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Down Syndrome-Associated Diabetes Is Not Due To a Congenital Deficiency in β Cells

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Aims/Hypothesis: We sought to establish whether the increased incidence of diabetes associated with Down syndrome was due to a congenital deficit in β cells.

Methods: The pancreas was obtained at autopsy from nondiabetic subjects with Down syndrome (n = 29) and age-matched nondiabetic control subjects without Down syndrome (n = 28). The pancreas sections were evaluated for the fractional β-cell area.

Results: No difference was found in the fractional β-cell area between the subjects with Down syndrome and the control subjects.

Conclusions/Interpretations: The increased incidence and prevalence of diabetes in individuals with Down syndrome is not due to an underlying congenital deficiency of β cells.

Freeform/Key Words: Down syndrome, β cell, diabetes

Down syndrome, caused by trisomy 21, is a common congenital condition, occurring in approximately 1 in 700 live births. Currently, 250,000 to 400,000 individuals have Down syndrome in the United States, with >5 million cases worldwide. In addition to causing a broad range of developmental anomalies [1, 2], Down syndrome is associated with an increased incidence of autoimmune diseases, including thyroid disorders [3, 4] and celiac disease [5–7], with a well-documented increased risk and prevalence of type 1 diabetes [8–11]. In addition, metabolic syndrome and type 2 diabetes occur at an increased frequency at a relatively early age in those with Down syndrome [12].

Development of an individual's complement of β cells begins during embryonic life and undergoes a rapid postnatal expansion, largely accomplished through replication of the existing β cells [13, 14]. It has been suggested that a risk factor for the development of diabetes might be a failure to establish a sufficient β-cell mass during infancy [15]. β-Cell replication requires nuclear translocation of the transcription factor nuclear factor of activated T cells (NFAT) [16]. Nuclear translocation of NFAT is activated by the phosphatase calcineurin and inhibited by NFAT kinases, which includes dual-specificity tyrosine phosphorylation-regulated kinase 1A [16, 17]. The Down syndrome critical region (DSCR) of chromosome 21 encodes a calcineurin inhibitor, Down syndrome critical region gene 1 (*DSCR1*), and the NFAT kinase dual-specificity tyrosine phosphorylation-regulated kinase 1A [16]. The intriguing hypothesis was posed that the presumptive increased generation of these 2 inhibitors of β-cell replication might suppress β-cell replication

Abbreviations: DSCR, Down syndrome critical region; *DSCR1*, Down syndrome critical region gene 1; NFAT, nuclear factor of activated T cells.

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during infancy, leading to a deficient β-cell mass and, thus, the increased risk of diabetes [16].

To address that hypothesis in humans, in the present study, we evaluated the β-cell area in pancreas specimens obtained at autopsy from child and adult nondiabetic individuals with Down syndrome compared with unaffected controls. Our findings reject the hypothesis that there is a deficiency of β cells in nondiabetic subjects with Down syndrome.

1. Materials and Methods

A. Autopsy Cases

Human pancreatic tissue was obtained at autopsy from 29 nondiabetic individuals with documented Down syndrome during life and 28 nondiabetic, age-matched control individuals without Down syndrome (Tables 1 and 2). The subjects were identified by retrospective analysis of the Mayo Clinic autopsy database. For inclusion in the present study, a full autopsy had to have been performed within 24 hours of death and a sample of pancreatic tissue of adequate size and quality stored. Subjects were excluded if the pancreas integrity had been compromised by either autolysis or acute pancreatitis. None of the subjects selected for inclusion in the present study had had a history of diabetes or any other disease known to affect the pancreas. The subject characteristics and diagnoses leading to death are presented in Tables 1 and 2. The institutional review board of the Mayo Clinic and the University of California, Los Angeles, approved the present study. Fasting blood glucose values in health were unavailable from the included subjects. Our presumption that neither the subjects with Down syndrome nor the control subjects had diabetes was determined by an absence of a history of diabetes in the previous medical records and an absence of diabetes documented in their final illness.

Because of the substantial changes in the fractional β-cell area during the rapid growth phase of childhood [16], the subjects were grouped into 3 age brackets: <5 years (Down syndrome, n = 14; control, n = 12), 5 to 15 years (Down syndrome, n = 5; control, n = 5), and >15 years (Down syndrome, n = 10; control, n = 11).

B. Pancreatic Tissue Processing

All autopsies were performed at the Mayo Clinic, where a sample of the tail of the pancreas measuring approximately $2.0 \times 1.0 \times 0.5$ cm in size was resected and, together with a sample of spleen, was fixed in formaldehyde before being embedded in paraffin. Next, 5-μm sections were obtained from these tissue blocks and stained for insulin (peroxidase staining) and hematoxylin for light microscopy. For immunohistochemical staining, the primary antibody used was guinea pig anti-insulin (1:200; Dako Laboratories, Carpinteria, CA; Research Resource Identification, AB_2617169).

