Homozygous Mutations in NEUROD1 Are Responsible for a Novel Syndrome of Permanent Neonatal Diabetes and Neurological Abnormalities

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OBJECTIVE—NEUROD1 is expressed in both developing and mature β-cells. Studies in mice suggest that this basic helix-loop-helix transcription factor is critical in the development of endocrine cell lineage. Heterozygous mutations have previously been identified as a rare cause of maturity-onset diabetes of the young (MODY). We aimed to explore the potential contribution of NEUROD1 mutations in patients with permanent neonatal diabetes.

RESULTS—Two homozygous mutations in NEUROD1 (c.427_428del and c.364dupG) were identified in two patients. Both mutations introduced a frameshift that would be predicted to generate a truncated protein completely lacking the activating domain. Both patients had permanent diabetes diagnosed in the first 2 months of life with no evidence of exocrine pancreatic dysfunction and a morphologically normal pancreas on abdominal imaging. In addition to diabetes, they had learning difficulties, severe cerebellar hypoplasia, profound sensorineural deafness, and visual impairment due to severe myopia and retinal dystrophy.

CONCLUSIONS—We describe a novel clinical syndrome that results from homozygous loss of function mutations in NEUROD1. It is characterized by permanent neonatal diabetes and a consistent pattern of neurological abnormalities including cerebellar hypoplasia, learning difficulties, sensorineural deafness, and visual impairment. This syndrome highlights the critical role of NEUROD1 in both the development of the endocrine pancreas and the central nervous system in humans. Diabetes 59:2326–2331, 2010

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Monogenic permanent neonatal diabetes (PNDM) is typically diagnosed within the first 6 months of birth in contrast to polygenic autoimmune type 1 diabetes, which is usually diagnosed later in childhood or in young adults (1,2). PNDM is both phenotypically and genetically heterogeneous. Most patients present with isolated diabetes, but in some cases diabetes appears in the context of a more complex multisystemic syndrome. Dominant mutations in three genes (KCNJ11, ABCC8, and INS) are the cause of PNDM in ~50% of cases, and in the majority diabetes is an isolated finding (3,4). Recessive mutations, autosomal or X-linked, have been described in 10 genes (ABCC8, GCK, EIF2AK3, FOXP3, IPF1, PTF1A, GLIS3, SLC2A2, SCL19A2, and WFS1). These are rare and often result in extrapancreatic features in addition to neonatal diabetes (3). The genetic cause remains unknown in up to 40% of patients with PNDM (4).

From a pathogenetic perspective, a number of different mechanisms can lead to PNDM. Firstly, β-cells may be present but not functional as in patients with activating mutations in KCNJ11 and ABCC8, the genes encoding the two subunits of the ATP-sensitive K⁺ channel (Kir6.2 and SUR1, respectively). Secondly, the number of β-cells may be reduced due to an increased destruction, either by apoptosis (INS and EIF2AK3) or as a consequence of an autoimmune insult (FOXP3). Finally, there may be a reduced number of β-cells as a result of impaired pancreatic development, affecting either the whole pancreas (IPF1 and PTF1A) or endocrine cells (GLIS3) specifically (3).

Pancreatic development is coordinated by a complex interplay of signaling pathways and transcription factors that determine early pancreatic specification as well as the later differentiation of exocrine and endocrine lineages (5,6). The basic helix-loop-helix (bHLH) transcription factor NEUROD1 (also known as BETA2) plays an important role in the development of the endocrine pancreas. NEUROD1 expression, along with NEUROG3 and INSM1, specifies the endocrine lineage (7). Neurod1⁻/⁻ mice fail to develop mature islets, leading to ketosis-prone diabetes and death within the first few days of life (8).

Heterozygous loss-of-function mutations in NEUROD1 have previously been identified as a very rare cause of maturity-onset diabetes of the young (MODY) and late-onset diabetes in humans, with only five families reported to date (9–12). We assessed the role of NEUROD1 in PNDM and describe two unrelated probands with homozy-
gous truncating NEUROD1 mutations who have PNDM and similar neurological abnormalities.

