Trisomy 21 Is a Cause of Permanent Neonatal Diabetes That Is Autoimmune but Not HLA Associated

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Identifying new causes of permanent neonatal diabetes (PNDM) (diagnosis <6 months) provides important insights into β-cell biology. Patients with Down syndrome (DS) resulting from trisomy 21 are four times more likely to have childhood diabetes with an intermediate HLA association. It is not known whether DS can cause PNDM. We found that trisomy 21 was seven times more likely in our PNDM cohort than in the population (13 of 1,522 = 85 of 10,000 observed vs. 12.6 of 10,000 expected) and none of the 13 DS-PNDM patients had a mutation in the known PNDM genes that explained 82.9% of non-DS PNDM. Islet autoantibodies were present in 4 of 9 DS-PNDM patients, but DS-PNDM was not associated with polygenic susceptibility to type 1 diabetes (T1D). We conclude that trisomy 21 is a cause of autoimmune PNDM that is not HLA associated. We propose that autoimmune diabetes in DS is heterogeneous and includes coincidental T1D that is HLA associated and diabetes caused by trisomy 21 that is not HLA associated.

Permanent neonatal diabetes (PNDM) is diagnosed before the age of 6 months, and a genetic diagnosis is possible for >82% of cases (1). Twenty-four causative PNDM genes have been identified (1–4), and four of these cause monogenic autoimmune PNDM that results from destruction of the β-cells very early in life (*FOXP3*, *IL2RA*, *LRBA*, and *STAT3*). Identifying novel causes of autoimmune neonatal diabetes can provide key insights into the development of autoimmunity and can provide new targets for therapeutic intervention.

Down syndrome (DS) is caused by trisomy of chromosome 21 and has an incidence of 1:700 to 1:1,100 live births (5). Large studies have shown childhood-onset autoimmune diabetes is four times more common in DS than in the general population and has an intermediate HLA association (6). There have been three reported cases of DS with diabetes diagnosed before 6 months (DS-PNDM) (7,8). However, it is not known whether DS was the cause of PNDM or a coincidental finding in these patients. The aim of our study was to use our large international cohort of PNDM patients to assess whether DS can aetiologically cause PNDM. We assessed clinical phenotype, islet autoantibodies, and polygenic risk of T1D in patients with monogenic PNDM and DS-PNDM.

RESEARCH DESIGN AND METHODS

Study Population

We defined PNDM as diabetes diagnosed before the age of 6 months, which is treated with continual insulin treatment. We studied 1,522 DNA samples from two international collections of PNDM patients, 1,360 recruited in Exeter and 162 recruited in Chicago. These either had a confirmed monogenic etiology (82.9% [*n* = 1,262]) or had been tested (and were negative) for all 24 known genes (17.1% [*n* = 260]). Clinical information was provided by the referring physician via a comprehensive referral form. This includes a section for the reporting of extra pancreatic clinical features.

Genetic Testing

Testing of the Known Genes

All individuals diagnosed in the first 6 months of life were tested for mutations either by rapid Sanger sequencing of

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*A complete listing of members of the International DS-PNDM Consortium can be found in Supplementary Data.

ABCC8, *KCNJ11*, and *INS* or, if no mutation was identified, via targeted DNA sequencing through next-generation sequencing (tNGS) of all 24 genes (1–4,9). This assay can detect single nucleotide variants, insertion-deletions, copy number variation, and structural variation (9).

Exclusion of Trisomy 21

We used SavvyCNV (10,11) to screen for trisomy 21 in the PNDM and autoimmune-PNDM cohorts where tNGS was undertaken (n = 445). This uses off-target reads from tNGS data to detect large copy number and structural variants.

Type 1 Diabetes Genetic Risk Score

The Type 1 Diabetes genetic risk score (T1D-GRS) was generated where there was sufficient DNA as previously described (12). Briefly, we genotyped single nucleotide polymorphisms (SNPs) tagging the top 10 risk alleles for type 1 diabetes (T1D) and summed their log-transformed odds ratios before dividing by the total number of alleles to obtain a numeric score. None of the SNPs used are on chromosome 21. We used T1D-GRS from 1963 gold standard T1D individuals (all diagnosed <17 years) from the Wellcome Trust Case Control Consortium as a representative sample for polygenic T1D (13). As part of the score generation, SNPs rs2187668 and rs7454108 were used to tag DR3 (DRB1*0301-DQA1*0501-DQB1*0201) and DR4-DQ8 (DRB1*04-DQA1*0301-DQB1*0302) alleles. These SNPs have been shown to be 98.6% sensitive and 99.7% specific for tagging DR3/DQ4-DQ8 (14). We used rs3129889 to tag HLA DRB1*15 (15).

