Perspectives in Diabetes

Activating Mutations in Kir6.2 and Neonatal Diabetes

New Clinical Syndromes, New Scientific Insights, and New Therapy

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Closure of ATP-sensitive K^+ channels (K_{ATP} channels) in response to metabolically generated ATP or binding of sulfonylurea drugs stimulates insulin release from pancreatic β-cells. Heterozygous gain-of-function mutations in the *KCJN11* gene encoding the Kir6.2 subunit of this channel are found in ~47% of patients diagnosed with permanent diabetes at <6 months of age. There is a striking genotype-phenotype relationship with specific Kir6.2 mutations being associated with transient neonatal diabetes, permanent neonatal diabetes alone, and a novel syndrome characterized by developmental delay, epilepsy, and neonatal diabetes (DEND) syndrome. All mutations appear to cause neonatal diabetes by reducing K_{ATP} channel ATP sensitivity and increasing the K_{ATP} current, which inhibits β-cell electrical activity and insulin secretion. The severity of the clinical symptoms is reflected in the ATP sensitivity of heterozygous channels in vitro with wild type > transient neonatal diabetes > permanent neonatal diabetes > DEND syndrome channels. Sulfonylureas still close mutated K_{ATP} channels, and many patients can discontinue insulin injections and show improved glycemic control when treated with high-dose sulfonylurea tablets. In conclusion, the finding that Kir6.2 mutations can cause neonatal diabetes has enabled a new therapeutic approach and shed new light on the structure and function of the Kir6.2 subunit of the K_{ATP} channel. *Diabetes* 54:2503–2513, 2005

Neonatal diabetes diagnosed within the first 3 months of life is usually a single gene disorder associated with altered β-cell number or function. Transient neonatal diabetes resolves by a median of 12 weeks and is generally associated with an abnormality of the imprinted region 6q24 (1). In contrast, permanent neonatal diabetes requires insulin treatment for life, and until recently the genetic etiology was largely unknown. Thus, the discovery that heterozygous gain-of-function mutations in *KCNJ11* cause permanent neonatal diabetes in almost half of cases is an important advance (2). *KCNJ11* encodes Kir6.2, the pore-forming subunit of the ATP-sensitive K^+ channel (K_{ATP} channel) (3). This article summarizes recent genetic, clinical, and functional work on Kir6.2 mutations that cause neonatal diabetes.

PHYSIOLOGICAL ROLES OF K_{ATP} CHANNELS

The physiological importance of K_{ATP} channels in insulin secretion was established >20 years ago (4). At substimulatory glucose concentrations, K^+ efflux through open K_{ATP} channels maintains the β-cell membrane at a hyperpolarized potential of around −70 mV, which keeps voltage-gated Ca^{2+} channels closed (5). Elevation of the blood glucose concentration increases glucose uptake and metabolism by the β-cell, producing changes in cytosolic nucleotide concentrations that cause K_{ATP} channel closure. This leads to a membrane depolarization that opens voltage-gated Ca^{2+} channels initiating β-cell electrical activity and Ca^{2+} influx, and the subsequent rise in $[Ca^{2+}]_i$ triggers exocytosis of insulin granules. Although glucose has additional (downstream) effects on insulin secretion (6), under physiological conditions K_{ATP} channel closure is a central step in glucose-stimulated insulin release. K_{ATP} channels are also the target for sulfonylurea drugs, which are widely used to treat type 2 diabetes. These drugs stimulate insulin secretion by binding to, and closing, K_{ATP} channels (7). Thus, sulfonylureas bypass β-cell metabolism but subsequently stimulate the same chain of events as glucose.

In addition to their well-characterized role in insulin secretion (5,8), K_{ATP} channels have many other functions (rev. in 9). They contribute to glucose homeostasis by controlling glucagon-like peptide 1 secretion from L-cells (10) and glucose uptake in skeletal muscle (11), and they mediate the counter-regulatory response to glucose via effects on hypothalamic neurons (12). They are also involved in the response to cardiac stress and ischemic preconditioning (13,14), regulate vascular smooth muscle tone (15), modulate electrical activity and transmitter release at brain synapses (16,17), and protect against seizures (18,19). In all these tissues, K_{ATP} channels couple

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CHI, congenital hyperinsulinism of infancy; DEND, developmental delay, epilepsy, and neonatal diabetes; K_{ATP} channel, ATP-sensitive K^+ channel; SUR, sulfonylurea receptor.

metabolism to electrical activity. Increased metabolism produces channel closure, membrane depolarization, and electrical activity, and conversely, metabolic inhibition opens K_{ATP} channels and suppresses electrical activity. In glucose-sensing tissues, K_{ATP} channels respond to changes in blood glucose concentration, but in many other tissues they open only under ischemic conditions or in response to hormonal stimulation.

Functional properties. K_{ATP} channels are subject to complex regulation by numerous cytosolic factors, the most important being the adenine nucleotides ATP and magnesium ADP (MgADP). Under physiological conditions, channel activity is determined by the balance between ATP, which blocks the channel, and MgADP, which reverses channel inhibition by ATP (8,9,20). Consequently, reciprocal changes in the intracellular concentrations of ATP and MgADP probably mediate the metabolic regulation of the K_{ATP} channel.

