age at the onset of diabetes in the proband; the cumulative risk of autoimmunity and/or diabetes by age 15 was 61% in siblings of probands diagnosed when younger than 10 years old compared with only 4.7% in those diagnosed after age 10 years ($P < 0.001$). The age of the proband at diagnosis, but not HLA haplotype sharing, was an independent determinant of sibling risk. This suggests that non-HLA genes or epigenetic/environmental factors that accelerate the progression of type 1 diabetes in the proband strongly affect risk in siblings.

Diabetes 2014;63:1041–1047 | DOI: 10.2337/db13-0994

Type 1 diabetes results from the autoimmune destruction of insulin-producing β-cells, a process that begins early in life. Islet autoantibodies are detectable by the age of 5 years—often by the age of 2 years—in children who go on to develop diabetes, and autoantibodies to insulin (generally the first to appear) have been detected as early as 6–12 months (1,2). Established islet autoimmunity is difficult to modulate, and therefore strategies aimed at primary prevention before the initiation of autoimmunity are needed. Trials of potential interventions at this stage will have to recruit children on the basis of genetic risk alone.

Recent whole-genome studies have resulted in an explosion of information regarding genetic susceptibility to type 1 diabetes, but the HLA region remains the most important genetic determinant. More than 90% of children with type 1 diabetes carry the HLA class II haplotypes *DRB1*03-DQB1*02:01* (DR3-DQ2) and/or *DRB1*04-DQB1*03:02* (DR4-DQ8), and the highest risk DR3-DQ2/DR4-DQ8 diplotype is present in 50% of cases of very early–onset diabetes (3). We and others have previously shown that the age of diagnosis of children with type 1 diabetes and the number of HLA haplotypes they share are determinants of risk of diabetes in their siblings (3,4). HLA class II determined risk can also be modulated by other factors close to or within the HLA region as well as by non-HLA genes (5).

Current trials to prevent the initiation of islet autoimmunity use HLA-based risk assessment to identify individuals eligible for inclusion, but they use different

Diabetes and Metabolism, School of Clinical Sciences, University of Bristol, Bristol, U.K.

Corresponding author: Kathleen M. Gillespie, k.m.gillespie@bristol.ac.uk.

Received 25 June 2013 and accepted 3 November 2013.

strategies to assign risk that depend on the potential toxicity of the planned intervention (6,7). The international Trial to Reduce IDDM in the Genetically at Risk (TRIGR) combined HLA-determined genetic susceptibility with a first-degree family history of type 1 diabetes to identify children with an estimated 15.4% risk of type 1 diabetes by age 10 who were suitable to include in a dietary modification trial (8). In the Finnish Diabetes Prediction and Prevention (DIPP study), high-risk HLA combinations identified children with no family history of type 1 diabetes who had a 36.4% (95% CI 20.0–52.8) risk of developing two or more antibodies and a 2.4% risk of diabetes within 5 years (9).

Other primary prevention trials are seeking to identify infants at much higher genetic risk. A study of 48 families with siblings matched for the high-risk *DR3-DQ2/DR4-DQ8* genotype participating from birth in the prospective Diabetes Autoimmunity Study in the Young (DAISY) reported a dramatically increased risk of islet autoimmunity and diabetes in 29 siblings who shared both extended high-risk haplotypes identical by descent (IBD) with the diabetic proband. In this group, the estimated risk of islet autoimmunity was 63% (95% CI 44–85) by age 7, and risk of diabetes was 55% (95% CI 30–80) by age 12 (10). This compares with a 7% risk of diabetes by age 12 in HLA DR/DQ identical siblings who were not IBD. This strategy for identifying a subgroup of DR3-DQ2/DR4-DQ8 siblings at "extreme risk" based on IBD haplotypes has been used to recruit children for the Pre-POINT study, a clinical trial assessing the efficacy of oral insulin given very early in life (7).