The T1D-GRS was available for 13 patients with DS-PNDM, 458 individuals with a confirmed nonautoimmune monogenic etiology (non-DS PNDM), and 40 individuals with a monogenic cause of autoimmune PNDM (autoimmune non-DS PNDM). There was no difference in clinical features between individuals with or without T1D-GRS.

Islet Autoantibody Testing

Islet autoantibodies GADA, IA-2A, and/or ZnT8A were measured either by local assays or from plasma on the sample received at the Exeter Clinical Laboratory using an automated ELISA-based assay as previously described (16). We used >97.5th centile of control subjects (n = 1,559) to define positivity for autoantibodies.

Statistical Analysis

The Mann-Whitney U and Kruskal-Wallis tests were used to compare continuous variables, and the Fisher exact test was used to compare categorical variables. Statistical analysis was undertaken in Stata14 (StataCorp, College Station, TX).

Ethics Approval

This study was approved by the Genetic Beta Cell Research Bank, Exeter, U.K., and The University of Chicago. Ethics approval was provided by the North Wales Research Ethics Committee, U.K. (Integrated Research Application System [IRAS] project identification no. 231760) and The University of Chicago Institutional Review Board (institutional review board no. 6858, identification no. 15617B).

RESULTS

Trisomy 21 Is Enriched in PNDM

To assess whether DS is enriched in our PNDM cohort, we compared the observed frequency in the PNDM cohort with the expected frequency based on the population prevalence. Using clinical data obtained at referral, we identified 13 of 1,522 (0.9%) individuals with DS-PNDM in our international PNDM cohort. In all 13 individuals, trisomy 21 was confirmed by karyotyping and analysis of tNGS data using SavvyCNV. We did not detect Trisomy 21 in any individual without a clinical diagnosis of DS. The observed frequency in our cohort was therefore 85 of 10,000 (95% CI 45–146), which is 6.7-fold higher than the expected population frequency of 12.6 of 10,000 (95% CI 12.4–12.8) (P = 0.007) (5) (Fig. 1).

Patients With DS-PNDM Do Not Have Mutations in Any of the 24 Genes Known to Cause Monogenic PNDM

We next assessed the presence of known monogenic etiology in patients with DS-PNDM. None of the 13 DS-PNDM individuals had pathogenic variants in the 24 known PNDM genes. In contrast, 1,262 of 1,522 (82.9%) of the non-DS PNDM had a monogenic etiology (Fig. 2) (P = 1.4 × 10^{-10}). Taken together, these two results strongly support trisomy 21 as a cause of PNDM.
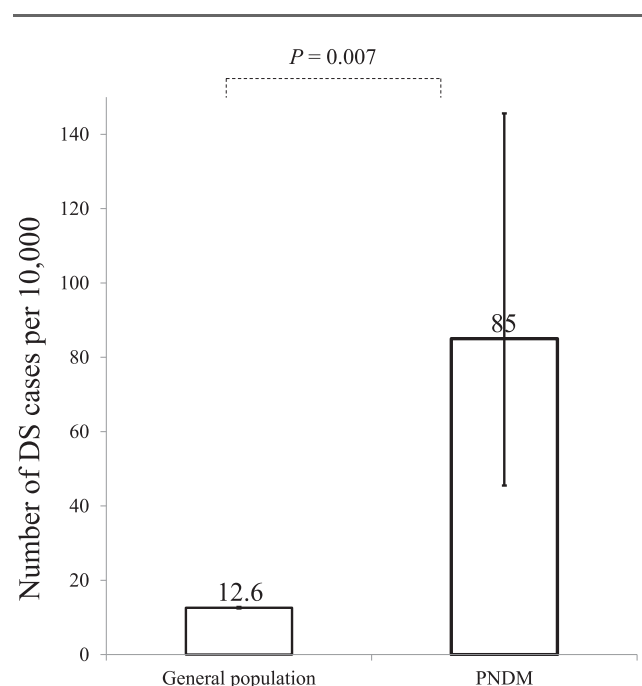

