Brief Genetics Report

Islet Autoimmunity in Children With Down's Syndrome

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There is an unexplained excess of type 1 diabetes and other organ-specific autoimmune diseases in children with Down's syndrome, but the immunogenetic characteristics of diabetes in Down's syndrome have not been investigated. We studied the frequency of islet autoantibodies in 106 children with Down's syndrome and no history of autoimmunity and analyzed HLA class II genotypes in 222 children with Down's syndrome, 40 children with Down's syndrome and type 1 diabetes, 120 age- and sex-matched children with type 1 diabetes, and 621 healthy control subjects. Co-occurrence of at least two islet autoantibody markers was observed in 6 of 106 nondiabetic children with Down's syndrome compared with 13 of 2,860 healthy age-matched children ($P < 0.001$). There was an excess of diabetes-associated HLA class II genotypes in children with Down's syndrome and type 1 diabetes compared with age- and sex-matched healthy control subjects ($P < 0.001$). Down's syndrome children with type 1 diabetes were, however, less likely to carry the highest risk genotype DR4-DQ8/DR3-DQ2 than children with type 1 diabetes from the general population ($P = 0.01$) but more likely to carry low-risk genotypes ($P < 0.0001$). The frequency of subclinical islet autoimmunity is increased in Down's syndrome, and susceptibility to type 1 diabetes in Down's syndrome is partially HLA mediated. Other factors, possibly including genes on chromosome 21, may increase the penetrance of type 1 diabetes in Down's syndrome. *Diabetes* 55: 3185–3188, 2006

Rare forms of a disease often help to explain the mechanisms underlying a more common form of the disorder. There is an increased frequency of clinically diagnosed type 1 diabetes and other organ-specific autoimmune diseases associated with type 1 diabetes in children with Down's syndrome. Individuals with Down's syndrome and diabetes may therefore provide critical insights into mechanisms underlying susceptibility to immune-mediated diabetes, but islet autoantibodies or HLA class II markers of type 1 diabetes have not been studied in children with Down's syndrome.

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GADA, GAD antibody; IA-2A, IA-2 antibody; WHO, World Health Organization.

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Down's syndrome is a common genetic defect (1:700 live births), affecting over 300,000 people in the U.S. and 30,000 in the U.K. (1,2). A considerably increased risk of diabetes has been consistently reported in children with Down's syndrome (3,4). In a questionnaire-based study of 20,362 patients with Down's syndrome in the U.K. and U.S., the prevalence of diabetes diagnosed before age 20 years in the Down's syndrome population was some six times higher than expected (5), and other studies suggest an approximate fourfold increase in childhood diabetes (3,6). A more recent population-based study of the prevalence of type 1 diabetes in Down's syndrome in Denmark demonstrated a fourfold increased risk of type 1 diabetes in children with Down's syndrome (7). Although classification was generally made on clinical grounds alone, most appear to have type 1 diabetes. For example, diabetes requiring insulin within a month of diagnosis and ketonuria has been shown to be 10 times more common in Down's syndrome than expected (4). Diabetes in Down's syndrome generally presents early in life; one study from the 1960s showed a peak onset at 8 years of age, compared with 14 years in contemporary cases of childhood diabetes (8). In a more recent study of 59 individuals with Down's syndrome and type 1 diabetes, 22% developed diabetes by the age of 2 years, as compared with 7% of those from the general population (9).

Although the children examined in the studies described above appear to have the clinical features of type 1 diabetes, no studies of the frequency of islet autoantibodies in Down's syndrome have been carried out. An autoimmune basis seems likely, since the risk of other organ-specific autoimmune diseases is also greatly increased in Down's syndrome. The prevalence of autoimmune thyroid disease is at least fourfold higher in children with Down's syndrome than in the general population (10,11), and celiac disease may be 10–40 times more common (12–14). Down's syndrome therefore appears to confer susceptibility to multiple forms of autoimmunity, although no mechanism has been proposed to explain this phenomenon. Some specific genes have been linked to different autoimmune diseases, for instance the HLA haplotype DRB1*03-DQA1*0501-DQB1*0201 (DR3-DQ2) is a major susceptibility haplotype for type 1 diabetes as well as thyroid disease and celiac disease. An excess of this haplotype in Down's syndrome could potentially contribute to increased risk of autoimmunity in Down's syndrome, but there are no published studies of HLA class II in children with Down's syndrome and diabetes.

