Permanent Neonatal Diabetes due to Mutations in \( \text{KCNJ11} \) 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-2\( \alpha \) 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 (\( \text{KCNJ11} \)) 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 \( \cdot \) kg\(^{-1} \) \( \cdot \) 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-2\( \alpha \) 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 \( \text{KCNJ11} \) 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.** \( \text{KCNJ11} \) 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 chemiluminesometric ([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 \( 30, 15, 0, 15, 30, 45, 60, \) and 90 minutes.
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 · kg⁻¹ · 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 V359M 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 microenesis, 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 tolbutamid (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 [V359M]) and are reported here. Oral tolbutamid 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 · kg⁻¹ · day⁻¹, the insulin requirement declined, and on 0.4 mg · kg⁻¹ · day⁻¹ glibenclamide, insulin was discontinued. Frequent capillary glucose measurements showed no deterioration as insulin was removed (Figs. 2 and 3). HbA₁c for patient N92 was 8.2% (reference value 4.0–6.2) at the start of the treatment.
<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Family Status</th>
<th>Mutation</th>
<th>Birth Weight (g)</th>
<th>Gestation (weeks)</th>
<th>Glucose (mmol/l)</th>
<th>Age of Diabetes Diagnosis (weeks)</th>
<th>Recent HbA1c (%)</th>
<th>C-peptide (nmol/l)</th>
<th>Paired Glucose (mmol/l)</th>
<th>Paired C-peptide (nmol/l)</th>
<th>Neurological Features</th>
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</table>

*Investigated at age 5 weeks, 4 months, and 16 months, respectively (10). NA, not available.
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 mg \( \cdot \) kg\(^{-1} \cdot \) 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 mg \( \cdot \) kg\(^{-1} \cdot \) 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.
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 expressing 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 extrapancreatic 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 HbA1c 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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REFERENCES