The K_{ATP} channel is a 4:4 complex of Kir6.x and sulfonylurea receptor (SUR) subunits (9,21). In most tissues, the pore-forming subunit is Kir6.2; binding of ATP to Kir6.2 results in channel closure (22). SUR acts as a regulatory subunit, conferring stimulation by Mg nucleotides and K-channel openers (such as diazoxide) and inhibition by sulfonylureas (22,23). Like the Kir subunit, SUR exists in more than one isoform, and variation in SUR subunit composition accounts for the different metabolic and drug sensitivities of K_{ATP} channels (7,9). SUR1 is found in pancreas and brain muscle, SUR2A in heart and skeletal muscle, and SUR2B in brain and smooth muscle.

Studies on genetically modified mice have provided valuable insights into the role of the K_{ATP} channel in β-cells (8). These have shown that targeted overactivity of β-cell K_{ATP} channels induces profound neonatal diabetes (24), whereas targeted suppression of K_{ATP} channel activity leads to hyperinsulinism (25). Complete knockout of Kir6.2 or SUR1 causes hyperinsulinism in neonates, but hypoinsulinism occurs in adult animals due to apoptotic loss of β-cell mass (9,26).

Further support for the importance of K_{ATP} channels in insulin secretion comes from the fact that naturally occurring loss-of-function mutations in either the human *SUR1* or Kir6.2 (*KCNJ11*) genes are the most common causes of congenital hyperinsulinism of infancy (CHI) (27). Some CHI mutations not only cause neonatal hyperinsulinism but also result in β-cell dysfunction and diabetes in adult life (28).

KIR6.2 AS A CANDIDATE GENE FOR NEONATAL DIABETES

Studies in isolated cells and tissues, animal models, and human genetics have firmly established the central role of the K_{ATP} channel in insulin secretion and suggested that Kir6.2 is a good candidate gene for neonatal diabetes. First, overexpression in β-cells of a mutant Kir6.2 with a reduced ATP sensitivity caused mice to develop severe neonatal diabetes (24). Second, the fact that loss-of-function mutations in Kir6.2 or SUR1 leads to CHI suggests that gain-of-function mutations in the same genes might cause diabetes. Gain- and loss-of-function mutations in the same ion channel gene have been shown to cause opposing phenotypes (29), and hyperglycemia and hypoglycemia can result from inactivating and activating mutations, respectively, in glucokinase (30). Third, as discussed more fully below, a common polymorphism in the Kir6.2 gene (E23K) is consistently associated with type 2 diabetes in both large-scale studies and meta-analyses (31–33), so a severe mutation might cause monogenic diabetes.

Kir6.2 mutations cause neonatal diabetes. Gloyn et al. (2) first reported that Kir6.2 mutations result in neonatal diabetes. They showed that heterozygous Kir6.2 mutations occurred in 10 of 29 patients diagnosed with diabetes before 6 months of age who required continuing insulin treatment after diagnosis. This study established that most Kir6.2 mutations were spontaneous, that some mutations were more prevalent, and that neurological features occurred in some patients. It also showed that the most common mutation, R201H, caused a reduced response of the K_{ATP} channel to ATP, consistent with a gain-of-function mutation, and that while patients with this mutation did not secrete insulin in response to glucose, they did respond to sulfonylureas. Subsequent studies have confirmed and extended these results.

Genetics. Heterozygous mutations in Kir6.2 are the most common cause of neonatal diabetes in multiple populations and ethnic groups, accounting for 40–64% of cases in large series of permanent neonatal diabetes (Table 1). To date, at least 63 patients with activating mutations in Kir6.2 have been described, comprising 21 different mutations in 49 families (2,34–44) (E.L. Edghill, S. Ellard, S. Flanagan, and A.T.H., personal communication). The distribution of these residues within the single exon *KCNJ11* gene is shown in Fig. 1. Four mutations (V59M, R201H, R201C, and Y330C) have been described in more than one family, with the most common being R201H and V59M. Since most (>90%) mutations arose spontaneously, positions 59 and 201 represent recurrent mutations rather than founder mutations within populations. At position 201, there is a CpG dinucleotide, which represents a hot spot for mutations in eukaryote genes. In seven families, there was autosomal dominant inheritance from either the mother or father (2,37,41,44). In a single family, there was a paternal germline mosaicism, resulting in the R201C mutation being detected in the leukocyte DNA of two half-siblings but not in that of the father (35). The possibility of paternal germline mosaicism should therefore be considered when counseling parents of a child with an apparently de novo mutation of the risk of subsequent children being affected.

Clinical features. There is a spectrum of phenotypes associated with activating mutations in Kir6.2 (Table 2). Almost all patients have neonatal diabetes, which may either be permanent or more rarely may remit (and hence be transient). Some patients have additional neurological features.

