Extremes of Clinical and Enzymatic Phenotypes in Children With Hyperinsulinism Caused by Glucokinase Activating Mutations

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OBJECTIVE—Heterozygous activating mutations of glucokinase have been reported to cause hypoglycemia attributable to hyperinsulinism in a limited number of families. We report three children with de novo glucokinase hyperinsulinism mutations who displayed a spectrum of clinical phenotypes corresponding to marked differences in enzyme kinetics.

RESEARCH DESIGN AND METHODS—Mutations were directly sequenced, and mutants were expressed as glutathionyl S-transferase–glucokinase fusion proteins. Kinetic analysis of the enzymes included determinations of stability, activity index, the response to glucokinase activator drug, and the effect of glucokinase regulatory protein.

RESULTS—Child 1 had an ins454A mutation, child 2 a W99L mutation, and child 3 an M197I mutation. Diazoxide treatment was effective in child 3 but ineffective in child 1 and only partially effective in child 2. Expression of the mutant glucokinase ins454A, W99L, and M197I enzymes revealed a continuum of high relative activity indexes in the three children (26, 8.9, and 3.1, respectively; wild type = 1.0). Allosteric responses to inhibition by glucokinase regulatory protein and activation by the drug RO0281675 were impaired by the ins454A but unaffected by the M197I mutation. Estimated thresholds for glucose-stimulated insulin release were more severely reduced by the ins454A than the M197I mutation and intermediate in the W99L mutation (1.1, 3.5, and 2.2 mmol/l, respectively; wild type = 5.0 mmol/l).

CONCLUSIONS—These results confirm the potency of glucokinase as the pancreatic β-cell glucose sensor, and they demonstrate that responsiveness to diazoxide varies with genotype in glucokinase hyperinsulinism resulting in hypoglycemia, which can be more difficult to control than previously believed. *Diabetes* 58:1419–1427, 2009

Hypoglycemia in infants with congenital hyperinsulinism has been associated with mutations that affect the regulation of insulin secretion by all three major classes of metabolic fuels: glucose, amino acids, and fatty acids (1–6). The most common of these disorders is caused by recessive mutations of the β-cell ATP-sensitive K$^+$ (K$_{ATP}$) channel; these mutations cause severe neonatal hypoglycemia that does not respond to medical therapy with diazoxide, a K$_{ATP}$ channel agonist, and often requires near-total pancreatectomy (7,8). Other genetic forms of congenital hyperinsulinism, such as dominant mutations of glutamate dehydrogenase, cause less severe disease, with hypoglycemia that may not be recognized until childhood or even adult life and that responds well to diazoxide therapy (4,9–11). In 1998, the first case of hyperinsulinism caused by a dominant gain-of-function mutation of glucokinase was reported (12). This remains one of the rarest forms of hyperinsulinism, and information on its clinical and biochemical manifestations is limited because only a few cases have been reported subsequently (13–19). Most of these cases have been identified because of family histories of hypoglycemia with dominant patterns of transmission, and most affected individuals were reported to have relatively mild disease that could be managed medically with diazoxide.

Glucokinase catalyzes the first step in glucose metabolism in pancreatic β-cells and liver (20). It exists as a monomer in three conformations that control catalytic function: a closed form, an open form, and a super open form (21). Transitions between these conformations are controlled by glucose concentration, giving a sigmoidal enzyme activity curve, as well as by allosteric modulators. Binding of novel glucokinase activator molecules, such as RO0281675, to the allosteric site increases glucokinase activity, resulting in both augmented hepatic glucose uptake and lowering of the β-cell threshold for glucose-stimulated insulin release (22). In the liver, glucokinase enzyme activity is inhibited by binding of glucokinase regulatory protein, which also leads to nuclear sequestration of the enzyme (23).

Glucokinase serves a critical physiological function as the β-cell glucose sensor. It determines the glucose threshold for insulin release because of the low affinity of the enzyme for its substrate, glucose (half-maximal activity, $S_{0.5}$, occurs at 7.5 mmol/l glucose). Heterozygous mutations that reduce enzyme activity cause a subtype of maturity-onset diabetes of the young 2 (MODY2), whereas, as noted above, heterozygous activating mutations cause hypoglycemia. Expression of these activating mutations shows increased affinity for glucose with elevations of

calculated enzyme activity indexes and lower calculated glucose thresholds for insulin release (24).

Based on the initial cases reported, glucokinase hyperinsulinism has been assumed to be a mild form of hypoglycemia that can easily be managed medically. However, one reported case with a more severe clinical phenotype of uncontrollable hypoglycemia suggests that the range of manifestations of glucokinase hyperinsulinism may be greater than has been appreciated (14). The purpose of this report is to describe three children with hyperinsulinism caused by de novo glucokinase mutations who exhibit marked differences in responsiveness to medical therapy that correlate with differences in enzyme activity indexes.

RESEARCH DESIGN AND METHODS

All clinical studies were approved by the institutional review board of the Children's Hospital of Philadelphia. Patient tests were performed in the Clinical Translational Research Center of the Children's Hospital of Philadelphia. Protein sensitivity and acute insulin response tests to calcium, leucine, and glucose were carried out as previously described (25,26). Insulin assays were performed using an enzyme-linked immunosorbent assay kit (Mercodia; Alpco Diagnostics, Salem, NH).

Identification of glucokinase gene mutations. Genomic DNA from peripheral lymphocytes was amplified by PCR using published primer sequences for glucokinase (Genbank accession no. M88011) (27). Products were sequenced using a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and analyzed using Sequencher 3.1 (Gene Codes, Ann Arbor, MI).