RESEARCH DESIGN AND METHODS

This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the local ethics committee, and written informed consent was obtained from the parents or guardians of each patient. Study population. We studied 44 probands with PNDM diagnosed before 6 months of age, who had been referred to the Molecular Genetics Laboratory at the Peninsula Medical School in Exeter, U.K. Mutations in KCNJ11, ABCC8, INS, and GCK had been excluded. The relevant clinical information was obtained from the medical records.

NEUROD1 gene analysis. Genomic DNA was extracted from peripheral leukocytes using standard procedures. The single coding exon of NEUROD1 was PCR amplified in three overlapping fragments using specific primers for each amplicon tagged with 5’ M13 tails to allow sequencing to be performed with a universal M13 primer (primers and conditions available upon request). Single-strand sequencing was carried out using standard methods on an ABI 3730 sequencer (Applied Biosystems, Warrington, U.K.). Sequences were compared with the published template ( accession no. NM_002500) using Mutation Surveyor (version 3.20; SoftGenetics). Sequence variants were tested for their presence in family members whenever DNA was available.

Homozygosity mapping. High-density single nucleotide polymorphism (SNP) genotyping was carried out on the Affymetrix human 10K Xba chip by Medical Solutions Nottingham (formerly GeneService) (Nottingham, U.K.). Processing of genomic DNA was performed in accordance with the Affymetrix protocol. In-house Perl scripts were developed to automatically identify genomic homozygous segments, defined by at least 20 consecutive homozygous SNPs marking a region that exceeded 3 cM (13).

RESULTS

Molecular genetics. Two novel homozygous mutations in NEUROD1, a single base pair duplication (c.364dupG) and a two-base pair CT deletion (c.427_428del), were identified in two unrelated probands. Both mutations result in a frameshift and a premature truncation of the C terminus of the expressed protein (p.Asp122Glyfs*12 and p.Leu143Alafs*55, respectively), leading to mutated proteins completely lacking the transactivation domain (Fig. 1). These mutations had not been previously documented and were not present in 200 alleles from healthy unrelated individuals. No mutations were identified in the remaining 42 patients.

The two homozygous probands inherited the mutation from their heterozygous parents (Fig. 2). In family A with the c.364dupG mutation, parents were known to be first cousins and, consistent with parental consanguinity, SNP genotyping analysis of the proband revealed a total genomic homozygosity value of 6.0% (13). The mutation-containing homozygous segment was the largest homozygous segment (46.6-Mb long) and spanned 2q31.1–2q36.1 delimited by the SNPs rs720032 to rs724149. In contrast, in family B, the parents of the patient with the homozygous c.427_428del mutation were not known to be related and, in keeping with this, total genomic homozygosity value was very low (0.3%). However, the mutation in both parents was inherited on an extended haplotype of 10.4 Mb between positions Chr2q31.1–32.1 (SNPs rs2884471–rs722385), suggesting that the mutation arose from a single common ancestor.

Clinical features. The two probands were diagnosed with permanent diabetes within the first 2 months of life and had presented with intrauterine growth retardation (birth weights 1,490 and 2,230 g at 34 and 38 weeks of gestation, respectively), reflecting reduced insulin secretion in utero. They had no evidence of pancreatic exocrine dysfunction and normal pancreatic size on abdominal scanning (see supplementary information, available in an online appendix [http://diabetes.diabetesjournals.org/cgi/content/full/db10-0011/DC1]). In addition to diabetes, they presented with a similar pattern of neurological abnormalities including moderate-to-severe developmental delay, profound sensorineural deafness, and visual impairment due to myopia and diffuse retinal dystrophy. Brain magnetic resonance imaging scans showed severe cerebellar hypoplasia with no other major intracranial abnormalities (Fig. 3 and supplementary information). A more detailed clinical description is given in Table 1.

There was limited availability of other family members for genetic and clinical testing. The diabetes status, age of diagnosis, treatment, and genetic testing result of family members are shown in Fig. 2. We assessed glucose tolerance in the four parents of the two probands who were proven heterozygous carriers of the mutations. In family A (c.364dupG mutation), the mother had been diagnosed with type 2 diabetes at 33 years, despite having a normal BMI, and was treated with gliclazide. In contrast, the father (also aged 33 years) had normal fasting (4–6 mmol/l) and postprandial (5–7 mmol/l) blood glucose levels on several occasions. In family B (c.427_428del mutation), the mother and father underwent standard oral glucose tolerance tests (aged 33 and 37 years, respectively) that confirmed normal glucose tolerance (6.2 and 4.8 mmol/l, respectively, at 2 h). No heterozygous family members in either family had any developmental delay or neurological features on clinical examination.
DISCUSSION

We report the first two cases of PNDM caused by homozygous mutations in \textit{NEUROD1}. The patients with this novel autosomal recessive syndrome not only had early-onset permanent diabetes but also presented with developmental delay, cerebellar hypoplasia, and hearing and visual impairment. This is the 13th gene in which mutations have been described in patients with permanent neonatal diabetes.

