Permanent Neonatal Diabetes Caused by Glucokinase Deficiency

Inborn Error of the Glucose-Insulin Signaling Pathway

Pål R. Njølstad,1,2 Jørn V. Sagen,1 Lise Bjørkhaug,2 Stella Odili,3 Naim Shehadeh,4 Doua Bakry,4 S. Umit Sarici,5 Faruk Alpay,5 Janne Molnes,1 Anders Molven,2,6 Oddmund Søvik,1 and Franz M. Matschinsky4

Neonatal diabetes can be either permanent or transient. We have recently shown that permanent neonatal diabetes can result from complete deficiency of glucokinase activity. Here we report three new cases of glucokinase-related permanent neonatal diabetes. The probands had intrauterine growth retardation (birth weight <1,900 g) and insulin-treated diabetes from birth (diagnosis within the first week of life). One of the subjects was homozygous for the missense mutation Ala378Val (A378V), which is an inactivating mutation with an activity index of only 0.2% of wild-type glucokinase activity. The second subject was homozygous for a mutation in the splice donor site of exon 8 (interrupting sequence 8 [IVS8] + 2T→G), which is predicted to lead to the synthesis of an inactive protein. The third subject (second cousin of subject 2) was a compound heterozygote with one allele having the splice-site mutation IVS8 + 2T→G and the other the missense mutation Gly264Ser (G264S), a mutation with an activity index of 86% of normal activity. The five subjects with permanent neonatal diabetes due to glucokinase deficiency identified to date are characterized by intrauterine growth retardation, permanent insulin-requiring diabetes from the first day of life, and hyperglycemia in both parents. Autosomal recessive inheritance and enzyme deficiency are features typical for an inborn error of metabolism, which occurred in the glucose-insulin signaling pathway in these subjects. Diabetes 52: 2854–2860, 2003

From the 1Department of Pediatrics, Haukeland University Hospital, University of Bergen, Norway; the 2Department of Medical Genetics and Molecular Medicine, Haukeland University Hospital, University of Bergen, Norway; the 3Department of Biochemistry and Biophysics, and Diabetes Research Center, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; the 4Division of Newborn Medicine, Department of Pediatrics, Gulhane Military Medical Academy, Ankara, Turkey; and the 5Department of Pathology, the 6Division of Newborn Medicine, Department of Pediatrics, Guhzne Military Medical Academy, Ankara, Turkey. Address correspondence and reprint requests to Pål R. Njølstad, MD, PhD, Department of Pediatrics, University of Bergen, N-5021 Bergen, Norway. E-mail: pal.njolsstad@uib.no.

RESEARCH DESIGN AND METHODS

Subjects. The screening included eight cases of PNDM, defined as a diagnosis of permanent diabetes before age 1 month. The probands of families 1–3 were identified by one of the authors (P.R.N.) by a PubMed literature search, after which the corresponding authors (N.S. and S.U.S.) were contacted. The other PNDM patients were from the Department of Pediatrics, University of Bergen (N89-1) or referred to us by other clinics (BR1, R1826, AA, and US4-1). We have not performed a systematic, population-based screening program for this study. Informed consent was obtained from the subjects or their parents. The studies were performed according to the Declaration of Helsinki and approved by ethical committees.

GENETIC STUDIES. The exons, flanking introns, and minimal promoter regions of the gene encoding glucokinase were screened for mutations by direct sequencing of the PCR products. In vitro mutagenesis was performed as described in Bjørkhaug et al. (5). The primers 5'-CGTGCTACCGGGGTGGCGCACATGTCCTGAG-3' (F1), 5'-CGAGCAGCATGGCGCAACGGCGGTAGACC-3' (R1), 5'-GCCCTGCCGACACCGGAAGGCGTGTTACGCGG-3' (F2), and 5'-GAGACTCGTCACCTGCCGCGGGAACGTCG-3' (R2) were used to introduce the appropriate nucleotide changes corresponding to the mutations A378V (F1 and R1) and G264S (F2 and R2).

