

Early-Onset, Coexisting Autoimmunity and Decreased HLA-Mediated Susceptibility Are the Characteristics of Diabetes in Down Syndrome

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OBJECTIVE—Down syndrome (DS) is associated with an increased risk of diabetes, particularly in young children. HLA-mediated risk is however decreased in children with DS and diabetes (DSD). We hypothesized that early-onset diabetes in children with DS is etiologically different from autoimmune diabetes.

RESEARCH DESIGN AND METHODS—Clinical and immunogenetic markers of autoimmune diabetes were studied in 136 individuals with DSD and compared with 194 age- and sex-matched individuals with type 1 diabetes, 222 with DS, and 671 healthy controls. HLA class II was analyzed by sequence-specific primed PCR. Islet autoantibodies were measured by radioimmunoassay.

RESULTS—Age at onset of diabetes was biphasic, with 22% of DS children diagnosed before 2 years of age, compared with only 4% in this age-group with type 1 diabetes in the general population ($P < 0.0001$). The frequency of the highest-risk type 1 diabetes-associated HLA genotype, DR3-DQ2/DR4-DQ8, was decreased in both early- and later-onset DSD compared with age-matched children with type 1 diabetes ($P < 0.0001$), although HLA DR3-DQ2 genotypes were increased ($P = 0.004$). Antibodies to GAD were observed in all five samples tested from children diagnosed at ≤ 2 years of age, and persistent islet autoantibodies were detected in 72% of DSD cases. Thyroid and celiac disease were diagnosed in 74 and 14%, respectively, of the DSD cohort.

CONCLUSIONS—Early-onset diabetes in children with DS is unlikely to be etiologically different from autoimmune diabetes occurring in older DS children. Overall, these studies demonstrate more extreme autoimmunity in DSD typified by early-onset diabetes with multiple autoimmunity, persistent islet autoantibodies, and decreased HLA-mediated susceptibility.

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Children with Down syndrome (DS) are at increased risk of thyroid (1), gut (2), and islet autoimmunity (3,4). In the only population-based study that has addressed the prevalence of DS in type 1 diabetes patients, a more than fourfold

increased prevalence was observed (5). It has been suggested that diabetes in children with DS presents particularly early in life; one study from the 1960s showed a peak onset of 8 years of age, compared with 14 years in cases of childhood diabetes (6). In a

previous study of DS and diabetes, 22% of participants had developed diabetes by 2 years of age, compared with only 7% of those with type 1 diabetes from the general population (7). A recent study of 159 DS children diagnosed with type 1 diabetes (DSD) demonstrated two peaks in diabetes incidence, one occurring before 2 years of age and the other in early adolescence. The mean age at onset in the 41,983 control subjects with type 1 diabetes was 8.42 years (8). These data suggest that diabetes occurring before 2 years of age in DS children may be etiologically different from type 1 diabetes. In the seminal study of type 1 diabetes pathology by Foulis et al. (9), three cases of DS and diabetes were described. A 14-year-old boy with longstanding diabetes and a 12-year-old boy with recent-onset diabetes both showed evidence of lymphocytic infiltration, with an absence of insulin staining in the 14-year-old boy, typical of type 1 diabetes. The third DS child, however, diagnosed with diabetes at 18 months, whose pancreas was analyzed within 2 weeks of diabetes diagnosis, displayed normal insulin staining with no morphological abnormality.

An age-related association between the HLA class II susceptibility haplotypes DRB1*04-DQB1*0302 (DR4-DQ8) and DRB1*03-DQB1*0201 (DR3-DQ2) and type 1 diabetes in the general population is well established, with an increased frequency in young children with type 1 diabetes (10,11). These haplotypes also appear to contribute to susceptibility to diabetes in DS, but to a lesser degree (12).

The aim of this study, therefore, was to test the hypothesis that diabetes diagnosed before 2 years of age does not have an autoimmune basis in a well-characterized cohort of individuals with DSD. Distinguishing whether insulin deficiency in these young children is caused by accelerated autoimmunity or an alternative mechanism, such as a β -cell secretory deficit, could have consequences for treatment or provide insights into a more aggressive autoimmune process in children with DS.