Figure 1—DS is enriched in our PNDM cohort. DS has a population prevalence of 12.6 of 10,000 (95% CI 12.4–12.8), whereas in our cohort of 1,522 patients with neonatal diabetes we have 13 cases, equivalent to 85 of 10,000 (40.4–144.3); P = 0.007.
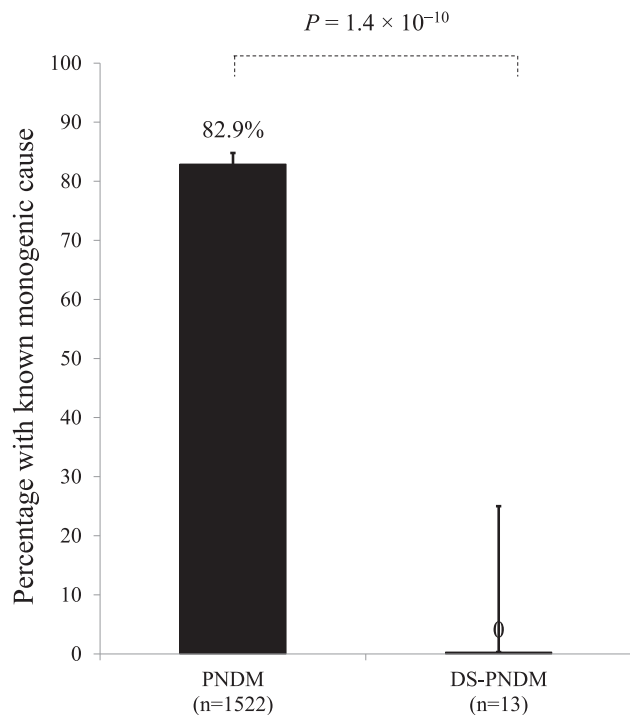
Figure 2—DS-PNDM is not caused by other known neonatal diabetes genes. The 24 known neonatal diabetes genes account for 82.9% of cases, while none of the 13 DS-PNDM case subjects had a mutation in a known gene ($P = 1.4 \times 10^{-10}$).

Islet Autoimmunity Is Common in DS-PNDM

To further assess the underlying etiology of DS-PNDM, we compared the clinical characteristics of the DS-PNDM patients with the PNDM cohort with a nonautoimmune monogenic cause (non-DS PNDM) ($n = 458$) or monogenic autoimmunity (autoimmune non-DS PNDM) ($n = 40$). We found that 44% (4 of 9) of the DS-PNDM individuals were positive for one autoantibody (all GADA and none IA2A or ZnT8A) with time from diabetes diagnosis to testing ranging from 4 months to 10 years (Table 1). This was similar to the autoimmune non-DS PNDM (46% [13 of 28]; $P = 1.0$) but higher than the non-DS PNDM (21 of 293 [7%]; $P = 0.004$) (Table 2). This supports an autoimmune etiology in DS-PNDM.

The median age of onset of diabetes was similar in all three groups (Table 2). The birth weight of the DS-PNDM cohort was low (-1.23 SDs). This was similar to that in the non-DS PNDM individuals (-1.74 SDs; $P = 0.87$) and lower than in those with autoimmune non-DS PNDM (-0.39 SDs; $P = 0.02$). Full clinical information for each subject with DS-PNDM and diabetes is given in Table 1.

DS-PNDM Is Genetically Distinct From Type 1 Diabetes

To further investigate the etiology of autoimmune diabetes in DS-PNDM, we assessed polygenic risk for T1D (T1D-GRS) in DS-PNDM and compared it with both T1D and monogenic PNDM. T1D-GRS in individuals with DS-PNDM was similar to that in control subjects without

diabetes (Fig. 3) (median T1D-GRS 0.61 vs. 0.55; $P = 0.33$), non-DS PNDM (0.61 vs. 0.55; $P = 0.48$), and autoimmune non-DS PNDM (0.61 vs. 0.57; $P = 0.65$) but lower than the T1D control group (0.61 vs. 0.69; $P = 0.0001$). While 10 of 13 individuals with DS-PNDM carried a copy of either DR3 or DR4, none had the highest-risk HLA DR3/DR4 genotype compared with 34% (666 of 1,963) of T1D control subjects ($P = 0.006$) (Table 1). Furthermore, 4 of 13 (36%) DS-PNDM subjects carried the HLA DRB1*15 allele, which is dominantly protective against T1D, compared with 2% (40 of 1,963) of T1D control subjects ($P = 0.0001$). This is similar to control subjects without diabetes who carry the protective HLA DRB1*15 allele (1,643 of 2,938, $P = 0.27$). These data suggest that DS-PNDM is not HLA associated and is therefore unlikely to be very-early-onset polygenic T1D.

