Homozygous mutations in *NEUROD1* are responsible for a novel syndrome of permanent neonatal diabetes and neurological abnormalities

*NEUROD1* in permanent neonatal diabetes

Oscar Rubio-Cabezas, MD, PhD (1, 2, *), Jayne A.L. Minton, PhD (1, *), Iren Kantor, MD (3, *), Denise Williams (4), Sian Ellard, PhD, FRCPATH (1), Andrew T. Hattersley, DM, FRCP (1).

(1) Institute of Biomedical and Clinical Science, Peninsula Medical School, University of Exeter, Exeter, United Kingdom
(2) Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Madrid, Spain
(3) Department of Pediatrics, Jósa András Hospital, Nyíregyháza, Hungary
(4) West Midlands Regional Clinical Genetics Service, Birmingham Women’s Hospital, Birmingham, United Kingdom

* O.R.-C., J.A.L.M. and I.K. contributed equally to the paper

**Corresponding author:**
Prof. Andrew T Hattersley
Email: andrew.hattersley@pms.ac.uk

Additional information for this article can be found in an online appendix at [http://diabetes.diabetesjournals.org](http://diabetes.diabetesjournals.org)

Submitted 5 January 2010 and accepted 20 June 2010.

This is an uncopyedited electronic version of an article accepted for publication in *Diabetes*. The American Diabetes Association, publisher of *Diabetes*, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of *Diabetes* in print and online at [http://diabetes.diabetesjournals.org](http://diabetes.diabetesjournals.org).
Objective: \textit{NEUROD1} is expressed in both the developing and the mature beta cells. Studies in mice suggest 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 \textit{NEUROD1} mutations in patients with permanent neonatal diabetes.

\textit{Research Design and Methods:} We sequenced the \textit{NEUROD1} gene in 44 unrelated patients with permanent neonatal diabetes of unknown genetic etiology.

\textit{Results:} Two homozygous mutations in \textit{NEUROD1} (c.427_428del and c.364dupG) were identified in two patients. Both mutations introduced a frameshift which 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.

\textit{Conclusions:} We describe a novel clinical syndrome that results from homozygous loss of function mutations in \textit{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 \textit{NEUROD1} in both the development of the endocrine pancreas and central nervous system in humans.

Monogenic permanent neonatal diabetes (PNDM) is typically diagnosed within the first six months after 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 multi-systemic syndrome. Dominant mutations in three genes (\textit{KCNJ11}, \textit{ABCC8} and \textit{INS}) are the cause of PNDM in approximately 50\% of cases and in the majority diabetes is an isolated finding (3, 4). Reccessive mutations, autosomal or X-linked, have been described in 10 genes (\textit{ABCC8}, \textit{GCK}, \textit{EIF2AK3}, \textit{FOXP3}, \textit{IPF1}, \textit{PTF1A}, \textit{GLIS3}, \textit{SLC2A2}, \textit{SCL19A2}, and \textit{WFS1}). These are rare and often result in extra-pancreatic 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, beta cells may be present but not functional as in patients with activating mutations in \textit{KCNJ11} and \textit{ABCC8}, the genes encoding the two subunits of the ATP-sensitive potassium channel (Kir6.2 and SUR1, respectively). Secondly, the number of beta cells may be reduced due to an increased destruction, either by apoptosis (\textit{INS}, \textit{EIF2AK3}) or as a consequence of an autoimmune insult (\textit{FOXP3}). Finally there may be a reduced number of beta cells as a result of impaired pancreatic development, affecting either the whole pancreas (\textit{IPF1},
PTF1A) or specifically endocrine cells (GLIS3) (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 5 families reported to date (9-12). We assessed the role of NEUROD1 in PNDM and describe two unrelated probands with homozygous 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/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, UK. 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 are available upon request). Single strand sequencing was carried out using standard methods on an ABI 3730 sequencer (Applied Biosystems, Warrington, UK). Sequences were compared to the published template (accession number NM_002500) using Mutation Surveyor v3.20 (SoftGenetics, PA, USA). Sequence variants were tested for their presence in family members whenever DNA was available.

