GATA6 Mutations Cause a Broad Phenotypic Spectrum of Diabetes From Pancreatic Agenesis to Adult-Onset Diabetes Without Exocrine Insufficiency

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We recently reported de novo *GATA6* mutations as the most common cause of pancreatic agenesis, accounting for 15 of 27 (56%) patients with insulin-treated neonatal diabetes and exocrine pancreatic insufficiency requiring enzyme replacement therapy. We investigated the role of *GATA6* mutations in 171 subjects with neonatal diabetes of unknown genetic etiology from a cohort of 795 patients with neonatal diabetes. Mutations in known genes had been confirmed in 624 patients (including 15 *GATA6* mutations). Sequencing of the remaining 171 patients identified nine new case subjects (24 of 795, 3%). Pancreatic agenesis was present in 21 case subjects (six new); two patients had permanent neonatal diabetes with no enzyme supplementation and one had transient neonatal diabetes. Four parents with heterozygous *GATA6* mutations were diagnosed with diabetes outside the neonatal period (12–46 years). Subclinical exocrine insufficiency was demonstrated by low fecal elastase in three of four diabetic patients who did not receive enzyme supplementation. One parent with a mosaic mutation was not diabetic but had a heart malformation. Extrapancreatic features were observed in all 24 probands and three parents, with congenital heart defects most frequent (83%). Heterozygous *GATA6* mutations cause a wide spectrum of diabetes manifestations, ranging from pancreatic agenesis to adult-onset diabetes with subclinical or no exocrine insufficiency. ***Diabetes*** **62:993–997, 2013**

Exome sequencing recently led to the discovery that heterozygous *GATA6* mutations are the most common cause of pancreatic agenesis, accounting for 15 of 27 patients with neonatal diabetes (NDM) requiring insulin treatment and exocrine pancreatic insufficiency requiring enzyme replacement therapy (1). This finding supports a previously unrecognized essential function of GATA6 in human pancreatic development. Extrapancreatic features are common and include biliary tract defects and gut and other endocrine abnormalities. Fourteen of the 15 patients had a congenital cardiac malformation, consistent with previous reports of *GATA6* mutations causing structural heart defects (2–4).

NDM is a rare disorder, with a reported incidence of 1:100,000 (5); a genetic diagnosis is possible in 60–75% of cases. The transient form (TNDM), which accounts for ~50% of cases, is most frequently due to abnormalities of the paternally expressed genes on chromosome 6q24 (6). Mutations in the potassium channel genes *KCNJ11* and *ABCC8* and in the *INS* gene encoding insulin also result in TNDM and are the most common causes of permanent neonatal diabetes (PNDM) (7–12).

Mutations in genes encoding transcription factors that are critical for pancreatic development can cause syndromic NDM. These include *HNF1B* (13), *GLIS3* (14), *RFX6* (15), *NEUROD1* (16), and *NEUROG3* (17). Each genetic subtype is characterized by specific extrapancreatic developmental defects.

Complete absence of the pancreas can result from biallelic *PDX1* or *PTF1A* mutations (18,19) or from heterozygous *GATA6* mutations (1). The PDX1 and PTF1A transcription factors are both expressed early during pancreatic development and actively regulate the differentiation of pancreatic precursors toward an endocrine or exocrine fate (20). Hypomorphic *PDX1* mutations have subsequently been identified in patients with PNDM without clinical symptoms of exocrine deficiency (21).

In this study, we undertook *GATA6* mutation screening in 171 subjects with NDM of unknown genetic etiology from a cohort of 795 patients with NDM and describe the spectrum of pancreatic and extrapancreatic phenotypes in 29 case subjects with *GATA6* mutations.