Neonatal diabetes. The median age of diagnosis is 6 weeks, with all patients being diagnosed before 6 months of age, except for four cases diagnosed in childhood or early adult life (Table 2). Most (79%) patients are diagnosed before 3 months, which has been suggested as a criterion for neonatal diabetes. A large Italian study of early-onset diabetes suggested that diagnosis by 6 months is the boundary between nonautoimmune diabetes and type 1 diabetes (45). Consistent with this idea, Kir6.2

TABLE 1
Prevalence of Kir6.2 mutations in patients selected on different clinical criteria (data from probands only)

Reporting Center	Number of Kir6.2 mutations in probands/total (%)	Criterion	Nationality of cohort	Reference(s)
Permanent diabetes diagnosed before 6 months				
Exeter, U.K.	17/42 (40%)	Diagnosed 0–6 months, GCK mutations excluded	International	2,34,35*
Lille/Paris, France	9/16 (56%)	Diagnosed 0–5 months, GCK mutations excluded	French	39
Rome, Italy	8/14 (57%)	Diagnosed 0–6 months	Italian	36
Bergen, Norway	7/11 (64%)	Diagnosed 0–6 months, GCK mutations excluded	International	37
Santiago, Chile/Exeter, U.K.	1/5 (20%)	Diagnosed <6 months	Chilean	40
Krakow, Poland/Exeter,U.K.	1/5 (20%)	Diagnosed <6 months	Polish	43
Jerusalem, Israel	1/1 (100%)	Diagnosed 2 months	Israeli	38
Paris, France	1/1 (100%)	Diagnosed 3 months	French	42
Total	45/95 (47%)			
Permanent diabetes diagnosed after 6 months				
Exeter, U.K.	0/76	Permanent insulin treated diabetes 6–24 months	U.K. Caucasian	34
Krakow, Poland/Exeter, U.K.	0/20	Permanent insulin treated diabetes 6–24 months	Polish	43
Rome, Italy	0/4	Permanent insulin treated diabetes 6–12 months	Italian	36
Lille/Paris, France	0/1	Permanent insulin treated diabetes 8.5 months	French	39
Exeter, U.K.	0/15	MODY X (Autosomal dominant diagnosed before 25 years) without mutations in the 6 known MODY genes	U.K.	2
Total	0/116 (0%)			
Transient neonatal diabetes				
Exeter, U.K.	3/11	Transient neonatal diabetes diagnosed at <6 months without abnormalities of 6q24	International	41
Lille/Paris, France	0/7	Transient neonatal diabetes without abnormalities of 6q24	French	39
Kyoto, Japan	1/1	Transient neonatal diabetes with family history of diabetes at different ages	Japanese	44
Total	4/19 (21%)			

*In addition to E.L. Edghill, S. Ellard, S. Flanagan, and A.T.H. GCK, glucokinase gene; MODY, maturity-onset diabetes of the young.

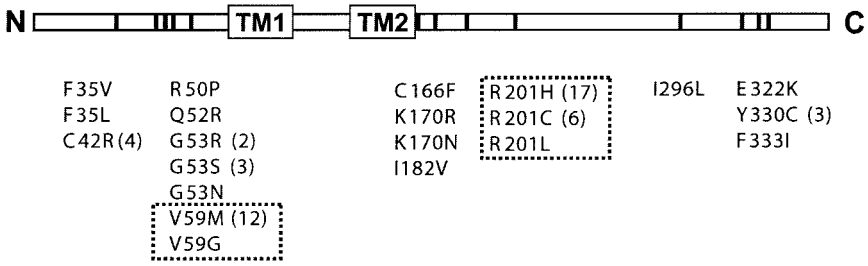

FIG. 1. The location of mutations causing neonatal diabetes within the linear sequence of Kir6.2. The numbers in brackets give the number of patients carrying the indicated mutation (no number is given if this is 1). Residues that represent the two common hot spots for mutations are boxed (2,33–44 and E.L. Edghill, S. Ellard, S. Flanagan, and A.T.H., personal communication). N, NH_2-terminal; C, COOH-terminal; TM1, first transmembrane domain; TM2, second transmembrane domain.

mutations were not detected in 101 patients with insulin-treated diabetes diagnosed between 6 and 24 months (Table 1). This suggests that screening for Kir6.2 mutations is appropriate for all patients diagnosed before 6 months but not for patients diagnosed later than this.

The majority of patients have low birth weights (median 2.58 kg) with 71% below the 10th centile for gestational age in keeping with a lower fetal insulin production resulting in reduced insulin-mediated growth in utero. Patients show rapid catch-up growth after insulin treatment.

Most patients with Kir6.2 mutations present with symptomatic hyperglycemia with median blood glucose concentrations of 33.5 mmol/l, and in many cases there is ketoacidosis. However, the relatively late age of diagnosis in many cases suggests there is not absolute insulin deficiency from birth. Patients require insulin treatment from diagnosis; this is permanent in all but five cases where there is evidence of remission with insulin able to be completely discontinued at least for 1 year. These patients were defined clinically as having transient neonatal diabetes (41,44).

The phenotype of diabetes due to Kir6.2 mutations differs from that found for other causes of neonatal diabetes. Permanent neonatal diabetes due to homozygous glucokinase mutations (46) or pancreatic aplasia due to homozygous IPF1 mutations (47,48) results in a more severe insulin deficiency as shown by a lower birth weight and a younger age at diagnosis. In keeping with pancreatic autoantibodies being rare in patients who are diagnosed with diabetes before 6 months (45), no patients with Kir6.2 mutations have pancreatic autoantibodies, but these are found in patients with FOXP3 mutations leading to IPEX syndrome (45). Transient neonatal diabetes resulting from the common abnormalities of the imprinted region on 6q24 differs from transient neonatal diabetes caused by Kir6.2 mutations. The former are born lighter (mean 2.1 vs. 2.6 kg) and diagnosed earlier (1.0 vs. 5.2 weeks), but remit earlier (16 vs. 76 weeks) (1,41).

