Brief Genetics Report

Permanent Neonatal Diabetes due to Mutations in *KCNJ11* Encoding Kir6.2

Patient Characteristics and Initial Response to Sulfonylurea Therapy

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Permanent neonatal diabetes (PND) can be caused by mutations in the transcription factors insulin promoter factor (IPF)-1, eukaryotic translation initiation factor-2α kinase 3 (EIF2AK3), and forkhead box-P3 and in key components of insulin secretion: glucokinase (GCK) and the ATP-sensitive K$^+$ channel subunit Kir6.2. We sequenced the gene encoding Kir6.2 (*KCNJ11*) in 11 probands with GCK-negative PND. Heterozygous mutations were identified in seven probands, causing three novel (F35V, Y330C, and F333I) and two known (V59M and R201H) Kir6.2 amino acid substitutions. Only two probands had a family history of diabetes. Subjects with the V59M mutation had neurological features including motor delay. Three mutation carriers tested had an insulin secretory response to tolbutamide, but not to glucose or glucagon. Glibenclamide was introduced in increasing doses to investigate whether sulfonylurea could replace insulin. At a glibenclamide dose of 0.3–0.4 mg · kg^{-1} · day^{-1}, insulin was discontinued. Blood glucose did not deteriorate, and HbA$_{1c}$ was stable or fell during 2–6 months of follow-up. An oral glucose tolerance test performed in one subject revealed that glucose-stimulated insulin release was restored. Mutations in Kir6.2 were the most frequent cause of PND in our cohort. Apparently insulin-dependent patients with mutations in Kir6.2 may be managed on an oral sulfonylurea with sustained metabolic control rather than insulin injections, illustrating the principle of pharmacogenetics applied in diabetes treatment. *Diabetes* 53:2713–2718, 2004

Neonatal diabetes may be defined as hyperglycemia diagnosed within the first 3 months of life (1). Transient neonatal diabetes is associated with abnormalities in chromosome 6 (2), whereas the permanent form may be caused by mutations in the genes encoding the transcription factors insulin promoter factor (IPF)-1 (3,4), eukaryotic translation initiation factor-2α kinase 3 (EIF2AK3) (5), forkhead box-P3 (6), and the glucose-sensing enzyme glucokinase (GCK) (7,8). Gloyn et al. (9) recently identified that the ATP-sensitive K$^+$ channel subunit Kir6.2 can cause PND. We here report nine new cases of PND associated with mutations in the gene *KCNJ11* encoding Kir6.2. In addition, we also show that oral therapy with a sulfonylurea drug should be considered in patients with mutations in Kir6.2.

RESEARCH DESIGN AND METHODS

The neither systematic nor population-based screening included 16 referred probands with PND from Norway, Israel, Italy, Turkey, and the U.S. (8,10). Informed consent was obtained from the subjects or their parents. The studies were performed according to the Declaration of Helsinki and approved by ethics committees.

Genetic studies. *KCNJ11* encoding Kir6.2 was sequenced as previously described (9). Microsatellite markers localized to four different chromosomes were used to confirm family relationships.

Clinical studies. A common protocol was carried out for all participating centers. The medical records of mutation carriers were reviewed and the patients subjected to general physical and neurological examinations. Electroencephalograms, electrocardiograms, and echocardiography were performed. Biochemical tests were done after an overnight fast. Assays were chemiluminometric (C-peptides), photometric hexokinase (glucose), and immunoinhibition (HbA$_{1c}$). Oral tolbutamide tests, meal challenge, and glucagon tests were done as follows: if multiple daily insulin injections were used, nighttime long-acting insulin was withheld. If the patient was on an insulin pump, basal insulin was set to 50%. Breakfast and morning insulin were withheld. For the tolbutamide test, blood was drawn at times −30, −15, 0, 15,
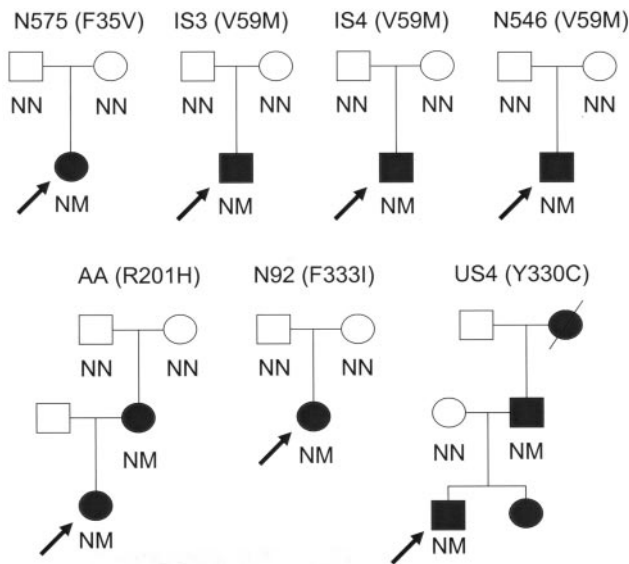
FIG. 1. Families with permanent neonatal diabetes and mutations in the *KCNJ11* gene encoding Kir6.2. The partial pedigrees and the predicted amino acid substitutions F35V, V59M, R201H, Y330C, and F333I are shown. Black squares and circles indicate subjects with neonatal diabetes. A slash mark indicates deceased subjects. Allele status is shown as no mutation (N) and mutation (M). Arrows denote the probands in each family. Amino acids are identified by their single-letter codes.