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RESEARCH DESIGN AND METHODS

Study populations

DS and diabetes. An international collection of clinical details and genetic and serum samples from children with DSD (Diaploidy) was established in 2010. In the U.K., a call for potential participants for a study of diabetes in children with DS was sent out by the Diabetes Research Network and Diabetes UK. Internationally, a call was sent out through the International Society for Pediatric and Adolescent Diabetes. All cases referred were accepted. By June 2012, 136 individuals with DS and a clinical diagnosis of type 1 diabetes had been registered (80 from the U.K., 30 from Austria and Germany, 7 from other European countries and Australia, and 19 from the State of Kuwait). Clinical data on age at diagnosis of diabetes, thyroid and celiac disease, family history of autoimmunity, treatment history, and current height and weight were collected by questionnaire. Three control groups were studied as follows.

1) DS controls. Blood samples were taken from 30 nondiabetic school-aged children with DS (15 male and 15 female, age range 4–21 years) during routine thyroid screening in the area covered by the Gloucestershire Health Authority, U.K. Aliquots of DNA samples from 83 children with DS had been collected as controls for a study of congenital heart disease in DS (13). DNA samples ($n = 109$) were also available for analysis from a population-based study of children with DS in Manchester, U.K. (14). There was no clinical evidence of diabetes in any of these children.

2) Type 1 diabetes controls. For the HLA analysis, two age-at-onset- and sex-matched children with type 1 diabetes for each child with DS and diabetes were randomly selected from the population-based Bart's Oxford (BOX) study of type 1 diabetes that has been ongoing since 1985 with 95% ascertainment (15). Age-at-onset data from 1,822 probands diagnosed before 21 years of age from this cohort were used to compare with age-at-onset data of the DSD cohort.

3) Healthy control subjects. HLA genotypes from 621 adult white U.K. control subjects with no history of autoimmune disease were provided by Steven Gough (Institute of Biomedical Research, University of Birmingham, Birmingham, U.K.) and have been described previously (16).

Ethical permission

Ethical permission had been granted for all studies described, and written informed consent was obtained from the participant, parent, or guardian, as appropriate, for all samples collected (MREC/02/6/26).

Genetic analysis

DNA samples were genotyped for all HLA class II HLA DRB1 and DQB1 haplotypes by PCR using a DYNAL reli SSO system (Life Technologies, Paisley, U.K.). DRB1*04 alleles were subtyped using a PCR with sequence-specific primers. Haplotypes were derived from established patterns of linkage disequilibrium. The established type 1 diabetes-associated haplotype HLA DRB1*0401-DQB1*0302 was abbreviated to DR4-DQ8, and HLA DRB1*03-DQB1*0201 was abbreviated to DR3-DQ2. Nonrisk haplotypes were described as X.

The analyses of HLA data were restricted to individuals with DSD diagnosed before 21 years of age to avoid the issue that some older individuals with DSD may have type 2 diabetes and to allow age matching with individuals participating in the BOX study of type 1 diabetes (11).

Islet autoantibody analysis

Antibodies to GAD65 (GADA), IA-2ic (IA-2A), and ZnT8RA/WA were measured by radioimmunoassay as previously described (15,17). The laboratory-defined assay sensitivities and specificities of GADA were 86 and 99%, and of IA-2A 72 and 93%, respectively, in the Third Diabetes Antibody Standardization Program (18). The interassay coefficient of variation was 9% at 14 WHO units/mL (GADA), 14% at 10 WHO units/mL (IA-2A), and 16% for ZnT8RA and 27% for ZnT8WA, both at 1.8 units/mL. Serum samples were available from 43 individuals with DSD. Due to the nature of the Diaploidy cross-sectional study design, serum samples collected at diagnosis were not available for analysis. Time from diagnosis ranged from 1 to 396 months (median 89 months); samples collected within 10 years of diagnosis were available from 23 individuals, and a further 20 samples were collected between 10 and 39 years from diagnosis. Positivity for islet autoantibodies would be supportive of an autoimmune etiology, whereas a negative postdiagnosis result could not be interpreted.