Neurological features. In addition to neonatal diabetes, it is becoming clear that neurological features, particularly developmental delay, muscle weakness, and epilepsy, are associated with some Kir6.2 mutations (2,34,35,37,39,42). The diabetes of these patients is similar to that found for patients without neurological features, but its management is more difficult due to marked communication problems and the risk that hypoglycemia can precipitate seizures in patients with epilepsy.

There has been some controversy about whether the neurological features associated with Kir6.2 mutations constitute a distinct syndrome or are a secondary consequence of diabetes or its treatment. There is now strong evidence in favor of the former. First, developmental delay is not a typical feature of neonatal diabetes from other causes (1,46,49). Second, the pattern of neurological features is distinct from that resulting from cerebral edema, which may occur in the treatment of diabetic ketoacidosis, or as a consequence of severe hypoglycemia. Third, the presence of contractures at birth (arthrogryposis) in some patients is indicative of a neurological defect in utero (2). Fourth, as discussed below, there is a strong genotype-phenotype relationship with the functional severity of the mutations in the heterozygous state correlating with differences in clinical phenotype. Finally, the neurological features are consistent with the tissue distribution of Kir6.2 in muscle, nerve, and brain (3,9). Thus, we believe it appropriate to define a novel syndrome that is associated with some Kir6.2 mutations. We propose this be known as developmental delay, epilepsy, and neonatal diabetes (DEND) syndrome (50).

Four patients (7%) have a very severe neurological phenotype that exhibits all the features of DEND syndrome (2,42). They show marked developmental delay, motor weakness, and epilepsy in addition to diabetes. Two children died as a consequence of the neurological features within the first 15 months of life (2,42). The other two are unable to talk, stand, or walk unaided at the ages of 5 and 18 (2). All four patients had generalized epilepsy before the age of 1. In all cases, electroencephalograms were abnormal with bilateral sharp waves, and two patients showed hypsarrhythmia. These patients also have mild dysmorphic features including a prominent metopic suture, bilateral ptosis, and a downturned mouth (the last two are both features of muscle weakness).

A less severe clinical picture, consisting of neonatal diabetes with developmental delay and/or muscle weakness but not epilepsy, is more common (14 of 51 patients) (2,34,35,37,39) (Table 2). We refer to this as intermediate DEND syndrome. Motor milestones are delayed 1–2 years but less severely than in patients with epilepsy, and there is a variable degree of motor weakness. The most marked feature of social delay is the late development of speech, which may not occur until 5 years.

Genotype-phenotype relationships. There is emerging evidence for a clear genotype-phenotype relationship for Kir6.2 mutations. Of the 24 patients with mutations at R201 described to date, all but 3 have nonremitting neonatal diabetes without neurological features (Table 2). Conversely, 10 of 13 patients with the V59M mutation have developmental delay and features consistent with intermediate DEND syndrome (Table 2). The mutations associated with full DEND syndrome are not found in less severely affected patients. A milder phenotype is associated with the C42R mutation; two of four patients did not develop diabetes until early adult life, one patient devel-

TABLE 2
Clinical features of patients with Kir6.2 mutations

Clinical presentation (*n*)	Age diabetes diagnosed (weeks)	Glucose at presentation (mmol/l)	Insulin dose (units/kg)	Birth weight (g)	Birth weight (centile)	Developmental delay and/or weakness	Epilepsy	Mutations	References
PNDM (35)	6 (0–260)	35.7 (11.2–69.5)	0.7 (0.25–2)	2,655 (1,440–3,300)	5 (0.1–60)	0/34	0/34	R201H (17/17) R201C (3/6), V59M (3/12), G53R (1/2), G53S (1/3), Y330C (1/3), F35L, F35V, R50P, K170R, K170N, R201L, E322K, F333I	2,34–40 and E.L. Edghill, S. Ellard, S. Flanagan, and A.T.H., personal communication
Intermediate DEND (14)	6 (0–18)	35.1 (16.3–70.0)	0.8 (0.3–0.5)	2,480 (2,070–3,260)	4 (1.4–37)	14/14	0/14	V59M (9/12) R201C (3/6) Y330C (2/3)	2,34,35,37,39 and E. L. Edghill, S. Ellard, S. Flanagan, and A.T.H., personal communication
DEND (4)	15 (5–26)	18.8 (18–34.5)	0.35 (0.3–0.5)	2,400 (1,850–2,550)	11 (2.5–29)	4/4	4/4 (100%) generalized epilepsy before 12 months	Q52R V59G C166F I296L	2,42
TNDM (4)	3 (1–15)	16.5 (15.2-25.8)	0 (0–0.6)	2,690 (1,535–3,570)	88 (0.1–94)	1/4	0/4	G53S (2/3) G53R (1/2) I182V	41
All (57)	6 (0–260)	33.5 (11.2–70.0)	0.7 (02)	2,580 (1,440–3,570)	5 (0.194)	19/57 (33%)	4/57 (7%)		