30, 45, 60, 90, 150, and 180 min for serum glucose and C-peptide measurements. Tolbutamide (25 mg/kg) was given orally. The meal challenge included one slice of white bread and 100 ml milk or yogurt or Boost High Protein (6 ml/kg, maximum 300 ml). Blood samples were obtained at times 0, 30, 60, and 90 min. Glucagon (0.03 mg/kg, maximum 1 mg) was given intravenously and blood drawn at times 0, 5, and 10 min for serum glucose and C-peptide measurements. For the treatment protocol, daily insulin requirement and capillary glucose before four to five meals were recorded 2 weeks before, during, and after the trial. If available, a continuous glucose monitoring system was used before, during, and after the trial. Glibenclamide was given with an initial dose of 0.05 mg/kg two times a day, and the dose was increased one to two times a week by 25–100%.

RESULTS

Of 16 case subjects referred with PND, 5 had mutations in *GCK*, which have been reported elsewhere (7,8). Heterozygous missense mutations were identified in the gene encoding Kir6.2 in 7 of 11 probands with *GCK*-negative PND and also in two parents (Fig. 1). The novel mutations TTT to GTT, TAC to TGC, and TTT to ATT, corresponding to the predicted amino acid substitutions Phe35Val (F35V), Tyr330Cys (Y330C), Phe333Ile (F333I), respectively, were identified in families N575 (F35V), US4 (Y330C), and N92 (F333I). The mutations CGT to CAT and GTG to ATG, corresponding to the previously described PND-associated amino acid substitutions Val59Met (V59M) and Arg201His (R201H), respectively, were identified in families N546 (V59M), IS3 (V59M), IS4 (V59M), and AA (R201H). The affected amino acid residues 35, 59, 201, 330, and 333 in the Kir6.2 protein are conserved in humans, mouse, and bullfrog. The mutations were not present in 100 nondiabetic Caucasian subjects. In two families, there was vertical transmission of the mutation and diabetes, which occurred either maternally or paternally. In five probands, the mutations arose de novo because the parents were healthy and did not have the mutation. Taken together, there is very strong evidence that the Kir6.2 mutations are the cause of PND in the seven probands.

The clinical features of the nine mutations carriers from the seven families are shown in Table 1. Diabetes was diagnosed at a median age of 6 weeks (range 1–24). All subjects presented with marked hyperglycemia (median 27 mmol/l; range 20–55). Autoantibodies associated with type 1 diabetes were not present. Basal serum C-peptide concentrations were <0.5 nmol/l, and glucagon-stimulated values were <0.66 nmol/l. Insulin doses before the treatment trial were 0.6 units $\cdot$ kg^{-1} $\cdot$ day^{-1} (median, range 0.3–2). Birth weights were all below the mean (median 2,580 g; range 2,100–3,260), and six patients were small for gestational age (<10th percentile). All had normal electrocardiograms and echocardiography. None of the patients had epilepsy. Three subjects, N546, IS3, and IS4, all with the V59M mutation, had (on neurological examination) neurological features with mildly retarded motor and mental development evident in the first years of life. One patient (N546) also had muscle weakness. His developmental delay involved failure to achieve motor, mental, and social milestones appropriate for his age and was judged appropriate for 9 months at 12 months of age. Two subjects (IS3 and IS4) had mildly delayed motor development and attention deficit hyperactivity disorder. One of these children (IS4) had pathological electroencephalogram, with few generalized bursts of spike and slow waves. One subject (IS3) had micropenis, but none of the probands had other dysmorphic features. Neither of the patients with neurologic features was reported to have more or longer episodes of hypoglycemia than a typical child with diabetes. Notably, the father in family US4 was evaluated by a geneticist at the age of 8–9 years and was noted as having motor and mental retardation, receiving special education. We have not been able to investigate this subject any further.