Data analysis

Differences in age at onset and frequencies of HLA class II genotypes in children

with DS and diabetes compared with age-matched children with type 1 diabetes were analyzed using the χ^2 test.

RESULTS

DS and diabetes: subject characteristics

Of 136 individuals with DSD, 69 (51%) were male. Data on clinical diagnosis of other autoimmune diseases were available on 92 subjects. Of these, 68 (74%) had coexisting thyroid disease and 11 (14%) had coexisting celiac disease. Seven of 92 (8%) had coexisting diagnoses of diabetes and thyroid and celiac disease.

Age-at-onset analysis in the DS and diabetes population

Of 118 patients with DSD diagnosed with diabetes before 21 years of age, 22% were diagnosed with diabetes before 2 years of age compared with 5% of 1,822 individuals with type 1 diabetes from the general population notified to the BOX study in the same age-group ($P < 0.0001$). As shown in Fig. 1, there was a biphasic pattern in age at diagnosis, with a peak at 1 year of age and another centered around 10 years of age.

HLA class II analysis

In the healthy control cohort, only 3% had the highest-risk diplotype (DR4-DQ8/DR3-DQ2), 13% had DR4-DQ8/X, 27% had DR3-DQ2/X, and 57% had no risk haplotypes. HLA class II frequencies in the DS control population were very similar to the healthy control population (Fig. 2A). As expected, the risk haplotypes were increased in 194 individuals with type 1 diabetes age- and sex-matched with the DSD population: 38% had DR4-DQ8/DR3-DQ2, 40% had DR4-DQ8/X, 17% had DR3-DQ2/X, and 5% had no risk haplotypes. Genetic samples were available from 97 individuals with DSD diagnosed before 21 years of age. HLA frequencies in the DSD cohort were intermediate between the type 1 diabetes and control cohorts. Specifically, 17 (17%) had the highest-risk diplotype (DR4-DQ8/DR3-DQ2); 23 (24%) and 31 (32%) had the moderate-risk DR4-DQ8 and DR3/DQ2 haplotypes, respectively, and 26 (27%) had no risk haplotypes. In contrast, 5% of 194 age- and sex-matched children with type 1 diabetes from the BOX study ($P < 0.0001$) and 64% of 222 DS individuals had no risk haplotypes (Fig. 2A). The frequency of the HLA DR3-DQ2/X diplotype (where X is

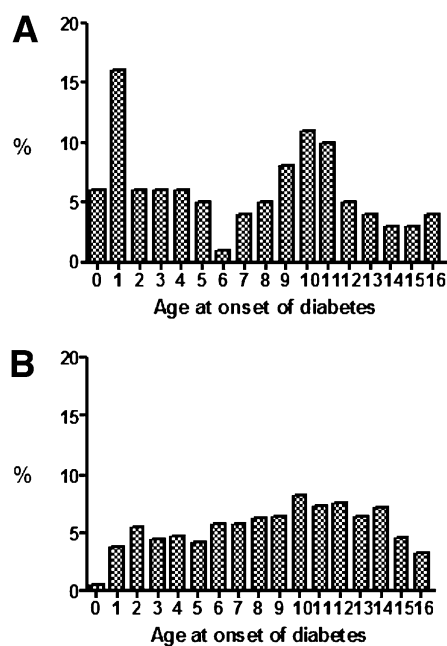


Figure 1—Age at diagnosis of diabetes in individuals with DS diagnosed with diabetes (A) compared with individuals with type 1 diabetes from the BOX study (B).

not DR4-DQ8 or DR3-DQ2) in DSD (32%), however, was increased relative to age-matched patients from the BOX study (17%, $P = 0.004$).