C. Morphometric Analysis

The pancreatic fractional β-cell area was determined by imaging the entire pancreatic section at $\times 40$ magnification ($4\times$ objective). The ratio of the β-cell area to the exocrine pancreatic area was digitally quantified, as previously described [18], using Image Pro Plus software (Image Pro Plus, version 4.5.1; Media Cybernetics, Silver Springs, MD). By digitally excluding the interlobular connective tissue, large blood vessels, and adipocytes, the analysis concentrated on the ratio of the pancreatic islets to the acinar tissue. Two independent observers (A.E.B. and W.S.) performed the analysis. If the interobserver measurements in a sample differed by $>5\%$, the sample was reevaluated.

D. Statistical Analysis

The data are presented as the mean $\pm$ standard error. The statistical calculations were performed using GraphPad Prism, version 5 (GraphPad Software, San Diego, CA).

Table 1. Clinical Characteristics of Subjects With Down Syndrome

Pt. No.	Sex	Age	Age (y)	BCA (%)	Cause of Death
Age <5 y					
1	Male	Newborn	0.00	1.57	Fetal distress
2	Female	4 d	0.01	5.02	Aspiration pneumonia
3	Female	12 d	0.03	3.33	Congenital heart disease
4	Female	3 mo	0.25	1.49	Congenital heart disease
5	Male	4 mo	0.33	1.93	Congenital heart disease
6	Male	4 mo	0.33	1.93	Congenital heart disease
7	Female	4 mo	0.33	1.10	Congenital heart disease
8	Female	5 mo	0.42	1.70	Congenital heart disease
9	Female	6 mo	0.50	3.24	Congenital heart disease
10	Female	7 mo	0.58	5.56	Congenital heart disease
11	Female	9 mo	0.75	2.07	Congenital heart disease
12	Female	9 mo	0.75	2.38	Congenital heart disease
13	Female	17 mo	1.42	3.43	Congenital heart disease
14	Male	22 mo	1.83	2.16	Chronic bronchiolitis
Mean			**0.54**	**2.64**	
SEM			**0.14**	**0.36**	
Age 5–15 y		NA			
1	Male		10	2.48	Congenital heart disease
2	Female		5	1.07	Congenital heart disease
3	Male		9	1.86	Congenital heart disease
4	Male		9	2.68	Congenital heart disease
5	Male		13	2.00	Respiratory insufficiency
Mean			**9.20**	**2.02**	
SEM			**1.28**	**0.28**	
>15 y		NA			
1	Female		19	1.53	Congenital heart disease
2	Female		33	1.22	Congenital heart disease
3	Male		34	2.29	Right ventricular hypertrophy
4	Male		38	3.61	Congenital heart disease
5	Female		47	0.79	Respiratory failure
6	Female		48	1.14	Bronchopneumonia
7	Female		56	1.39	Hemorrhage
8	Male		57	2.70	Bronchopneumonia
9	Male		62	0.96	Bronchopneumonia
10	Female		62	0.88	Bronchopneumonia
Mean			**45.60**	**1.65**	
SEM			**4.52**	**0.29**	

Abbreviations: BCA, beta cell area; NA, not applicable; Pt. No., patient number; SEM, standard error of the mean.

2. Results

A. Age

In each of the 3 defined age brackets, the ages of the Down syndrome group were matched with the ages of the control group [Down syndrome vs control, age <5 years, 0.54 ± 0.14 vs 0.57 ± 0.13 years; age 5 to 15 years, 9.2 ± 1.3 vs 9.6 ± 1.4 years; age >15 years, 45.6 ± 4.5 vs 40.5 ± 4.5 years; Fig. 1 and Fig. 2(A)].

B. Fractional β-Cell Area

No difference was found in the fractional β-cell area between the subjects with Down syndrome and the control subjects in the 3 defined age brackets studied [Down syndrome vs control, age <5 years, 2.64% ± 0.36% vs 2.86% ± 0.37%; age 5 to 15 years, 2.02% ± 0.28% vs 1.84% ± 0.52%; age >15 years, 1.65% ± 0.29% vs 1.99% ± 0.24%; Fig. 1 and Fig. 2(B)].