Data are median (range), unless otherwise indicated. When there is more than one patient with the mutation, the number with that phenotype and the total number with the mutation are shown. The percentage is given in brackets. Data from ref. 44 are not included. TNDM, transient neonatal diabetes.

oped diabetes at age 3 years, and another exhibited transient neonatal diabetes (44). However, although these data suggest a strong correlation between phenotype and genotype, the association is not absolute, as shown by differences in the degree to which β-cell function is impaired in patients with the R201H (2,38,43) and C42R (44) mutations and the absence of developmental delay in three patients with the V59M mutation. This implies that genetic background and environmental factors influence the clinical phenotype, as found for other monogenic subtypes of diabetes (51,52).

The reason that some Kir6.2 mutations give rise to transient neonatal diabetes is unknown, but possible explanations include a reduced insulin requirement at the time of remission; compensation at the level of the β-cell, pancreas, or whole body that is able to overcome the effects of the channel defect; or changes in β-cell turnover due to the mutation in the gene encoding Kir6.2.

Structural location of mutations. Figure 2 maps the mutations associated with neonatal diabetes onto a structural model of Kir6.2 (53). Residues associated with neonatal diabetes alone (transient neonatal diabetes or permanent neonatal diabetes) lie within the putative ATP-binding site (R50, I192, R201, and F333) or are located at the interfaces between Kir6.2 subunits (F35, F35, C42 and E332) or between Kir6.2 and SUR1 (G53). Mutations that cause additional neurological features occur at residues that lie at some distance from the ATP-binding site. Thus, Q52 sits within the cytosolic part of the NH_2-terminal domain, which is thought to contribute to functional coupling of SUR1 to Kir6.2 (54,55). Residue V59 lies within the "slide helix," a domain implicated in the opening and closing of the pore (53,56). C166 lies at the cytosolic end of the outer transmembrane helix close to the helix-bundle crossing suggested to form an inner gate to the channel (57), and I296L lies within the permeation pathway at the mouth of the transmembrane pore in a region postulated to play a role in gating (53,58).

Functional properties of mutations. All mutations studied to date produce a marked decrease in the ability of ATP to block the K_{ATP} channel when expressed in heterologous systems (2,41,44,50,59,60). This reduction in ATP sensitivity means the channel will open more fully at physiologically relevant concentrations of ATP (1–5 mmol/l), leading to an increase in the K_{ATP} current. In pancreatic β-cells, an increase in K_{ATP} current will hyperpolarise the membrane, suppressing electrical activity, Ca^{2+} influx, and insulin secretion, and thereby causing diabetes (48).

The molecular mechanism by which the ATP sensitivity of the Kir6.2 subunit is reduced varies between mutations. Studies to date suggest that most mutations associated with neonatal diabetes alone impair ATP-dependent channel inhibition without much change in the fraction of time the channel spends in the open state in the absence of ATP (the intrinsic open probability) (59,60). This is consistent with the fact that most of these mutations lie within the predicted ATP-binding site (Fig. 2). Mutations associated with neurological features, however, markedly bias the channel toward the open state, thus increasing the intrinsic open probability (59,50,60). This indirectly reduces the ability of ATP to block the channel because ATP stabilizes the long-closed state of the channel, which is now less frequent (61).