Sulfonylureas stimulate insulin secretion by binding to the β-cell's high-affinity sulfonylurea receptor and closing the ATP-sensitive K$^+$ channels by an ATP-independent mechanism (11). Initial studies in subjects with activating Kir6.2 mutations showed a response to intravenous tolbutamide (9), and we therefore examined the response to an oral sulfonylurea. For practical reasons, initial studies were completed on three patients (probands in families N92 [F333I], AA [R201H], and N546 [V59M]) and are reported here. Oral tolbutamide tolerance tests revealed a maximum serum C-peptide increment from 0.16 to 0.39 for N92 (online appendix 1 [available from http://diabetes.diabetesjournals.org]), from 0.10 to 0.60 for AA, and from <0.01 to 0.01 for N546 (not shown). Corresponding plasma glucose decreased from 6.8 to 3.9 (N92), from 11.9 to 5.7 (AA), and from 3.8 to 3.1 mmol/l (N546). This finding prompted us to introduce orally administered glibenclamide. The results for N92 are shown in Fig. 2. Basal and bolus insulin were given with continuous subcutaneous insulin infusion as needed. The parents measured capillary glucose before every meal and at bedtime and recorded the patient's daily insulin requirement. At a glibenclamide dose of 0.1 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}, the insulin requirement declined, and on 0.4 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} glibenclamide, insulin was discontinued. Frequent capillary glucose measurements showed no deterioration as insulin was removed (Figs. 2 and 3). HbA$_{1c}$ for patient N92 was 8.2% (reference value 4.0–6.2) at the start of the treatment

TABLE 1
Clinical characteristics of subjects with PND screened for mutations in the gene encoding Kir6.2

	N575	N546	IS3	IS4	AA	AA	US4	US4	N92
Ethnicity	Caucasian	Caucasian	Arabic	Arabic	Hispanic	Hispanic	Caucasian	Caucasian	Caucasian
Family status	Proband	Proband	Proband	Proband	Proband	Mother	Proband	Father	Proband
Mutation	F35V	V59M	V59M	V59M	R201H	R201H	Y330C	Y330C	F333I
Birth									
Weight (g)	2,950	3,260	2,100	2,710	2,600	2,400	2,580	2,100	2,410
Percentile	10	25–50	<3	3–10	5–10	<5	10–25	10–25	<3
Gestation (weeks)	42	39	40	42	38	38	36	38	40
Findings on presentation									
Glucose (mmol/l)	26	20	20	27	44	48	55	22	55
Age of diabetes diagnosis (weeks)	12	1	6	6	2	24	6	<1	10
Current status									
Age (years)	5	1	9	5	6	26	3	25	2
Percentile for height	50	10	45	20	25	10	10–25	<5	50
Percentile for weight	50	50	25	15	10	<5	75–90	10	25
Insulin dose (units · kg^{-1} · day^{-1})	0.55	0	1.2	0.9	0	2	0.45	0.84	0
Sulfonylurea (mg · kg^{-1} · day^{-1})	0	0.3	0	0	0.4	0	0	0	0.4
Recent HbA$_{1c}$ (%)	7.2	6.8	8.7	11.9	6.1	NA	8.2	10	6.3
C-peptide (nmol/l)	NA	<0.01	<0.01	0.39	0.5	NA	NA	0.36/0.07/0.0*	0.17
Paired glucose (mmol/l)	NA	7.3	5.5–8.8	10.7	7.7	NA	NA	20.6/14.3/23.4*	7.6
Peak C-peptide with glucagon (nmol/l)	NA	NA	<0.165	0.66	0.5	NA	NA	NA	0.22
Neurological features	No	Yes	Yes	Yes	No	No	No	Yes	No