However, a smaller, more recent study of 14 HLA DR3-DQ2/DR4-DQ8 IBD siblings in the Dutch Kolibrie cohort failed to replicate this extreme risk: only 2 of the 14 developed type 1 diabetes by the age of 15 (11). The aims of our study were therefore to evaluate the risks of autoimmunity and diabetes associated with the extended DR3-DQ2/DR4-DQ8 diplotype in a larger group of U.K. families with more than 25 years follow-up in the population-based Bart's-Oxford (BOX) study of childhood diabetes and to identify other factors that might contribute to the discrepant results of previous studies.

RESEARCH DESIGN AND METHODS

Subjects

The participants in this study were siblings of probands who had been notified to the BOX study, which, since 1985, has recruited more than 95% of the families of children who have developed type 1 diabetes before the age of 21 years in the former Oxford Health Authority Region of the U.K. (12). The background population is 95% Caucasian and the remainder originates mainly from the Indian subcontinent (data from the Office of Population Censuses and Surveys for 1991). All cases of type 1 diabetes were referred by diabetes specialists based on diagnosis according to World Health Organization criteria (13) and a clinical requirement for insulin treatment from diagnosis. Patients with secondary diabetes, known genetic subtypes including maturity onset diabetes of the young, or clinical type 2 diabetes were not included in the study.

Between 1985 and 2002 we recruited to the study 2,134 siblings from 1,745 families. HLA genotype data were available for 1,440 siblings (67%). Of these, 191 had the high-risk HLA *DR3/DR4* genotype. Haplotype sharing was uncertain in 53 proband/sibling pairs. Therefore, 138 siblings were included in the analysis (Fig. 1).

Clinical Data and Follow-up

Clinical data and family history of diabetes, including type of diabetes and age at diagnosis, were collected by interview upon entry to the study. The median time between diagnosis of the proband and the enrollment of siblings was 0.2 years (range 0.1–2.5 years). Of 138 siblings, 7 had developed diabetes before entry to the study. Families were followed by annual visit, telephone call, or postal questionnaire, and the diabetes status of every family member was ascertained on each occasion. A further 12 siblings developed diabetes during follow-up. Serum samples used to determine islet autoantibodies were collected routinely from consenting unaffected siblings at entry to the study, with repeat sampling if any family member was positive for one or more autoantibody. At least one sample was tested for autoantibodies in 94 of 131 siblings (72%) who were nondiabetic at entry to the study. The first available sample was measured in each sibling, and all those in this study who were found to be positive for islet autoantibodies were identified using the first sample. The median age at collection

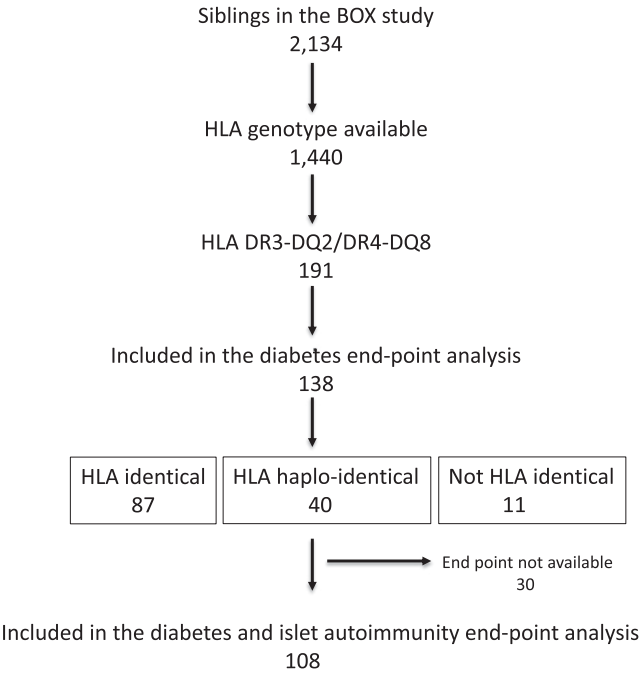


Figure 1—Characteristics of participants included in the study.

of the first sample was 12.9 years (interquartile range [IQR] 8.1–16.4), 13.2 years at collection of the last sample (IQR 8.4–16.8), and 25.4 years (IQR 20.6–29.9) at last contact.