The aims of this study were to determine whether there is evidence of increased islet autoimmunity in children with Down's syndrome (1), whether the frequency of diabetes-associated HLA class II genotypes is increased in children with Down's syndrome (2), and whether HLA

class II associations in Down's syndrome and diabetes are the same as those observed in type 1 diabetes (3).

RESEARCH DESIGN AND METHODS

The study populations included 222 children with Down's syndrome, 40 children with Down's syndrome and type 1 diabetes, 120 age- and sex-matched children with type 1 diabetes, and 621 healthy control subjects. Ethical permission was granted for all studies described, and informed consent from the child and/or parents was obtained for all samples.

Down's syndrome. Blood samples were taken from 30 nondiabetic school-aged children with Down's syndrome (15 male and 15 female, 4–21 years of age) during routine thyroid screening in the area covered by the Gloucestershire Health Authority, U.K. Aliquots of DNA samples from 83 children with Down's syndrome had been collected as part of a study of congenital heart disease in Down's syndrome (15). Samples were also available (109 DNA and 106 serum) for analysis from a population-based study of children with Down's syndrome in Manchester, the Hester Adrian Research Centre cohort (16). There was no clinical evidence of diabetes in any of these children.

Down's syndrome and diabetes. A mouth-swab sample was obtained from 29 children with Down's syndrome and diabetes, all members of the Down's Syndrome Association. An additional 11 samples were identified through local and pediatric clinics nationwide. The total group comprised 21 males and 19 females diagnosed within the age range of 8 months to 21 years (median 9 years, mode 1 year).

Type 1 diabetic control subjects. The control population of 120 type 1 diabetic control subjects (3 age- and sex-matched control subjects for every child with Down's syndrome and type 1 diabetes) was randomly selected from the Bart's Oxford Family Study, a prospective population-based study of type 1 diabetes. This study has recruited parents and siblings of patients with type 1 diabetes, diagnosed before age 21 years, from within the Oxford Regional Health Authority area in England since 1985 (17).

General population control subjects. HLA genotypes from 621 adult U.K. Caucasian control subjects from the general population with no history of autoimmune disease were kindly sent to us by Professor Steven Gough at the Institute of Biomedical Research, University of Birmingham, and have been described previously (18).

Islet autoantibody analysis. Antibodies to insulin, GAD65, and IA-2 were measured by radioimmunoassay as previously described (17). These assays had laboratory-defined sensitivities and specificities of 86 and 99% (GAD antibody [GADA]), 72 and 93% (IA-2 antibody [IA-2A]), and 56 and 97% (islet autoantibody), respectively, in the Third Diabetes Autoantibody Standardization Program workshop. The interassay coefficient of variation was 9% at 14 World Health Organization (WHO) units/ml (GADA), 14% at 10 WHO units/ml (IA-2A), and 24% at 0.6 units (islet autoantibody).

Genetic analysis

Individuals with Down's syndrome and Down's syndrome with type 1 diabetes. Details of DNA extraction methods and HLA class II analysis have been published previously (19). Briefly, mouth-swab extractions were carried out using a guanadinium chloride/phenol:chloroform method, and DNA was "salted out" from blood. DNAs were quantified using OD_{260} readings for blood DNA and picoGreen analysis for mouth-swab DNA. Low-yield DNA samples from mouth swabs underwent whole-genome amplification by primer extension preamplification. HLA analysis was carried out by PCR using sequence-specific primers or a DYNAL reli SSO system. The haplotype HLA DRB1*04-DQB1*0302 has been abbreviated to DR4-DQ8 and HLA DRB1*03-DQB1*0201 to DR3-DQ2.

Data analysis. Antibody positivity was defined as levels above the 97.5th centile of 2,860 schoolchildren control subjects (17). Frequencies of islet autoantibodies in children with Down's syndrome compared with control subjects and frequencies of HLA class II genotypes in children with Down's syndrome and diabetes compared with children from the general population with type 1 diabetes were analyzed using the χ^2 test.

