Diazoxide-Unresponsive Congenital Hyperinsulinism in Children With Dominant Mutations of the β-Cell Sulfonylurea Receptor SUR1

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OBJECTIVE—Congenital hyperinsulinemic hypoglycemia is a group of genetic disorders of insulin secretion most commonly associated with inactivating mutations of the β-cell ATP-sensitive K$^+$ channel (K$_{ATP}$ channel) genes *ABCC8* (SUR1) and *KCNJ11* (Kir6.2). Recessive mutations of these genes cause hyperinsulinism that is unresponsive to treatment with diazoxide, a channel agonist. Dominant K$_{ATP}$ mutations have been associated with diazoxide-responsive disease. We hypothesized that some medically uncontrollable cases with only one K$_{ATP}$ mutation might have dominant, diazoxide-unresponsive disease.

RESEARCH DESIGN AND METHODS—Mutations of the K$_{ATP}$ genes were identified by sequencing genomic DNA. Effects of mutations on K$_{ATP}$ channel function in vitro were studied by expression in COSm6 cells.

RESULTS—In 15 families with diazoxide-unresponsive diffuse hyperinsulism, we found 17 patients with a monoallelic missense mutation of SUR1. Nine probands had de novo mutations, two had an affected sibling or parent, and four had an asymptomatic carrier parent. Of the 13 different mutations, 12 were novel. Expression of mutations revealed normal trafficking of channels but severely impaired responses to diazoxide or MgADP. Responses were significantly lower compared with nine SUR1 mutations associated with dominant, diazoxide-responsive hyperinsulinism.

CONCLUSIONS—These results demonstrate that some dominant mutations of SUR1 can cause diazoxide-unresponsive hyperinsulinism. In vitro expression studies may be helpful in distinguishing such mutations from dominant mutations of SUR1 associated with diazoxide-responsive disease. *Diabetes* 60:1797–1804, 2011

Inactivating mutations of the β-cell ATP-sensitive K$^+$ channel (K$_{ATP}$ channel) are the most common cause of hypoglycemia due to congenital hyperinsulinism (1). These mutations occur in either of the two subunits of the K$_{ATP}$ channel, SUR1 and Kir6.2, which are encoded by two adjacent genes on chromosome 11p, *ABCC8* and *KCNJ11* (2,3). Infants with recessive mutations of these genes typically have complete loss of K$_{ATP}$ channel function affecting all of their β-cells. Recessive

K$_{ATP}$ mutations can also cause focal hyperinsulinism through a mechanism of postzygotic loss of heterozygosity for the maternal 11p region, leading to isodisomy for a paternally transmitted mutation (4). Recessive *ABCC8* and *KCNJ11* mutations are usually null mutations or are amino acid substitutions that prevent trafficking of channels to the plasma membrane, thus leading to persistent plasma membrane depolarization and insulin release (5,6). Because diazoxide suppresses insulin secretion by acting as a K$_{ATP}$ channel agonist to prevent membrane depolarization, most children with such mutations of the K$_{ATP}$ genes are not responsive to treatment with diazoxide.

In contrast to these well-recognized recessive mutations of the K$_{ATP}$ channel, we and others have described children who have mutations of *ABCC8* and *KCNJ11* that are expressed in dominant fashion (7–9). In most of these cases, the hypoglycemia could be controlled with diazoxide, consistent with retention of residual channel activity. All of the dominant K$_{ATP}$ mutations associated with hyperinsulinism involve amino acid changes which, in the cases tested, could be shown by in vitro expression studies to permit normal trafficking of mature channels to the plasma membrane. However, the resultant channels had impaired responses to agonists, such as MgADP and diazoxide (7).