Kinetic analysis of glucokinase. Wild-type and mutant forms of human β-cell glucokinase were generated and expressed as glutathionyl S-transferase (GST) fusion proteins in E. coli; the kinetic properties of the purified proteins were determined as previously described (6). We performed four serial experiments. The wild-type GST-glucokinase preparation was newly made for the current study. Kinetic data may vary as a function of the chemical nature.
and concentration of the sulphydryl reagent used in the kinetic analysis. In our studies, 2 mmol/l dithiothreitol was used in the standard kinetic assay. Nonlinear kinetics using the Hill equation were applied. The relative activity index (AI) was calculated as previously described with some modifications as AI = \((k_{cat}/S_{0.5}n_{H})(2.5/2.5 + 4ATP/K_{m}(S_{0.5}n_{H})\), where \(k_{cat}\) is the turnover rate, \(S_{0.5}\) is the concentration of glucose needed to achieve the half-maximal rate of phosphorylation, \(n_{H}\) is Hill coefficient for cooperativeness with glucose, and \(ATP/K_{m}\) is the ATP concentration required for glucokinase activity to be half maximum when glucose is in excess (6). This number indicates the in situ phosphorylation capacity of the enzyme at 5 mmol/l blood glucose. An intracellular ATP concentration of 2.5 mmol/l was assumed. The relative activity index was normalized to a basal blood glucose of 5 mmol/l to account for glucokinase expression.

**Mathematical modeling.** A minimal mathematical model was used to assess the impact of the G264S, IVS8 mutations of glucokinase, in both the homozygous and heterozygous state, on the glucose-stimulated insulin secretion rate (GSIR) (6,16). The modeling was modified to account for adaptation of both alleles in homozygous and heterozygous cases by using the theoretically plausible expression (\(S_{0.5}/2)(S_{0.5}/2 + 2S_{0.5}/(2n_{H})\), rather than an empirical factor of 0.2 per mmol/l glucose change. \(S\) is the glucose level at threshold and “2” indicates that half-maximal induction is achieved at glucose \(S_{0.5}\).

**Structural analysis.** A predicted model of the three dimensional structure of wild-type human glucokinase (1 glk; Rasnol Windows version 2.7.2.1) was used to inspect the spatial relations for the previously published homozygous missense mutations causing PNDM (M210K and T228M) (4,9) together with the present missense mutations G264S and A378V.

**RESULTS**

**Clinical data.**

**Family 1.** Family 1 is presented in Fig. 1, Table 1, and a preliminary report (7). The Turkish proband (T1-1) of central Anatolian (Caucasian) ancestry was a girl born at 33 weeks of gestation after a pregnancy complicated with oligohydramnios. Her birth weight was 1,550 g (10th centile) and her birth length was 43.5 cm (25th–50th centile). As a neonate, this girl suffered from respiratory failure that required intermittent mechanical ventilation. Hyperglycemia (13.4 mmol/l [241 mg/dl]) was present on the first day of life and increased rapidly to 24.6 mmol/l (450 mg/dl), after which insulin was administrated intravenously in a dosage of 2.4 units/kg daily. She had no digestive problems. Serum C-peptide was not detectable and neither markers for type 1 diabetes (islet cell antibodies and HLA type) nor transient neonatal diabetes (uniparental disomy for chromosome 6) were found (7). At present, this 4-year-old girl weighs 16.8 kg, her height is 98.5 cm, and she is being treated with insulin (0.60 units/kg) daily. Her recent fasting serum glucose varied between 3.0 mmol/l (54 mg/dl) and 10.3 mmol/l (185 mg/dl) and her HbA1c was 8.6% (reference value for HbA1c in the analytic laboratory was 4–5.7%). She shows no signs of diabetic complications, and her psychomotor development has been normal. Her parents were first cousins. The mother was diagnosed with gestational diabetes at age 33 years. The mother’s recent fasting serum glucose was 6.9 mmol/l (125 mg/dl) and her HbA1c was 5.5%. She is presently not taking any antidiabetic medication. The father had mild diabetes diagnosed at age 39 years. His recent fasting serum glucose was 8.6 mmol/l (155 mg/dl) and his HbA1c was 7.6%. His illness is treated with diet only.