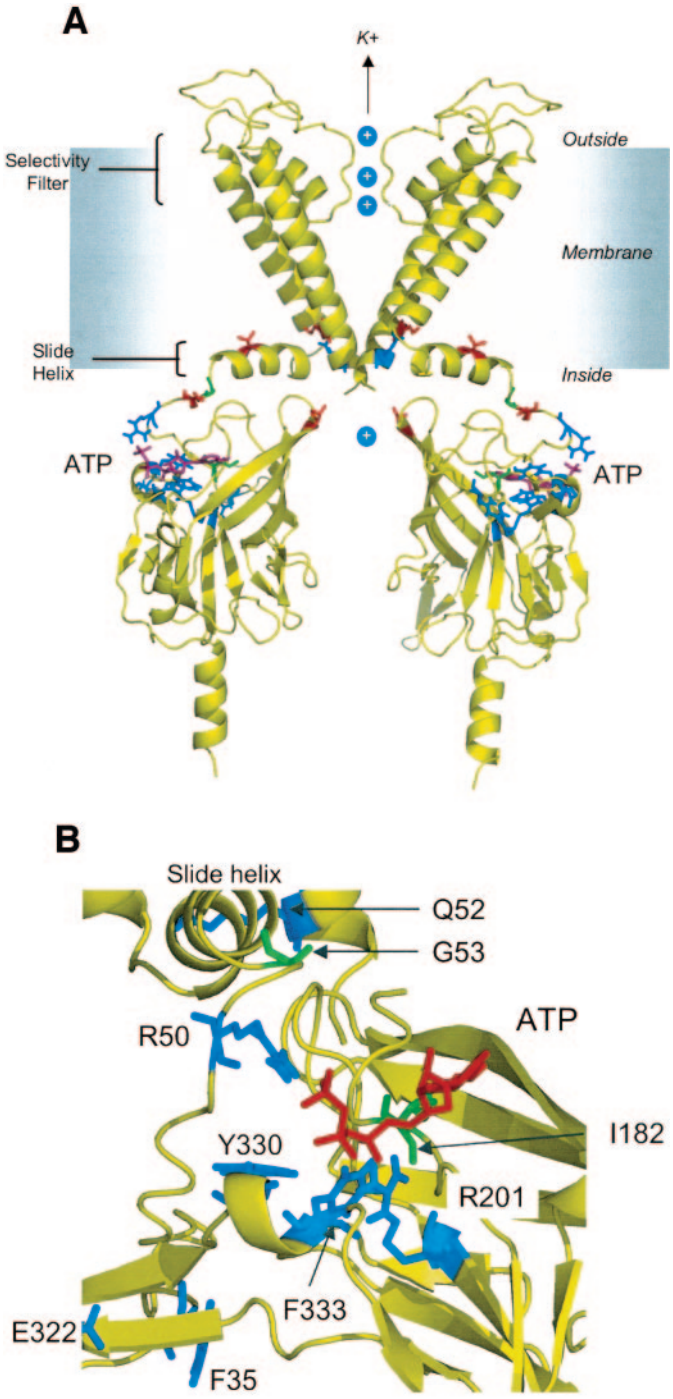

FIG. 2. *A*: Structural model of Kir6.2 (53) viewed from the side. For clarity, only two transmembrane domains and two separate cytosolic domains are shown. Residues mutated in permanent neonatal diabetes are shown in blue, in transient neonatal diabetes in green, and in DEND syndrome in red. ATP (purple) is docked into its binding site. *B*: Close-up of the putative ATP-binding site. Residues mutated in permanent neonatal diabetes are shown in blue, in transient neonatal diabetes in green, and ATP is shown in red.

To date, most functional studies have been conducted in Mg^{2+}-free solutions, as it is easier to study exactly how Kir6.2 mutations affect K_{ATP} channel ATP sensitivity in the absence of the stimulatory effects of Mg-nucleotides. However, it is also important to examine the effect of Kir6.2

mutations in the presence of Mg^{2+} because Mg^{2+} is always present in the cell and stimulation by Mg-nucleotides will shift the ATP sensitivity to higher ATP concentrations. Recent studies suggest that the reduction in K_{ATP} channel ATP sensitivity is greater in the presence of Mg^{2+} and that K_{ATP} currents recorded at physiological levels of MgATP (>1 mmol/l) are strikingly larger for DEND syndrome mutations than transient neonatal diabetes mutations, consistent with the severity of the clinical phenotype (41,50,60). Thus, it appears that, in addition to reducing the inhibitory effect of ATP at Kir6.2, some mutations enhance MgATP stimulation mediated via SUR1 (50,60), perhaps by influencing interactions between Kir6.2 and SUR1. Finally, all experiments to date have been performed by expression in heterologous systems, such as *Xenopus* oocytes. Thus, studies using β-cells, neurons, and animal models are now needed.

The importance of heterozygosity. All neonatal diabetes–associated mutations are heterozygous, so both wild-type and mutant Kir6.2 are expressed in the same cell. Because Kir6.2 is a tetramer (21), there will be a mixed population of channels, each containing between zero and four mutant subunits. The ATP sensitivity of any individual channel in this population will depend on the number of mutant subunits it contains and the extent to which each subunit contributes to the overall ATP sensitivity. This contribution may vary according to whether the mutation affects ATP binding or the intrinsic open probability. Binding of a single ATP molecule closes the K_{ATP} channel (62). This means that if a mutation influences just ATP binding, only channels with four mutant subunits will have a markedly reduced ATP sensitivity. If wild-type and mutant Kir6.2 subunits distribute according to binomial theory, homomeric mutant channels will only account for one-sixteenth of channels in the heterozygous population; thus, the shift in ATP sensitivity will be small. Conversely, mutations that influence the intrinsic open probability will affect 15 of 16 channels (albeit to different extents), as each heteromeric channel will have at least one mutant subunit. Thus, the shift in ATP sensitivity will be larger. This may help explain why mutations that affect the intrinsic open probability produce a more severe clinical phenotype than those that affect the ATP-binding site. Studies are now required to determine whether this idea is correct and if wild-type and mutant subunits distribute as binomial theory predicts.

FROM GENETICS TO THERAPY

For patients with neonatal diabetes resulting from Kir6.2 mutations, diagnosis of the genetic etiology of their disease has revolutionized therapy. K_{ATP} channels that are insensitive to ATP as a consequence of Kir6.2 mutations can still be closed by sulfonylureas and glinides that bind to the SUR subunit and close the channel directly (7). Thus, sulfonylurea tablets can be considered as an alternative to insulin injections.

Patients with Kir6.2 mutations have all the clinical characteristics of being insulin dependent. They present with ketoacidosis and C-peptide levels similar to those found in type 1 diabetes. Even when stimulated by glucagon, serum C-peptide concentration is very low (typically <200 pmol/l). Consequently, they were previously treated with insulin therapy. However, initial physiological studies in three patients showed that although there was no response to intravenous glucose, insulin secretion was observed within minutes of receiving intravenous tolbutamide (2). Furthermore, a 46-year-old patient has had life-long good glycemic control after being treated with sulfonylurea tablets from diagnosis (2).