\textit{NEUROD1}, a tissue-specific member of the bHLH family of transcription factors, is expressed in developing pancreatic islets and in mature B-cells. It forms a heterodimer with the ubiquitous bHLH transcription factor E47 that binds to specific E-box motifs on specific promoters.

FIG. 2. Extended pedigrees of the two families showing inheritance of \textit{NEUROD1} mutations (family A, c.364dupG [A]; family B, c.427_428del [B]). Genotype is shown underneath each symbol; M and N denote mutant and wild-type alleles, respectively. Directly below the genotype is the age of the individual at testing or the age at diagnosis of diabetes if diabetic, followed by the most recent treatment for diabetes. Subjects who were genotyped were tested for diabetes. Squares represent male family members, and circles represent female subjects. Black-filled symbols denote patients with neonatal diabetes, and gray-filled symbols represent patients with later-onset diabetes. A dash denotes information not applicable or not available. An arrow denotes the proband in each family. OHA, oral hypoglycemic agents.

FIG. 3. Magnetic resonance imaging of the brain in a proband from family A demonstrating the typical neuroimaging findings of \textit{NEUROD1}-PNDM. A: Sagittal T1-weighted image. B: Coronal T2-weighted image. There is significant cerebellar hypoplasia, particularly of cerebellar vermis inferiorly. Unusually, the posterior fossa is well formed. Supratentorial midline structures and myelination are normal.
target genes, including INS, GCK, and ABCC8, to regulate their expression (14–16). The two homozygous NEUROD1 mutations both introduce a frameshift that results in truncated proteins lacking the transactivation domain, which has been shown to be important for the interaction of NEUROD1 with its main coactivator, p300 (17). These are likely to have no biological activity, as shown previously for a different frameshift mutation (c.616dupC, p.His206Profs*38) identified in a patient with NEUROD1-MODY (9). The two patients have a remarkably consistent phenotype (Table 1), with clinical features in keeping with the known expression and biology of this transcription factor, and this provides further evidence that the homozygous mutations in NEUROD1 are causative.

Both patients have neonatal diabetes but a normal pancreas on scanning and no evidence of exocrine dysfunction. This is consistent with the central role of NEUROD1 in islet development. Mice lacking Neurod1 die shortly after birth from severe diabetic ketoacidosis (8). Histological examination of the Neurod1-deficient pancreas shows an impaired islet morphogenesis with a reduction in the number of endocrine cells— especially β-cells (8).

In addition to diabetes, our two patients presented with a similar pattern of neurological features, including developmental delay, cerebellar hypoplasia, and visual and hearing impairment. This is in keeping with the abundant expression of NEUROD1 in the developing and mature nervous system. Interestingly, the initial Neurod1-null mice that rapidly died from diabetes had

### Table 1
Clinical features of the two patients with homozygous NEUROD1 mutations

|            | Case A (c.0.364dupG) | Case B (c.0.427_428del) |
|------------|----------------------|-------------------------|
| Sex        | Female               | Female                  |
| Country of origin | Pakistan          | Hungary                 |
| Parental consanguinity | Yes (first cousins) | No                      |
| Birth information |           |                         |
| Gestational age (weeks) | 34             | 38                      |
| Birth weight (g) | 1,490            | 2,230                   |
| Birth weight (SDS) | -2.06           | -1.92                   |
| Diabetes |           |                         |
| Age at diagnosis (weeks) | 8              | 4                       |
| Blood glucose (mmol/l) | 31.8             | 24.0                    |
| Ketosis | Yes                   | No                      |
| C-peptide | N/A                  | Undetectable            |
| Exocrine function | Normal         | Normal                  |
| Pancreas size | Normal (MRI scan)  | Normal (CT scan)        |
| Current insulin dose (units · kg⁻¹ · day⁻¹) | 1.1             | Not known               |
| Neurological features |            |                         |
| Developmental delay | Yes            | Yes                     |
| Cerebellar hypoplasia | Severe cerebellar hypoplasia on MRI | Severe cerebellar hypoplasia on MRI |
| Sensori-neural deafness | Yes (hearing aids [80 dB loss]) | Yes (hearing aids) |
| Visual impairment | Severe myopia, diffuse retinal dystrophy (ERG reduced to approx. 25%) | Moderate myopia, pigmental epithelial atrophy and enlarged fovea |
| Seizures | No epilepsy; two hypoglycemic seizures (at 7 and 15 years) | No epilepsy |