HLA Class I and II Genotyping

HLA class II *DRB1* and *DQB1* and HLA class I *A* and *B* genotyping was carried out by PCR using a DYNAL Reli SSO system (Invitrogen, Paisley, U.K.). The type 1 diabetes–associated haplotype *HLA-DRB1*04-DQB1*03:02* was abbreviated to DR4-DQ8 and HLA-*DRB1*03-DQB1*02:01* was abbreviated to DR3-DQ2. IBD siblings were recognized by analyzing the transmission of extended haplotypes from HLA class II to HLA class IA from both parents to affected and unaffected children (10). This will be described as extended HLA identity.

Islet Autoantibodies

Samples from all participants were tested for islet cell antibodies and autoantibodies to glutamate decarboxylase, islet antigen-2, and insulin, as previously described (14). Data on antibodies to zinc transporter 8 were not available from this cohort. Samples were judged to be antibody positive if levels were at or above the 97.5th percentile of levels in a control population of 2,860 schoolchildren from the same region (15). The islet cell antibody assay was shown to have 81% sensitivity and 86% specificity in the First Immunology of Diabetes Society Combined Antibody Workshop (16). Radiobinding assays for autoantibodies to glutamate decarboxylase, islet antigen-2, and insulin had sensitivities of 91, 74, and 58%, respectively, with 99% sensitivity in the first Diabetes Antibody Standardization Proficiency Evaluation (17).

Data Analysis

Risks of diabetes and autoimmunity in siblings were estimated by survival analysis using the log-rank test to compare groups based on HLA sharing and age at diagnosis of diabetes in the proband. Islet autoimmunity was defined as more than two autoantibodies above the 97.5th percentile. Cumulative risks were calculated for clinical diagnosis of diabetes and a composite end point of diabetes and/or islet autoimmunity. For the analyses of diabetes risk, time to event was defined from birth to the development of diabetes or date of last contact; for risk of diabetes and/or autoimmunity time to event was defined as birth to the development of diabetes or first detection of two or more antibodies or date of last sample. Time-to-event outcomes for combined predictive markers (HLA haplotype sharing and age at diagnosis of the diabetic proband) were analyzed using Cox proportional hazards models. As samples for autoantibody testing were not available from birth in this cohort, the cumulative risks of positivity for two or more islet autoantibodies were calculated using the following assumptions: *1*) all siblings who were antibody negative had not previously been positive for two or more antibodies, *2*) all siblings with at least two autoantibodies remained in this category until diagnosis of diabetes or the date of last contact (end of follow-up), and *3*) the date of collection of the first antibody-positive sample was used as the initiation of islet autoimmunity. The risk of the composite end point of diabetes and autoimmunity could not be calculated in 30 individuals for whom no serum sample was available and who remained nondiabetic at last contact. A total of 108 individuals were therefore included in that analysis.

RESULTS

Of 138 informative HLA DR3-DQ2/DR4-DQ8 siblings, 87 (63%) shared both haplotypes with their diabetic proband, 40 (29%) shared one haplotype, and 11 (8%) shared neither. Of the siblings analyzed, 69 (50%) were from families in which the diabetic proband was younger than age 10 years at the time of diagnosis (median 5.4 years [IQR 2.5–7.7 years]), and the remaining 69 were from families in which the proband was diagnosed after this age (median 13.4 years [IQR 11.4–15.2 years]). The analysis included 34 siblings from multiplex families in which more than one first-degree relative had type 1 diabetes. The characteristics of the cohort are shown in Table 1.

Table 1—Characteristics of the cohort

	HLA-identical siblings (*n* = 87)	Haplo-identical siblings (*n* = 40)	Nonidentical siblings (*n* = 11)
Sex (*n*)			
Male	37	26	2
Female	50	14	9
Age at last contact, years	22.3 (15.6–28.2)	23.6 (19.5–30.5)	26 (21.0–28.4)
Age of diagnosis of the proband, years	9.2 (3.9–13.3)	11 (6.1–13.8)	13.3 (10.5–13.5)
Sibling with diabetes (*n*)	15	3	1
Age at diagnosis of sibling, years	5.7 (3.3–10.9)	6.6 (4.7–7.5)	1.8
Multiplex family (two or more affected first-degree relatives) (*n*)	15	7	3

Data are median (IQR) unless otherwise indicated.