*Investigated at age 5 weeks, 4 months, and 16 months, respectively (10). NA, not available.
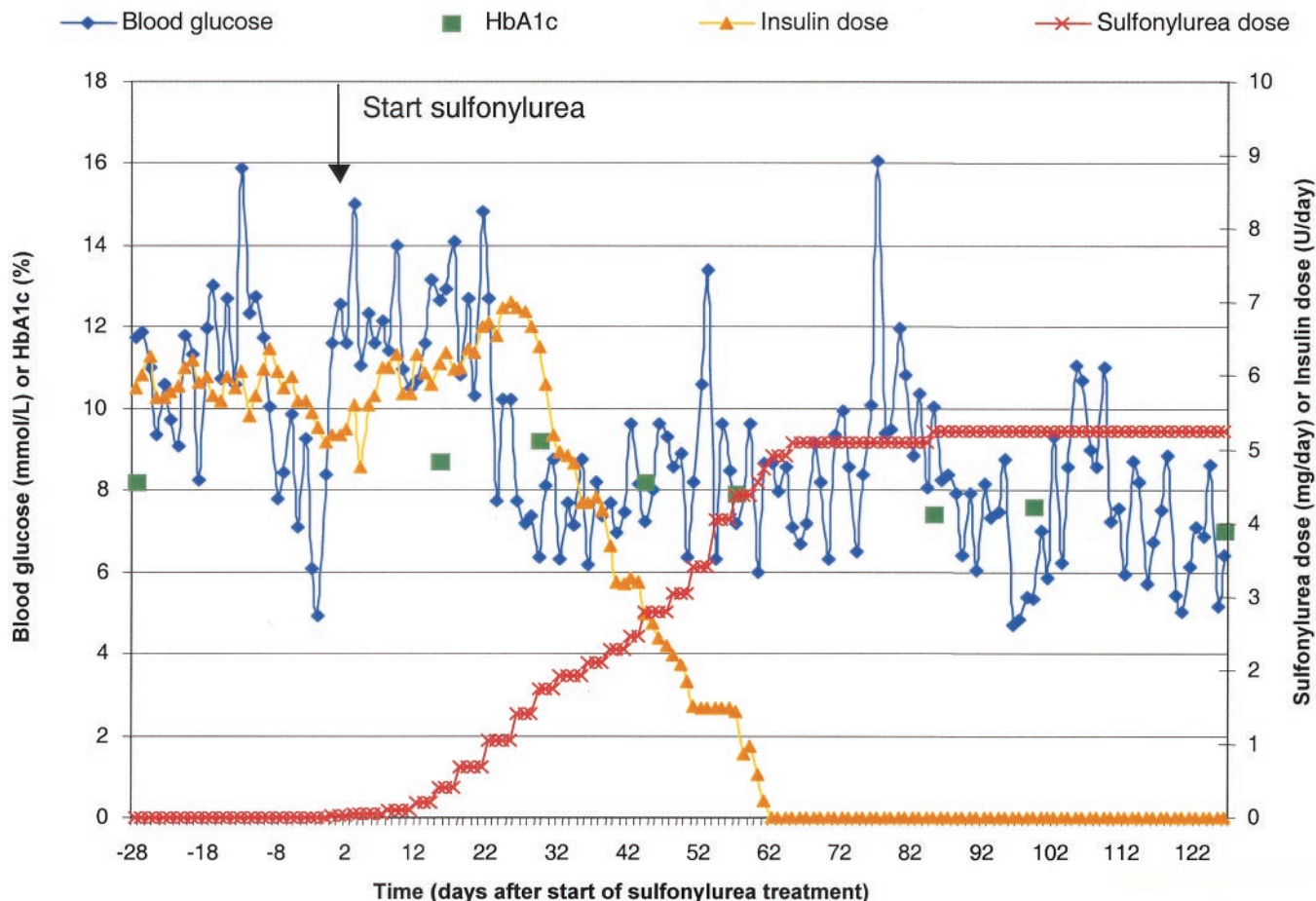
FIG. 2. Sulfonylurea treatment in an apparently insulin-dependent infant with PND caused by a mutation in Kir6.2 (N92 carrying the mutation F333I). Measurements of capillary glucose, HbA$_{1c}$, administered dose of sulfonylurea, and insulin requirement are presented. Each point of capillary glucose (blue) is an average of five to six daily measurements done by a standard glucose meter at home. HbA$_{1c}$ (green) was measured at the hospital. An arrow indicates when orally administered sulfonylurea (glibenclamide) was initiated. The dose was increased every 3 days. The parents reduced the insulin dose (orange) in parallel, according to the capillary glucose measurements at home. As revealed by HbA$_{1c}$ measurements, the metabolic control did not deteriorate 6 months after insulin was discontinued.