Biochemical characterization of ins454A, W99L, and M197I mutants. Recombinant human islet wild-type glucokinase, the three patient mutants (ins454A, W99L, and ins454A), known instability mutant E300K, and a series of designed mutations (M197A, D, E, F, K, L, T, and V) were generated using methods previously described (28). Briefly, the mutations were cloned for expression as glucokinase fusion proteins containing a COOH-terminal glutathionyl S-transferase (GST). GST-glucokinase was produced in *Escherichia coli* and then purified from crude extracts to near homogeneity by affinity chromatography using glutathione-agarose (Sigma, St. Louis, MO). Characterization of GST cleaved enzymes was also performed by cleavage of GST with Factor Xa. The product was then purified again using glutathione-agarose and Factor Xa with benzamidine Sepharose 6B to yield the cleaved proteins.

Kinetic analysis of the expressed forms of glucokinase was performed using the protocols developed previously (13,16), both in the presence and absence of the glucokinase activator compound RO0281675. The activity index, an expression of the proposed enzyme's in situ phosphorylation capacity, and the theoretical threshold for glucose-stimulated insulin release were calculated as previously described (13,20). Kinetic analysis was also performed in the presence of recombinant human glucokinase regulatory protein, a competitive inhibitor of glucokinase, with and without sorbitol-6-phosphate, a structural analog of fructose-6-phosphate that enhances glucokinase regulatory protein inhibition (16). For this purpose the assay was performed with glucose at 3 mmol/l for wild type and the glucose concentration adjusted based on $S_{0.5}$ of the different mutants in both the presence and the absence of 10 μmol/l sorbitol-6-phosphate.

The stability of mutant and wild-type GST-glucokinase to low glucose and high temperature was tested as previously described by spectrophotometric enzyme assay (16,29). The stability of W99L was measured using tryptophan fluorescence by comparing the quantum (photonic) yield of wild-type glucokinase to the mutant glucokinase protein solution (30).

Structural analysis. Structural modeling of M197I, W99L, ins454A, and published activating mutations was performed using the structure of the super-open form of glucokinase and closed form determined by Kamata et al. (21). Modeling was performed using a SwissPdb viewer (31).

Patients. Child 1 is a 17-year-old male subject who had a large-for-gestational-age birth weight of 4.8 kg at term. Hypoglycemia was detected in the first hour after delivery, and high rates of intravenous glucose infusion were required to control blood glucose levels (18 mg · kg^{-1} · min^{-1}; normal <5–6). Plasma insulin levels at times of hypoglycemia were found to be elevated (8–15 μU/ml; normal <3). He was not fully responsive to diazoxide, defined as being able to fast >12 h with blood glucose of >70 mg/dl. However, treatment with a high dose of diazoxide (20 mg · kg^{-1} · day^{-1}) and feedings every 4 h prevented symptoms of hypoglycemia, although plasma glucose levels re-
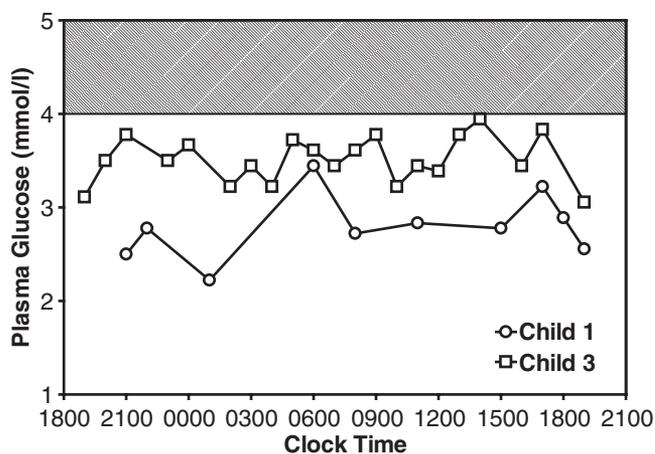

FIG. 1. Diurnal patterns of plasma glucose concentrations in child 1 and child 3. Plasma glucose was measured before meals and after overnight fasting conditions. Shaded area indicates the normal range of plasma glucose (4.0–5.0 mmol/l, 72–90 mg/dl). Child 1 was 4 years old, 2 years after near-total pancreatectomy; child 3 was 15 years old. Neither child was on medical therapy. Mean glucose was 50 ± 2 mg/dl in child 1 and 63 ± 1 mg/dl in child 3. $P < 0.0001$ between children.

mained largely between 2.8 and 3.3 mmol/l (50–60 mg/dl). There were no family members with hypoglycemia.

At 1 year of age, he was referred to the Children's Hospital of Philadelphia because of persistently low plasma glucose values of 2.2–3.3 mmol/l (40–60 mg/dl). A trial of octreotide gave only transient improvement in control of hypoglycemia (32). Because of persistent hypoglycemia at 23 months of age, a 95% pancreatectomy was performed; however, this failed to control hypoglycemia. Diazoxide treatment was retried, but plasma glucose fell to 3.3 mmol/l (60 mg/dl) after only 5 h of fasting. Treatment consisted of feedings given frequently, day and night. Despite the low levels of plasma glucose, hypoglycemic symptoms rarely occurred, mainly triggered by exercise or high-carbohydrate feedings. His parents report that his hypoglycemia appeared to improve after age 11 years, and nighttime feedings were stopped. However, they also report that he now has occasional hyperglycemia after meals, suggestive of mild glucose intolerance. Currently, child 1 has normal school performance.

Child 2 is a boy with a normal term birth weight of 3.2 kg. Hypoglycemia was not recognized until age 6 years, when he became unarousable a few hours after eating and was found to have a blood glucose of 48 mg/dl (2.7 mmol/l). There was no family history of hypoglycemia. Treatment with 10 mg · kg^{-1} · day^{-1} of diazoxide failed to control hypoglycemia. He was suspected to have an insulinoma because of the apparent new onset of hypoglycemia. Endoscopic ultrasound suggested a lesion in the head of the pancreas that was found to be a lymph node at surgery; biopsies of the neck and body of the pancreas were normal. Octreotide failed to control hypoglycemia. Successful control of hypoglycemia has required a combination of diazoxide (15 mg · kg^{-1} · day^{-1}) plus a low rate of continuous dextrose overnight by gastrostomy tube (2 mg · kg^{-1} · min^{-1}). School performance was considered to be normal.