RESEARCH DESIGN AND METHODS

Subjects. Patients were selected from an international cohort of 795 case subjects from 71 countries with diabetes diagnosed before 6 months of age. Pancreatic agenesis, defined as diabetes needing insulin and pancreatic exocrine insufficiency requiring enzyme supplementation therapy, was diagnosed in 39 of 795 case subjects. We previously studied 27 of these 39 case subjects with pancreatic agenesis (1). A genetic cause was identified in 624 of the 795 case subjects; these include 15 patients with pancreatic agenesis who are heterozygous for a *GATA6* mutation (1). Mutations in *PTF1A* and *PDX1* were excluded in all the patients with pancreatic agenesis.

We sequenced *GATA6* in the remaining 171 patients in whom no genetic etiology had been identified. These included 24 case subjects with pancreatic agenesis (NDM requiring insulin and pancreatic exocrine insufficiency requiring enzyme supplementation therapy). NDM without exocrine insufficiency was reported in 147 patients: 126 with PNDM and 21 with TNDM. Mutations in *ABCC8*, *KCNJ11*, *INS*, and chromosome 6q24 abnormalities had been excluded.

Clinical information was provided by the referring clinicians via a NDM request form (available at www.diabetesgenes.org) or from clinical notes. The study was conducted in accordance with the Declaration of Helsinki principles with informed parental consent given on behalf of children.

Molecular genetic analysis. We screened the coding sequence and ~50 bp of flanking sequence of *GATA6* using Sanger sequencing as described previously (primers) (1). Sequencing reactions were run on an ABI3730 capillary machine (Applied Biosystems, Warrington, U.K.) and analyzed using Mutation Surveyor v3.98 (SoftGenetics, State College, PA) (*GATA6* nucleotide reference NM_005257.3).

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The bioinformatics tools SIFT, PolyPhen-2, and Align GVGD were used to predict the effect of novel variants on the GATA6 protein (protein reference NP_005248.2).

Microsatellite analysis of parent/proband trios using the PowerPlex kit (Promega, Southampton, U.K.) was used to confirm apparently de novo mutations. The parental origin of de novo mutations in pedigrees with inherited mutations was investigated when grandparental samples were available by analyzing microsatellites D18S1149, D18S1104, D18S1067, D18S1001, D18S869, and D18S1002.

RESULTS

GATA6 mutations were identified in 9 of 171 probands and five parents. Six of the probands have pancreatic agenesis. In total, we have now identified *GATA6* mutations in 21 of 39 (54%) patients with pancreatic agenesis (6 case subjects in this study and 15 reported by Lango Allen et al. [1]). Three of 147 patients (2%) with NDM but not on exocrine supplementation were heterozygous for *GATA6* mutations; 2 have permanent diabetes, and 1 has transient episodes of hyperglycemia.

***GATA6* mutations.** The nine novel mutations include three missense changes (p.C447R, p.G469E, and p.R479G) and four splicing, one frameshift, and one nonsense mutation. In total, 23 different *GATA6* mutations have been identified in patients with diabetes (p.R456C was present in two unrelated patients) (Fig. 1). All missense mutations affect highly conserved residues within the second zinc-finger domain, which is needed for DNA binding and protein-protein interaction. None of the mutations were present in the dbSNP137, 1000 Genomes (March 2012 release, based on 1,197 individuals), or Exome Variants Server (June 2012 release, based on 6,503 individuals) databases. They were predicted to affect protein function by at least two of the bioinformatics predictive tools used (Supplementary Table 1). Three mutations affect conserved splice sites, and in silico analysis suggested aberrant splicing for the intronic c.1303–10C>G, c.1516+4A>G, c.1429–8T>G, and c.1429–41_1441del mutations (see Supplementary Table 2).

Parental DNA samples were available for eight of nine previously unreported patients. Mutations were shown to have arisen de novo in three patients and been inherited from a parent in five case subjects (see Fig. 2). The analysis of grandparental samples in families ISPAD 207, ISPAD 208, ISPAD 209, and ISPAD 212 showed that the mutation had arisen de novo in the parent on the grandmaternal allele in three case subjects and on the grandpaternal allele in the remaining one. The level of the *GATA6* mutation in one parent (ISPAD 208-2) was estimated at ~20% in leukocyte DNA.