RESULTS

Of the 106 children with Down's syndrome tested, GADAs were present in 8, IA-2A in 5, and insulin autoantibodies in 9 children. Two or more islet autoantibodies were present in 6 of 106 children with Down's syndrome compared with 13 of 2,860 healthy schoolchildren ($P < 0.001$) (Table 1). As demonstrated in Fig. 1, levels of GADA in the Down's syndrome samples were particularly high; all eight GADA-positive samples had levels >99.0th centile (28 WHO units/ml), and seven were >99.5th centile (46 WHO units/ml). When all data were analyzed using the 99.0th centile as the threshold, 2 of 106 individuals were positive for two or more islet autoantibodies compared with 8 of 2,860 healthy schoolchildren ($P = 0.005$).

TABLE 1
The prevalence of GADA, IA-2A, and insulin autoantibodies (IAA) in 106 children with Down's syndrome and 2,860 healthy schoolchildren

	Down's syndrome children	Healthy schoolchildren
n	106	2,860
No markers	90 (84.9)	2,667 (93.3)
Single marker		
GADA	2 (1.9)	59 (2.0)
IA-2A	3 (2.8)	60 (2.1)
islet autoantibody	5 (4.7)	61 (2.1)
Two markers		
GADA/IA-2A	2 (1.9)	2 (0.07)
GADA/IAA	4 (3.8)	4 (0.14)
IA-2A/IAA	0	2 (0.07)
Three markers	0	5 (0.17)

Data are *n* (%).

Comparison of HLA class II genotypes showed similar distributions of diabetes-associated genotypes in children with Down's syndrome and healthy control subjects (Table 2). The frequency of the most strongly associated type 1 diabetes–associated HLA class II genotype (DR4-DQ8/DR3-DQ2) was increased in children with Down's syndrome and type 1 diabetes ($P < 0.001$), indicating that type 1 diabetes in Down's syndrome has the same HLA associations as type 1 diabetes in the general population. Of children with Down's syndrome and diabetes, 70% were positive for either DR4-DQ8/DR3-DQ2, DR4-DQ8/X, or DR3-DQ2/X compared with only 44% of the healthy control population ($P < 0.01$). None of the children with Down's syndrome and type 1 diabetes were positive for HLA DRB1*02-DQB1*0602 (DR2-DQ6).

Direct comparison of the frequency of DR4-DQ8/DR3-DQ2 in the Down's syndrome and type 1 diabetic group ($n = 40$) and an age- and sex-matched type 1 diabetic population ($n = 120$) indicated that only 25% were DR4-DQ8/DR3-DQ2 positive compared with 43% ($P = 0.01$), and more children with Down's syndrome and type 1 diabetes are negative for diabetes-associated HLA class II haplotypes (X/X) ($P < 0.0001$).

DISCUSSION

We have shown that islet autoimmunity is increased in children with Down's syndrome. Two or more markers of islet autoimmunity were present in 6 of 106 children with Down's syndrome as compared with 13 of 2,860 healthy schoolchildren ($P < 0.001$), and high levels of GADA were observed in all eight children who tested positive.

Since relatively little is known about the HLA class II status of people with Down's syndrome, we first wished to exclude the possibility that an excess of autoimmunity-associated HLA haplotypes might explain the increased risk of autoimmunity observed in Down's syndrome. Our data reveal that the HLA class II haplotypes DR4-DQ8 and DR3-DQ2 are not overrepresented in Down's syndrome. This is in agreement with earlier studies of HLA class I in children with Down's syndrome. One study found no differences between HLA-A and -B between 50 couples and