Child 3 is a 16-year-old boy with a large-for-date birth weight of 4.9 kg at 41 weeks of gestation. Hypoglycemia was detected in the first hours after birth, requiring treatment with intravenous dextrose at 12 mg · kg^{-1} · min^{-1}. There was no history of hypoglycemia in the parents or in two older siblings. Hyperinsulinemic hypoglycemia was documented but was considered to have resolved after 2 months of treatment with prednisone. There was no further suspicion of hypoglycemia until 14 years of age, when he suffered two brief seizures, each occurring 3 h after breakfast. Plasma glucose was found to be 2.2 mmol/l (40 mg/dl). Hyperinsulinemic hypoglycemia was diagnosed. A high dose of diazoxide at 900 mg (15 mg · kg^{-1} · day^{-1}) was required to maintain fasting plasma glucose levels >3.9 mmol/l (>70 mg/dl). School performance was considered normal, although not on a par with his siblings.

RESULTS

Figure 1 shows the diurnal profile of plasma glucose levels in child 1 and child 3 (at ages 4 and 15 years, respectively). Glucose values ranged between narrow limits, rarely rising into the normal range even after meals, but also rarely
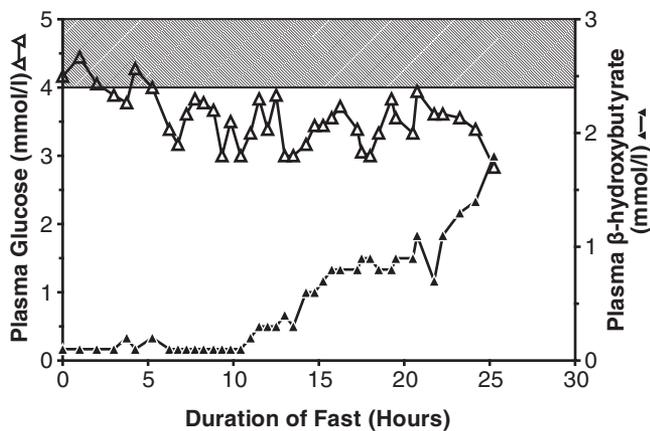
FIG. 2. Plasma glucose and β-hydroxybutyrate responses to fasting in child 2. Mean plasma glucose was 64 ± 1 mg/dl. β-Hydroxybutyrate values increased from 0.1 to 1.8 mmol/l, whereas fasting plasma glucose levels decreased. At the end of the fast, plasma C-peptide was suppressed (0.16 nmol/l, normal 0.26–1.32 nmol/l). Glucose response to glucagon stimulation was 36 mg/dl. Reference normals when fasting blood glucose <50 mg/dl: β-hydroxybutyrate >2.5 mmol/l, and glycemic response to glucagon >30 mg/dl. △, plasma glucose (SureStep bedside meter); ▲, β-hydroxybutyrate (Precision Xtra bedside meter).

falling very low, even during 12 h of overnight fasting (child 3). Child 1, whose hypoglycemia was more difficult to manage, had significantly lower mean plasma glucose levels (mean ± SE) than child 3 (50 ± 2 vs. 63 ± 1 mg/dl, respectively; $P < 0.0001$). As shown in Fig. 2, child 2 fasted for 24 h with a stable plasma glucose ranging from 3 to 4 mmol/l (54–72 mg/dl) before dropping to 2.8 mmol/l (50 mg/dl). During the fast he developed a significant ketonemic response, albeit lower than normally seen at this level of blood glucose (β-hydroxybutyrate >2.5 mmol/l).

Table 1 shows the responses in child 1 and child 3 to stimulation tests of insulin secretion. Both children showed exaggerated acute insulin responses to intravenous glucose. In child 3, acute insulin responses to calcium and leucine were normal, in contrast to the hyperresponsiveness to calcium and leucine seen in patients with K_{ATP} channel or glutamate dehydrogenase mutations, respectively. In further contrast to patients with K_{ATP} or glutamate dehydrogenase hyperinsulinism, hypoglycemia was not provoked by oral protein tolerance tests in either child 1 or child 3. All three children had normal serum concentrations of cholesterol and triglyceride.

Review of the surgical specimen of resected pancreas from child 1 showed normal islet distribution, shape, and size from head to tail. There were occasional large endocrine cell nuclei that occupied an area at least three times as large as the surrounding endocrine cell nuclei, similar to the histological features seen in recessive K_{ATP} hyper-

TABLE 1
Acute insulin response to insulin secretagogues and plasma lipid concentrations

	Child 1	Child 2	Child 3	Normal
Glucose AIR (pmol/l)	483	—	474	318 ± 228
Calcium AIR (pmol/l)	—	—	−24.0	0.6 ± 11.4
Leucine AIR (pmol/l)	—	—	30.0	12.0 ± 66
Cholesterol (mmol/l)	2.9	4.3	4.3	2.8–5.8
Triglycerides (mmol/l)	0.29	0.61	1.7	0.4–1.9

Reference normal values for acute insulin response tests are from Ferry et al. (25) and Grimberg et al. (26). AIR, acute insulin response.

insulinism. The surgical biopsies from child 2 showed normal pancreas.

Genetic mutation analysis. In child 1, mutation analysis of genomic DNA identified a heterozygous novel missense mutation, ins454A (nt 13631-364 ins CGG). In child 2, a glucokinase mutation was suspected because of the stability of his hypoglycemia (Fig. 2); he was found to have a heterozygous novel W99L missense mutation (nt 296 G>T). In child 3, a mutation in glucokinase was also suspected because of the stability of hypoglycemia; he had a heterozygous novel M197I mutation (nt 591 G>T). The mutations were not detected in any of the children's parents and were not found in 100 normal chromosomes. No mutations were found in other hyperinsulinism genes, including ABCC8, KCNJ11, and GLUD1.