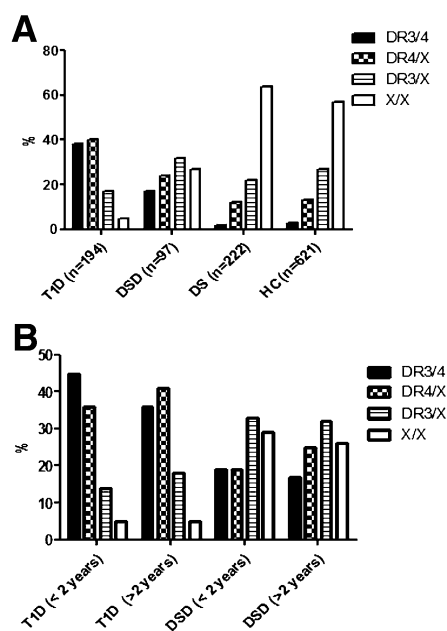


Figure 2—A: The frequency (%) of type 1 diabetes-associated haplotypes in DSD children relative to age-matched children with type 1 diabetes (T1D), DS alone, and healthy control subjects (HC). B: The HLA characteristics of diabetes in children with DS by age at onset compared with type 1 diabetes.

There was no difference in HLA-mediated risk in DS children who had developed diabetes before and after 2 years of age (Fig. 2B), indicating that diabetes in the early-onset cases is unlikely to be etiologically distinct from the diabetes found in older DS children.

Islet autoantibodies

Despite the extended diabetes duration at the time many samples were collected, islet autoantibodies were detected in 72% of the DSD patients for whom serum was available (Table 1). Furthermore, all five samples from DSD children diagnosed before 2 years of age were positive for GADA.

CONCLUSIONS

In this study, we hypothesized that some early-onset cases of diabetes in DS children are not autoimmune. A biphasic distribution in age at onset of diabetes in children with DS previously observed in a European study of 159 children with DS and diabetes compared with 42,000 age-matched individuals with type 1 diabetes (8) was confirmed in our study. We also demonstrated, in the largest analysis to date, that type 1 diabetes-associated HLA genotypes are decreased in children with DSD. To account for this difference in HLA frequencies, we hypothesized that diabetes in some children diagnosed before 2 years of age may be etiologically different from autoimmune type 1 diabetes. Analysis of HLA data by age at onset, however, did not support this hypothesis. This shows that DS children with early-onset diabetes are unlikely to have an etiologically distinct form of diabetes. Two children diagnosed within the first month of life may have an alternative etiological basis for their diabetes; the remaining children were diagnosed at, or after, 6 months of age, consistent with type 1 diabetes (19). Autoimmunity was supported by data obtained from a postdiagnosis analysis of islet autoantibodies; antibodies to GAD were detected in all five serum samples tested from children diagnosed with diabetes before 2 years of age. Although we cannot rule out the possibility that some individuals with DS and early-onset diabetes have an etiologically distinct form of diabetes, we suggest that this is rare and may present in the first 6 months of life.

Our previous study of diabetes in 40 DS children (12) suggested that the frequency of autoimmune diabetes-associated HLA class II genotypes was increased in DSD

but to a lesser extent than might be expected. We confirmed, within a substantially enlarged sample, that the frequency of autoimmune-related HLA genotypes was decreased with a concomitant increase in nonautoimmune-related genotypes in children with DSD compared with age-matched children with type 1 diabetes. In young European populations with type 1 diabetes, 5–10% of individuals do not carry DR4-DQ8 and/or DR3-DQ2 (10,11), but this proportion was increased to 27% in our similarly aged cohort of patients with DSD. This difference was not explained by the inclusion of 19 children with DS and diabetes from the State of Kuwait, a population where HLA-mediated susceptibility to diabetes may be different, as the pattern was the same when these individuals were removed from the analysis. This increased penetrance of low-risk HLA class II haplotypes in DSD children mirrors the trend observed in the general population as type 1 diabetes incidence is increasing (20–23). Understanding how autoimmunity occurs in the absence of HLA risk genotypes in children with DS could therefore provide important insights into disease mechanisms in the general population.

