

ORIGINAL ARTICLE

Activating Mutations in the Gene Encoding the ATP-Sensitive Potassium-Channel Subunit Kir6.2 and Permanent Neonatal Diabetes

Anna L. Gloyn, D.Phil., Ewan R. Pearson, M.R.C.P., Jennifer F. Antcliff, B.Sc., Peter Proks, D.Phil., G. Jan Bruining, M.D., Annabelle S. Slingerland, M.D., Neville Howard, M.D., F.R.A.C.P., Shubha Srinivasan, M.B., B.S., M.R.C.P., José M.C.L. Silva, M.D., Janne Molnes, M.Sc., Emma L. Edghill, M.Sc., Timothy M. Frayling, Ph.D., I. Karen Temple, F.R.C.P., Deborah Mackay, Ph.D., Julian P.H. Shield, M.D., F.R.C.P.C.H., Zdenek Sumnik, M.D., Adrian van Rhijn, M.D., Jerry K.H. Wales, D.M., F.R.C.P.C.H., Penelope Clark, Ph.D., F.R.C.Path., Shaun Gorman, M.R.C.P., Javier Aisenberg, M.D., Sian Ellard, Ph.D., M.R.C.Path., Pål R. Njølstad, M.D., Ph.D., Frances M. Ashcroft, Ph.D., and Andrew T. Hattersley, D.M., F.R.C.P.

From the Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, United Kingdom (A.L.G., E.R.P., E.L.E., T.M.F., S.E., A.T.H.); the University Laboratory of Physiology, Oxford University, Oxford, United Kingdom (J.F.A., P.P., F.M.A.); Sophia Children's Hospital, Rotterdam, the Netherlands, (G.J.B., A.S.S.); the Institute of Endocrinology and Diabetes, Children's Hospital at Westmead, Westmead, Australia (N.H., S.S.); Piaui State University Medical School, Teresina, Piaui, Brazil (J.M.C.L.S.); the Institute for Clinical Medicine and Molecular Medicine, University of Bergen, Bergen, Norway (J.M., P.R.N.); Wessex Clinical Genetics Service and the Division of Human Genetics, Southampton University and Hospitals, National Health Service Trust, Southampton, United Kingdom (I.K.T.); Wessex Regional Genetics Laboratories, Salisbury District Hospital, Salisbury, United Kingdom (D.M.); Royal Hospital for Children, Bristol, United Kingdom (J.P.H.S.); Second Department of Pediatrics and Second Faculty of Medicine, Charles University, Prague, Czech Republic (Z.S.); Meander Medical Center, Amersfoort, the Netherlands (A.R.); Academic Unit of Child Health, Sheffield Children's Hospital, Sheffield, United Kingdom (J.K.H.W.); Regional Endocrine Laboratory, Birmingham, United Kingdom (P.C.); St. Luke's Hospital, Bradford, United Kingdom (S.G.); and the Division of Pediatric Endocrinology and Diabetes, Hackensack University Medical Center, Hackensack, N.J. (J.A.). Address reprint requests to Dr. Hattersley at Diabetes and Vascular Medicine, Institute of Biomedical and Clinical Science, Peninsula Medical School, Barrack Rd., Exeter EX2 5AX, United Kingdom, or at a.t.hattersley@ex.ac.uk.

Dr. Pearson, Ms. Antcliff, and Dr. Proks contributed equally to the article.

N Engl J Med 2004;350:1838-49.
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ABSTRACT

BACKGROUND

Patients with permanent neonatal diabetes usually present within the first three months of life and require insulin treatment. In most, the cause is unknown. Because ATP-sensitive potassium (K_{ATP}) channels mediate glucose-stimulated insulin secretion from the pancreatic beta cells, we hypothesized that activating mutations in the gene encoding the Kir6.2 subunit of this channel (*KCNJ11*) cause neonatal diabetes.

METHODS

We sequenced the *KCNJ11* gene in 29 patients with permanent neonatal diabetes. The insulin secretory response to intravenous glucagon, glucose, and the sulfonylurea tolbutamide was assessed in patients who had mutations in the gene.

RESULTS

Six novel, heterozygous missense mutations were identified in 10 of the 29 patients. In two patients the diabetes was familial, and in eight it arose from a spontaneous mutation. Their neonatal diabetes was characterized by ketoacidosis or marked hyperglycemia and was treated with insulin. Patients did not secrete insulin in response to glucose or glucagon but did secrete insulin in response to tolbutamide. Four of the patients also had severe developmental delay and muscle weakness; three of them also had epilepsy and mild dysmorphic features. When the most common mutation in Kir6.2 was coexpressed with sulfonylurea receptor 1 in *Xenopus laevis* oocytes, the ability of ATP to block mutant K_{ATP} channels was greatly reduced.

CONCLUSIONS

Heterozygous activating mutations in the gene encoding Kir6.2 cause permanent neonatal diabetes and may also be associated with developmental delay, muscle weakness, and epilepsy. Identification of the genetic cause of permanent neonatal diabetes may facilitate the treatment of this disease with sulfonylureas.

NEONATAL DIABETES MAY BE DEFINED as insulin-requiring hyperglycemia that is diagnosed within the first three months of life. It may be either transient, resolving within a median of three months, or permanent, in which case insulin treatment is required for life.¹ Substantial progress has been made in our understanding of transient neonatal diabetes, with the majority of cases being attributable to an abnormality in an imprinted region of chromosome 6.² In most patients, the cause of permanent neonatal diabetes is unknown; homozygous and compound heterozygous mutations in the gene encoding glucokinase account for a minority of cases,³⁻⁶ and the genes for some very rare, multisystem conditions that include neonatal diabetes have been identified.⁷⁻⁹

ATP-sensitive potassium (K_{ATP}) channels play a central role in glucose-stimulated insulin secretion from pancreatic beta cells: insulin secretion is initiated by closure of the channels and inhibited by their

opening (Fig. 1).¹⁰ The beta-cell K_{ATP} channel is an octameric complex of four pore-forming, inwardly rectifying potassium-channel subunits (Kir6.2) and four regulatory sulfonylurea-receptor subunits (SUR1).¹¹ Both Kir6.2 and SUR1 are required for correct metabolic regulation of the channel: ATP closes the channel by binding to Kir6.2, and magnesium nucleotides (Mg-ADP and Mg-ATP) stimulate channel activity by interacting with SUR1. Sulfonylureas stimulate insulin secretion in type 2 diabetes by binding to SUR1 and closing K_{ATP} channels by an ATP-independent mechanism.¹⁰