Diabetes phenotype. The median age at diagnosis of diabetes for the 24 probands with *GATA6* mutations was 2 days (IQR 1–7 days) and median birth weight was 1,588 g (SDS = −3.22, <1st percentile). Pancreatic imaging had been undertaken in 18 of 21 pancreatic agenesis case subjects and showed complete absence (n = 8) or marked hypoplasia (n = 10) of the pancreas. Measurement of fecal elastase or fecal fat in 15 case subjects showed severe exocrine pancreatic insufficiency.

The two probands with PNDM but not requiring treatment with pancreatic enzyme supplementation were diagnosed at 1 day (c.1303–1G>T) or 1 week (p.C447R) of age, and both have been treated with insulin since diagnosis. Although they had never received enzyme supplements, fecal elastase measurement in ISPAD 208 showed exocrine insufficiency (<15 μg/g, normal range >200 μg/g).

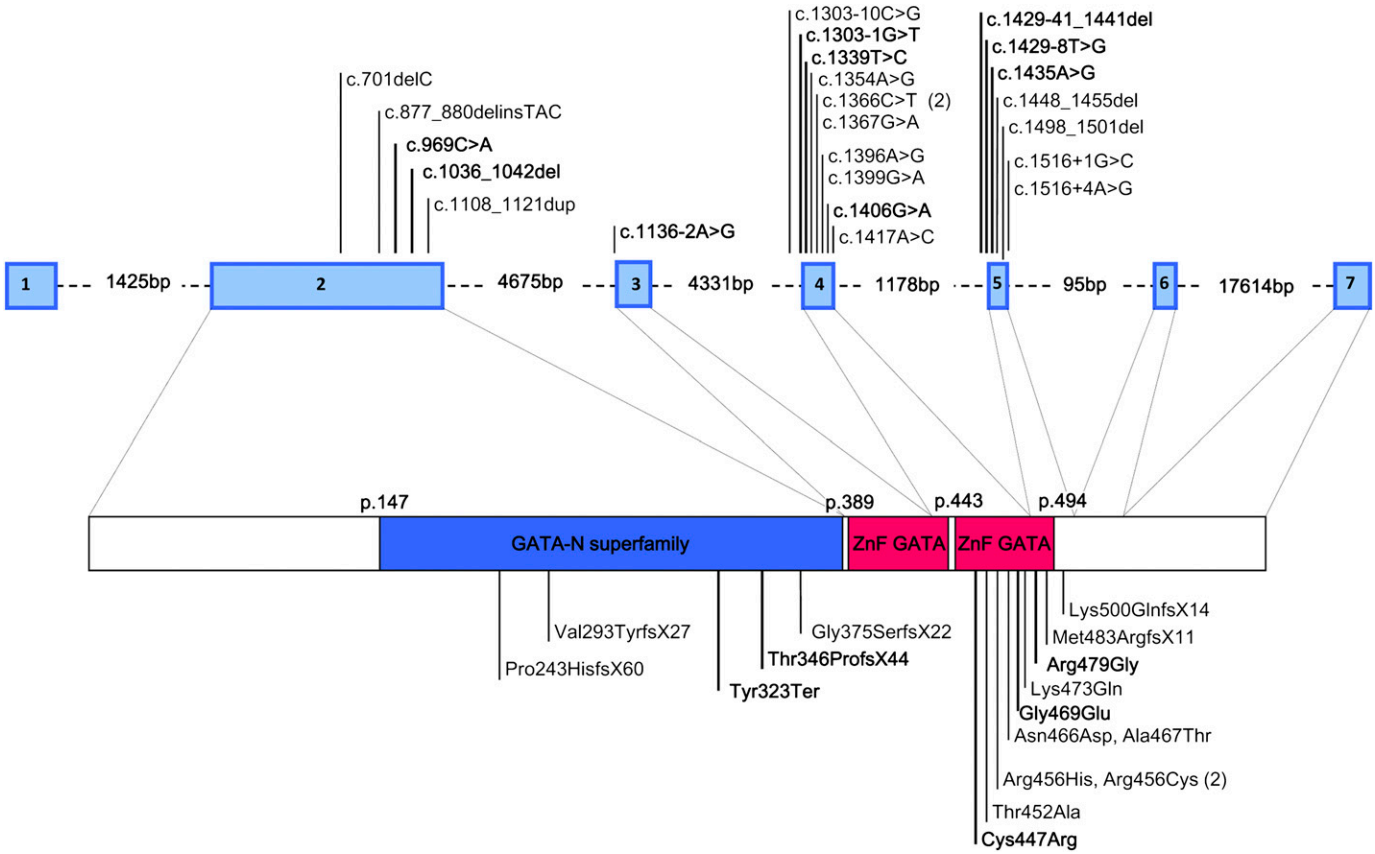

FIG. 1. Mutations diagram. Genomic and protein positions of the 23 *GATA6* mutations. The nine novel mutations are highlighted in bold.