CT, computed tomography; ERG, electroretinography; MRI, magnetic resonance imaging; SDS, SD score.

### Table 2
Comparison of the major features seen in Neurod1-deficient mice (refs. 8 and 18–22) and NEUROD1-deficient patients with homozygous NEUROD1 mutations

| Mouse model | Patient features |
|-------------|------------------|
| Endocrine pancreas | Early-onset ketosis-prone diabetes | Permanent neonatal diabetes |
| Exocrine pancreas | Failure of mature islets development | Normal |
| Enteroendocrine cells | Postnatal-onset acinar cell polarity defects (indirect effect?) | Not known |
| Cerebral cortex | Normal | Normal |
| Dentate gyrus (hippocampus) | Seizures | No epilepsy |
| Cerebellum | Severe hypoplasia | Severe hypoplasia |
| Retina | Decreased granule cells | Ataxia |
| Inner ear | Blindness | Myopia |
|            | Decreased synapses and loss of outer nuclear layer | Retinal dysfunction |
|            | Deafness and imbalance | Sensorineural deafness |
|            | Shortened cochlear duct, sensory epithelia abnormalities, and degeneration of acoustic ganglions |  |
no obvious anatomic and histologic abnormalities of the brain (8). However, it is possible to explore the role of Neurod1 in the nervous system by rescuing Neurod1-null mice either by expressing a transgene encoding the mouse Neurod1 gene under the insulin promoter (18) or by crossing the null mutation into a different genetic background to reduce the severity of the diabetes (19). The rescued Neurod1-null mice show a similar neuronal phenotype consisting of impaired balance, ataxic gait, circling, and swaying head movement as a result of impaired cerebellum development (18–20). Furthermore, rescued Neurod1-deficient mice have abnormal hearing and vision as a result of severe sensory neuronal defects in the inner ear and neural retina, respectively (20–22). The main feature seen in the mouse that was not present in our patients was epilepsy (19). The remarkable similarity between the NEUROD1-deficient patients and the Neurod1-deficient mice (Table 2) strongly supports a similar biological role of this transcription factor across species.

Homozygous mutations in PTF1A, which encodes another bHLH transcription factor, also cause a syndrome of neonatal diabetes and cerebellar hypoplasia/agenesis (23). However, in this condition the pancreatic phenotype is not limited to the islets; affected patients have pancreatic hypoplasia/aplasia. In keeping with the islets representing <1% of the endocrine pancreas, the size of the pancreas was found to be normal in our two patients with homozygous NEUROD1 mutations. This suggests that shared developmental pathways are important during development in the pancreas and the cerebellum.

Although heterozygous loss-of-function mutations in NEUROD1 have previously been identified as a very rare cause of diabetes in humans (9–12), diabetes was present in only one of four heterozygous mutation–carrying parents. Their age at the time of the study ranged from 33 to 39 years and does not exclude the possibility of developing diabetes later in life. In addition, incomplete penetrance has been described in some of the families with NEUROD1 diabetes (9). Homozygous mutations in other known MODY genes, namely GCK and IPF1, have previously been associated with isolated PNDM and isolated pancreatic agenesis, respectively (24,25). We have shown that homozygous mutations in another MODY gene are also associated with a more severe phenotype of neonatal diabetes.

In conclusion, homozygous mutations in NEUROD1 constitute a rare novel autosomal recessive cause of neonatal diabetes with severe neurological abnormalities. This confirms the important role that NEUROD1 plays in the development of both the pancreas and the nervous system in humans.

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