We hypothesized that activating mutations in the gene encoding the Kir6.2 subunit of the beta-cell K_{ATP} channel (*KCNJ11*) cause monogenic diabetes, because inactivating mutations in this gene lead to uncontrolled insulin secretion and congenital hyperinsulinism.¹² The contrasting phenotypes of permanent neonatal diabetes and hyperinsulinism are seen with inactivating and activating mutations, re-

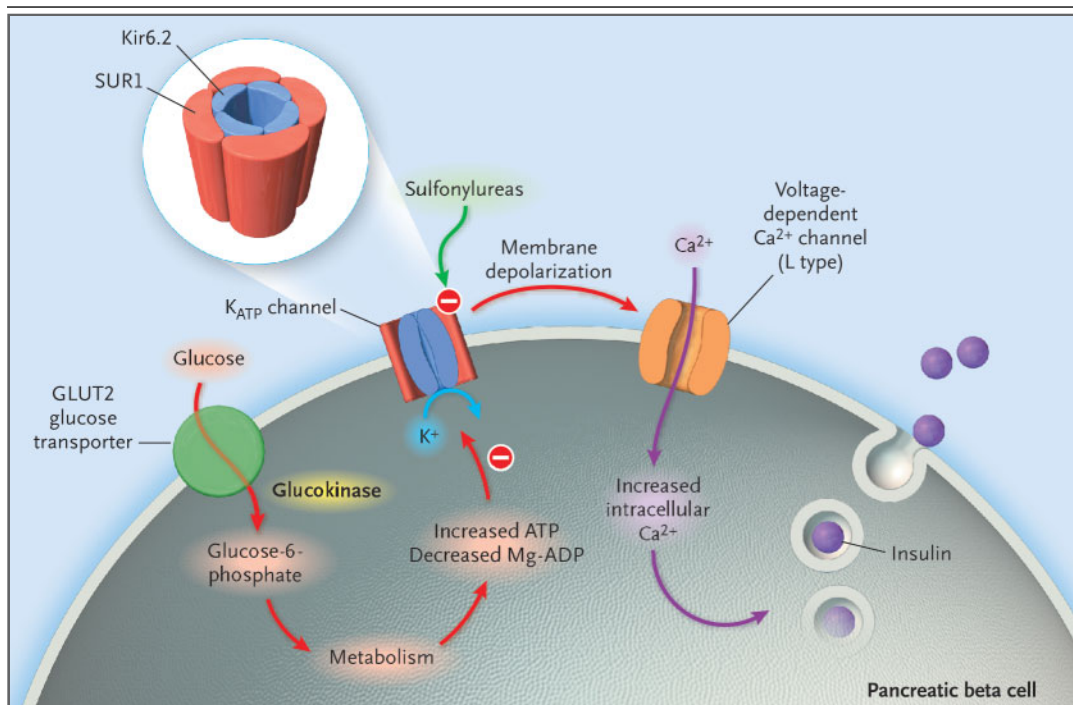


Figure 1. Schematic Representation of the Pancreatic Beta Cell, Illustrating the Role of the ATP-Sensitive Potassium (K_{ATP}) Channel in Insulin Secretion.

Glucose enters the beta cell by way of the GLUT2 glucose transporter. Once inside the cell, glucose is metabolized, leading to changes in the intracellular concentration of adenine nucleotides that inhibit the K_{ATP} channel and thus cause channel closure. The K_{ATP} channel consists of four sulfonylurea-receptor (SUR1) subunits and four Kir6.2 subunits in an octameric structure. Channel closure leads to membrane depolarization, which subsequently activates voltage-dependent calcium (Ca^{2+}) channels, leading in turn to an increase in intracellular Ca^{2+} , which triggers insulin exocytosis. Sulfonylureas initiate secretion by directly binding to the SUR1 subunits of K_{ATP} channels and causing channel closure. Mg-ADP denotes magnesium ADP.

METHODS

spectively, of the gene encoding glucokinase.^{3,13,14} Strong support for our hypothesis comes from the observation that transgenic mice with overactive beta-cell K_{ATP} channels have profound neonatal diabetes.¹⁵ We therefore sequenced the gene encoding Kir6.2 in patients who had permanent neonatal diabetes or dominantly inherited maturity-onset diabetes or dominantly inherited maturity-onset diabetes of the young (MODY).

PATIENTS

We sequenced the DNA of 29 probands with permanent neonatal diabetes, mainly from the International Society for Pediatric and Adolescent Diabetes (ISPAD) Rare Diabetes Collection. Patients were registered in the collection or were recruited for the