DISCUSSION

Our study identifies trisomy 21 as a rare cause of autoimmune PNDM. We have shown that DS is sevenfold enriched in our PNDM cohort and that none of the DS-PNDM individuals have a mutation in any of the 24 known PNDM genes. The antibody data support an autoimmune etiology in DS-PNDM that is not associated with increased polygenic susceptibility to T1D. DS-PNDM is rare in people with DS, suggesting that trisomy 21 is a low-penetrance cause of PNDM.

We found that PNDM caused by trisomy 21 can be linked to β-cell autoimmunity, as shown by the presence of islet antibodies in four of nine cases (17). Islet antibodies are seen in HLA-associated T1D and also in autoimmune non-DS PNDM, which is not HLA associated (12). The proportion of patients with DS-PNDM who were autoantibody positive was similar to that in non-DS PNDM caused by monogenic autoimmunity. The islet antibodies are unlikely to be due to maternal transfer of antibodies, as measurement for 3 of the 4 GADA-positive patients was done after 9 months (18,19).

Our data suggest prenatal onset of β-cell dysfunction/ destruction in DS-PNDM. We found that the birth weight of the DS-PNDM individuals was reduced (median z score -1.23), lower than the birth weight seen in DS without PNDM (z score for boys -0.47 and girls -0.07). Interestingly, the birth weight was similar to that in non–DS-PNDM patients whose lower birth weight is due to reduced insulin secretion in utero. Global immunological changes in individuals with DS have been identified including defects in T-cell regulation and thymus development that are evident in newborns (20). These data taken together suggest prenatal onset of autoimmunity and pancreatic dysfunction.

We found that individuals with DS-PNDM do not have increased polygenic risk of T1D even though they develop diabetes before 6 months of age. This is contrary to T1D in which the HLA association is increased with younger age of diagnosis (21). Furthermore, 4 of 13 (31%) individuals carry the DRB1*15 haplotype, which provides dominant

Table 1—Features of the DS-PNDM cohort

Patient	Sex	Ethnicity	Maternal age at birth (years)	Patient current age	Birth weight z score[a]	Age at diabetes diagnosis	Treatment	HbA1c, % (mmol/L)[b]	Autoantibodies tested	Autoantibody status	Diabetes duration at antibody testing	T1D-GRS (centile of T1D)[c]	HLA DR genotype[d]	Other conditions
1	Male	Caucasian	ND	23 years	−0.79	7 weeks	Insulin	9.5 (80)	ND	ND	NA	0.37 (0.1)	DRB1*15/X	Hypothyroidism, ASD
2	Female	Hispanic	ND	14 years	−3.99	1 week	Insulin	8.1 (65)	ND	ND	NA	0.70 (53.5)	DR4/DR4	Hypothyroidism, hearing and vision problems
3	Male	Caucasian	33	1 years	−1.02	2 days	Insulin	ND	GADA, IA-2A, ZnT8A	Negative	1 week	0.69 (48.2)	DR3/DR3	Tetralogy of Fallot, macroglossia
4	Male	Caucasian	ND	28 years	ND	3 months	Insulin	8.5 (69)	GADA, IA-2A, ZnT8A	GADA positive	10 years	0.52 (2.5)	X/X	Seizures
5	Male	Arabic	37	1 years	ND	4 months	Insulin	8.8 (73)	GADA, IA-2A, ZnT8A	GADA positive	4 months	0.66 (33.9)	DR3/DR3	—
6	Male	Hispanic	40	9 months	−2.65	2 weeks	Insulin	5.7 (39)	GADA, IA-2A, ZnT8A	Negative	2 months	0.64 (25.4)	DR4/X	Subclinical hypothyroidism, macroglossia, cardiac malformation
7	Male	Mixed (East Asian/Caucasian)	43	20 years	0.80	3 days	Insulin	8.8 (73)	GADA	GADA positive	8 years	0.63 (20.6)	DR3/X	Hypothyroidism, celiac disease exocrine insufficiency
8	Male	Arabic	ND	9 years	−1.23	8 weeks	Insulin	9.3 (78)	GADA, IA-2A	GADA positive	5 years	0.37 (0.1)	DRB1*15/X	Macroglossia
9	Female	Turkish	ND	15 years	ND	3 weeks	Insulin	8.1 (65)	GADA, IA-2A	Negative	1 day	0.61 (14.3)	DR3/X	Hypothyroidism
10	Female	Hispanic	ND	Died aged 7 months	−3.30	3 days	Insulin	6.6 (49)	ND	ND	NA	0.49 (0.9)	DR4/DRB1*15	—
11	Male	Caucasian	35	6 years	−0.84	1 day	Insulin	6.6 (49)	GADA, IA-2A, ZnT8A	Negative	5 months	0.48 (0.8)	DR3/DRB1*15	Transient hypothyroidism, VSD
12	Male	Caucasian	ND	17 years	0.19	18 days	Insulin	9.8 (84)	ND	ND	ND	0.69 (48.2)	DR4/X	Seizures, hypothyroidism, frequent infections (low IgM), hepatic abscess