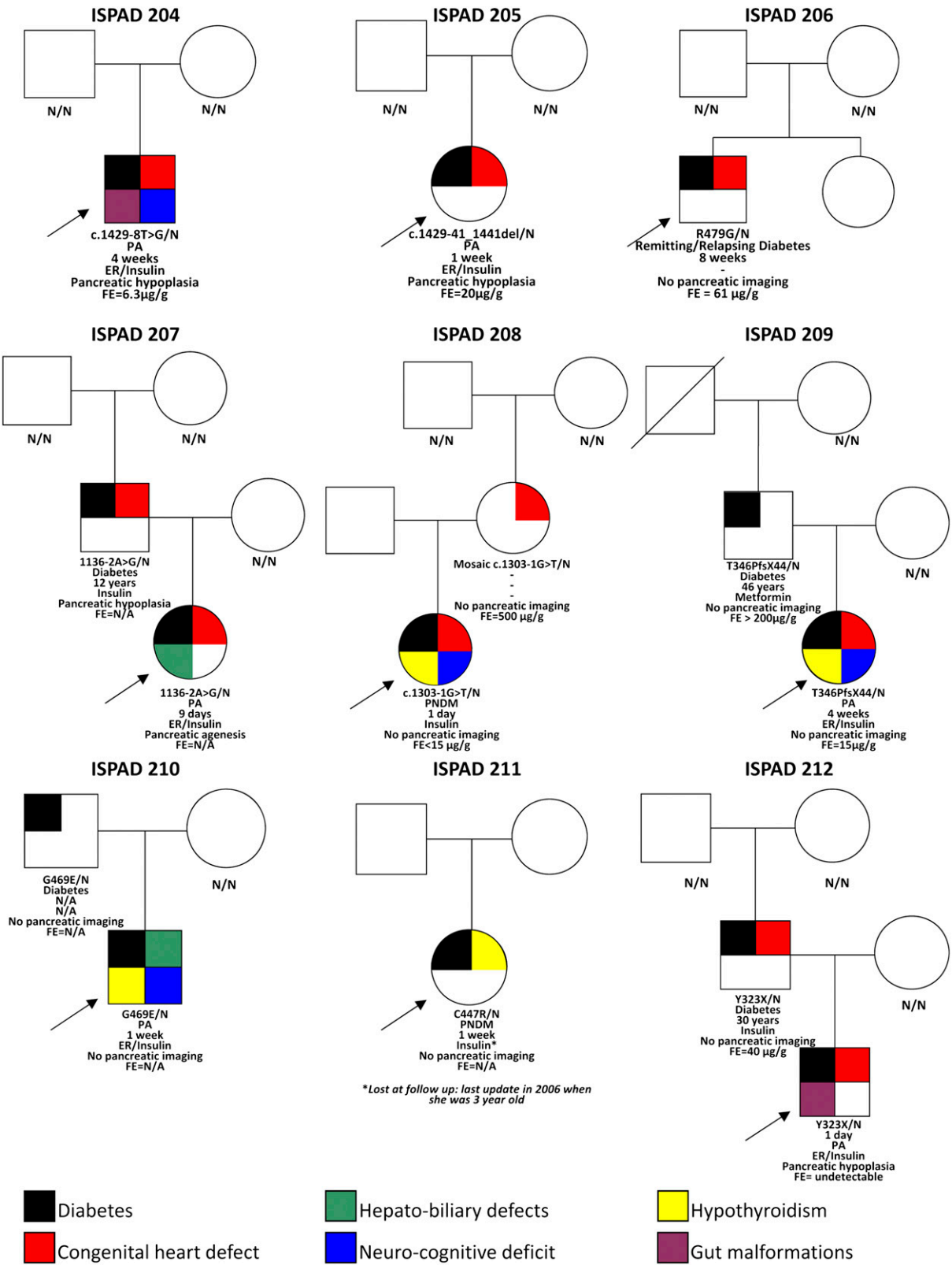

FIG. 2. Pedigrees. Partial pedigrees of the nine novel case subjects reported. Arrows indicate probands. The genotype is shown underneath each symbol. N/N denotes no mutation identified. Below the genotype is the type of diabetes (PA, pancreatic agenesis), age of diagnosis, treatment (ER, enzyme replacement), pancreatic imaging result, and fecal elastase (FE) measurement (N/A, not available). Arrows indicate the proband. Colors indicate phenotypic features: black, diabetes; red, congenital heart defect; green, hepatobiliary defects; blue, neurocognitive defects; yellow, hypothyroidism/hypopituitarism; purple, gut malformations.

The patient with the de novo p.R479G mutation (ISPAD 206) was diagnosed with diabetes at age 8 weeks (glucose 28 mmol/L) and treated with insulin for 1 week during an enteroviral meningitis infection. During the past 4 years, he has twice presented with hyperglycemia, during a rotavirus infection and after surgery to repair his congenital heart defect. Now 5 years of age, his fecal elastase level is low (61 μg/g, normal range >200 μg/g), but he is not receiving insulin or pancreatic enzyme supplementation treatment.

Five patients had inherited their *GATA6* mutation from a parent (Fig. 2). The parent with a mosaic mutation is not diabetic at 43 years of age, but the four heterozygotes were diagnosed with diabetes at 12–46 years of age and treated with insulin (three case subjects) or metformin. The parent diagnosed at 12 years of age tested negative for GAD autoantibodies and is receiving a nonreplacement dose of insulin (0.35 units/kg/day). Fecal elastase levels were measured in three parents and showed subclinical exocrine insufficiency (40 μg/g) in one of them (ISPAD 212-02).

Extrapancreatic features. All the patients with NDM and three of five parents with a *GATA6* mutation also show extrapancreatic features (see Table 1 and Fig. 2). Congenital heart defects are most common, present in 21 of 24 probands and three parents (83%), and required surgical correction in 19 case subjects.

Additional extrapancreatic features in the probands include congenital hypothyroidism (n = 6), hepatobiliary malformations (in particular gallbladder agenesis and biliary atresia, n = 5), and gut abnormalities (intestinal malrotation and hernias, n = 6). Eleven patients also have a significant neurocognitive deficit. Three parents had cardiac malformations, but no other features were reported.

DISCUSSION

This study of a large cohort of patients with NDM confirms that *GATA6* mutations are the major cause of pancreatic agenesis (1). Familial studies showed that heterozygous *GATA6* mutations can also cause adolescent/adult-onset diabetes. Four parents were diagnosed with diabetes at 12–46 years of age and treated with insulin (n = 3) or metformin, and none had received pancreatic enzyme supplements. Fecal elastase measurement in two diabetic parents showed subclinical exocrine insufficiency in one and a normal level in the other. Our data therefore extend the diabetic phenotypic spectrum of *GATA6* mutations from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency.