There are limitations to this work. Although it is the largest existing cohort of DSD individuals from whom serum and DNA are available, the Diaploidy study is relatively small. This is, however, a difficult group to recruit as co-occurrence of both conditions is rare. The cohort is not population based, and definitive studies of incidence are therefore not possible. The analysis of islet autoantibodies years after diagnosis is not ideal, as antibody levels tend to fall post-diagnosis, although antibodies to GAD are known to be the most persistent (24). Indeed, in this study, at least one islet autoantibody was detectable in 75% of post-diagnosis samples, with multiple islet autoantibody positivity detectable in serum from eight individuals >10 years after diagnosis.

There is a wide variation in reported prevalence rates of thyroid disorders in the DS population. The prevalence of autoimmune thyroid disease has been reported to be at least fourfold higher in children with DS than in the general population (25–27), but a recent longitudinal study suggests that that this may be an overestimation (28). Celiac disease may be 10 times more common in DS populations (2,29). Our study suggests that individuals with DS are at risk for extreme autoimmunity;

Table 1—Residual islet autoantibody positivity in 43 individuals with DSD from whom serum was available

Time from diagnosis	Three islet antibodies	Two islet antibodies	GADA alone	IA-2A alone	ZNT8R/W alone	Negative
<10 years (%)	2 (11)	4 (21)	7 (37)	0	0	6 (31)
>10 years (%)	2 (8)	6 (25)	9 (38)	1 (4)	0	6 (25)

co-occurrence of clinically diagnosed thyroid disease and diabetes was observed in 74% and clinically diagnosed celiac disease and diabetes was observed in 14% of individuals with DSD. This was based on data collected by questionnaire. The precise etiology of thyroid disease is therefore unclear, and data on antithyroid antibodies at diagnosis are unavailable.

Overall, a clinical picture of DSD is emerging with earlier-onset diabetes, co-existence of other organ-specific autoimmune diseases with persistent islet autoantibodies, and decreased HLA-mediated susceptibility. Why might this be? Overexpression of type 1 diabetes-associated genes on chromosome 21 combined with generalized immunological dysfunction in DS appears probable. A genome-wide association study identified (30) and replicated (31) a chromosome 21q22.3 type 1 diabetes-associated locus. The candidate gene is the ubiquitin-associated and SH3 domain-containing A (*UBASH3A*), which is expressed in spleen and peripheral blood lymphocytes (32) and regulates T-cell signaling (33,34). Overexpression of *UBASH3A* may therefore provide one candidate for the increased frequency of autoimmune disease in DS. Immune cell dysfunction in DS is well established. A smaller thymus in DS children has been reported several times, (35,36) and total lymphocyte numbers, including CD4 and CD8 T-cell subsets are decreased, particularly in the first 2 years of life. Recent analysis of protein and gene expression in surgically removed thymuses from 14 DS patients compared with 42 age-matched control subjects showed reduced expression of AIRE, a chromosome 21 gene product that regulates ectopic expression of tissue-specific antigens in thymic medullary epithelial cells, a crucial mechanism for thymic T-cell selection (37). This mechanism could contribute to the increased risk of multiple autoimmunity and the earlier onset of diabetes that we have observed.

In conclusion, diabetes in DS children is associated with a lower frequency of high-risk HLA class II susceptibility genes

than children matched for age at onset of diabetes with type 1 diabetes from the general population, but this is not caused by a subset of children with an etiologically different early-onset form of diabetes. HLA DR3-DQ2/X combinations are increased in DSD children, but this does not fully explain their increased frequency of endocrine autoimmunity. Our data show high rates of coexisting organ-specific autoimmunity with a high prevalence of residual islet autoimmunity and lower frequencies of class II HLA diabetes susceptibility haplotypes in DSD. Understanding how this occurs may provide insights into the mechanisms underlying type 1 diabetes in the general population.

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R.J.A., K.L.M., J.B., and J.P.H.S. researched data and wrote the manuscript. A.J.W. and P.J.B. coordinated sample collection and analysis and contributed to discussion. R.W.H., T.R.R., E.S., and M.M.A.-R. coordinated sample collection and analysis. K.M.G. researched data, conducted analyses, and wrote the manuscript. All authors reviewed, edited, and discussed the draft manuscript. K.M.G. 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.

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