**Family 2.** Family 2 is presented in Fig. 1, Table 1, and a preliminary report (8). These Israeli parents of Arabic ancestry were related, as the mother was the father’s first cousin once removed. The male proband (IS1-1) was child number two of three siblings. He showed IUGR and was
TABLE 1
Clinical characteristics and genotypes of subjects with permanent neonatal diabetes screened for mutations in the glucokinase gene

<table>
<thead>
<tr>
<th>ID</th>
<th>Parents (glucose intolerance)</th>
<th>Birth weight (g)</th>
<th>Birth weight (centile)</th>
<th>Gestation age (weeks)</th>
<th>Age at diagnosis (days)</th>
<th>Blood glucose* (mmol/l)</th>
<th>Insulin treatment (units·kg⁻¹·day⁻¹)</th>
<th>Glucokinase mutation</th>
<th>Insulin treatment (units·kg⁻¹·day⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N17-1</td>
<td>Both</td>
<td>1,670</td>
<td>&lt;3</td>
<td>36</td>
<td>1</td>
<td>16.8</td>
<td>0.8</td>
<td></td>
<td>1.1</td>
<td>M210K/M210K</td>
</tr>
<tr>
<td>TO/nd-1</td>
<td>Both</td>
<td>1,650</td>
<td>&lt;3</td>
<td>38</td>
<td>1</td>
<td>39.6</td>
<td>2</td>
<td></td>
<td>1.4</td>
<td>T22SM/T22SM</td>
</tr>
<tr>
<td>T1-1</td>
<td>Both</td>
<td>1,550</td>
<td>10</td>
<td>33</td>
<td>1</td>
<td>13.4</td>
<td>2.4</td>
<td></td>
<td>0.6</td>
<td>A378V/A378V</td>
</tr>
<tr>
<td>IS1-1</td>
<td>Both</td>
<td>1,900</td>
<td>&lt;3</td>
<td>40</td>
<td>11</td>
<td>57.0</td>
<td>1.2</td>
<td></td>
<td>TDU</td>
<td>IVSS + 2/IVSS + 2</td>
</tr>
<tr>
<td>IS2-1</td>
<td>Mother*</td>
<td>1,870</td>
<td>&lt;3</td>
<td>38</td>
<td>2</td>
<td>12.0</td>
<td>TDU</td>
<td></td>
<td>0.9</td>
<td>IVSS + 2/G264S</td>
</tr>
<tr>
<td>BR1</td>
<td>Father only</td>
<td>3,100</td>
<td>—</td>
<td>38</td>
<td>30</td>
<td>29.7</td>
<td>TDU</td>
<td></td>
<td>0.4</td>
<td>None</td>
</tr>
<tr>
<td>R1826</td>
<td>Mother only</td>
<td>—</td>
<td>38</td>
<td>Birth</td>
<td>—</td>
<td>—</td>
<td>TDU</td>
<td></td>
<td>—</td>
<td>None</td>
</tr>
<tr>
<td>AA</td>
<td>Mother only</td>
<td>2,400</td>
<td>—</td>
<td>38</td>
<td>Birth</td>
<td>—</td>
<td>TDU</td>
<td></td>
<td>—</td>
<td>None</td>
</tr>
<tr>
<td>US4-1</td>
<td>Mother only</td>
<td>2,100</td>
<td>10–25</td>
<td>36</td>
<td>5</td>
<td>13.3</td>
<td>TDU</td>
<td></td>
<td>1.0</td>
<td>None</td>
</tr>
<tr>
<td>N89-1</td>
<td>Father only</td>
<td>1,440</td>
<td>&lt;3</td>
<td>40</td>
<td>1</td>
<td>16.1</td>
<td>TDU</td>
<td></td>
<td>0.6</td>
<td>None</td>
</tr>
</tbody>
</table>

Blood glucose measurement given is for first measurement. *Father diseased, thus unavailable for analysis. ID, identity used in the original publication; TDU, treated but dose unknown; TR, this report; —, unknown.