Cumulative Risk of Diabetes by Extended HLA Identity and Age at Diagnosis of the Proband

By the time of analysis, 19 of the 138 DR3-DQ2/DR4-DQ8 siblings had developed type 1 diabetes. Of these, 15 (11%) were HLA identical, 3 (2%) were haplo-identical, and 1 (1%) did not share either high-risk haplotype with the proband. For 16 diabetic siblings, diabetes had been diagnosed in the proband before age 10 years, and for 3 siblings the proband had been diagnosed after age 10.

The cumulative risk of diabetes by age 15 was 16.6% (95% CI 8.6–24.6) in the HLA-identical siblings, compared with 6% (95% CI 0–12.4) in those sharing one or no haplotype (P = 0.095); cumulative risk was 22.5% (95% CI 12.5–32.5) in the siblings of probands diagnosed when younger than age 10, compared with 3% (95% CI 0–6.8) in those whose proband was diagnosed after age 10 (P = 0.001). Among the 25 siblings from multiplex families, the risk of diabetes was 13.4% (95% CI 6.3–18.9).

As shown in Fig. 2*A*, the effect of extended HLA haplotypes on cumulative risk of diabetes varied with the age of the proband at onset of type 1 diabetes. Among the 50 HLA-identical DR3-DQ2/DR4-DQ8 siblings from families in which the proband was diagnosed before age 10, the cumulative risk of diabetes by age 15 was 25% (95% CI 13–38), compared with 5.4% (95% CI 0–13) in the 37 siblings from families in which the proband was diagnosed after age 10 (P = 0.009).

Cumulative Risk of Diabetes and/or Islet Autoimmunity

Of the 108 siblings included in this analysis, 8 had progressed to the combined end point of islet autoimmunity (more than two antibodies) and/or diabetes by age 5, 17 progressed by age 10, and 23 progressed by age 15. The overall cumulative risk of islet autoimmunity and/or diabetes by age 15 was 26% (95% CI 16–35%); it was 31% (18–44) in HLA-identical siblings, compared with 18% (4.5–31) in those sharing one or no haplotype (P = 0.113). In 53 siblings from families in which the proband was diagnosed before age 10, the risk of islet autoimmunity and/or diabetes was 52% (33–70%) by age 15, compared with 8.6% (0–17) risk by age 15 in 55 siblings from families in which the proband was diagnosed after age 10 ($P < 0.001$).

As shown in Fig. 2*B*, the age of the proband at the onset of type 1 diabetes modulated the combined risk of autoimmunity and/or diabetes. Among the 36 HLA-identical DR3-DQ2/DR4-DQ8 siblings from families in which the proband was diagnosed before age 10, the cumulative risk of autoimmunity and/or diabetes by age 15 was 61% (95% CI 13–38) compared with 4.7% (95% CI 0–16) in the 29 siblings from families in which the proband was diagnosed after age 10 ($P < 0.0001$).

Cox regression analysis showed that the age of the proband when diabetes was diagnosed was an independent determinant of risk of diabetes and of the combined end point of autoimmunity and/or diabetes, but HLA haplotype sharing was not. Coming from a family in which the proband was diagnosed before age 10 was associated with 5.9-fold increased risk of diabetes (95% CI 1.7–20.5; P = 0.006) and 7.4-fold increased risk of the combined end point (95% CI 2.7–20.3; $P < 0.001$), whereas being HLA identical with the proband was associated with a hazard ratio of 1.9 (95% CI 0.6–5.7; P = 0.265) for the development of diabetes and a hazard ratio of 1.6 (95% CI 0.6–3.8; P = 0.322) for the combined end point.