Biochemical analysis of expressed ins454A, W99L, and M197I glucokinase mutations. Table 2 shows the enzymatic activities of the purified mutant forms of glucokinase. The ins454A mutation resulted in a markedly increased affinity for glucose (decreased glucose $S_{0.5}$). The glucose $S_{0.5}$ values in child 2 and child 3 were reduced to a lesser degree. The turnover rate, k_{cat}, of the ins454A mutation was similar to wild-type glucokinase; however, k_{cat} for the GST-glucokinase fusion M197I mutant was ~60% of wild type, and k_{cat} for W99L was increased compared with wild type. Hill numbers for all three mutations were only slightly reduced compared with wild type. Kinetic variables for the GST-glucokinase fusion proteins were similar to their cleaved counterparts (data not shown). For all mutations, the calculated enzyme activity index was increased compared with wild type, consistent with a gain of glucokinase enzyme function. This was especially true for child 1, whose ins454A mutation resulted in an activity index >25 times normal, consistent with his poorer response to diazoxide therapy compared with child 2 and child 3.

Because enzyme stability can potentially affect glucokinase activity, we examined the effect of incubating ins454A and M197I mutant enzymes under conditions of low glucose and high temperature (Fig. 3A and B). The stability of enzyme activity of the two mutants after incubation at low glucose concentrations was similar to that of wild-type glucokinase and was much greater than that of the known diabetes-associated instability mutant E300K (Fig. 3A). The thermal stability of the ins454A and M197I mutant proteins was intermediate between that of wild-type and E300K glucokinase (Fig. 3B). The thermal stability of W99L was investigated by tryptophan fluorescence under similar glucose conditions (30) and was also intermediate between that of wild type and the E300K instability mutant (data not shown).

As shown in Table 2, the ins454A GST-glucokinase mutant was unresponsive to the glucokinase activator compound RO0281675, suggesting that its activity was already maximal. The response of the W99L and M197I GST-glucokinase mutants to the activator was similar to wild type. The latter finding may be attributable to the M197I mutation being 19 Å away from the activator site and therefore unlikely to interfere with activator binding. Another plausible explanation is the moderate degree of enzyme activation leaving room for further activation by the glucokinase activator, as in the case of W99L.

Figure 3C and D shows the response of two of the mutant glucokinase enzymes to inhibition by hepatic glucokinase regulatory protein. W99L and M197I GST-glu-

TABLE 2
Kinetic characteristics of glucokinase ins454A, W99L, and M197I

Mutants	Yield (mg/l)	k_{cat} (sec^{-1})	Glucose S$_{0.5}$ (mmol/l)	ATP K_m (mmol/l)	n$_H$ (Hill number)	Activity index	Activation by GKA (fold)	EC$_{50}$ for GKA (μmol/l)
ins454A	10.9 ± 3.1	53.2 ± 2.9	1.1 ± 0.1	0.3	1.2	39.5 ± 1.7	No activation	N/A
W99L	19.9 ± 1.7	85.6 ± 3.1	2.9 ± 0.1	0.4	1.6	13.3 ± 0.8	11.8	4.3
M197I	62.1 ± 0.3	38.1 ± 4.5	2.6 ± 0.2	1.5	1.6	4.8 ± 0.3	11.9	4.9
M197L	34.5	54.5	5.4	1.0	1.4	3.8	13.9	6.2
M197V	25.1	44.7	2.6	0.5	1.7	8.0	13.1	3.5
M197A	36.4	53.3	3.2	0.3	1.7	7.0	14.7	3.8
M197T	26.7	53.9	4.1	0.5	1.6	4.4	18.3	5.5
M197F	40.3	21.2	54.6	0.2	1.3	0.1	8.6	6.1
M197K	36.4	3.78	86.7	0.2	1.1	0.03	13.6	9.3
M197D	27.7	10.3	197.0	0.3	1.1	0.03	6.0	2.8
M197E	6.82	21.0	56.2	1.3	0.2	0.1	9.0	2.7
Wild type	43.4 ± 3.8	62.3 ± 4.8	7.6 ± 0.2	0.4	1.7	1.5 ± 0.1	15.8 ± 0.6	6.9 ± 0.4

Kinetic data of purified expressed GST-tagged glucokinase with hyperinsulinism-causing ins454A (child 1), W99L (child 2), and M197I (child 3) mutations. EC$_{50}$ relates to the activity index. GKA, glucokinase activator; EC$_{50}$, half-maximal effective concentration.

cokinase mutants had similar responses to glucokinase regulatory protein as wild type (W99L results not shown). The ins454A mutant showed little response to inhibition by glucokinase regulatory protein.