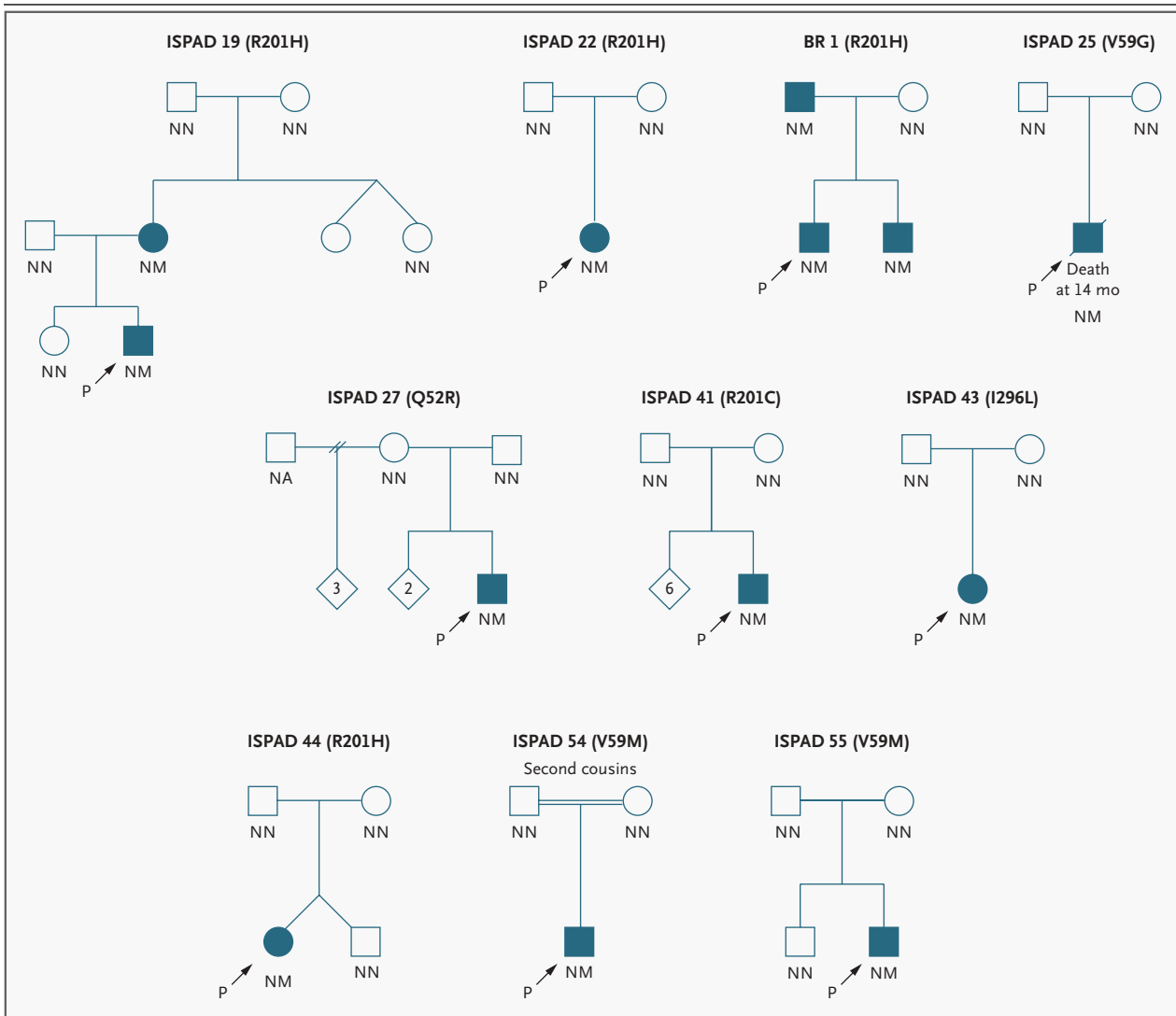


Figure 2. Diabetes Status and Mutations in the Gene Encoding Kir6.2 in 10 Families.

These partial pedigrees show families with the Q52R, V59G, V59M, R201C, R201H, and I296L mutations. In all the pedigrees for which parental DNA was available, family relationships were confirmed by a panel of 10 microsatellites. In ISPAD pedigrees 19, 22, 27, 41, 43, 44, 54, and 55, spontaneous mutations explain the absence of permanent neonatal diabetes in the parents and its presence in a child. Squares represent male family members, circles female family members, and diamonds sex not defined; blue circles and squares represent persons with neonatal diabetes; a slash mark indicates deceased. The numbers inside diamonds indicate the number of unaffected siblings. A two-letter code for allele status is shown underneath each symbol: N denotes no mutation, M mutation, and NA not available for testing. P and an arrow denote the proband in each family (the first affected member recruited for this study). Amino acids are denoted by their single-letter codes.

study between September 2001 and October 2003. Patients with abnormalities in chromosome 6q24, mutations in the gene encoding glucokinase, exocrine pancreatic insufficiency, and pancreatic agenesis were excluded. We also sequenced the DNA of 15 probands with MODY from families in the United Kingdom in whom mutations in the six known MODY-associated genes had been ruled out.¹⁶ Written informed consent was obtained from all the patients or their parents.

MUTATIONAL ANALYSES

The coding region and the intron–exon boundaries of *KCNJ11* were amplified from genomic DNA by the polymerase chain reaction with the use of previously described primers¹⁷ in addition to fragment 5R 5'CTGTGGTCCTCATCAAGCTG3', fragment 6F 5'GCTGAGGAGGACGGACGTTAC3', and fragment 6R 5'CCACATGGTCCGTGTGTACACACG3'. The products were sequenced by standard methods. Family relationships were confirmed with the use of a panel of 10 microsatellite markers.

CLINICAL STUDIES

All patients with mutations in the gene encoding Kir6.2 underwent clinical examinations, including detailed developmental and neurologic assessments by a consultant pediatrician or physician, and their medical records were reviewed. Electrocardiograms were examined for evidence of arrhythmias and for measurement of the QT interval. All physiological tests were performed after the patients had fasted overnight. A glucagon stimulation test was performed as follows: 15 μ g of glucagon per kilogram of body weight (maximal dose, 1 mg) was given intravenously at time 0, and blood samples for measurement of C-peptide were obtained at –10, –5, 0, 2, 4, 6, 8, 10, 15, and 20 minutes. The highest C-peptide value was then recorded. A tolbutamide-modified, frequently sampled intravenous glucose-tolerance test was performed as previously described.¹⁸ After base-line sampling, a bolus of 0.3 g of glucose per kilogram was given intravenously, followed by a bolus of 3 mg of tolbutamide per kilogram 20 minutes later. We calculated the peak incremental insulin response after the glucose bolus and after the tolbutamide bolus.

FUNCTIONAL STUDIES

Wild-type mouse Kir6.2 or Kir6.2 in which histidine replaced arginine at position 201 (R201H) was coexpressed with rat SUR1 (containing exon 17) in

Xenopus laevis oocytes, and K_{ATP} currents were recorded as previously described.^{19,20} To simulate the effect of heterozygosity, we injected oocytes with SUR1 and a 1:1 mixture of Kir6.2 and Kir6.2-R201H messenger RNA (mRNA). ATP concentration–response curves were fitted according to the Hill equation: $I \div I_C = 1 \div [1 + ([ATP] \div IC_{50})^h]$, where I is the K_{ATP} current, I_C is the current in the absence of nucleotide, $[ATP]$ is the ATP concentration, IC_{50} is the ATP concentration at which inhibition is half maximal, and h is the Hill coefficient. Data are given as means \pm SE.