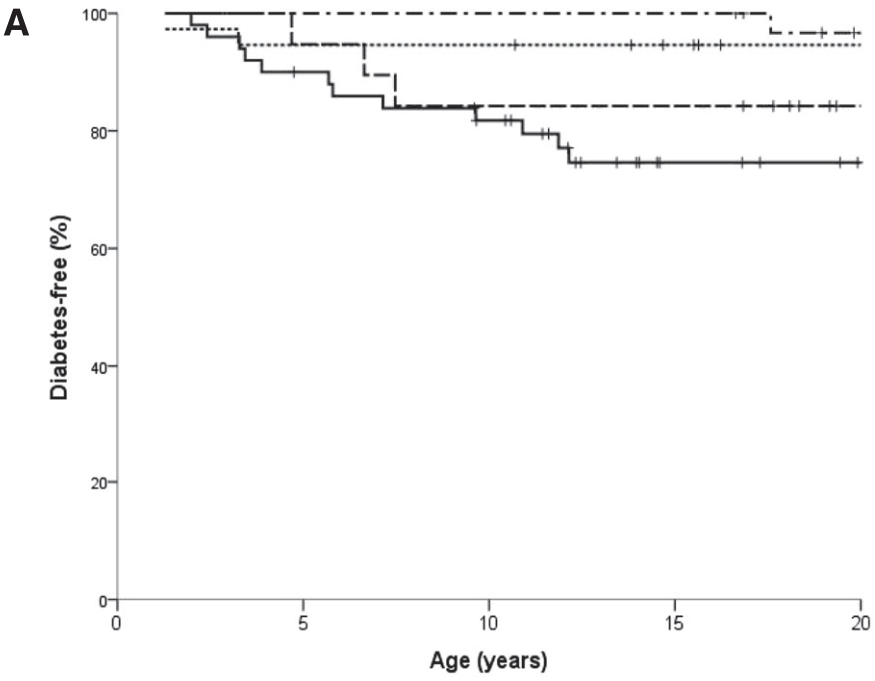
DISCUSSION

In this study we examined the risk of diabetes and islet autoimmunity in HLA DR3-DQ2/DR4-DQ8 siblings of children with type 1 diabetes in relation to the number of extended HLA haplotypes shared with the proband and the age at which the proband's diabetes was diagnosed. We observed that increased risk was concentrated in the subgroup of siblings who were HLA identical to the proband (sharing both haplotypes) compared with siblings who shared one or no haplotype. We have shown for the first time that risk is higher in the DR3-DQ2/DR4-DQ8 siblings of children whose diabetes was diagnosed at a young age and that this factor is the dominant determinant.

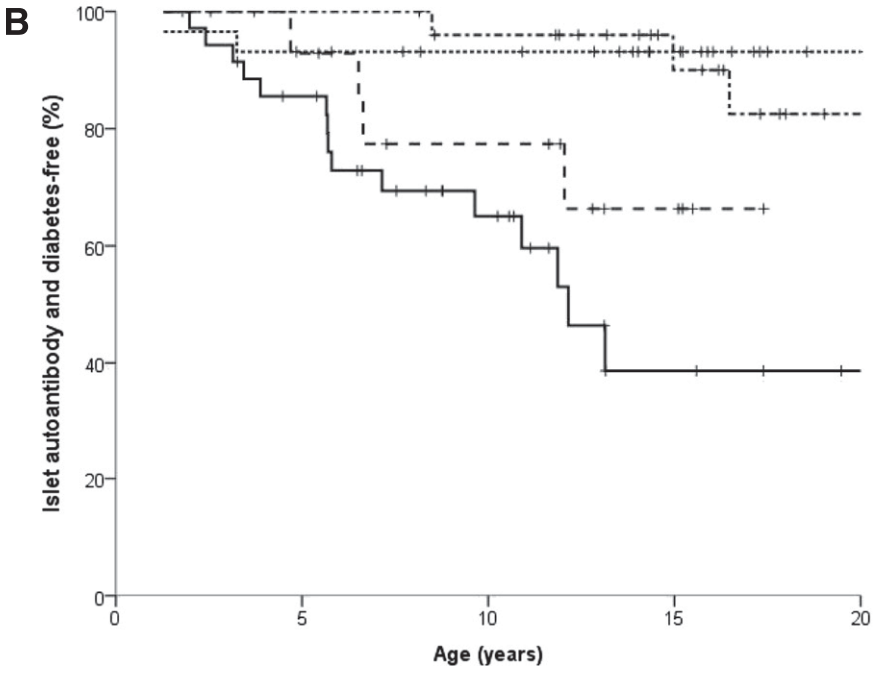
The existing literature in this area has yielded conflicting results. Using an approach similar to that adopted by us, a study of 48 families from the DAISY cohort reported that HLA DR3-DQ2/DR4-DQ8 siblings IBD with the proband for both extended HLA haplotypes had a 55% risk (95% CI 30–80) of developing diabetes by age 12 (10). The same study also found a 63% risk (95% CI 41–84) of autoimmunity by age 7 and an 85% risk (95% CI 61–100) by age 15 in this group, compared with 20% risk (95% CI 0–42) by age 15 for those who shared only one or no haplotype. However, a smaller, more recent study of 14 DR3-DQ2/DR4-DQ8 siblings sharing the IBD haplotypes failed to replicate these findings after up to 12 years' follow-up (11).