Homozgyosity mapping. High-density single nucleotide polymorphism (SNP) genotyping was carried out on the Affymetrix human 10K Xba chip by Medical Solutions Nottingham (formerly GeneService), UK. Processing of genomic DNA was performed as per 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 2-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 (Figure 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 (Figure 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 rs726032 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 two months of life and had presented with intrauterine growth retardation (birth weights 1490 and 2230 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 in the online appendix available at [http://diabetes.diabetesjournals.org](http://diabetes.diabetesjournals.org)). 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 MRI scans showed severe cerebellar hypoplasia with no other major intracranial abnormalities (Figure 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 is shown in Figure 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 glicazide. In contrast, the father (also aged 33) 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) that confirmed normal glucose tolerance (6.2 and 4.8 mmol/L at 2 hours, respectively). No heterozygous family members in either family had any developmental delay or neurological features on clinical examination.

**CONCLUSIONS**
We report the first two cases of PNDM caused by homozygous mutations in **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. NEUROD1, a tissue-specific member of the basic helix-loop-helix (bHLH) family of transcription factors, is expressed in the developing pancreatic islets as well as in mature beta cells. It forms a heterodimer with the ubiquitous bHLH transcription factor E47 that binds to specific E-box motifs on specific target genes, including INS, GCK, and ABCC8, to regulate their expression (14-16). The two homozygous NEUROD1 mutations both introduce a frameshift that result 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 for the homozygous mutations in NEUROD1 being causative.

Both patients have neonatal diabetes but a normal pancreas on scanning and no evidence of an 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 beta 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 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 which 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 as affected patients have pancreatic hypoplasia/aplasia. In keeping with the islets representing less than 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 been previously 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 been previously 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
NEUROD1 in permanent neonatal diabetes

A 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.

Author Contributions: O.R.C. researched clinical data and wrote manuscript. J.A.L.M. researched molecular genetic data and reviewed manuscript. I.K. and D.W. researched clinical data and reviewed manuscript. S.E. and A.T.H. reviewed/edited manuscript.

ACKNOWLEDGMENTS
We thank Ann-Marie Patch from the Peninsula Medical School for her excellent support with homozygosity mapping analysis. This work was funded by the European Union FP6 Integrated programme 2006-2010 EURODIA (LSHM-CT-2006-518153) and the Welcome Trust (Grants No. 067463/Z/2/Z and 083270/Z/07/Z). ORC is supported by an “Ayuda para contratos post-Formación Sanitaria Especializada” from the “Instituto de Salud Carlos III” (FIS CM06/00013). SE is employed as a core member of staff within the NIHR funded Peninsula Clinical Research Facility. ATH was a Wellcome Trust Research Leave Fellow.

Disclosure—The authors declare no conflict of interest.

REFERENCES


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

<table>
<thead>
<tr>
<th></th>
<th>Case A (c.364dupG)</th>
<th>Case B (c.427_428del)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Country of origin</td>
<td>Pakistan</td>
<td>Hungary</td>
</tr>
<tr>
<td>Parental consanguinity</td>
<td>Yes (first cousins)</td>
<td>No</td>
</tr>
<tr>
<td>Birth information:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Gestational age (weeks)</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td>- Birth weight (grams)</td>
<td>1490</td>
<td>2230</td>
</tr>
<tr>
<td>- Birth weight (SDS)</td>
<td>-2.06</td>
<td>-1.92</td>
</tr>
<tr>
<td>Diabetes mellitus:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Age at diagnosis (weeks)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>- Blood glucose (mmol/L)</td>
<td>31.8</td>
<td>24.0</td>
</tr>
<tr>
<td>- Ketosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>- C-peptide</td>
<td>N/A</td>
<td>Undetectable</td>
</tr>
<tr>
<td>- Exocrine function</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>- Pancreas size</td>
<td>Normal (MRI scan)</td>
<td>Normal (CT scan)</td>
</tr>
<tr>
<td>- Current insulin dose</td>
<td>1.1 U/kg/day</td>
<td>Not known</td>
</tr>
<tr>
<td>Neurological Features:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Developmental delay</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>- Cerebellar hypoplasia</td>
<td>Severe cerebellar hypoplasia on MRI</td>
<td>Severe cerebellar hypoplasia on MRI</td>
</tr>
<tr>
<td>- Sensori-neural deafness</td>
<td>Yes (Hearing aids 80dB loss)</td>
<td>Yes (Hearing aids)</td>
</tr>
<tr>
<td>- Visual impairment</td>
<td>Severe myopia, diffuse retinal dystrophy (ERG reduced to approx. 25%)</td>
<td>Moderate myopia, pigmental epithelial atrophy, enlarged fovea</td>
</tr>
<tr>
<td>- Seizures</td>
<td>No epilepsy. Two hypoglycemic seizures (at 7 and 15 years).</td>
<td>No epilepsy</td>
</tr>
</tbody>
</table>