Biochemical analysis of additional mutants at residue 197. As shown in Table 2, to evaluate the function of the M197 residue, eight additional mutations were designed that substituted a range of amino acids from hydrophobic
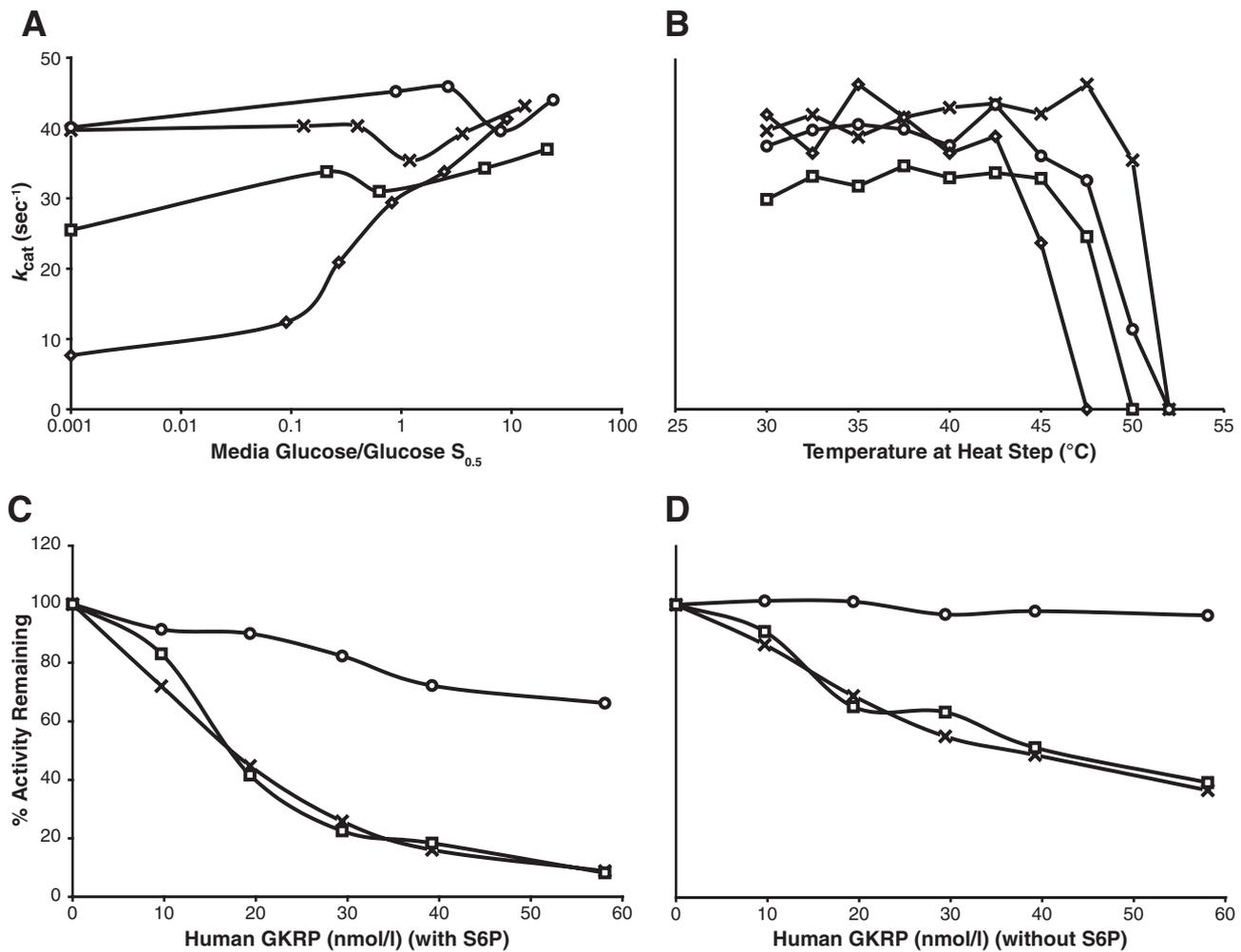

FIG. 3. Thermal stability of GST-tagged wild-type and mutant glucokinase (*A* and *B*). Response of GST-glucokinase mutants to inhibition with glucokinase regulatory protein (GKRP) (*C* and *D*). GST-glucokinase levels were 22 nmol/l in all tests. *A*: Effect of glucose concentration on expressed GST-tagged glucokinase with ins454A and M197I hyperinsulinism mutations and the known E300K instability mutant. For M197I, the studies were not extended below the glucose S$_{0.5}$ of 2.6 mmol/l. *B*: Effect of temperature on mutant glucokinase enzyme activities. For these assays, enzymes were incubated for 30 min with glucose levels at their respective S$_{0.5}$. *C* and *D*: Inhibition of glucokinase activity by glucokinase regulatory protein measured in the presence and absence of sorbitol-6-phosphate (S6P), respectively. ○, ins454A; □, M197I; ◇, E300K; X, wild type.

to hydrophilic at this site. Generally, substitution of hydrophobic residues for the normal methionine resulted in increased affinity for glucose and increased enzyme activity indexes, whereas changes to hydrophilic or amphipathic residues reduced enzyme activity.

DISCUSSION

The results of these studies demonstrate that congenital hyperinsulinism in these three children was caused by three novel activating mutations of glucokinase, ins454A, W99L, and M197I. Expression of the mutations demonstrated changes in glucokinase kinetics consistent with increased enzyme activity. Increases in calculated glucokinase activity indexes ranged from 25 times normal in child 1, with the ins454A mutation, to three times normal in child 3, with the M197I change. These differences in enzyme activation correlated with the more severe diazoxide-unresponsive hypoglycemia of child 1; the intermediate, partially diazoxide-responsive hypoglycemia of child 2; and the milder diazoxide-responsive hypoglycemia of child 3.

As shown in Fig. 4A, the mutations at positions 99, 197, and 454 of glucokinase are located on the reverse side of the enzyme, opposite the catalytic cleft. The ins454A mutation is adjacent to three previously reported glucokinase activating mutations (V455M, A456V, and V452L), whereas the W99L mutation is located at the same position as a previously reported W99R activating mutation (Table 3 summarizes known glucokinase hyperinsulinism mutations). Most of the 11 glucokinase activating mutations that have been identified occur in or close to the region of the enzyme that interacts with allosteric activator compounds, such as RO0281675, and with the inhibitory glucokinase regulatory protein. Heredia et al. (33,34) have shown that some of these mutations (T65I and A456V) increase glucose binding to the enzyme, whereas the others (W99R, Y214C, and V455M) facilitate enzyme isomerization into the active form. The two mutations that enhance glucose binding also abrogate interaction with glucokinase regulatory protein, suggesting that these mutations cause increased glucokinase activity by favoring maintenance of the closed, active form of the enzyme and preventing opening of the catalytic cleft into the inactive form of the enzyme. The ins454A glucokinase mutation in child 1 appears to fit into this category of mutations, which makes the enzyme unresponsive to both inhibition by glucokinase regulatory protein and allosteric activation by RO0281675. Loss of inhibition by glucokinase regulatory protein does not seem to be a major determinant of clinical phenotype (34) because the Y214C mutation retains sensitivity to glucokinase regulatory protein but was reported to have diazoxide-unresponsive hypoglycemia similar to ins454A (Table 3).