RESULTS

MUTATIONAL ANALYSES

We identified six novel, heterozygous mutations in the gene encoding Kir6.2 in 10 of the 29 probands

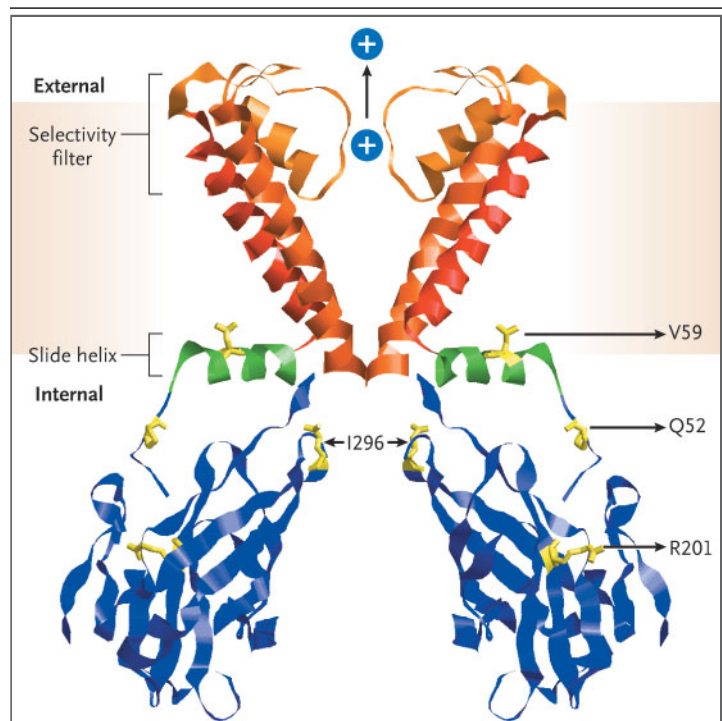


Figure 3. Illustration of Two Kir6.2 Subunits, Showing the Mutations Identified in Patients with Permanent Neonatal Diabetes.

The illustration is based on the crystal structure of the potassium channel KirBac1.1.²¹ The alpha-helical slide helix is shown in green, the selectivity filter in orange, and the C-terminal beta sheets in blue. The residues affected by mutations in patients with neonatal diabetes (Q52, V59, R201, and I296) are shown in yellow. Amino acids are denoted by their single-letter codes. The shaded band represents the cell membrane, and the blue circles the potassium ion. The labels “External” and “Internal” refer to areas outside and inside the cell.

Table 1. Clinical Characteristics of Patients with Mutant Kir6.2.*

Characteristic	ISPAD 19		ISPAD 22		ISPAD 41		ISPAD 44		ISPAD 54		BR 1		ISPAD 55		ISPAD 25		ISPAD 27		ISPAD 43		
	Proband	Mother	Proband	F	Proband	M	Proband	F	Proband	M	Proband	Brother	Father	Proband	M	Proband	M	Proband	M	Proband	F
Mutation†	R201H	R201H	R201H	R201C	R201H	R201H	R201H	R201H	V59M	R201H	R201H	R201H	R201H	V59M	V59G	Q52R	Q52R	Q52R	Q52R	Q52R	I296L
Birth																					
Weight (g)	2550	2280	2280	2200	2125	1440	3080	3120	2500	2700	1850	2400	2550	2700	1850	2400	2550	2400	2550	2550	2550
Percentile for weight‡	9	0.4	1.3	1.5	2.5	<0.1	16	18	NA	1.4	29	11	2.5	1.4	29	11	2.5	11	11	2.5	2.5
Gestation (wk)	38	40	39	38	38	37	40	40	NA	41	33	37	40	41	33	37	40	37	37	40	40
Findings on presentation																					
Clinical abnormality	Hyperglycemia	Ketoacidosis	Ketoacidosis	Hyperglycemia	Ketoacidosis	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia	Hyperglycemia
Glucose (mmol/liter)	15	54	54	36	33	16	23	30	NA	35.1	18	34.5	18.8	35.1	18	34.5	18.8	34.5	34.5	18.8	18.8
Pancreatic auto-antibodies	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Age diabetes diagnosed (wk)	Birth	6	6	4	15	Birth	<4	<3	12	5	1	5	26	5	1	5	5	5	5	26	26
Current status																					
Age (yr)	8	36	3	12	2	2	5	3	46	2	4	2	17	2	4	4	1.2 (died); 0.8 (last assessed)	3	3	<3	<3
Percentile for height	75	10	10	98	10-25	75	10	10	<5	85	1.5	3	<3	85	1.5	3	3	3	3	<3	<3
Percentile for weight	91	75	25	98	10-25	>98	10	10	6	50	1	3	<3	50	1	3	3	3	3	<3	<3
Insulin dose (U/kg/day)	0.6	0.6-1.0	0.6-1.0	1.6	0.7-1.0	1.3	0.7	0.7-1.3	NA§	0.3	0.3-0.4	0.2-0.5	0.2-0.4	0.3	0.3-0.4	0.2-0.5	0.2-0.5	0.2-0.5	0.2-0.5	0.2-0.4	0.2-0.4
Glycosylated hemoglobin (%)	8.7	7.4-10.0	7.4-10.0	7.0	7.7	9.2	NA	NA	NA	7.0	9.0-11.0	9.0	9.0-10.0	7.0	9.0-11.0	9.0	9.0	9.0	9.0	9.0-10.0	9.0-10.0
C-peptide (pmol/liter)	<170	<200	<200	<100	NA	<94	<200	<200	400¶	613	603	<165	60	613	603	<165	<165	<165	<165	60	60
Paired glucose (mmol/liter)	18.1	12.0	12.0	16.3	16.3	13.0	13.0	13.8	6.1	5.7	16.2	17.2	18.8	5.7	16.2	17.2	17.2	17.2	17.2	18.8	18.8
Peak C-peptide with glucagon (pmol/liter)	<170	<200	<200	NA	NA	NA	NA	NA	NA	NA	<165	<165	NA	NA	<165	<165	<165	<165	<165	NA	NA

Neurologic features											
Muscle weakness	No	No	No	No	No	No	No	No	No	Yes	Yes
Developmental delay											
Motor	No	No	No	No	No	No	No	Yes (appropriate for 8–11 mo at 23 mo of age)	Yes (appropriate for 6–7 mo at 4 yr of age)	Yes (appropriate for 3–6 mo at 12 mo of age)	Yes (appropriate for 1–2 yr at 17 yr of age; unable to walk)
Mental	No	No	No	No	No	No	No	Yes (appropriate for 18 mo at 23 mo of age)	Yes (speech and social skills appropriate for 7 mo at 4 yr of age)	Yes, but not formally assessed	Yes (no speech; fully dependent on parents at 17 yr of age)
Epilepsy	No	No	No	No	No	No	No	No	Yes	Yes	Yes
Age at onset (mo)	NA	NA	NA	NA	NA	NA	NA	NA	3	2	11
Seizure type	NA	NA	NA	NA	NA	NA	NA	NA	Tonic-clonic	Myoclonic	Generalized complex
Electroencephalogram	NA	NA	NA	NA	NA	NA	NA	Normal	Hypsarrhythmia, multifocal sharp waves, slow delta waves	Multifocal sharp waves, slow delta waves	Bifrontal sharp waves
Dysmorphic features	No	No	No	No	No	No	No	No	Downturned mouth, bilateral ptosis, prominent metopic suture, contractures in legs at 4 yr	Downturned mouth, bilateral ptosis, contractures mainly in legs at birth	Downturned mouth, bilateral ptosis, generalized contractures at birth (arthrogryposis)

