Brief Genetics Report

HLA Genotyping Supports a Nonautoimmune Etiology in Patients Diagnosed With Diabetes Under the Age of 6 Months

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Children with permanent diabetes are usually assumed to have type 1 diabetes. It has recently been shown that there are genetic subgroups of diabetes that are often diagnosed during the neonatal period but may present later. A recent Italian study proposed that type 1 diabetes is rare before 6 months of age. We aimed to examine genetic susceptibility to type 1 diabetes in patients diagnosed with diabetes before the age of 2 years. We analyzed HLA class II genotypes, markers of autoimmune diabetes, in 187 children with permanent diabetes diagnosed at <2 years of age. Of the 79 subjects diagnosed at <6 months of age, 41% (95% CI 0.30–0.51) had type 1 diabetes–associated high-risk genotypes, a proportion similar to that in healthy population control subjects (44%, $P = 0.56$). This group included 32 patients with mutations in the *KCNJ11* gene, which encodes Kir6.2 (44% high-risk HLA class II genotypes), and 47 in whom the etiology of diabetes was unknown (38% high-risk HLA class II genotypes). Of 108 patients diagnosed between 6 and 24 months of age, 93% (0.86–0.99) had high-risk HLA class II genotypes compared with 44% of the population control subjects ($P < 0.0001$). We conclude that infants diagnosed with diabetes before 6 months of age are unlikely to have autoimmune type 1 diabetes and are most likely to have a monogenic etiology.
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Appropriate treatment regimens for diabetes require accurate characterization of disease (1). For patients diagnosed in infancy, this may be complicated; several different etiologies may cause diabetes, including type 1 diabetes and permanent neonatal diabetes (PNDM). Type 1 diabetes results from autoimmune destruction of insulin-producing β-cells and is characterized by the presence of multiple islet autoantibodies and high-risk HLA haplotypes for type 1 diabetes. HLA DRB1*04-DQB1*0302 and/or HLA DRB1*03-DQB1*0201 are observed in >90% of affected children and in only 40% of the general population (2). The study of HLA genotypes may therefore help define which patients diagnosed in infancy are likely to have type 1 diabetes.

PNDM is typically defined as diabetes requiring permanent insulin treatment that is diagnosed in the first 3 months of life (3). Recent advances in determining the etiology of PNDM have demonstrated that heterozygous-activating mutations in the *KCNJ11* gene encoding the ATP-sensitive K^+ channel subunit Kir6.2 account for ~40–64% of PNDM cases (4). To date, all activating *KCNJ11* mutations have been identified in subjects diagnosed under the age of 6 months (5–11). Some of these patients are diagnosed outside the defined neonatal period of 3 months and therefore highlight a need to investigate the etiologies of diabetes in infancy. The identification of patients with *KCNJ11* mutations is important, as most respond well to sulfonylureas and achieve better glycemic control following transfer from insulin to sulfonylurea tablets (6,8,12–14).

A recent Italian study suggested that children diagnosed with diabetes before 6 months of age do not have the genetic characteristics of autoimmune diabetes (15). Many patients from this cohort have since been diagnosed with *KCNJ11* mutations (10), but the etiology of diabetes in the remainder is uncertain. To determine the likelihood of type 1 diabetes in children diagnosed in infancy, we investigated the frequency of high-risk HLA class II genotypes in a cohort of 187 subjects diagnosed with permanent diabetes under the age of 2 years, 32 with *KCNJ11* mutations and 155 in whom mutations have been excluded.

RESEARCH DESIGN AND METHODS

A total of 187 subjects were recruited worldwide. The inclusion criteria for this study were a diagnosis of diabetes before 24 months of age requiring continual insulin treatment from diagnosis. The physician's classification was type 1 diabetes in the majority of cases. This cohort included 104 U.K. samples, of whom 58 subjects were recruited from the 1972–1981 British Diabetic Association Under 2s cohort (16) and 19 from the Bart's Oxford study of childhood diabetes (17). Of the 187 samples, 79 were diagnosed at 0–6 months of age (22 of U.K. origin), 45 were diagnosed at 7–12 months (21 of U.K. origin), 32 were diagnosed at 13–18 months (30 of U.K. origin), and 31 were diagnosed at 19–24 months (31 of U.K. origin). Consent was obtained from all patients or their parents.