The novel M197I amino acid substitution reported here has a unique location that is remote from the other glucokinase activating mutations. As shown in Fig. 4B and C, the M197I mutation alters a methionine residue that moves in and out of a hydrophobic pocket during the transition between the active and inactive forms of glucokinase. The importance of this hydrophobic lock-and-key interaction is demonstrated by the series of designed mutations of M197 (Table 2). Substitutions with isoleucine or other hydrophobic amino acids retained or enhanced glucokinase activity. However, substitutions with hydrophilic amino acids essentially inactivated the enzyme.

These observations have been confirmed in a recent report by Pal and Miller (35). The M197I mutation indicates that activating mutations of glucokinase need not be restricted to the allosteric domain, where all previous defects have been located, and suggests that additional mutation sites are likely to be identified in patients with glucokinase hyperinsulinism.

Glucokinase mutations have been found infrequently in mutation analysis of patients with congenital hyperinsulinism, accounting for only 5 of 167 patients in recently reported series (18) and only 3 of 212 cases we have analyzed. Table 3 summarizes the major features of hyperinsulinism caused by previously reported activating mutations of glucokinase and the three additional mutations described in this report. Familial cases account for 76% of the 29 known patients. This contrasts with the high proportion of de novo cases in glutamate dehydrogenase hyperinsulinism and other dominant disorders, suggesting that many cases are not identified in the absence of a family history of hypoglycemia. Only 3 of the 29 patients had surgery, suggesting a relatively mild hypoglycemia phenotype, especially in the familial cases. Many of the familial cases escaped recognition of their hypoglycemia disorder until beyond the neonatal period or even into adult life. In all of the familial cases, treatment with the β-cell K_{ATP} channel agonist diazoxide was reported to have been successful in controlling hypoglycemia. Child 3 (M197I mutation) fits this milder, diazoxide-responsive phenotype, although it should be noted that unusually high doses of diazoxide were needed to maintain even low-normal levels of plasma glucose. However, similar to child 2 (W99L), incomplete responsiveness to diazoxide was also apparent in some of the reported cases in the six families with the mild hypoglycemia phenotype (W99R, A456V).

In contrast, children with Y214C and ins454A mutations had a much more severe form of hypoglycemia. Our child 1 with the ins454A mutations could not be controlled on diazoxide, even at a very high dose of $20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. In the case of the Y214C mutation, diazoxide responsiveness is unknown because only a low dose was tried. Both of these children required surgery and continued to have poorly controlled hypoglycemia, despite near-total pancreatectomy. These cases make it apparent that the clinical manifestations of hyperinsulinism in some glucokinase mutations can be as severe and as unresponsive to diazoxide treatment as in children with hyperinsulinism caused by recessive mutations of the K_{ATP} channel subunits sulfonylurea receptor 1 and Kir6.2.