* NA denotes not available.
 † Amino acids are denoted by their single-letter codes.
 ‡ Percentiles for birth weight were calculated according to United Kingdom growth charts.²²
 § This patient was treated with tolbutamide.
 ¶ C-peptide was measured while the patient was being treated with tolbutamide.
 || The paired glucose values are the glucose concentrations measured at the same time as the corresponding C-peptide concentrations.

who had permanent neonatal diabetes. The mutations were a glutamine-to-arginine substitution at position 52 (Q52R), a valine-to-glycine substitution at position 59 (V59G), a valine-to-methionine substitution at position 59 (V59M), an arginine-to-histidine substitution at position 201 (R201H), an arginine-to-cysteine substitution at position 201 (R201C), and an isoleucine-to-leucine substitution at position 296 (I296L). No mutations were found in any of the probands who had MODY. The R201H missense mutation was identified in 4 of these 10 probands, and the V59M missense mutation was detected in 2. In all the families, neonatal diabetes was seen only in persons who had Kir6.2 mutations, and all family members who did not have these mutations were not diabetic (Fig. 2).

In two families (ISPAD 19 and BR 1), neonatal diabetes had been transmitted from an affected parent to his or her offspring. Because both maternal and paternal transmission can occur, imprinting of this locus is unlikely. In nine cases, DNA was available from both unaffected parents, and paternity was established; the mutations were shown to have arisen spontaneously. None of the mutations were present in 100 nondiabetic subjects from the United Kingdom.

Figure 3 shows the location of mutated residues in Kir6.2. All the mutated residues are conserved among humans, rats, mice, and bullfrogs. The arginine residue at position 201 is conserved among 10 members of the family of Kir channels, a finding that supports the possibility that this residue has a critical role in channel function. In addition, we identified several previously recognized polymorphisms (a glutamic acid-to-lysine substitution at position 23 [E23K], a silent alanine-to-alanine substitution at position 190 [A190A], a silent leucine-to-leucine substitution at position 267 [L267L], a leucine-to-valine substitution at position 270 [L270V], an isoleucine-to-valine substitution at position 337 [I337V], a silent lysine-to-lysine substitution at position 381 [K381K], and a serine-to-cysteine substitution at position 385 [S385C]).

CLINICAL CHARACTERISTICS

The clinical characteristics of patients with mutations are shown in Table 1. There were two subgroups of patients: those who had only diabetes and those who had diabetes and shared neurologic abnormalities. Diabetes and low birth weight reflect impaired intrauterine and postnatal insulin secretion and were similar in the two subgroups of patients.

Diabetes

Diabetes was diagnosed at a mean age of 7 weeks (range, birth to 26 weeks). At diagnosis, all the patients had marked hyperglycemia (glucose concentration, 270 to 972 mg per deciliter [15 to 54 mmol per liter]), and three had ketoacidosis. None of the patients had elevated concentrations of autoantibodies associated with type 1 diabetes, and the C-peptide concentration was usually less than 200 pmol per liter. The median dose of insulin was 0.8 U per kilogram (range, 0.3 to 1.3). Only one patient (the proband's father in family BR 1) was not treated with insulin. He had received tolbutamide since childhood, and at 46 years of age, he had good control of the disease with this medication (fasting glucose concentration, 110 mg per deciliter [6.1 mmol per liter]; C-peptide concentration, 400 pmol per liter).

Low Birth Weight

Low birth weight was a feature of all the patients; in 7 of 12 (58 percent) the birth weight was at or below the 3rd percentile. Patients who did not have neurologic symptoms (Table 1) showed marked catch-up growth after birth, and their weights and heights were normally distributed on follow-up after a mean of 9.3 years.

Neurologic Features

Three of the patients (the probands in families ISPAD 25, ISPAD 27, and ISPAD 43) had very similar neurologic abnormalities, which suggested extrapancreatic phenotypes associated with their Kir6.2 mutation (Table 1). All three had marked developmental delay, muscle weakness, epilepsy, and dysmorphic features as well as neonatal diabetes. Another patient (the proband in family ISPAD 55) had an intermediate phenotype involving severe developmental delay and muscle weakness in addition to neonatal diabetes, but no other neurologic features. No cause other than their Kir6.2 mutation was found for their neurologic problems. All children had normal karyotypes. The other patients had normal development, indicating that not all mutations in Kir6.2 are associated with neurologic abnormalities.

Developmental Delay

All four patients with common neurologic features had marked developmental delay involving failure to achieve motor, intellectual, and social milestones appropriate for their age. The motor delay was the most marked of these features; the oldest child was

unable to walk unaided at the age of 17 years, and all four children showed motor development that was consistent with that of children half their chronological age or younger. There was muscular weakness on neurologic examination in all four cases. The creatine kinase concentration was normal in all of them. Muscle-biopsy specimens obtained from two of the patients were normal, and electromyography performed in two confirmed that nerve conduction was normal. In one patient action potentials of decreased duration and amplitude suggested a myopathy.

Social and language development was also markedly delayed in these four patients. None of them had microcephaly, and magnetic resonance imaging (MRI) and computed tomographic studies showed no reduction in the size of the cortex or cerebellum. No structural abnormalities were seen, apart from small, nonspecific, generalized patches throughout the white matter in one patient (the proband in family ISPAD 43) on an MRI scan obtained when she was 14 years of age.

Epilepsy

Generalized seizures, either complex or myoclonic, were observed in three patients (the probands in families ISPAD 25, ISPAD 27, and ISPAD 43) beginning in the first year of life. The seizures responded to antiepileptic medication (vigabatrin in two patients and sodium valproate in one). The seizures preceded clinically recognized episodes of hypoglycemia. All electroencephalograms showed generalized abnormal activity with bilateral sharp waves. One patient had marked hypsarrhythmia, which responded to vigabatrin.

Dysmorphic Features

All three patients with epilepsy had mild dysmorphic features (see Supplementary Appendix 1, available with the full text of this article at www.nejm.org). Their appearance was characterized by a prominent metopic suture, a downturned mouth, and bilateral ptosis. All three patients had limb contractures, which were diagnosed at birth in two and at four years of age in one.