Table 1—Continued

Patient	Sex	Ethnicity	Maternal age at birth (years)	Patient current age	Birth weight z score[a]	Age at diabetes diagnosis	Treatment	HbA1c, % (mmol/L)[b]	Autoantibodies tested	Autoantibody status	Diabetes duration at antibody testing	T1D-GRS (centile of T1D)[c]	HLA DR genotype[d]	Other conditions
13	Male	Caucasian	ND	1 year	−1.85	Birth	Insulin	7.0 (53)	GADA, ZnT8A	Negative	ND	0.68 (44.5)	DR4/DR4	Hyperthyroidism, bowel obstruction, speech and vision problems

ASD, atrial septal defect; NA, not applicable; ND, no data; VSD, ventral septal defect. [a] z score adjusted for sex and gestational age. [b] Latest HbA1c reported by clinician. [c] T1D-GRS based on top 10 risk alleles; centile of T1D refers to the centile of the T1D control population that this score corresponds with. [d] X denotes any HLA DR haplotype other than DR3, DR4, or DRB1*15.

protection against T1D and is very rarely seen in patients with T1D (40 of 1,963 [2%]). Two of these also carried DR3 or DR4; however, inheritance with the DRB1*15 allele nullifies the risk allele (22). The DS-PNDM cohort is of mixed ethnicities (6 Caucasian, 3 Middle Eastern, 3 Hispanic, and 1 mixed), but population stratification does not account for the difference in HLA alleles between DS-PNDM cases and T1D; the HLA DR3/DR4 diplotype is strongly predisposing and the DRB1*15 allele is strongly protective for T1D across these populations (23–25). These data support that DS-PNDM is not HLA associated.

A previous study showed that DS with diabetes had intermediate HLA association (7). The highest-risk HLA diplotype—DR3/DR4—was present in half as many DS diabetes cases compared with T1D (17% of DS diabetes, 38% of T1D, 3% of control subjects). We propose that this is explained by diabetes in DS in these studies reflecting a mixture of two subtypes of autoimmune diabetes: one caused by trisomy 21–related immune dysregulation (not HLA associated), and the other coincidental polygenic T1D (HLA associated) in which trisomy 21–related immune dysregulation is not playing as strong a role. A mixture of two aetiologies is also reflected by the observation that age of onset of diabetes in DS is biphasic, with a peak at 1 year and another at 10 years of age, the latter coinciding with non-DS T1D.

We have shown a sevenfold increase in the prevalence of diabetes before the age of 6 months in DS. Previous authors (6) have shown an increase of diabetes between 0.5 and 18 years of approximately fourfold. The difference in prevalence means patients are far less likely to get diabetes before 6 months (7 of 100,000) compared with 0.5–18 years (700 of 100,000).