The observations in the present three cases identify clinical features that may be useful for distinguishing glucokinase hyperinsulinism. A notable feature in the cases presented here was the remarkable stability of their hypoglycemia (Figs. 1 and 2), consistent with a resetting of the threshold for insulin release at a value lower than normal. This contrasts with other forms of hyperinsulinism in infants, where blood glucose concentrations can fall without interruption to extremely low values. Insulin responses to secretagogues may also help to distinguish glucokinase hyperinsulinism from other disorders. The glucokinase activating mutations in our three cases are not associated with hyperresponsiveness to calcium (as seen in K_{ATP} hyperinsulinism) or leucine (as seen in glutamate dehydrogenase hyperinsulinism), did not predispose to protein-induced hypoglycemia (as seen in K_{ATP} and glutamate dehydrogenase hyperinsulinism), but are accompa-
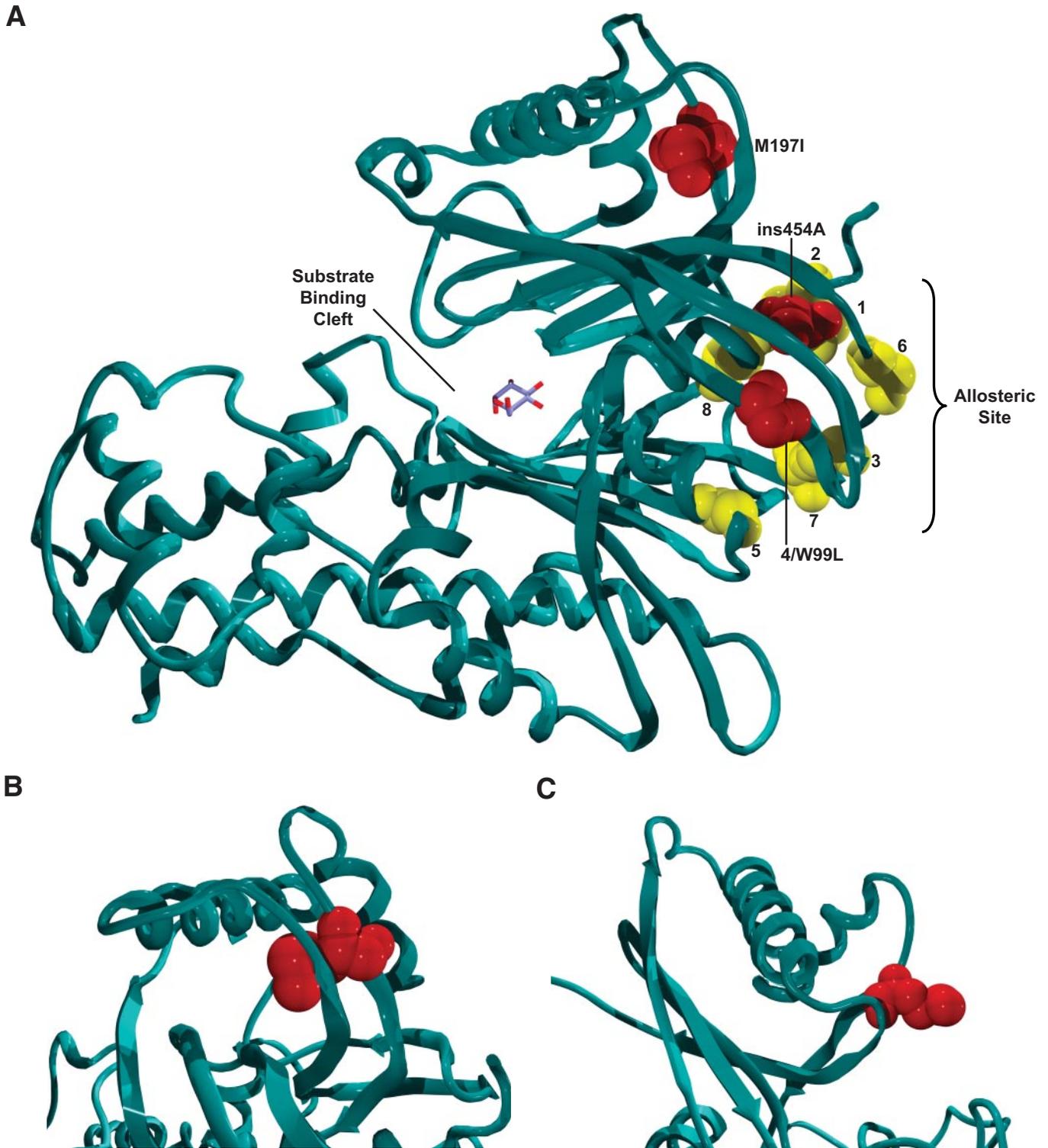
FIG. 4. *A*: Location of glucokinase mutations in the active (closed) form of the enzyme crystallographic structure. *B*: Location of the M197I residue tucked within a hydrophobic pocket of the active conformation of glucokinase. *C*: Location of the M197I residue projecting into the hydrophilic space of the inactive conformation of the crystal.

nied by increased acute insulin response to glucose (which is blunted in K_{ATP} hyperinsulinism).

Information about islet morphologic abnormalities in patients with glucokinase hyperinsulinism remains limited. In child 1, a small proportion of islet cells showed nucleomegaly similar to, but to a lesser extent than, that seen in diffuse K_{ATP} hyperinsulinism. The increase in islet size described by Cuesta-Munoz et al. (14) in a child

with a Y214C glucokinase mutation was not apparent in our child. No abnormalities were noted in the pancreas of the mother of the child with the T65I mutation (16). Although further study may identify specific histological features, current information suggests that it may not be easy to distinguish glucokinase hyperinsulinism from K_{ATP} hyperinsulinism or, possibly, from normal pancreas.

TABLE 3
Comparison of clinical and biochemical features of published glucokinase activating mutants associated with hyperinsulinism

	V455M	A456V	T65I	W99R	Y214C	G68V	S64Y	V452L	ins454A	W99L	M197I
Number of families	1	2	1	1	—	1	—	—	—	—	—
de novo case subjects	—	—	—	1	1	—	1	1	1	1	1
Total cases ($n = 29$)	5	5	2	3	1	8	1	1	1	1	1
Birth weight (kg)	2.9–4.1	2.4–3.8	3.1	3.1, 4.0	4.4	1.9–3.7	4.3	5.9	4.9	3.2	4.9
Age at diagnosis											
Neonatal ($n = 8$)	—	1	1	2	1	—	1	1	1	—	—
Childhood ($n = 7$)	2	1	—	—	—	3	—	—	—	1	—
Adolescence ($n = 4$)	1	—	—	—	—	2	—	—	—	—	1
Adulthood ($n = 10$)	2	3	1	1	—	3	—	—	—	—	—
Severity of hypoglycemia											
Mild or untreated ($n = 12$)	1	3	—	1	—	7	—	—	—	—	—
Diazoxide treated ($n = 13$)	4	2	1	2	—	—	1	1	—	1	1
Diazoxide and octreotide treated ($n = 1$)	—	—	—	—	—	1	—	—	—	—	—
Required surgery ($n = 3$)	—	—	1	—	1	—	—	—	1	—	—
Pretreatment plasma glucose (normal > 3.9 mmol/l)	1.3–2.5	2.1–3.5	2.2–3.0	2.0–3.5, 2.1	0.1–2.6	1.6–3.3	2.0	2.6–3.3	1.7–2.8	2.6–3.7	2.6–3.6
Response to diazoxide	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Partial	Yes
$S_{0.5}$ (normal = 7.55 mmol/l)*	3.0	2.0	1.8	4.5	1.2	1.9	1.5	2.6	1.1	2.9	2.6
Relative activity index*	5.2	17	3.1	4.1	130	16	22	11	26	8.9	3.1
Original clinical report reference no.	12	13	14	15	16	17	18	19	—	—	—

*Values from published reports.