born by Caesarian section after 40 gestational weeks at a birth weight of 1,900 g ( <3rd centile) and a length of 45.0 cm ( <3rd centile). At age 11 days, he was admitted to the hospital in a very severe condition with fever, dehydration, and a serum glucose of 57 mmol/l (1,026 mg/dl), which varied initially between 52 and 77 mmol/l (945 and 1,400 mg/dl). He was subsequently treated with insulin (1.2 units/kg) daily. No ketoacidosis was present. His further psychomotor development has been normal. GAD and insulinoma-associated protein 2 (IA2) antibodies were not detectable. The father was initially regarded as healthy. During the investigation, however, it was revealed that he had mild diabetes with fasting serum glucose of 8.4 mmol/l (151 mg/dl). His HbA1c was 6.3% (reference value for HbA1c in the analytic laboratory was 4–6.3%). During her pregnancies, the mother had gestational diabetes that was treated with diet alone. Presently, she has diabetes with a fasting serum glucose of 7.2 mmol/l (130 mg/dl), whereas her sister (born at term, birth weight 4,300 g [95th centile]) had normal glucose tolerance. The proband’s elder brother (36 weeks’ gestation, birth weight 1,950 g [ <3rd centile]) had a recent fasting serum glucose of 7.2 mmol/l (130 mg/dl), whereas his sister (born at term, birth weight 4,300 g [ >97th centile]) had a recent fasting serum glucose of 5.3 mmol/l (95 mg/dl).

Family 3. The male proband (IS2-1) was number three of five siblings (8). His mother was both first and third cousin of the father of the proband in Family 2, and she was also the third cousin once removed of the mother of the proband in Family 2 (Fig. 2). The proband’s parents were not known to be related. The proband presented with IUGR and was born by vaginal delivery at a gestational age of 38 weeks. His birth weight was 1,870 g ( <3rd centile) and his birth length was 44 cm (3rd centile) (Table 1). At age 2 days, he had hyperglycemia (12.0 mmol/l [216 mg/dl]) without ketoacidosis. His psychomotor development has been normal. He was treated with insulin from day 3 of life. At present, he is age 18 years and is treated with insulin daily (0.9 units/kg). His HbA1c is presently 9.4% (reference value for HbA1c in the analytic laboratory was 4–6.3%). The father died at age 49 years from hepatic failure, but had no history of diabetes. His mother was diagnosed with gestational diabetes during her pregnancy. She is now age 56 years and has developed manifest diabetes (fasting serum glucose 10.1 mmol/l [182 mg/dl]; HbA1c, 7.0%). Her diabetes is being treated with diet. The siblings had birth weights of 3,550 (55th centile), 4,300 (96th centile), 2,450 (6th centile), and 3,260 g (45th centile), and their recent fasting serum glucose levels were 5.6, 5.5, 6.8, and 6.0 mmol/l (101, 99, 122, and 108 mg/dl), respectively.

Screening for the glucokinase gene. The clinical features of the probands (Table 1) could suggest a diagnosis of glucokinase-related PNDM, so we screened them for mutations in this gene.

Family 1. In this family, we screened for GCK by direct sequencing and found a novel missense mutation in exon 9 (nucleotide 1,139: GCT to GGT) of GCK, resulting in the substitution of alanine for valine at amino acid residue 378 of the glucokinase protein (designated c.1,139 C→T, A378V). Residue 378 is strictly conserved among glucokinase enzymes from man to Drosophila. The proband was homozygous, whereas her parents were heterozygous for the mutation. The mutation was not found in 91 individuals of Norwegian ancestry.

Families 2 and 3. Because of the known relationship between families 2 and 3 (Fig. 1), we first performed a linkage analysis using the available microsatellite markers to map the GCK gene (Fig. 2). Because the haplotype pattern suggested a homozygous and a heterozygous GCK mutation in the probands of families 2 and 3, respectively, we therefore sequenced this gene in all available samples. In family 2, we identified a splice site mutation in the second nucleotide of the donor splice site of exon 8, designated IVSS + 2T→G. The proband was homozygous and the parents were heterozygous for this mutation. Family 3 was related to family 2 through the mother of the proband in family 3. The mother had inherited family 2’s specific mutation IVSS + 2T→G. The proband shared this mutation in addition to another GCK mutation in exon 7 (nucleotide 790: −GGC to −AGC), resulting in the substitution of glycine for serine at amino acid residue 264 of the glucokinase protein (designated c.790 G→A, G264S). Residue 264 is strictly conserved from man to Drosophila.
Neither IVS8 + 2T→G nor G264S were identified in 91 Norwegian subjects.