TABLE 1
Phenotypic manifestations in 29 case subjects with a *GATA6* mutation (24 probands and 5 parents)

Clinical features	Patients with *GATA6* mutation, n = 29
Pancreatic exocrine insufficiency	
Enzyme supplementation	21 (72%)
Enzyme supplementation or low fecal elastase	25* (93%)
Extrapancreatic features	
Cardiac malformation	24 (83%)
Cardiac malformation requiring surgery	19 (66%)
Hypothyroidism/hypopituitarism	7 (24%)
Gallbladder agenesis/biliary atresia	5 (17%)
Significant neurocognitive deficit	11 (38%)
Gut abnormalities	6 (21%)

Data are n (%). *25 of 27; 2 patients were not tested.

This study describes 29 subjects (24 probands and 5 parents) with *GATA6* mutations from an international cohort of 795 subjects with NDM Within this large cohort, *GATA6* mutations account for at least 3% of NDM cases. Of 24 probands with *GATA6* mutations, 21 had pancreatic agenesis, and *GATA6* mutations were present in 21 of 39 (54%) patients with pancreatic agenesis. Mutations were also identified in three patients with NDM who were not receiving enzyme supplementation; fecal elastase was measured in two case subjects and showed subclinical exocrine insufficiency.

The mutation in the four diabetic parents appeared heterozygous in leukocyte DNA samples, consistent with a spontaneous mutation in a parental germ cell rather than a postzygotic mosaic mutation in the developing embryo. One parent was shown to be a somatic mosaic (suggesting a postzygotic event) and was not diabetic at 43 years of age, although she had surgery to correct a patent ductus arteriosus as a child. Her risk of developing diabetes will depend on the mutational load in the pancreas.

These data confirm previous evidence of the essential function of *GATA6* in the development of the human pancreas. In mouse models, *GATA6* expression was significantly increased during endocrine differentiation (22), suggesting that it might also be involved in the last stages of β-cell development. The variation in the pancreatic phenotype is also seen with heterozygous mutations in *HNF1B*, another β-cell transcription factor associated with early pancreatic development (23).

Consistent with the initial discovery of *GATA6* mutations as a rare cause of isolated congenital heart defects (2–4), cardiac malformations were the most common extrapancreatic feature (83% of case subjects with a *GATA6* mutation) in our cohort. Studies in mouse models have confirmed the role of GATA6 as one of the master regulators of heart formation during embryo development (24). Our study extends the phenotypic spectrum of *GATA6* mutations from isolated congenital heart defects to pancreatic agenesis with cardiac malformations and adult-onset diabetes with/without heart defects.

In addition to congenital heart defects, a wide range of extrapancreatic malformations were observed in patients with *GATA6* mutations. The most common features were hypothyroidism, hepatobiliary malformations (mostly gallbladder agenesis and biliary atresia), and gut abnormalities (mostly congenital hernias). All these defects affect organs of endodermal origin, suggesting a defect in early embryonic differentiation. *Gata6*$^{-/-}$ mice die early during embryo development (embryonic day 6.5–7.5) and show a specific defect in endodermal differentiation, including severely downregulated expression of endodermal markers such as Gata4, Hnf4a, Hnf3b, and Afp (25). Our data suggest a role for GATA6 in the development of endodermal-derived organs in humans too.

This study shows that *GATA6* mutations cause a variable diabetic phenotype, ranging from pancreatic agenesis to adult-onset diabetes. Most, but not all, patients have exocrine insufficiency and other extrapancreatic features. Further work is needed to establish the prevalence of *GATA6* mutations as a cause of monogenic diabetes presenting outside the neonatal period.

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E.D.F. researched the molecular genetics data, contributed to discussion, and wrote the manuscript. C.S.-S. and M.H.S. researched the clinical data, contributed to discussion, and reviewed the manuscript. S.E.F. researched the molecular genetics data, contributed to discussion, and reviewed the manuscript. The International NDM Consortium authors identified the patients, researched the clinical data, and reviewed the manuscript. A.T.H. directed the research, contributed to discussion, and reviewed the manuscript. S.E. directed the research, contributed to discussion, and wrote the manuscript. A.T.H. 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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