Figure 5 shows the effects of different glucokinase activating mutations on predicted glucose threshold for insulin release, based on the relative activity indexes of the expressed proteins in vitro. Cases reported to be diazoxide unresponsive (ins454A and Y214C) have very low glucose thresholds, as does the A456V mutation,
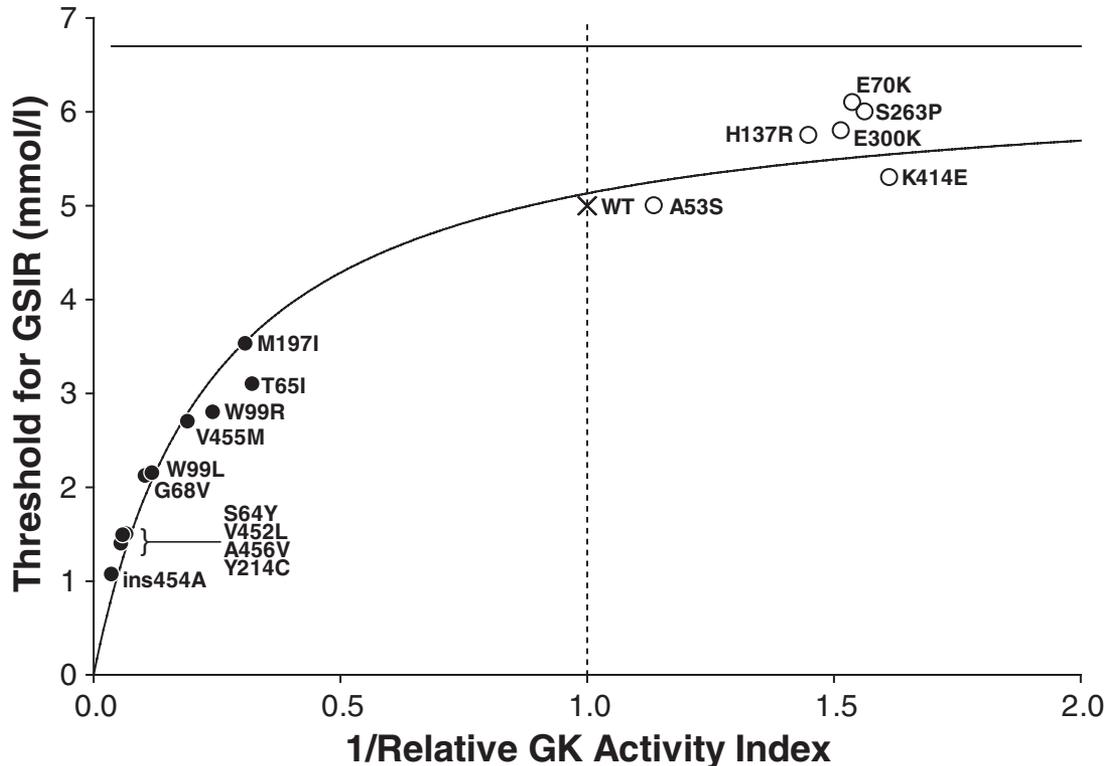

FIG. 5. Calculated thresholds for glucose-stimulated insulin release (GSIR) in activating and inactivating mutations of glucokinase. Thresholds are plotted against the inverse of the mutant enzyme activity index relative to wild-type (WT) enzyme. Because relative expression of the glucokinase forms is affected by enzyme affinity for glucose and the ambient glucose concentration, the wild-type enzyme dominates the estimated threshold for the heterozygous inactivating defects, but the mutant enzyme dominates the threshold for activating mutations. Thus, the threshold for inactivating mutations plateaus at ~7 mmol/l, whereas the calculated threshold for severe activating mutations approaches zero as the relative activity increases. For purposes of consistency, all kinetic data in the figure are from the laboratory of F.M.M. Threshold and activity indexes were calculated per Gloyn et al. (24). ●, glucokinase hyperinsulinism mutations; ○, MODY2 mutations; X, wild type. GK, glucokinase.

which in one patient was not completely responsive to diazoxide. In contrast, the better responses to diazoxide in child 2 and child 3 correlate with higher calculated glucose thresholds. It should be noted that the range of plasma glucose levels in our three patients and in the reports of other glucokinase hyperinsulinism cases (Table 3) tends to be higher than their predicted glucose thresholds (Fig. 5). This may partly reflect the effects of counterregulatory responses to hypoglycemia. An additional potential problem in correlating data on glucokinase kinetics with clinical features in patients is that some of the changes in enzyme properties exert opposing effects. One example of this phenomenon is the V62M mutation, which has been associated with MODY2 diabetes (36). When expressed in vitro, this mutation has an increased activity index consistent with causing hypoglycemia, rather than diabetes. The increased instability of this mutant form of glucokinase may counterbalance its enhanced activity and explain why the mutation results in a net loss of function in vivo (36). Similarly, although the ins454A, W99L, and M197I mutations have increased activity indexes, they also have slightly reduced stability. Moreover, the impact of the reduced affinity of M197I for ATP in vivo is uncertain. Given these problems, efforts to understand the in vivo and in vitro phenotypes of glucokinase hyperinsulinism mutations will require that accurate data be obtained on the clinical features of affected individuals. For example, these data should especially include careful documentation of the ability of diazoxide treatment to completely normalize plasma glucose levels and accurate estimates of the in vivo glucose "set point," as illustrated in Figs. 1 and 2.

In summary, these three cases of congenital hyperinsulinism caused by activating mutations of glucokinase emphasize the key role that this enzyme plays in setting the glucose threshold of the pancreatic islets. These children had de novo mutations, which made them difficult to recognize. Clues to their diagnosis included persistent, but stable, hypoglycemia and exaggerated insulin responses to intravenous glucose stimulation. These cases indicate that the spectrum of hyperinsulinism attributable to glucokinase activating mutations can range from mild and intermediate cases, which can be managed medically with diazoxide, to severe cases that are diazoxide unresponsive and may require additional treatment, including near-total pancreatectomy, to control hypoglycemia. These cases also illustrate the potentials and limitations of new approaches to develop glucokinase activator drugs for the treatment of type 2 diabetes.

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