Other cases of neonatal diabetes. We screened five other patients with PNDM (clinical details given in Table 1). Pathogenic mutations in GCK were not identified in any of these patients.

Kinetic analysis of recombinant glucokinase. We prepared recombinant wild-type, A378V and G264S glucokinase in E. coli and compared the kinetic properties of the purified GST fusion proteins (Table 2) by previously described methods (6,9). The A378V proteins had a relative activity index (I_{GKB}) that was only 0.2% of that of wild-type glucokinase. The k_{cat} of A378V glucokinase was practically the same as that of the wild-type, and the glucose S_{0.5} was increased 76-fold. ATP \textit{K}_{m} was increased 27-fold. In contrast, the I_{GKB} of recombinant G264S proteins was near normal (0.86 of wild-type). Hence, G264S glucokinase k_{cat} was nearly equal to that of wild-type, and the glucose S_{0.5} was only moderately elevated (129% of wild-type). G264S glucokinase ATP \textit{K}_{m} was 130% of wild-type glucokinase. Thus, A378V severely impairs in vitro glucokinase activity and is the likely cause of PNDM and maturity-onset diabetes of the young (MODY) in family 1. By contrast, G264S has only a modest effect on the enzyme in vitro, if any. We did not test the effect of the IVS8 + 2T→G on RNA expression or glucokinase activity, but if intron 8 is not removed there is an in-frame stop codon positioned at nucleotides 479–481 of intron 8. This would give rise to a mutant glucokinase protein of 455 amino acids lacking residues 340–465 of the normal protein, but with an addition of 160 residues encoded by intron sequences.

**TABLE 2**

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Wild-type</th>
<th>A378V</th>
<th>G264S</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{cat}$ (s^{-1})</td>
<td>64.2 ± 3.06</td>
<td>55.4 ± 4.69</td>
<td>63.5 ± 2.70</td>
</tr>
<tr>
<td>S_{0.5} (mmol/l)</td>
<td>7.56 ± 0.31</td>
<td>576 ± 12.1</td>
<td>9.76 ± 0.74</td>
</tr>
<tr>
<td>nH</td>
<td>1.77 ± 0.04</td>
<td>0.94 ± 0.02</td>
<td>1.57 ± 0.05</td>
</tr>
<tr>
<td>ATP \textit{K}_{m} (mmol/l)</td>
<td>0.37 ± 0.01</td>
<td>9.92 ± 0.31</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>Relative activity index</td>
<td>1</td>
<td>0.002</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Data are means ± SE and represent the means of the kinetic analyses of four independent expressions of wild-type and mutant GST-glucokinase. Note that the Hill coefficient (nH) and the relative activity index are unitless.
G264 is localized on the outer part of the enzyme and not involved in binding glucose and MgATP (Fig. 4). The residue or close to the active site (M210, A378) of the enzyme residues are localized in the substrate-binding cleft (T228) mutations M210K and T228 mol/l (4,9), was modeled. The G264S, together with the previously described inactivation Structural analysis.

Mathematical modeling and pathophysiological implications. A modified minimal mathematical model (9) was used to quantitate the impact of A378V and G264S on GSIR and glucose homeostasis (Fig. 3). According to this model and based on the results of the current control data set, 28.7% of the total ß-cell glucose phosphorylation rate (BGPR) is necessary to initiate insulin secretion in the control subjects, with a threshold defined as 5 mmol/l. WT/IVS8 + 2T→G or WT/A378V had thresholds of 6.9 mmol/l, IVS8 + 2T→G/G264S had a threshold of 9.5 mmol/l, and WT/G264S had a threshold of 5.6 mmol/l. With 50 mmol/l glucose, there was practically no glucokinase-dependent glucose metabolism with the homozygous cases. The other affected heterozygous parents had predicted thresholds for GSIR of ~7 mmol/l, characteristic of patients with MODY2.

Structural analysis. A theoretical structural analysis of the residues of the inactivating mutations A378V and G264S, together with the previously described inactivation mutations M210K and T228 mol/l (4,9), was modeled. The residues are localized in the substrate-binding cleft (T228) or close to the active site (M210, A378) of the enzyme involved in binding glucose and MgATP (Fig. 4). The residue G264 is localized on the outer part of the enzyme and not in the immediate vicinity of the substrate binding site.