HLA genotypes on 621 adult U.K. Caucasian control subjects from the general population with no history of autoimmune disease have been described previously (18). All control subjects gave written informed consent.

Sequencing of *KCNJ11*. All subjects were sequenced for *KCNJ11* as previously described (5–7,11 and personal communication with A.L. Gloyn, Diabetes Research Laboratories, University of Oxford, Oxford, U.K.).

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PNDM, permanent neonatal diabetes.

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TABLE 1
The frequency of HLA class II genotypes in 32 individuals with a defined mutation in *KCNJ11* and in 155 negative for mutations in KCNJ11 46 diagnosed before and 109 after the age of 6 months

	KCNJ11 Mut +ve	*KCNJ11* Mut –ve (0–6 months)	Kir6.2 Mut –ve (>6 months)	Healthy control subjects
n	32	47	108	621
Genotype				
DR4-DQ8/DR3-DQ2	1 (3%)	4 (9%)	54 (50%)	17 (3%)
DR4-DQ8/X	6 (19%)	7 (15%)	18 (16%)	85 (14%)
DR3-DQ2/X	7 (22%)	7 (15%)	30 (28%)	167 (26%)
X/X	13 (40%)	21 (44%)	2 (2%)	249 (40%)
DR2-DQ6/ DR2-DQ6 or DR2-DQ6/X	5 (16%)	8 (17%)	4 (4%)	103 (17%)

Control data are provided for 621 healthy Caucasians. The genotypes HLA DRB1*04-DQB1*0302/ HLA DRB1*03-DQB1*0201, HLA DRB1*04-DQB1*0302/X, and HLA DRB1*03-DQB1*0201/X are described as DR4-DQ8/DR3-DQ2, DR4-DQ8/X, and DR3-DQ2/X, respectively, where X is not DRB1*02-DQB1*0602 (DR2-DQ6). +ve, mutation present; −ve, mutation not detected by sequencing.

HLA class II genotyping. HLA-DRB1, -DQA1, and -DQB1 genotyping was performed using a Dynal reverse SSOP method (Dynal Biotech, Wirral, U.K.). Details of HLA class II genotypes in the 77 individuals from the Bart's Oxford study/British Diabetic Association Under 2s study and 621 U.K. control subjects were already available (7,18). In the absence of parental samples, haplotypes were identified on the basis of well-established patterns of linkage disequilibrium between HLA DRB1 and DQB1.

For the purposes of this study, the genotypes HLA DRB1*04-DQB1*0302/X or HLA DRB1*03-DQB1*0201/X (where X was not DRB1*02-DQB1*0602) were defined as high risk. This includes the highest risk genotype HLA DRB1*04-DQB1*0302/HLA DRB1*03-DQB1*0201. All other genotypes including those containing the protective DRB1*02-DQB1*0602 were defined as low risk.

Statistical analysis. To examine differences in the frequencies of HLA genotypes, χ^2 analysis was used. All data were stored according to existing data protection regulations.

RESULTS

As previously reported, 32 of the 187 (17%) patients had heterozygous mutations in the *KCNJ11* gene. All subjects with a *KCNJ11* mutation were diagnosed with diabetes before 6 months of age (median 1.15 months [range 0.23–6]).

The overall results obtained are shown in Table 1. Of 32 individuals with a Kir6.2 mutation, 1 (3%) had the highest-risk HLA DRB1*04-DQB1*0302/HLA DRB1*03-DQB1*0201 genotype, whereas 16% had protective genotypes. These data are remarkably similar to those of the healthy control population, with frequencies of 3 and 17%, respectively. Of 47 children diagnosed under the age of 6 months with no mutation in the *KCNJ11* gene, 4 (9%) carried the highest-risk genotype for type 1 diabetes and 17% were positive for protective genotypes, also very similar to the healthy control population. This is in marked contrast to 108 children diagnosed at >6 months of age, where 50% had the highest-risk HLA genotype and only 4% had protective genotypes. These data, including mutation status, country of origin, HLA data, and age at diagnosis, are available in an online appendix (available at http://diabetes.diabetesjournals.org.