PHYSIOLOGICAL STUDIES

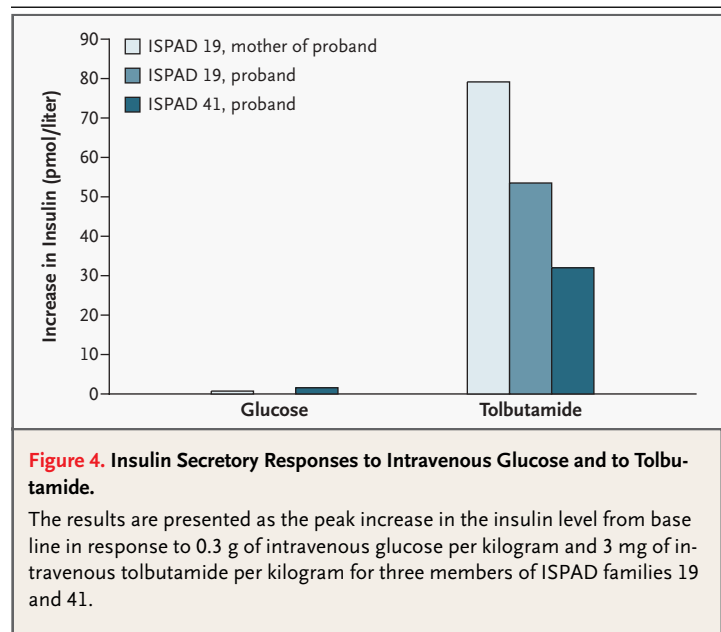
Both during fasting and after glucagon stimulation, the serum C-peptide concentration was generally less than 200 pmol per liter, despite marked hyperglycemia — a finding consistent with profound beta-cell dysfunction (Table 1). Serum C-peptide exceeded the lower limit of the normal range in only

three patients (two insulin-treated children and the adult whose diabetes was well controlled with tolbutamide) (Table 1). Three patients, who had mutations affecting residue 201, had only minimal insulin secretion in response to intravenous glucose but did secrete insulin in response to tolbutamide (Fig. 4).

FUNCTIONAL ANALYSIS OF THE R201H MUTATION

When wild-type Kir6.2 was coexpressed with SUR1 in *X. laevis* oocytes, K_{ATP} currents were almost undetectable because of inhibition by high intracellular ATP concentrations, but they could be activated by azide, which lowers cytosolic ATP concentrations (Fig. 5A). In contrast, significant resting currents were recorded from oocytes expressing Kir6.2-R201H-SUR1 ($P < 0.01$ for the comparison with wild-type Kir6.2). These currents were further activated by azide ($P < 0.05$ for the comparison with wild-type Kir6.2) and were blocked by tolbutamide, indicating that they flow through K_{ATP} channels (Fig. 5B and 5C). These data suggest that metabolism causes less blockade of Kir6.2-R201H-SUR1 channels than it does of wild-type K_{ATP} channels.

To explore the mechanisms underlying these findings, we examined the nucleotide sensitivity of wild-type and mutant channels in inside-out patches (Fig. 5D). Kir6.2-R201H-SUR1 channels were considerably less sensitive than wild-type channels to intracellular ATP; mutant channels were half maximally blocked at an ATP concentration of



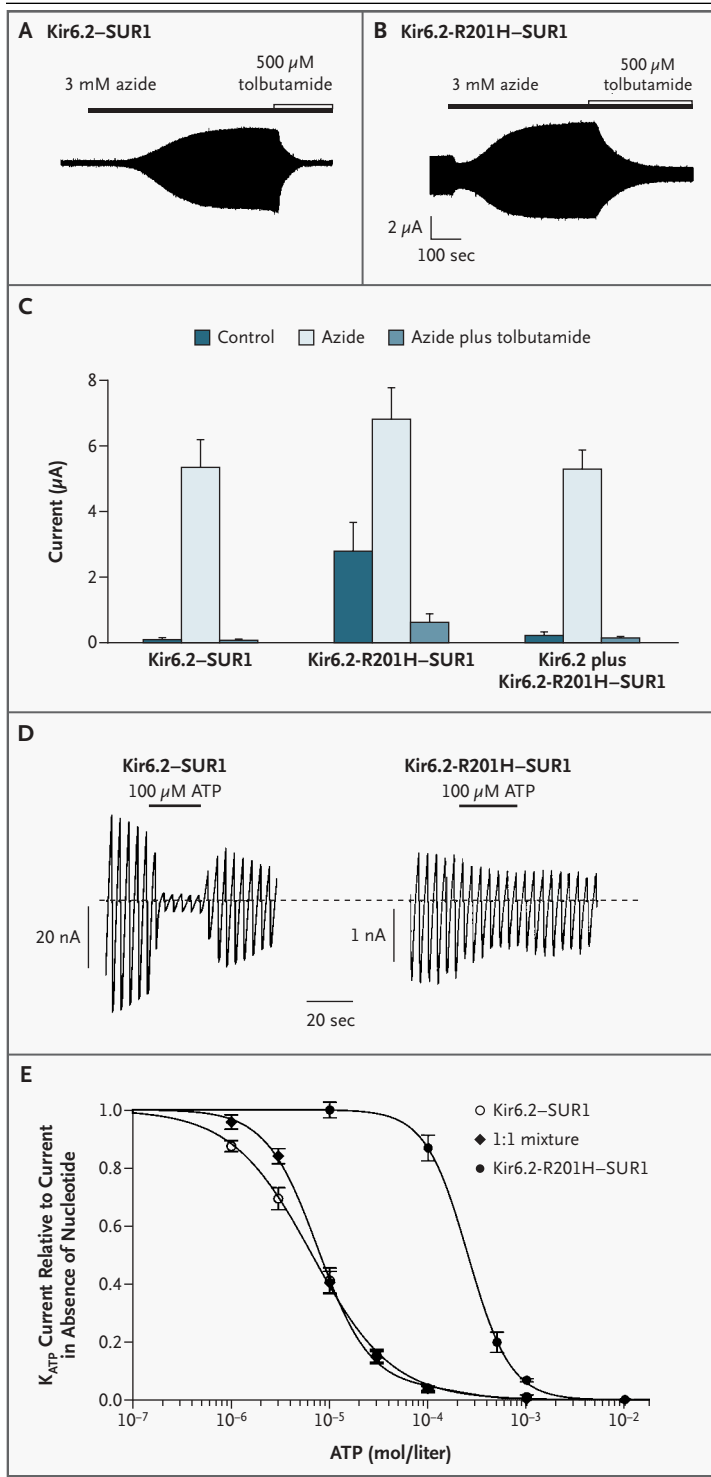


Figure 5. The Effects of Metabolic Inhibition, a Sulfonylurea, and Intracellular ATP on Currents in Wild-Type and Mutant ATP-Sensitive Potassium (K_{ATP}) Channels.