Table 2. Clinical Characteristics of Control Subjects

Pt. No.	Sex	Age	Age (y)	BCA (%)	Cause of Death
Age <5 y					
1	Female	7 d	0.02	2.92	Pneumothorax
2	Female	18 d	0.05	2.94	Duodenal atresia
3	Female	2.5 mo	0.21	2.75	Sudden infant death syndrome
4	Female	3 mo	0.25	4.54	Respiratory failure
5	Male	4 mo	0.33	4.38	Congenital heart disease
6	Male	4 mo	0.33	3.99	Congenital heart disease
7	Female	7 mo	0.58	4.71	Congenital heart disease
8	Female	8 mo	0.67	2.07	Congenital heart disease
9	Female	9 mo	0.75	1.07	Congenital heart disease
10	Female	11 mo	0.92	1.82	Congenital heart disease
11	Female	14 mo	1.17	2.02	Bronchopneumonia
12	Male	18 mo	1.50	1.16	Acute nonlymphoblastic leukemia
Mean			**0.57**	**2.86**	
SEM			**0.13**	**0.37**	
Age 5–15 y		NA			
1	Female		5	1.67	Congenital heart disease
2	Male		9	1.34	Congenital heart disease
3	Male		9	3.87	Acute lymphoblastic leukemia
4	Male		12	0.93	Acute encephalopathy
5	Male		13	1.39	Hepatitis
Mean			**9.60**	**1.84**	
SEM			**1.40**	**0.52**	
Age >15 y		NA			
1	Female		19	2.39	Bronchopneumonia
2	Female		19	1.29	Accidental poisoning
3	Female		33	3.76	Lymphoma
4	Male		33	1.03	Respiratory arrest
5	Male		34	2.17	Teratocarcinoma
6	Male		38	1.57	Cardiac arrhythmia
7	Female		47	1.31	Respiratory arrest
8	Male		48	2.78	Acute myocardial infarction
9	Female		48	2.14	Breast adenocarcinoma
10	Female		62	1.81	Bronchopneumonia
11	Male		64	1.67	Chronic ischemic heart disease
Mean			**40.45**	**1.99**	
SEM			**4.53**	**0.24**	

Abbreviations: BCA, beta cell area; NA, not applicable; Pt. No., patient number; SEM, standard error of the mean.

3. Discussion

We examined pancreas across a range of individuals with Down syndrome to test the hypothesis that the pancreatic β-cell area would be decreased and thus predispose these subjects to development of type 2 diabetes.

The intriguing hypothesis proposed by Shen *et al.* [16] was that the β-cell mass in those with Down syndrome would be deficient owing to suppression of the usual high β-cell replication in infancy through an increased dosage of genes in the DSCR. An alternative cause of the decreased β-cell mass in Down syndrome could be the low birth weight commonly present with this syndrome [19]. A low birth weight predicts an increased risk of type 2 diabetes [14], and a low β-cell mass arises after induced placental dysfunction in animal studies [20, 21]. However, the fractional pancreatic β-cell area was normal in children with Down syndrome.

Just as with all studies, the present study had limitations that should be considered. The sample size was small; thus, small differences could have been overlooked. None of the pancreata examined in the present study came from individuals with diabetes. It is