Overall, individuals diagnosed under 6 months of age, irrespective of *KCNJ11* mutation status, had very similar frequencies of type 1 diabetes–associated genotypes (41% [95% CI 0.3–0.51]) compared with the healthy control population (44%, $P = 0.56$). In contrast, 93% (101 of 108) (95% CI 0.86–0.96) of children diagnosed with diabetes over the age of 6 months, who screened negative for a *KCNJ11* mutation, had at least one high-risk haplotype for type 1 diabetes with a similar distribution in the three 6-month intervals: 6–12 months (89%), 12–18 months (94%), and 18–24 months (97%), all different from those <6 months ($P < 0.00001$ for all) (Fig. 1).

Since the role of HLA class II genotypes in susceptibility to type 1 diabetes is less well characterized in non-European populations, we analyzed European Caucasians with permanent diabetes separately and showed that the results were very similar to those observed in the international cohort. The European Caucasian subjects diagnosed with diabetes at <6 months of age ($n = 50$) had the same frequency of high-risk HLA class II genotypes as normal population (44%, $P = 0.99$), whereas those diagnosed at >6 months ($n = 92$) had a higher prevalence (93%) of high susceptibility HLA compared with those diagnosed at <6 months ($P < 0.00001$).

DISCUSSION

We have shown that HLA class II high-risk genotypes for susceptibility to type 1 diabetes are much more common in subjects diagnosed with diabetes over the age of 6 months than in those diagnosed before 6 months. The frequency of high-risk HLA class II genotypes in patients diagnosed before 6 months, regardless of *KCNJ11* mutation status, was similar to that found in control subjects. Individuals diagnosed before 6 months of age are therefore unlikely to have type 1 diabetes, and even those who do not have mutations in the *KCNJ11* gene are likely to have other forms of monogenic diabetes.

Our data support a previous elegant study (15) of HLA class II genotypes in 111 Italian subjects diagnosed under 12 months of age, which suggested that individuals diagnosed with diabetes under the age of 6 months frequently have protective HLA genotypes for type 1 diabetes. They also showed that patients diagnosed before 6 months of age were less likely to have β-cell autoantibodies ($n = 46$) and more likely to have low birth weight (small for gestational age) than patients diagnosed after 6 months. Low birth weight is an indicator of reduced insulin secretion in utero and is consistent with a genetic cause of reduced insulin secretion in utero. This has been described with known monogenic causes of neonatal diabetes (19) such as heterozygous-activating *KCNJ11* mutations (6) and homozygous glucokinase mutations (20). In contrast, there is evidence that HLA genotypes associated with type 1 diabetes are associated with high birth weight (21).

Although this is the largest study of HLA genotypes in patients aged between 0 and 2 years, it is difficult to be certain of an absolute cutoff at 6 months. The fact that the prevalence of high-risk HLA is slightly lower in those