A complex interaction between multiple genes on chromosome 21 may be responsible for autoimmunity in DS. The autoimmune regulator gene, AIRE, which is in the minimal region for DS on chromosome 21, regulates the ectopic expression of tissue-restricted antigens in the thymus to expose developing T cells to self-peptides; those that are strongly reactive are removed or reprogrammed (26). Mutations in AIRE cause autoimmune polyendocrine syndrome type 1, which commonly includes endocrine autoimmunity (27). AIRE has been shown to be aberrantly expressed in individuals with DS. A study of infant (0–6 months) thymi removed during cardiac surgery found that AIRE mRNA and protein expression was elevated in DS case subjects ($n = 5$) versus non-DS control subjects ($n = 5$) (28). The mRNA expression of two genes under AIRE's control in medullary thymocytes, INS (encoding insulin) and CHRNA1 (encoding a subunit of muscle acetylcholine receptor), was also increased. Furthermore, this study found an increased overall number of medullary thymocytes expressing AIRE, which the authors propose could be linked to an effect on thymocyte turnover; AIRE is known to promote terminal differentiation in medullary thymocytes (29,30). AIRE has multiple complex roles in thymic function (including Treg selection, antigen expression, cell differentiation, antigen

Table 2—Comparison of DS-PNDM with non-DS PNDM and autoimmune non-DS PNDM

Characteristic	DS-PNDM (n = 13)	Non-DS PNDM (n = 458)	Autoimmune non-DS PNDM (n = 40)
Age at diabetes diagnosis (weeks)	2.3 (0.4, 7.5)	7 (3, 12)	4 (1, 13.5)
Female sex	23% (3 of 13)	43% (197 of 458)	12.5% (5 of 40)*
Birth weight z score†	−1.23 (−2.65, −0.84) [n = 10]	−1.74 (−2.55, −0.82) [n = 378]	−0.39 (−1.06, 0.29) [n = 30]‡
Positive for ≥1 autoantibodies§	4 of 9 (44%)	21 of 293 (7%)‖	13 of 28 (46%)
T1D-GRS	0.61 (0.49, 0.66)	0.55 (0.51, 0.61)	0.57 (0.52, 0.62)

T1D-GRS: centiles based on control subjects with T1D. Age at diabetes diagnosis, birth weight z score, and T1D-GRS are given as median (interquartile range). Other than where indicated, data were similar for all cohorts ($P > 0.1$). *Low due to males with IPEX (immuno-dysregulation polyendocrinopathy enteropathy X-linked) syndrome ($n = 25/40$). †Adjusted for sex and gestational age. ‡DS-PNDM vs. autoimmune non-DS PNDM, $P = 0.02$. §One or more positive titer for GADA, IA-2A, or ZnT8A. ‖DS-PNDM vs. non-DS PNDM, $P = 0.006$.

presentation, and chemokine production [31]), and increased expression of AIRE was counterintuitively postulated to result in an alteration of the balance of these processes and therefore impaired thymic selection and reduced central tolerance resulting in increased autoimmunity.

Two larger studies of the DS thymus found that *AIRE* mRNA expression was reduced twofold in DS thymi and that genes under AIRE's control in medullary thymocytes had reduced expression (32,33). They found that the number of medullary thymocytes positive for AIRE was also reduced. Intriguingly, Giménez-Barcons et al. (33) found that all three copies of *AIRE* present in DS cases were equally expressed, albeit at reduced total levels, suggesting overcompensation by transcriptional repression of all alleles. The patients in these studies were older
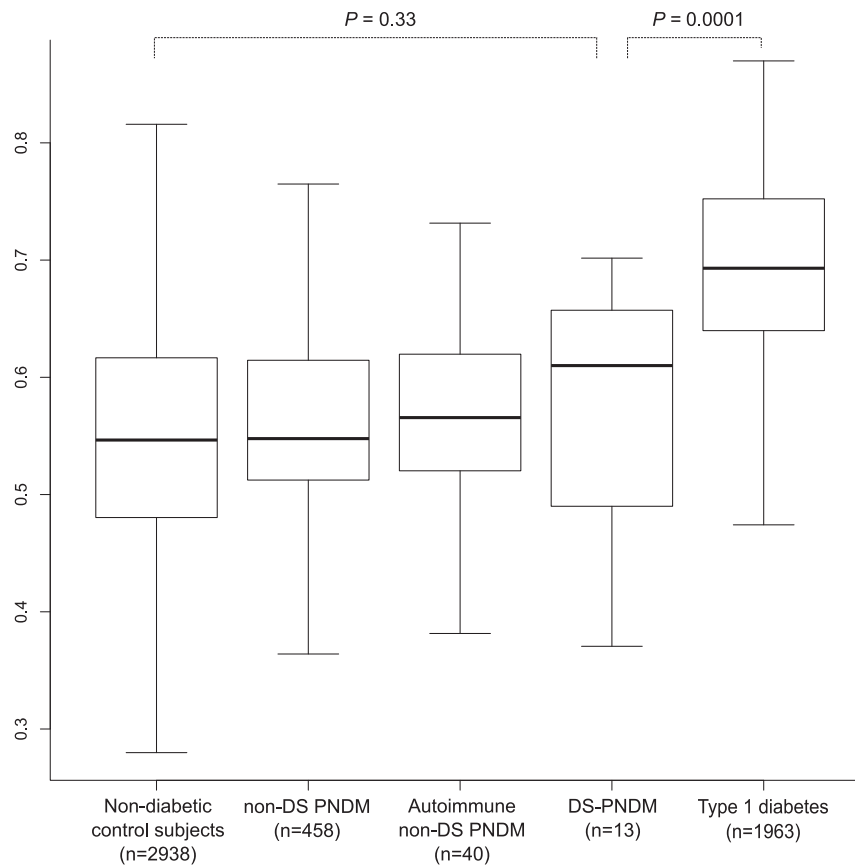