Whole-cell currents were recorded in a two-electrode voltage clamp from intact oocytes expressing Kir6.2-SUR1 (Panel A) or Kir6.2-R201H-SUR1 (Panel B) in response to voltage steps of ± 20 mV from a holding potential of -10 mV. The external solution consisted of 90 mM potassium chloride, 1 mM magnesium chloride, 1.8 mM calcium chloride, and 5 mM HEPES (pH 7.4 with potassium hydroxide), plus 3 mM azide and 500 μ M tolbutamide as indicated. Panel C shows the mean whole-cell current evoked by a voltage step from -10 to -30 mV in control solution, then after steady state was reached with 3 mM azide, then in the continued presence of azide plus 500 μ M tolbutamide. Oocytes were injected with messenger RNA encoding SUR1 plus either Kir6.2 (six oocytes), Kir6.2-R201H (seven), or a 1:1 mixture of Kir6.2 and Kir6.2-R201H (seven). The T bars represent standard errors. Panel D shows the K_{ATP} current recorded in response to voltage ramps from -110 to $+100$ mV from an inside-out patch excised from an *X. laevis* oocyte co-expressing SUR1 and either wild-type or mutant Kir6.2, as indicated. The dashed line indicates the zero current level. The pipette solution consisted of 140 mM potassium chloride, 1.2 mM magnesium chloride, 2.6 mM calcium chloride, and 10 mM HEPES (pH 7.4 with potassium hydroxide). The internal solution consisted of 107 mM potassium chloride, 1 mM potassium sulfate, 1 mM calcium chloride, 10 mM EGTA (egtazic acid), 10 mM HEPES (pH 7.2 with potassium hydroxide), and 100 μ M ATP, as indicated. Panel E shows the relation between the ATP concentration and the K_{ATP} current, expressed relative to the current in the absence of nucleotide, for Kir6.2-SUR1 in six oocytes (open circles), Kir6.2-R201H-SUR1 in six oocytes (solid circles), and a 1:1 mixture of Kir6.2-R201H and Kir6.2 coexpressed with SUR1 in six oocytes (diamonds). The lines are the best fit of the Hill equation to the mean data: for Kir6.2-SUR1, the concentration at which inhibition is half maximal (IC_{50}) is 6.6 μ M, and the Hill coefficient (h) is 1.1; for Kir6.2-R201H-SUR1, IC_{50} is 245 μ M and h is 2. For the 1:1 mixture, the line is the best fit to the following equation: $a(1 \div [1 + ([ATP] \div 245 \mu M)^2]) + (1-a)(1 \div [1 + ([ATP] \div IC_{50})^h])$, where $[ATP]$ is the ATP concentration, IC_{50} is 7.6 μ M, h is 1.6, and a (the fraction of the homomeric R201H channels) is 0.04.

$262 \pm 33 \mu M$, as compared with an ATP concentration of $7 \pm 1 \mu M$ for Kir6.2-SUR1 channels ($P < 0.001$) (Fig. 5E). However, mutant channels were activated by Mg-ADP to a similar extent. The single-channel conductance and the fraction of time the channel

spends in the open state (the “open probability”) were normal.

To simulate the effect of heterozygosity, we injected oocytes with SUR1 and a 1:1 mixture of Kir6.2 and Kir6.2-R201H mRNA. The resting current of oocytes injected with the 1:1 mixture was slightly, but not significantly, greater (0.27 ± 0.07 nA) than that of oocytes with the wild-type channel (0.13 ± 0.05 nA) ($P = 0.12$) (Fig. 5C). The K_{ATP} currents of mutant channels were further activated by azide and blocked by tolbutamide, to an extent

similar to that in the wild-type channel. The ATP sensitivity was also close to that of the wild-type channel, with an IC_{50} value of $7.6 \pm 0.4 \mu M$ (Fig. 5E). However, the K_{ATP} currents of mutant channels were significantly larger than those of wild-type channels at ATP concentrations of $1 \mu M$ ($P=0.002$) and $3 \mu M$ ($P=0.008$), due to the difference in the Hill coefficient.

DISCUSSION

Our findings show that heterozygous activating mutations in the gene encoding the Kir6.2 subunit of the K_{ATP} channel can cause both familial and sporadic neonatal diabetes. This genetic subtype may be a relatively common cause of permanent neonatal diabetes, since we found it in 34 percent of probands. Some patients with mutations in the gene encoding Kir6.2 have marked developmental delay, muscle weakness, and epilepsy, in addition to neonatal diabetes. These observations point to the critical role of K_{ATP} channels in pancreatic beta cells and suggest a role in human muscle and brain.

The evidence that these mutations are causal is very strong. They cosegregated with diabetes in the two families with vertical transmission, and the nonfamilial cases of diabetes were associated with spontaneous mutations (since the mutation was not present in the normoglycemic parents). Approximately 1 in 10^6 people is likely to have a spontaneous mutation in a gene of this size, so nine such mutations is highly unlikely to be a chance observation. The most common mutation (resulting in the R201H substitution) occurs within a CpG dinucleotide, a "hot spot" for mutations in mammalian genes. This probably explains the recurrent finding of the R201H mutation in unrelated families from different countries. Since the majority of the mutations are spontaneous, a family history of diabetes is frequently not present.

Diabetes was diagnosed at a mean age of seven weeks and within the first three months of life in 10 of the 13 patients. Although three patients presented with ketoacidosis, it is likely that at least some patients had minimal secretion of endogenous insulin, since in most patients the disease was not diagnosed immediately after birth, and some had detectable C-peptide concentrations. Patients with Kir6.2 mutations may show some overlap with type 1 diabetes in terms of clinical features, but none of our patients had beta-cell autoantibodies. The extent to which mutations in the gene encoding Kir6.2

account for antibody-negative type 1 diabetes requires investigation.

The severe intrauterine growth retardation found in the patients with Kir6.2 mutations is consistent with greatly reduced or absent insulin secretion in utero and is also seen in patients with glucokinase deficiency, loss of an imprinted region of chromosome 6q24 (which results in transient neonatal diabetes), and pancreatic agenesis.^{2,3,7,23} Marked postnatal catch-up growth is a feature of these conditions and was also observed in the patients with Kir6.2 mutations who did not have neurologic abnormalities.