DISCUSSION

We have previously shown that PNDM can result from complete deficiency of glucokinase activity (4). That study and the results presented here indicate that glucokinase mutations leading to inactive enzyme can cause PNDM and support a description of this form of PNDM as a specific syndrome (Online Mendelian Inheritance in Man #606176). Glucokinase-related PNDM has now been described in patients from Europe (Norway and Italy) and the Middle East (Turkey and Israel) and it is therefore likely that similar cases will be found in other populations.

The clinical picture of our new patients is quite similar to that of the first two cases. The patients had moderate or severe IUGR, birth weights of 1,550–1,900 g, and severe hyperglycemia and required subsequent exogenous insulin shortly after birth. This profile fits with the key role played by glucokinase in the regulation of insulin secretion in humans with glucokinase-related diabetes (MODY2) and in mice lacking one or both Gck genes (10,11). That insulin is a potent fetal growth factor is illustrated by our three cases and Gck−/− mice, which are also born growth retarded (11). Our three patients also demonstrate that the fetal growth effect of insulin is most pronounced in the last trimester. Thus, the premature proband of family 1 had a birth weight at the 10th centile, whereas the probands of family 2 and 3 were born at term with birth weights <3rd centile. Although the missense mutation G264S had significant enzyme activity, we believe this mutation is pathogenic as the residue G264 is strictly conserved and there are other members of family 3 at risk with a MODY2 phenotype. Moreover, the compound heterozygous proband of family 3 had severe hyperglycemia at day 2 of life and a birth weight of only 1,870 g, characteristics that are compatible with two defective alleles.

In this regard, it is interesting to compare fetuses with two mutated GCK alleles with those having a GCK mutation on one allele only (12). If a normal fetus is subjected to a diabetic environment (mother heterozygous for a GCK mutation), the combination of intrauterine hyperglycemia and augmented fetal insulin secretion leads to a birth weight increase of ~0.5 kg. When the mother and the fetus are both heterozygous, the reduced insulin secretion in the fetus might in theory balance the effect of the maternal hyperglycemia. Hence, the fetus will have a birth weight in the normal range. Should the fetus, but not the mother, be heterozygous for a GCK mutation, the birth weight is lower by ~0.5 kg. If the mother is heterozygous and the infant is homozygous, as in our cases, a nonoperating glucokinase renders the infant insensitive to maternal hyperglycemia and severe IUGR may ensue (mean birth weight 1,728 g in the present three and previous two cases) (4).

Which cases of neonatal diabetes should be screened for glucokinase mutations? We would suggest screening primarily diabetic neonates with IUGR of moderate or severe degree who have glucose-intolerant parents. If these criteria are not fulfilled, the absence of glucokinase mutations would not be surprising (Table 1) (13–15). It is noteworthy that four of the five cases of glucokinase deficiency had hyperglycemia within the first 2 days of life, illustrating the insulin secretion defect subsequent to the glucokinase deficiency. The proband of family 2 was
referred to the hospital in a very severe situation at day 11, suggesting he had hyperglycemia shortly after birth as well.

Is it possible to predict the phenotype of patients with glucokinase mutations from the genotypes? We believe our patients with homozygous or compound heterozygous GCK mutations are important for understanding more of the mechanisms for glucokinase as the glucose sensor. The corresponding residues of the missense mutations M210K, T228M, and A378V are localized either in the cleft leading to or close to the active site (M210 and A378, B and D), of the enzyme. The residue of the mutation G264S (C, space fill) is, however, present on the outer part of the enzyme suggesting near normal enzymatic activity (C). The NH2- and COOH-terminals are indicated with N and C, respectively. A and B are oriented in the same way, as are C and D.

Glucokinase deficiency may be regarded as a recessively inherited inborn error of metabolism, with heterozygous carriers having a mild phenotype (MODY2) (18) and homozygous carriers being associated with PNDM, as a particular severe phenotype.
ACKNOWLEDGMENTS
This study was supported by grants from the University of Bergen, Haukeland University Hospital, the Norwegian Diabetes Association, the Giertsen Fund, Aventis and Pfizer (P.R.N. and O.S.), the U.S. Public Health Service (NIDDK-19525 and -22122; F.M.M.).