Figure 3—The T1D-GRS in DS-PNDM. Patients with DS-PNDM ($n = 13$) had a lower score than T1D control subjects ($n = 1,963$), and their scores were similar to those with the known monogenic forms of non-DS PNDM and autoimmune non-DS PNDM ($n = 458$ and $n = 40$, respectively; $P = 0.52$) and control subjects without diabetes ($n = 2,938$; $P = 0.33$). The central line within the box represents the median, and the upper and lower limits of the box represent the interquartile range. The whiskers are the most extreme values within $1.5\times$ the interquartile range from the first and second quartiles.

than those in the study by Skogberg et al. (28) (2 months–12 years); therefore, a potential temporal relationship of *AIRE* expression in DS thymus, possibly linked to AIRE's role in differentiation and turnover of thymocytes (29,30), could explain the difference. None of the cases were reported to have diabetes; however, 11 of 19 reported by Giménez-Barcons et al. had organ-specific autoimmunity (hypothyroidism, Graves disease, or celiac disease).

Additional genes contained in chromosome 21 have been linked to the increased incidence of autoimmune diabetes in DS. The *UBASH3A* gene, also on chromosome 21, has been associated (and the association replicated) with T1D and has a role in the regulation of T cells (34). *UBASH3A* downregulates T-cell receptor–induced NFκB signaling (35). NFκB signaling regulates multiple aspects of the innate and adaptive immune system including the expression of IL2 (36), a pleiotropic cytokine whose roles include the regulation of self-tolerance (37). Furthermore, a gene cluster containing four interferon receptors (*IFNAR1, IFNAR2, IFNGR2,* and *IL10RB*) is on chromosome 21 and it has been shown that increased interferon signaling is a hallmark of DS (38). RNA sequencing experiments showed that interferon-related factors were consistently overexpressed in DS lymphocytes compared with controls. Increased interferon signaling has been implicated in multiple autoimmune diseases, including T1D (39–42).

To our knowledge, this is the largest study of DS-PNDM. Study of additional individuals will provide further insight into the underlying mechanism of DS-PNDM. An interesting follow-up would be to look at the T1D-GRS in an older cohort of DS individuals with diabetes to assess whether genetic risk for T1D increases with older age at onset. Furthermore, we were unable to test all islet autoantibodies in all individuals close to diagnosis or perform immunophenotyping on their leukocytes. This would provide further understanding of the autoimmunity in DS-PNDM.

In conclusion, we have shown that trisomy 21 is a cause of PNDM. The underlying mechanism of the diabetes is likely to be due to autoimmunity against the β-cells. We propose that diabetes in DS is heterogeneous and consists of a subtype with diagnosis very young (including PNDM) that is autoimmune but not HLA mediated and a second type that is similar to T1D in the non-DS population and has a strong HLA association. Extended genetic testing of known monogenic diabetes genes for individuals with DS-PNDM beyond the most common forms—activating mutations in *ABCC8* or *KCNJ11*—may not be needed.

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