A number of patients with Kir6.2 mutations have been able to discontinue their insulin injections completely and obtain as good, or better, glycemic control using oral sulfonylureas (2,37,38,40,43). Continuous glucose monitoring, and the insulin response to intravenous glucose, suggest that patients with Kir6.2 mutations taking sulfonylureas have glucose-responsive insulin secretion (43). Importantly, these patients require high doses of sulfonylureas: e.g., 0.4–1.0 mg/kg glibenclamide compared with a maximum suggested dose of 0.33 mg/kg for a 60-kg adult with type 2 diabetes. Mutations that affect the open probability of the channel (i.e., that cause neurological symptoms) are less sensitive to inhibition by sulfonylureas in vitro (59,61). Thus, even higher drug doses may be necessary in these patients. Long-term follow-up studies are now required to assess whether the response to sulfonylureas is maintained; to date, the longest a patient has remained off insulin is 1 year, although over one-third have been able to reduce their dose of sulfonylureas while maintaining good control.

The neurological symptoms found in DEND syndrome probably arise as a consequence of enhanced activity of K_{ATP} channels in tissues other than the β-cell, such as muscle and/or nerve. Although insulin therapy can control blood glucose levels, it cannot alleviate the extrapancreatic consequences of enhanced K_{ATP} channel activity. Theoretically, sulfonylureas should also close K_{ATP} channels in extrapancreatic tissues and thereby ameliorate the neurological symptoms. However, the extent to which neurological features respond to sulfonylureas is still uncertain; it will be influenced by many factors, such as the extent to which the drug crosses the blood-brain barrier, the severity of the mutation, the plasticity of the brain, and the SUR subtype the channel contains. With respect to the latter, the choice of sulfonylurea may be important. Most subjects to date have been tested with glibenclamide, which binds to both SUR1 and SUR2 and can therefore block all types of K_{ATP} channels (7). It is important to remember that while an SUR1-selective sulfonylurea (gliclazide and tolbutamide) will close β-cell K_{ATP} channels, it will not bind to the SUR2 receptors of muscle and brain K_{ATP} channels (7). Thus, nonspecific sulfonylureas may be the better choice when attempting to alleviate neurological symptoms.

IMPLICATIONS

Clinical implications. The high prevalence of Kir6.2 mutations in permanent neonatal diabetes means that all children <6 months of age diagnosed with diabetes should be tested for Kir6.2 mutations at diagnosis and assessed for neurological features. Details of genetic testing are available at many centers, including www.diabetesgenes.org. Finding a mutation offers the possibility of discontinuing insulin and implementing sulfonylurea therapy while maintaining good glycemic control (2,37,38,40,43). Early

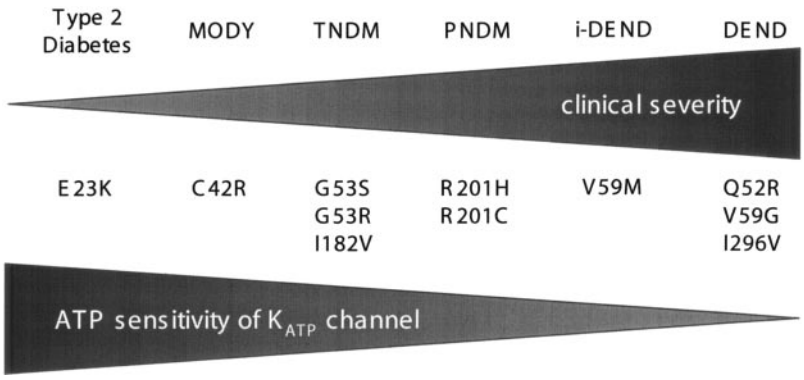

FIG. 3. Relationships between phenotype, genotype, and functional severity of Kir6.2 mutations and polymorphisms. Graphic indicating how the clinical severity of the disease is associated with specific mutations and how this may reflect the extent of reduction in the ATP-sensitivity of the mutated K_{ATP} channel. i-DEND, intermediate DEND syndrome; MODY, maturity-onset diabetes of the young (includes childhood and early adult onset diabetes); PNDM, permanent neonatal diabetes; TNDM, transient neonatal diabetes.

treatment with sulfonylureas may also limit neurological damage, even when it is unable to control the diabetes. Long-term surveillance and reporting of both short-term and long-term outcomes of all patients with Kir6.2 mutations receiving sulfonylureas is vital, as insulin injections remain a good treatment for diabetes and an alternative therapy must be clearly superior or help alleviate extrapancreatic symptoms.

Genetic implications. The demonstration that Kir6.2 mutations cause neonatal diabetes has implications for future genetic studies. It shows that genes in which common polymorphisms predispose to polygenic type 2 diabetes are excellent candidates for severe mutations resulting in monogenic diabetes and vice versa. It provides another example of activating and inactivating mutations in the same gene producing opposing phenotypes, in this case hypoglycemia and hyperglycemia. This supports the idea that genes associated with hyperinsulinism are good candidates for diabetes and vice versa. It emphasizes the value of selecting proteins known to play important roles in β-cell function as candidate genes, especially when genetic manipulation in experimental animals results in diabetes or hyperglycemia.