Our study of 138 informative families undergoing regular follow-up represents the largest study of IBD extended HLA haplotypes and sibling risk to date. As reported in the DAISY cohort, we found that HLA-identical DR3-DQ2/DR4-DQ8 siblings were at increased risk but the magnitude of risk was less. The estimates of diabetes/autoimmunity by age 15 were similarly high in both studies despite different definitions of islet autoimmunity: the DAISY study used single islet autoantibody positivity while the BOX study used more than two autoantibodies, a more accurate predictor of diabetes.

We have shown that this risk was concentrated in siblings of probands diagnosed when younger than 10 years old, while there was little association with the degree of haplotype sharing in siblings of children diagnosed after age 10. The influence of the age of the proband at the onset of diabetes on the risk among first-degree relatives has been described previously; the risk of progression to diabetes in monozygotic twins of



50	45	38	23	HLA-identical & proband diagnosis <age 10
37	35	35	32	HLA-identical & proband diagnosis ≥ age 10
19	18	16	16	Haplo- /non -identical & proband diagnosis < age 10
32	29	29	29	Haplo- /non -identical & proband diagnosis ≥ age 10



36	28	15	4	HLA-identical & proband diagnosis <age 10
29	26	23	16	HLA-identical & proband diagnosis ≥ age 10
17	13	9	4	Haplo- /non -identical & proband diagnosis < age 10
26	24	24	15	Haplo- /non -identical & proband diagnosis ≥ age 10

Figure 2—Cumulative risks of diabetes (*A*) and diabetes and/or islet autoimmunity (*B*) in DR4-DQ8/DR3 siblings subdivided by HLA sharing with the proband and age at diagnosis of the proband. Survival curves are presented for HLA-identical siblings of probands diagnosed before (solid line) and after (dotted line) age 10 years and haplo- or nonidentical siblings of probands diagnosed before (dashed line) or after (dashed-dotted line) age 10 years.

probands diagnosed before 15 years of age was almost twice that of twins of probands diagnosed after age 25, and twins of patients diagnosed when younger than 10 years old were estimated to have a 50% risk of developing diabetes within 6 years (18). Although the investigators noted that this increased risk did not seem to be related to HLA, the frequency of the high-risk HLA DR2-DQ2/DR4-DQ8 diplotype was relatively low: only 15 of 79 twin pairs (19%) diagnosed before the age of 25 years were positive for HLA DR2-DQ2/DR4-DQ8, compared with 5 of 21 twin pairs (24%) diagnosed later. We also previously showed that the overall risk of diabetes was higher in siblings of probands diagnosed with type 1 diabetes very early in life (3). Given the high frequency of the HLA genotype DR3-DQ2/DR4-DQ8 we observed in early-onset type 1 diabetes, as have others (19), our interpretation was that increased HLA susceptibility in siblings of probands with early-onset diabetes was the dominant mediator of increased risk of diabetes. Our current study disproves this.