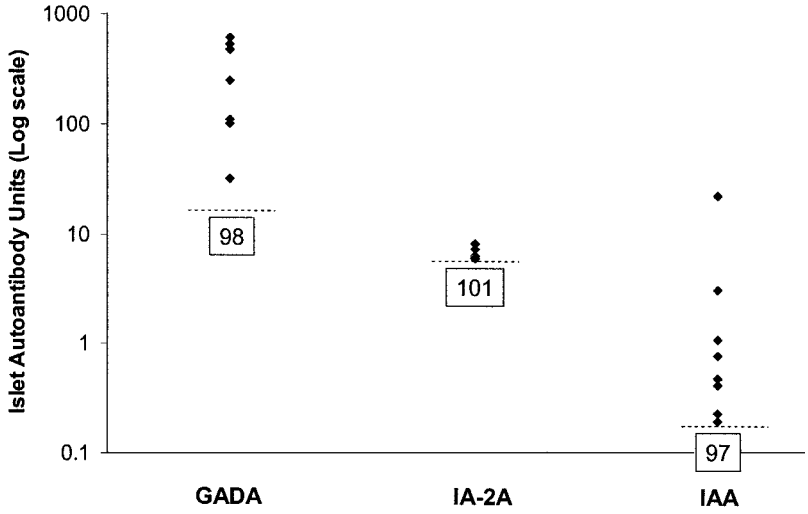

FIG. 1. The distribution of GADA, IA-2A, and insulin in 106 children with type 1 diabetes. The threshold for the 97.5th centile for each antibody is indicated by a dashed line. The numbers in boxes represent the number of children with levels of islet autoantibodys below the 97.5th centile.

their offspring with Down's syndrome, compared with 50 control families and 464 blood donors (20). Similarly, Soubiran et al. (21) examined 30 couples with a child with Down's syndrome typed for HLA-A and -B antigens compared with 20 control families and 176 blood donors, and no significant differences were reported. The excess of susceptibility to autoimmunity in Down's syndrome is therefore not attributable to differences in HLA.

Diabetes-associated HLA class II haplotypes are however increased in children with Down's syndrome and type 1 diabetes ($P < 0.001$), showing that type 1 diabetes in Down's syndrome has the same HLA associations as type 1 diabetes in the general population, suggesting that it shares the same etiology. The frequency of the highest risk HLA class II genotype was however almost twice as high in the type 1 diabetic control group as in the Down's syndrome and diabetic group (43 vs. 25%, respectively) ($P = 0.01$), and more children in the Down's syndrome and diabetes group were negative for diabetes-associated HLA class II haplotypes compared with age-matched control subjects with type 1 diabetes. No child with Down's syndrome and diabetes was positive for the protective haplotype HLA class II DRB1*02-DQB1*0602.

One limitation of this study is the number of Down's syndrome and diabetes samples available for analysis. The combination of Down's syndrome and diabetes is relatively rare. Robust prevalence data recently available for Denmark demonstrated that of 1.23 million births between 1981 and 2000, only 8 individuals were identified with both Down's syndrome and type 1 diabetes (7). If a similar frequency exists in the U.K., we have identified >10% of the possible national cohort. To confirm this, we are currently initiating a nationwide collection of samples from all individuals with Down's syndrome and type 1 diabetes in the U.K.

Our data are consistent with the possibility that a gene, or genes, on chromosome 21 may increase the penetrance of type 1 diabetes in Down's syndrome. A recent Scandanavian genome scan for genes associated with type 1 diabetes identified an area of interest on chromosome 21 (22). Recent fine mapping of this region in 253 Danish families supported the existence of a susceptibility gene for type 1 diabetes on chromosome 21q21.11-q22.3 (23). Taken together, these data support the need for further studies to identify genetic variants on chromosome 21 that may increase the penetrance of type 1 diabetes in Down's syndrome.

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TABLE 2
The frequency of HLA class II genotypes in 40 individuals with Down's syndrome and diabetes, 222 individuals with Down's syndrome, 120 sex- and age-matched individuals with type 1 diabetes, and 621 healthy control subjects

	Healthy control subjects	Down's syndrome	Down's syndrome and type 1 diabetes	Type 1 diabetes
n	621	222	40	120
Genotype				
DR4-DQ8/DR3-DQ2	17 (3)	4 (2)	10 (25)	51 (43)
DR4-DQ8/X	85 (13)	27 (12)	7 (17)	37 (31)
DR3-DQ2/X	167 (27)	49 (22)	11 (28)	21 (17)
X/X	249 (40)	94 (42)	12 (30)	10 (8)
DR2-DQ6/ DR2-DQ6 or DR2-DQ6/X	103 (17)	48 (22)	0 (0)	1 (1)

Data are *n* (%). 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 HLA DRB1*02-DQB1*0602 (DR2-DQ6).

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