SDS: Standard deviations score  
ERG: Electroretinography
Table 2. Comparison of the major features seen in *Neurod1* deficient mice (8, 18-22) and *NEUROD1* deficient patients with homozygous *NEUROD1* mutations

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Patients features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endocrine pancreas</strong></td>
<td>▪ Early-onset ketosis-prone diabetes</td>
</tr>
<tr>
<td></td>
<td>▪ Failure of mature islets development</td>
</tr>
<tr>
<td></td>
<td>▪ Striking reduction in both beta and alpha cells</td>
</tr>
<tr>
<td><strong>Exocrine pancreas</strong></td>
<td>▪ Postnatal-onset acinar cell polarity defects (indirect effect?)</td>
</tr>
<tr>
<td><strong>Enteroendocrine cells</strong></td>
<td>▪ Lack of secretin- and cholecystokinin-producing cells (remaining enteroendocrine cells normal)</td>
</tr>
<tr>
<td><strong>Cerebral cortex</strong></td>
<td>▪ Normal</td>
</tr>
<tr>
<td><strong>Dentate gyrus (hippocampus)</strong></td>
<td>▪ Seizures</td>
</tr>
<tr>
<td></td>
<td>▪ &gt;95% decrease of granule cells</td>
</tr>
<tr>
<td><strong>Cerebellum</strong></td>
<td>▪ Severe hypoplasia</td>
</tr>
<tr>
<td></td>
<td>▪ Impaired coordination, ataxia</td>
</tr>
<tr>
<td></td>
<td>▪ Decrease of granule cells</td>
</tr>
<tr>
<td><strong>Retina</strong></td>
<td>▪ Blindness</td>
</tr>
<tr>
<td></td>
<td>▪ Decreased synapses, loss of outer nuclear layer</td>
</tr>
<tr>
<td><strong>Inner ear</strong></td>
<td>▪ Deafness, imbalance</td>
</tr>
<tr>
<td></td>
<td>▪ Shortened cochlear duct, sensory epithelia abnormalities, degeneration of acoustic ganglions</td>
</tr>
</tbody>
</table>

▪ Permanent neonatal diabetes

▪ Normal

▪ Not known

▪ Normal

▪ No epilepsy

▪ Severe hypoplasia

▪ Ataxia

▪ Myopia

▪ Retinal dysfunction

▪ Sensorineural deafness
FIGURE LEGENDS

Figure 1. Schematic organization of NEUROD1 protein and effect of the two mutations on its structure. Numbers refer to the amino acids bordering the functional domains. Both mutations result in the generation of a truncated protein lacking the transactivation domain. The abnormal protein sequence between the frameshift and the termination codon is colored in grey.

Figure 2. Extended pedigrees of the two families showing inheritance of NEUROD1 mutations. Squares represent male family members, and circles represent female subjects. Black filled symbols denote patients with neonatal diabetes and grey filled symbols represent patients with later onset diabetes. Subjects who were genotyped were tested for diabetes. 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. OHA means oral hypoglycemic agents. A dash denotes information not applicable or not available. An arrow denotes the proband in each family.

Figure 3. MRI of the brain in proband from Family A demonstrating the typical neuroimaging findings of NEUROD1-PNMD (Panel A: Sagittal T1-weighted image. Panel 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.
FIGURE 2

Family A (c.364dupG)

Family B (c.427_428del)

FIGURE 3

A

B