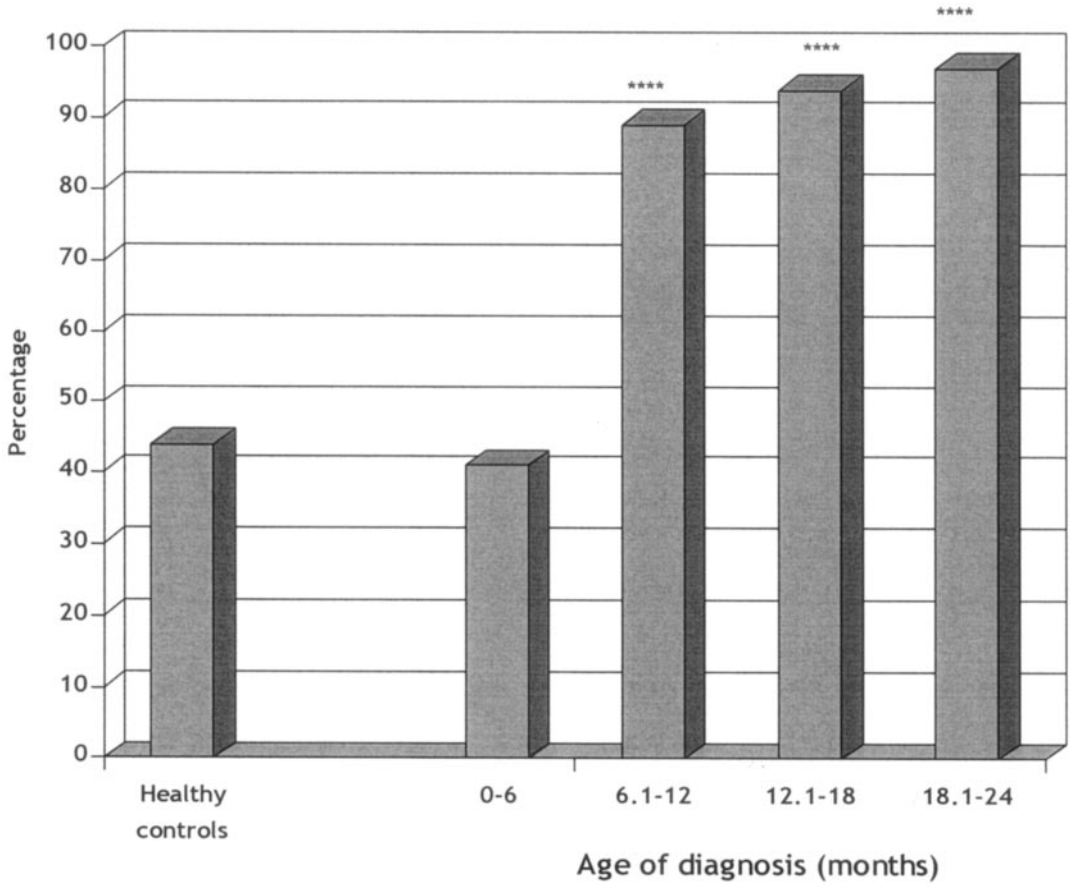

FIG. 1. The prevalence of high-risk HLA class II genotypes (DR4-DQ8/DR3-DQ2, DR4-DQ8/X, and DR3-DQ2/X, where X is not DR2-DQ6) in all subjects from the international cohort (n = 187) diagnosed with diabetes under 24 months of age and in the normal control population. The group diagnosed under 6 months was compared with the three age-groups over 6 months. ****P < 0.00001.

diagnosed between 6 and 12 months of age compared with those between 12 and 24 months of age indicates that there may be a few patients with nonautoimmune diabetes in the 6- to 12-month age range.

Our data emphasize that, as expected, there is not a role for HLA class II genotyping in the classification of type 1 diabetes on an individual basis. While the absence of a high-risk HLA genotype makes type 1 diabetes unlikely, the presence of high-risk HLA does not exclude nonautoimmune diabetes. Approximately 40% of patients with *KCNJ11* mutations have high-risk HLA genotypes, similar to their prevalence in the general population.

The risk associated with type 1 diabetes HLA haplotypes differs between continents (22). In this study, we assumed that HLA-DRB1*04-DQB1*0302 and HLA-DRB1*03-DQB1*0201 are high risk in all populations. We addressed this potential source of error by analyzing subjects of European Caucasian origin separately and obtained results similar to those of the international cohort.

We have shown that the frequency of HLA class II high-risk genotypes is significantly different in subjects with insulin-treated permanent diabetes diagnosed over and under 6 months of age. Those diagnosed under 6 months of age have a distribution of high-risk genotypes similar to that of the normal population. Our study confirms that children diagnosed with diabetes under 6 months of age are unlikely to have type 1 diabetes and should be screened for *KCNJ11* gene mutations.

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