protocol, 7.9% when insulin was discontinued, and 6.3% after 6 months off insulin. For subject AA, insulin was weaned and discontinued over a period of 2 weeks with a current glibenclamide dose of $0.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. HbA$_{1c}$ was 7.1% (reference value 4.0–6.4) before the trial and 6.1% (within normal range) after 3 months off insulin. For N546, insulin was reduced and discontinued after 8 weeks with a current glibenclamide dose of $0.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. HbA$_{1c}$ was 6.1% (reference value 4.0–6.2) before the trial, 7.1% during the trial, and 6.8% 2 months after the trial. Hence, the metabolic control on oral treatment with a sulfonylurea seemed equivalent compared with treatment with continuous subcutaneous insulin infusion. The patients remain well and off insulin 6 months (N92), 4 months (AA), and 2 months (N546) into follow-up.

An oral glucose tolerance test in patient N92 (F333I) after insulin was discontinued showed an increment in C-peptide from 0.74 to a maximum of 1.34 nmol/l (not shown), indicating that glucose-stimulated insulin release was restored to some extent on sulfonylurea treatment. This is in line with the clinical observations that three 24-h metabolic profiles revealed wide excursions, but essentially the same pattern before and after sulfonylurea therapy (Fig. 3).

DISCUSSION

Recently, it has been shown (9) that PND can result from mutations in Kir6.2 that reduce the ability for ATP to close the ATP-sensitive K$^+$ channel. This study supports that Kir6.2 mutations can cause PND. We found that 7 of 16 probands with PND had a mutation in *KCNJ11* encoding Kir6.2. Because 5 of the 16 probands had mutations in *GCK*, our findings support mutations in Kir6.2 being the most common cause of PND. Kir6.2-related PND has now been described in patients from Europe, the Middle East, Australia, and North and South America. In this study, five of seven case subjects had de novo mutations, which appear to be a common feature of the syndrome (9). This is an important clinical point as PND due to homozygous or compound heterozygous mutations in *IPF-1* or *GCK* are associated with heterozygous mutations in the glucose-intolerant parents (3,4,7,8).

The phenotypic expression of the nine patients presented here is similar to that reported for the first 10 subjects (9). Also our patients did not have type 1 diabetes–associated antibodies and had comparable low levels of basal and glucagon-stimulated C-peptide (9). Moreover, six of the new case subjects were small for gestational age
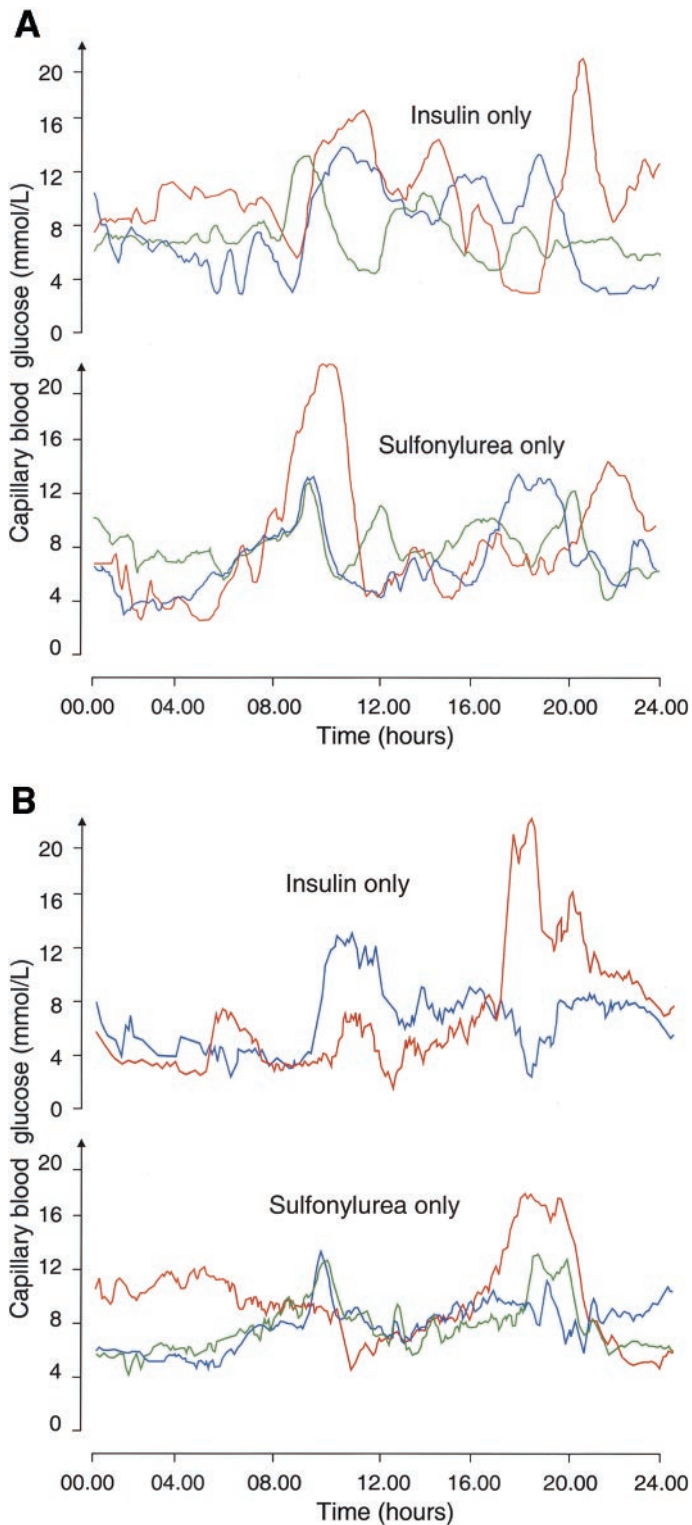
A