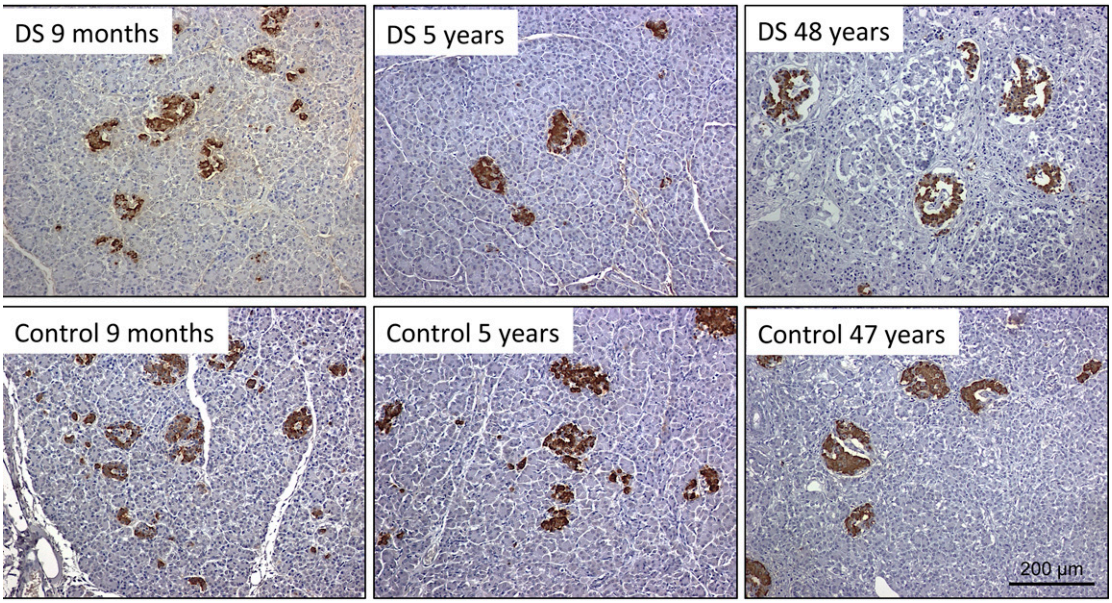

Figure 1. Representative images from pancreatic sections of (upper panels) individuals with Down syndrome (DS) and (lower panels) age-matched control subjects. Insulin-positive β cells are shown in brown (3,3′-diaminobenzidine) with hematoxylin counterstain. In early childhood (left panels), the islet density was greater, with more small clusters of insulin-positive cells compared with later childhood (middle panels) or adulthood (right panels). No difference was found in the β-cell mass between subjects with and without DS. Images were taken at ×200 magnification (20× objective). Scale bar = 200 μm.

plausible that abnormally decreased β-cell growth is present only in the subset of individuals with an increased risk of diabetes. We did not have the whole pancreas available to evaluate nor, therefore, the pancreas mass. It is possible that if the pancreata were much smaller in those with Down syndrome, the total β-cell mass would be decreased, despite a normal β-cell fractional area. We were unable to find any reports of pancreatic size in those with Down syndrome. We were unable to assess the β-cell replication, because the Ki67 marker was unreliable in these sections of pancreas from blocks stored long term.

However, other potential contributors to the increased predisposition to the development of diabetes in the setting of Down syndrome exist. Functional β-cell defects can arise as a consequence of excessive 21 gene dosage [22]. Individuals with Down syndrome might be more susceptible to enteroviral infections, which might predispose these individuals to the development of type 1 diabetes [23]. Epigenetic dysregulation of β cells in those with Down syndrome has also been proposed [24]. The high prevalence of obesity in those with Down syndrome is a predisposing factor for type 2 diabetes and, through endoplasmic stress, might increase the risk of the development of type 2 diabetes [25].

4. Conclusion

The pancreata from this nondiabetic group of subjects with Down syndrome did not have a deficit in the fractional β-cell area. Rather than a low number of β cells as the underlying trigger, the increased risk of type 1 diabetes in those with Down syndrome likely relates to defects in the immune system that increases the frequency of other related autoimmune disorders such as celiac disease and Hashimoto thyroid disease. Furthermore, the increased risk for the development of type 2 diabetes is presumably related to the increased incidence of central adiposity and metabolic syndrome.

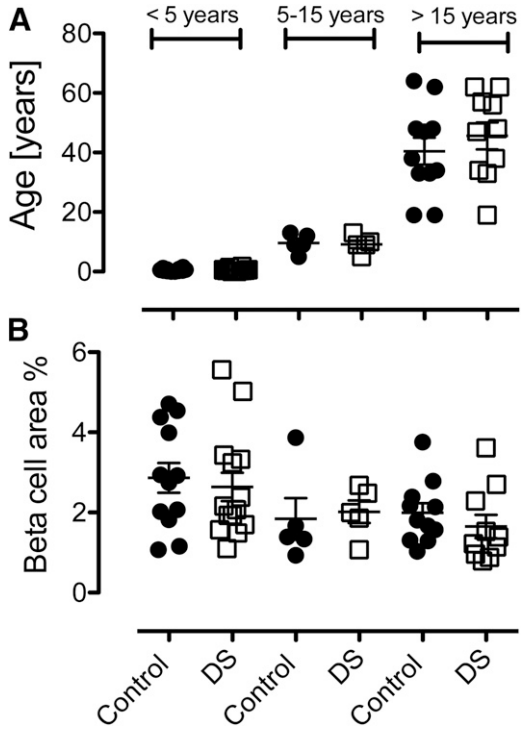

Figure 2. Age and fractional β-cell area percentage for nondiabetic subjects with Down syndrome (DS) and age-matched nondiabetic controls. (A) In each of the 3 defined age brackets, no difference was found between the ages of the Down syndrome group and the ages of the control group (Down syndrome vs control, age <5 years, 0.54 ± 0.14 vs 0.57 ± 0.13 years; age 5 to 15 years, 9.20 ± 1.28 vs 9.60 ± 1.40 years; age >15 years, 45.60 ± 4.52 vs 40.45 ± 4.53 years). (B) No difference was found in the fractional β-cell area between the subjects with Down syndrome and the control subjects in the 3 defined age brackets studied (Down syndrome vs control, age <5 years, 2.64% ± 0.36% vs 2.86% ± 0.37%; age 5 to 15 years, 2.02% ± 0.28% vs 1.84% ± 0.52%; age >15 years, 1.65% ± 0.29% vs 1.99% ± 0.24%).

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