Rapid progression resulting in early onset of type 1 diabetes is associated with less preserved β-cell function (20), suggesting a more aggressive autoimmune response. To date, most genetic studies of early-onset diabetes have focused on HLA class I and II, particularly DR3/4-DQ8, *A*24:02, B*18:01*, and *B*39:06* (19,21,22), as well as class I C/natural killer cell receptor interactions (23), yet our study strongly indicates that important factors outside the HLA modulate risk. These are likely to include other genes and potentially other epigenetic and environmental modulators. Our data support a recent report from the Nationwide Italian Twin Study highlighting the importance of exposure during fetal or neonatal life over genetics in their effect on concordance rates (24). There is increasing focus on maternal and intrauterine conditions that modulate genetic risk of type 1 diabetes (25). Potential factors include viral infections (26), maternally derived microbiome (27), weight gain in early life (28), and maternal/fetal cell transfer in pregnancy (29). Further studies focused on the determinants of early-onset autoimmune diabetes are required.

A limitation of our study is that islet autoantibody testing did not start in infancy and was not carried out at defined intervals in all families. We therefore had to make a number of assumptions, including use of the date of the first islet autoantibody–positive sample as the date of antibody appearance, although antibodies may have been present for many years. This assumption is likely to have resulted in an underestimation of the risk of islet autoantibody positivity in younger siblings. In addition, we may have missed transient antibody positivity that would have been detected in a prospective study from birth. A further consideration is that data on antibodies to zinc transporter 8 were not available in this cohort. Therefore, our estimates probably represent the lower limits of the true risk of islet autoimmunity.

This study represents an important clarification of increased risk of diabetes in IBD siblings of children with type 1 diabetes with long-term follow-up. It highlights the importance of integrating clinical data such as age of the proband at onset of diabetes and family history in estimating individual risk. It also offers a simple way of stratifying risk in *DR3-DQ2/DR4-DQ2* siblings to identify those at extremely high genetic risk for future therapeutic trials.

Acknowledgments. The authors are grateful to the Bart's-Oxford study families for participating in this research project and to Kyla Chandler and Saba Rokni, University of Bristol, for excellent technical assistance.

Funding. This study was funded by grants from Diabetes UK, the Wellcome Trust, and the North Bristol NHS Trust.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. K.M.G. wrote the manuscript; researched data; coordinated sample collection and analysis; reviewed, edited, and discussed the manuscript; and contributed to discussion. R.J.A., I.W., and A.J.K.W. researched data; coordinated sample collection and analysis; reviewed, edited, and discussed the manuscript; and contributed to discussion. P.J.B. wrote the manuscript; carried out the statistical analysis; reviewed, edited, and discussed the manuscript; and contributed to discussion. P.J.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Ziegler AG, Bonifacio E; BABYDIAB-BABYDIET Study Group. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. Diabetologia 2012;55:1937–1943
2. Parikka V, Näntö-Salonen K, Saarinen M, et al. Early seroconversion and rapidly increasing autoantibody concentrations predict prepubertal manifestation of type 1 diabetes in children at genetic risk. Diabetologia 2012; 55:1926–1936
3. Gillespie KM, Gale EA, Bingley PJ. High familial risk and genetic susceptibility in early onset childhood diabetes. Diabetes 2002;51:210–214
4. Tarn AC, Thomas JM, Dean BM, et al. Predicting insulin-dependent diabetes. Lancet 1988;1:845–850
5. Nejentsev S, Howson JM, Walker NM, et al.; Wellcome Trust Case Control Consortium. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. Nature 2007;450:887–892
6. Knip M, Virtanen SM, Akerblom HK. Infant feeding and the risk of type 1 diabetes. Am J Clin Nutr 2010;91:1506S–1513S
7. Achenbach P, Barker J, Bonifacio E; Pre-POINT Study Group. Modulating the natural history of type 1 diabetes in children at high genetic risk by mucosal insulin immunization. Curr Diab Rep 2008;8:87–93
8. Akerblom HK, Krischer J, Virtanen SM, et al.; TRIGR Study Group. The Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study: recruitment, intervention and follow-up. Diabetologia 2011;54:627–633
9. Siljander HT, Simell S, Hekkala A, et al. Predictive characteristics of diabetes-associated autoantibodies among children with HLA-conferred disease susceptibility in the general population. Diabetes 2009;58:2835–2842
10. Aly TA, Ide A, Jahromi MM, et al. Extreme genetic risk for type 1A diabetes. Proc Natl Acad Sci U S A 2006;103:14074–14079
11. Eerligh P, Koeleman BP, Lie BA, Roep BO, Thorsby E. No extreme genetic risk for type 1 diabetes among DR3/4-DQ8 siblings sharing both extended HLA haplotypes with their diabetic proband. Tissue Antigens 2011;77:338–340