Mutations in the gene encoding Kir6.2 probably cause decreased secretion of insulin from beta cells by conferring reduced sensitivity to ATP, which is predicted to result in gain of channel function. Functional analysis of the most common mutation, R201H, showed that the homozygous mutation led to markedly reduced sensitivity to ATP. When wild-type and mutant subunits were coexpressed to simulate the heterozygous state, the ATP sensitivity of the resulting mixed population of heteromeric channels was similar to that of the wild type, except at low ATP concentrations. However, we expect that there will be a small population of homomeric R210H channels with lower ATP sensitivity (about 6 percent of the channels if the number of mutant subunits in the tetrameric channel is binomially distributed). Indeed, such ATP-insensitive channels were observed at the single-channel level (data not shown). Although this current is difficult to measure at high ATP concentrations, it may be sufficient to keep the beta cell hyperpolarized even in the presence of glucose, thereby reducing electrical activity and insulin release. Our results indicate that very small changes in the resting K_{ATP} current, due to small changes in ATP sensitivity, can impair insulin secretion sufficiently to cause diabetes in humans. This finding is consistent with some²⁴ but not all²⁵ *in vitro* studies that suggest that a Kir6.2 polymorphism in which lysine replaces glutamic acid at position 23 (E23K) shows small changes in ATP sensitivity.

A molecular model of the C terminal of Kir6.2²⁶ predicts that R201 lies close to the phosphate tail of ATP and that it interacts with the alpha phosphate of ATP. The concept of a critical role for residue 201 in ATP binding is supported by the finding that the ATP sensitivity of K_{ATP} channels is reduced when the arginine at position 201 is mutated to histidine and other residues.^{27,28} Three mutations (Q52R,

V59M, and V59G) are found in the slide helix; residue 52 lies at one end of the helix, and residue 59 lies midway along its length (Fig. 3). The position of the slide helix implies a role in the regulation of channel gating,²¹ but it lies distant from the predicted ATP-binding site. Additional work is required to elucidate the mechanism by which the mutations that lie in the slide-helix domain effect disease.

Identification of mutations in the gene encoding the K_{ATP} -channel subunit Kir6.2 may have important implications for the treatment of affected patients. Our functional studies in vitro suggest that if the K_{ATP} channel could be closed by an ATP-independent mechanism (e.g., by sulfonylureas), insulin secretion might be restored. Patients with mutations affecting position 201 did have clear, but subnormal, insulin responses to intravenous tolbutamide. This observation suggests that the pathophysiological condition in humans mirrors the findings in vitro, raising the possibility of novel treatment strategies based on sulfonylureas (or other specific K_{ATP} -channel inhibitors) in these apparently insulin-dependent patients. Of note, one patient with an R201H mutation (the proband's father in family BR 1), who had always been treated with tolbutamide, had C-peptide levels in the normal range and good glycemic control. Further investigation is needed to determine whether the identification of a mutation in the gene encoding Kir6.2 will permit treatment to be given in the form of oral agents rather than subcutaneously injected insulin.

It is unlikely that the severe developmental delay, muscle weakness, and epilepsy seen in a subgroup of the patients with Kir6.2 mutations result from diabetes or its treatment. Severe developmental delay and persistent epilepsy are rare in children with neonatal diabetes^{2,3,6,7}; moreover, in our study, neurologic diagnosis preceded clinically recognized episodes of hypoglycemia, and the contractures seen at birth in two of the patients suggest that neurologic dysfunction was present in utero. Kir6.2 is the pore-forming subunit of K_{ATP} channels in skeletal muscle and neurons throughout the brain^{29,30}; hence, altered activity of these channels could cause developmental delay, muscle weakness, and epilepsy. Studies in animal models to assess skeletal muscle and the neurologic effects of gain-of-function mutations have not been performed; however, loss of function of the K_{ATP} channel can result in hypoxia-induced seizures,³¹ and overexpression of

SUR1 in forebrain reduces the susceptibility to seizures.³² Analysis of additional patients will be necessary to determine whether Kir6.2 mutations give rise to a discrete syndrome characterized by developmental delay, epilepsy, and neonatal diabetes.

The position and type of the mutation may influence the phenotype. All eight patients with mutations at residue 201 had diabetes but no neurologic abnormalities. The V59M variant is associated both with isolated diabetes and with marked developmental delay in addition to diabetes. This kind of genetic behavior — one in which the same mutation is associated with different phenotypes in different families — has been observed with other genes, such as *LMNA*.³³

That heterozygous mutations of Kir6.2 cause diabetes confirms that the K_{ATP} -channel-dependent pathway is critical for insulin secretion and that other, K_{ATP} -channel-independent pathways are unable to compensate for its loss. Mutations with a less severe functional effect or modification by the genetic background might lead to transient neonatal diabetes or to diabetes that becomes manifest after the neonatal period. Indeed, we and others have shown that the common Kir6.2 polymorphism E23K is associated with a slightly increased susceptibility to type 2 diabetes.^{24,34,35}

In conclusion, activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 cause neonatal diabetes, and in some patients, neurologic abnormalities. The preliminary finding that tolbutamide partly compensates for the effect of the most common mutation on insulin secretion offers hope that in at least some cases the diabetes may be effectively treated with sulfonylurea tablets.

Supported in part by funds from the Wellcome Trust and Diabetes UK (to the Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter); by grants from the Royal Society, the Wellcome Trust, and the Medical Research Council (to the Laboratory of Physiology, Oxford University, Oxford); by a grant from the Dutch Growth Foundation (to Dr. Slingerland); and by grants from the University of Bergen and Haukeland University Hospital (to the Institute for Clinical Medicine and Molecular Medicine, University of Bergen, Bergen). Dr. Hattersley is a Wellcome Trust Clinical Research Leave Fellow, Dr. Pearson is a Wellcome Trust Clinical Research Fellow, and Dr. Ashcroft is the Royal Society GlaxoSmithKline Research Professor.

We are indebted to the International Society for Pediatric and Adolescent Diabetes, which set up the ISPAD Rare Diabetes registry; to the Child Health and Well-Being Fund, Rotterdam, the Netherlands, which funded its establishment; to Peter Turnpenny and Julia Rankin for their advice; and to the Royal Devon and Exeter National Health Service Health Care Trust for their continued support.

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