We have recently completed genetic analyses on a large group of diazoxide-unresponsive congenital hyperinsulinism patients seen at the Children's Hospital of Philadelphia. In most cases with identified mutations, the children either had diffuse disease with recessively inherited mutations of the K$_{ATP}$ genes or had focal lesions isodisomic for a paternally derived recessive K$_{ATP}$ mutation. However, in a subset of diffuse, diazoxide-unresponsive cases, only a single mutation in *ABCC8* was detected. We hypothesize that these are dominantly acting mutations that cause a diazoxide-unresponsive form of hyperinsulinism. This report describes the clinical features of the affected children and evidence from in vitro expression studies that distinguish the diazoxide-unresponsive from the diazoxide-responsive forms of dominant K$_{ATP}$ hyperinsulinism.

RESEARCH DESIGN AND METHODS

The case subjects described in this study come from a large group of children with hyperinsulinism who were referred to The Children's Hospital of Philadelphia between 1990 and 2010. The diagnosis of hyperinsulinism was based on previously described criteria: fasting hypoglycemia accompanied by inadequate suppression of plasma insulin, inappropriately low plasma free fatty acid and plasma β-hydroxybutyrate concentrations, and an inappropriate increase in serum glucose levels after administration of glucagon injection at the time of hypoglycemia (10,11). Age of onset was defined as the age of first symptoms of hypoglycemia. Patients were defined as being unresponsive to diazoxide if hypoglycemia could not be controlled by treatment with 15 mg/kg/day

diazoxide for a minimum of 5 days (i.e., able to keep blood glucose >70 mg/dL for more than 8–10 h of fasting). Most of these diazoxide-unresponsive patients required surgical pancreatectomy.

Mutation analysis. Genomic DNA was isolated from peripheral blood samples of patients and family members (5 PRIME, Gaithersburg, MD). Where peripheral blood was not available, saliva was collected via the Oragene DNA Self Collection kit (DNA Genotek, Kanata, Ontario, Canada) and extracted according to the manufacturer's protocol. DNA was extracted from surgical pancreatic specimens using the DNA/RNA Allprep kit (Qiagen, Valencia, CA). The coding sequences and intron/exon splice junctions of the *ABCC8* and *KCNJ11* genes were amplified and directly sequenced on an ABI 3730 capillary DNA analyzer (Applied Biosystems, Carlsbad, CA). Resulting chromatograms were analyzed with the Sequencher 4.9 program (Gene Codes, Ann Arbor, MI). The nucleotides of the *ABCC8* gene and the translated amino acids were numbered according to the sequence reported by Nestorowicz et al. (2) that includes the alternatively spliced exon 17 sequence (NCBI accession no. L78224). Putative mutations were analyzed with mutation prediction software SIFT (12) and Polyphen (13) and were also screened in DNA from 50 control subjects (Coriell Cell Depository, Camden, NJ) to rule out the possibility of rare polymorphisms.

Exonic dosage assays. TaqMan Copy Number assays (Applied Biosystems/Life Technologies, Carlsbad, CA) were used to test for intragenic deletions/insertions that may have been missed by direct sequencing. Custom assays were designed for exons in which no suitable predesigned assay was found. Real-time PCR was performed on genomic DNA using an ABI Prism 7900HT Sequence Detection system and analyzed using SDS software (version 2.3; Applied Biosystems/Life Technologies). Applied Biosystems CopyCaller Software (version 1.0) was used to determine the copy number of each exon in each sample. Assays were run in quadruplicate for case subjects with two control samples and a negative control subject for each assay.