B

FIG. 3. Frequent capillary glucose measurements in two patients with PND caused by mutations in the gene encoding Kir6.2. A continuous glucose measuring system was recorded in subjects N92 (F333I) (*A*) and AA (R201H) (*B*) on 3 consecutive days (one color for each day) before the trial (on insulin only) and 1 month after insulin was discontinued (on sulfonylurea only).

due to intrauterine growth retardation, and all nine exhibited severe hyperglycemia and subsequent exogenous insulin requirement within 6 months after birth. This phenotype resembles a transgenic mouse model express-

ing a β-cell–specific Kir6.2 protein with reduced ATP sensitivity in which the animals develop hypoinsulinemia, severe hyperglycemia, and ketoacidosis shortly after birth (12). Notably, the patients described here and in Gloyn et al. (9) were diagnosed later and had higher birth weights than subjects with PND due to homozygous GCK mutations (7,8). A similar relationship is seen when the transgenic models are compared: knockout mice for GCK (13) are growth retarded at birth and die somewhat earlier than the Kir6.2 mice mentioned above, which typically live until day 5. Taken together, the data suggest a less severe insulin secretory defect in Kir6.2-mutated than in GCK-deficient patients, at least initially.

Our present case subjects, together with those in the recent report (9), suggest the description in some subjects of a specific syndrome of neonatal diabetes with extra-pancreatic features of muscle weakness and developmental delay. Some patients with the mutation V59M may have neurological features in addition to diabetes, representing a subgroup of PND and supporting a role for Kir6.2 in muscle and cerebral development and function (14). It is interesting that our three probands with neurological affection all had the same mutation. A controlled trial in which an identical neurological evaluation is blindly performed in matched non-Kir6.2 PND patients is required to prove a neurological component in Kir6.2-mutated subjects.

Which cases of neonatal diabetes should be examined for Kir6.2 mutations? Since the subjects here and in Gloyn et al. (9) were negative for type 1 diabetes–associated antibodies and were diagnosed within 24 weeks after birth, we will suggest screening primarily neonates with an antibody-negative form of diabetes diagnosed before 6 months of age.

Although we have not proven that responsiveness to sulfonylurea treatment is a specific characteristic of patients with mutations in Kir6.2, our treatment trial suggests that oral sulfonylureas can replace subcutaneously injected insulin in children with PND due to Kir6.2-activating mutations for up to 6 months. We have not established that the present treatment is better than insulin given as a continuous subcutaneous insulin infusion. It is, however, very interesting that continuous glucose monitoring systems were comparable during and after insulin treatment and that HbA_{1c} in one patient was stable and in two subjects declined from mildly to moderately elevated values, when the children were on insulin only, to values close to the normal range 4–6 months after insulin was discontinued. We found 0.3–0.4 mg · kg^{-1} · day^{-1} to be a suitable glibenclamide dose based on no episodes of hypoglycemia and stable metabolic control. Long-term follow-up and studies of more case subjects including other mutations are needed to evaluate our experience. The present case subjects illustrate the pharmacogenetic principle applied to some diabetic patients, i.e., how genetic definition of the etiology can be used to select optimal treatment (15).

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