12. Lambert AP, Gillespie KM, Thomson G, et al. Absolute risk of childhood-onset type 1 diabetes defined by human leukocyte antigen class II genotype: a population-based study in the United Kingdom. J Clin Endocrinol Metab 2004;89:4037–4043
13. World Health O. *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Organization, 1985
14. Williams AJ, Bingley PJ, Moore WP, Gale EA; ENDIT Screening Group. European Nicotinamide Diabetes Intervention Trial. Islet autoantibodies, nationality and gender: a multinational screening study in first-degree relatives of patients with Type I diabetes. Diabetologia 2002;45:217–223
15. Bingley PJ, Bonifacio E, Shattock M, et al. Can islet cell antibodies predict IDDM in the general population? Diabetes Care 1993;16:45–50
16. Verge CF, Stenger D, Bonifacio E, et al. Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. Diabetes 1998;47:1857–1866
17. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: first assay proficiency evaluation. Diabetes 2003;52:1128–1136
18. Redondo MJ, Yu L, Hawa M, et al. Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. Diabetologia 2001;44:354–362
19. Caillat-Zucman S, Garchon HJ, Timsit J, et al. Age-dependent HLA genetic heterogeneity of type 1 insulin-dependent diabetes mellitus. J Clin Invest 1992;90:2242–2250
20. Mortensen HB, Swift PG, Holl RW, et al.; Hvidoere Study Group on Childhood Diabetes. Multinational study in children and adolescents with newly diagnosed type 1 diabetes: association of age, ketoacidosis, HLA status, and autoantibodies on residual beta-cell function and glycemic control 12 months after diagnosis. Pediatr Diabetes 2010;11:218–226
21. Valdes AM, Erlich HA, Carlson J, Varney M, Moonsamy PV, Noble JA. Use of class I and class II HLA loci for predicting age at onset of type 1 diabetes in multiple populations. Diabetologia 2012;55:2394–2401
22. Nakanishi K, Inoko H. Combination of HLA-A24, -DQA1*03, and -DR9 contributes to acute-onset and early complete beta-cell destruction in type 1 diabetes: longitudinal study of residual beta-cell function. Diabetes 2006; 55:1862–1868
23. Mehers KL, Long AE, van der Slik AR, et al. An increased frequency of NK cell receptor and HLA-C group 1 combinations in early-onset type 1 diabetes. Diabetologia 2011;54:3062–3070
24. Nisticò L, Iafusco D, Galderisi A, et al.; Study Group on Diabetes of the Italian Society of Pediatric Endocrinology and Diabetology. Emerging effects of early environmental factors over genetic background for type 1 diabetes susceptibility: evidence from a Nationwide Italian Twin Study. J Clin Endocrinol Metab 2012;97:E1483–E1491
25. Stene LC, Gale EA. The prenatal environment and type 1 diabetes. Diabetologia 2013;56:1888–1897
26. Laitinen OH, Honkanen H, Pakkanen O, et al. Coxsackievirus B1 Is Associated With Induction of β-cell autoimmunity that portends type 1 diabetes. Diabetes 2013 Aug 23 [Epub ahead of print]
27. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107: 11971–11975
28. Couper JJ, Beresford S, Hirte C, et al. Weight gain in early life predicts risk of islet autoimmunity in children with a first-degree relative with type 1 diabetes. Diabetes Care 2009;32:94–99
29. Nelson JL, Gillespie KM, Lambert NC, et al. Maternal microchimerism in peripheral blood in type 1 diabetes and pancreatic islet beta cell microchimerism. Proc Natl Acad Sci U S A 2007;104:1637–1642