The demonstration that Kir6.2 mutations can cause neonatal diabetes illustrates the value of genetic studies of children with diabetes diagnosed before 6 months of age and indicates that such patients who lack Kir6.2 mutations provide an excellent resource for new genetic studies. Potentially, these may identify novel genes involved in insulin secretion and/or provide information about critical stages in β-cell development and function. One obvious candidate gene for such studies would be SUR1. There is also the interesting possibility that mutations that cause even less increase in the K_{ATP} current may be associated with diabetes in later life, as observed for the C42R mutation (44). Thus, other diabetic phenotypes, including maturity-onset diabetes of the young and atypical diabetes, should be examined for mutations in Kir6.2 to assess if there is a milder monogenic phenotype than neonatal diabetes. In this respect, it is interesting that the common E23K variant in Kir6.2 is consistently associated with type 2 diabetes in large-scale association studies (see below) (31–33).

In the past, monogenic studies have concentrated on large families with multiple affected members, as typically found in maturity-onset diabetes of the young. However, Kir6.2 mutations causing neonatal diabetes are predominantly de novo. Although it is not possible to map de novo mutations using reverse genetics, it is possible to rapidly establish that these mutations are likely to be causal when an affected proband born to unaffected parents has a mutation not present in the parents. The probability of such a result occurring by chance is very low, as the spontaneous mutation rate for any given nucleotide in an individual is estimated to be 1×10^{9}.

Association of the E23K variant in Kir6.2 with type 2 diabetes. Multiple large genetic studies consistently show a moderate association of the E23K variant in Kir6.2 with type 2 diabetes, with an ~20% increased risk associated with inheriting the E23 allele (31–33). Some of the smaller studies (<500 cases) did not detect an association (63), probably because they were underpowered for an effect of this size. However, the meta-analyses of all studies performed to date is highly significant ($P < 10^{-6}$) (32,33), and because the frequency of K allele is ~40% in Caucasians (63), it constitutes a significant population risk. The genetic studies do not, in themselves, conclusively establish that the E23K poymorphism is the causal variant because of strong linkage disequilibrium across the gene. For example, European Caucasians who inherit the less common K allele also inherit the rarer allele at the A1369S polymorphism in the adjacent SUR1 gene (32).

Functional studies suggest that overactivity of the K_{ATP} channel is likely to underlie the association of the E23K variant in Kir6.2 with type 2 diabetes. However, the mechanism by which this is achieved is controversial. A twofold reduction in the ATP sensitivity of Kir6.2/SUR1 channels (64) and Kir6.2/SUR2A channels (65) has been reported when E is mutated to K, yet a third study found little effect on ATP block (66). Minor variations in experimental protocol or DNA species (human versus mouse) might account for this difference. It has also been shown that the E23K mutation enhances activation of Kir6.2/SUR1 currents by Mg-nucleoside diphosphates (67) and by long-chain acyl CoAs (66), both of which lead to a small reduction in apparent inhibition by MgATP. Consequently, it is speculated that the K allele is associated with a small increase in the β-cell K_{ATP} current. In addition, recent studies suggest the E23K allele substantially reduced the ATP sensitivity of skeletal muscle K_{ATP} (Kir6.2/SUR2A) channels at acid pH (65), implying the K_{ATP} current will be enhanced during muscle ischemia and exercise. This could contribute to impaired glucose homeostasis by reducing glucose uptake in muscle.

The functional effects of the E23K polymorphism on glucose homeostasis in humans are equally controversial. Although some studies support the idea that the E23K variant is associated with reduced insulin secretion

(32,68), others have failed to detect such an association (69,70,71). It has also been suggested that the E23K variant may mediate its effect via changes in glucagon secretion (70). It is likely that the different findings of these studies relate to the relatively small numbers of the patients examined and the differences in age of the subjects, as the positive studies are in older patients. More studies are clearly needed to clarify these issues.

CONCLUSIONS

Kir6.2 mutations are associated with a range of phenotypes from transient neonatal diabetes to full DEND syndrome. The mutations show a strong genotype-phenotype relationship, and the disease phenotype is correlated with the extent of reduction in K_{ATP} channel ATP sensitivity and thus with the magnitude of the whole-cell K_{ATP} current. Mutations that cause a large reduction in ATP sensitivity will impair electrical activity in multiple tissues, whereas those that cause less reduction in ATP sensitivity will produce a smaller increase in the resting K_{ATP} current and result in neonatal diabetes alone (Fig. 3). The discovery that Kir6.2 mutations cause neonatal diabetes has resulted in a major change in treatment, with most patients being able to improve their glycemic control when insulin injections are replaced with high-dose sulfonylurea tablets. Although considerable prior knowledge of the structure and function of Kir6.2 existed, the finding of naturally occurring mutations has also offered new scientific insights; thus, it has confirmed the location of the ATP-binding site and identified new domains associated with channel gating (I296L, ref. 50) or SUR1 interactions (F333, ref. 60). These recent studies therefore reinforce and extend our previous understanding of the structure and function of the Kir6.2 subunit of the K_{ATP} channel and offer a novel therapeutic option to patients who previously faced life-long insulin injections.

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