We would like to thank the members of the families for their participation in this study, and Dr. Graeme I. Bell for comments on the manuscript.

REFERENCES
   Horm Res 53 (Suppl. S1):7–11, 2000

2. Temple IK, Gardner RJ, Mackay DJ, Barber JC, Robinson DO, Shield JP: 
   Transient neonatal diabetes: widening the understanding of the etiopathogenesis of diabetes. 
   Diabetes 49:1350–1366, 2000

   agenesis attributable to a single nucleotide deletion in the human IPF-1 
   gene coding sequence. 

   Massa O, Barbetti F, Undlien DE, Shiota C, Magnuson MA, Molven A, Matschinsky FM, Bell GI: 
   Neonatal diabetes mellitus due to complete glucokinase deficiency. 

5. Bjorkhaug L, Sagen JV, Thorsby P, Svik O, Molven A, Njolstad PR: 
   Hepatocyte nuclear factor-1a gene mutations and diabetes in Norway. 
   J Clin Endocrinol Metab 88:920–931, 2003

6. Davis EA, Cuesta-Munoz A, Raoul M, Buettger C, Sweet I, Moates M, 
   Magnuson MA, Matschinsky FM: Mutants of glucokinase cause hypoglycaemia 
   and hyperglycaemia syndromes and their analysis illuminates 
   fundamental quantitative concepts of glucose homeostasis. 
   Diabetologia 42:1175–1186, 1999

7. Sarici SU, Alpay F, Dundarzy MR, Gyknay E: Neonatal diabetes mellitus: 
   patient report and review of the literature. J Pediatr Endocrinol Metab 
   14:451–454, 2001

8. Shehadeh N, Gershoni-Baruch R, Mandel H, Nutenko I, Etrzioni A: 
   Congenital permanent diabetes: a different type of diabetes? 

9. Mahalingam B, Cuesta-Munoz A, Davis EA, Matschinsky FM, Harrison RW, 
   Weber FT: Structural model of human glucokinase in complex with glucose 
   and ATP: implications for the mutants that cause hypo- and hyperglycaemia. 
   Diabetes 48:1698–1705, 1999

    knockouts reveal a critical requirement for pancreatic β-cell glucokinase 
    in maintaining glucose homeostasis. Cell 83:69–78, 1995

    Kimura S, Kadowaki T: Insulin effect during embryogenesis determines 
    fetal growth: a possible molecular link between birth weight and susceptibility 

12. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S: 
    Mutations in the GCK gene of the fetus result in reduced birth weight. 

13. Prisco F, Iafusco D, Franzese A, Sulli N, Barbetti F: MODY 2 presenting as 
    neonatal hyperglycaemia: a need to reshape the definition of “neonatal diabetes”? 
    Diabetologia 43:1331–1332, 2000

    gene mutations are not a common cause of permanent neonatal diabetes in 

15. Gloyn AL, Ellard S, Shield JP, Temple IK, Mackay DJ, Polak M, Barrett T, 
    Hattersley AT: Complete glucokinase deficiency is not a common cause of 
    permanent neonatal diabetes. Diabetologia 45:290, 2002

16. Matschinsky FM: Regulation of pancreatic β-cell glucokinase: from basics 

17. Grimsby J, Sarabu R, Corbett WL, Haynes NE, Bizzarro PT, Coffey JW, 
    Guertin KR, Hilliard DW, Kester RF, Mahaney PE, Marcus L, Qi L, Spence CL, 
    Tengi J, Magnuson MA, Chu CA, Dvorozniak MT, Matschinsky FM, 
    Grippo JP: Allosteric activators of glucokinase: potential role in diabetes 

    Stoffel M, Takeda J, Passa P, Permutt MA, Beckmann JS, Bell GI, Cohen D: 
    Familial hyperglycemia due to mutations in GCK: definition of a subtype of diabetes mellitus. 

19. Alberti KGMM, Zimmet P: Definition, diagnosis and classification of diabetes mellitus 
    and its complications. I. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. 
    Diabet Med 15:539–553, 1998