Functional analysis of mutant channels. Point mutations were introduced into hamster SUR1 cDNA in the pECE plasmid using the QuikChange mutagenesis kit (Agilent Technologies, Santa Clara, CA) and confirmed by DNA sequencing (5). COSm6 cells were transfected with wild-type or mutant SUR1 and wild-type rat Kir6.2 cDNA (in pCDNAI) using FuGene6 and subjected to analysis by Western blot, immunofluorescence staining, and a quantitative chemiluminescence assay 48–72 h post-transfection as previously described (14). For these experiments, a SUR1 tagged with a FLAG-epitope at the NH$_2$-terminus (denoted as flag-SUR1 hereinafter) was used. The FLAG tag has been shown not to affect channel expression and function (15). For Western blotting, cells were lysed in 20 mmol/L Hepes, pH 7.0/5 mmol/L EDTA/150 mmol/L NaCl/1% Nonidet P-40 with protease inhibitors. Proteins were separated by SDS-PAGE (8%), transferred to nitrocellulose, probed with M2 anti-FLAG antibody (Sigma, St. Louis, MO) followed by horseradish peroxidase (HRP)-conjugated anti-mouse secondary antibodies, and visualized by enhanced chemiluminescence (Super Signal West Femto; Pierce). For surface staining, living cells were incubated with anti-FLAG antibody for 1 h at 4°C. Cells were then fixed with cold (−20°C) methanol for 10 min, incubated with Cy-3 conjugated anti-mouse secondary antibodies for 30 min at room temperature, and viewed with an Olympus Fluoview confocal microscope. For chemiluminescence assays, cells were fixed with 2% paraformaldehyde for 30 min at 4°C, preblocked in PBS/0.1% BSA for 30 min, incubated in M2 anti-FLAG antibody for an hour, washed 4 × 30 min in PBS/0.1% BSA, incubated in HRP-conjugated anti-mouse for 20 min, and washed again 4 × 30 min in PBS/0.1% BSA. Chemiluminescence was quantified using a TD-20/20 luminometer (Turner Designs) following 5-s incubation in Power Signal Elisa Femto luminol solution (Pierce).

Functional properties of channels were studied using inside-out patch-clamp recordings (14). COSm6 cells transfected with K$_{ATP}$ channel subunits and the green fluorescent protein (to identify transfected cells) cDNAs were plated onto coverslips and recorded 48–72 h posttransfection using micropipettes with resistance ~1.0–1.5 MΩ. The bath and pipette solution (K-INT) contained the following: 140 mmol/L KCl, 10 mmol/L K-HEPES, and 1 mmol/L K-EGTA, pH 7.3. ATP and ADP were added as the potassium salt. Currents were measured at membrane potential of −50 mV and inward currents shown as upward deflections. Data were analyzed using pCLAMP software (Molecular Devices, Sunnyvale, CA). The MgADP or diazoxide response was calculated as the current in K-INT solution plus 0.1 mmol/L ATP, 0.5 mmol/L ADP or 0.2 mmol/L diazoxide, and 1 mmol/L free Mg^{2+} relative to that in plain K-INT solution.

Statistical analyses. To compare the differences in diazoxide and MgADP stimulation between the diazoxide-responsive and diazoxide-unresponsive groups, a nonparametric Mann-Whitney U test was performed using GraphPad InStat (version 3.0b for Macintosh; GraphPad Software, San Diego, CA).

Consent. Written informed consent was obtained from patients' parents for this study. The study was reviewed and approved by The Children's Hospital of Philadelphia Institutional Review Board.

Illustrative case subject. The proband in Family 2 (Fig. 1), now aged 6 years, was the 1,760-g large-for-gestational-age (LGA) product of a 28-week pregnancy, delivered by cesarean section. During the pregnancy, the proband's mother was treated with insulin for diabetes resulting from a subtotal pancreatectomy at 3 months of age for congenital hyperinsulinism (see below). Hypoglycemia was identified at birth (blood glucose 21 mg/dL), initially attributed to the maternal diabetes. The hypoglycemia remained difficult to control (maximal glucose infusion rate of 37 mg/kg/min). A diagnosis of hyperinsulinism was made based on multiple elevated insulin levels at times of hypoglycemia (99, 94, and 125 μU/mL). Diazoxide treatment (17.6 mg/kg/day) was begun at 4 days of age but failed to control the hypoglycemia. Addition of octreotide (33 μg/kg/day) and 1 mg/day glucagon infusion were also ineffective. On day of life 27, the infant was transferred to the Children's Hospital of Philadelphia for pancreatectomy because of intractable hypoglycemia. At surgery, biopsies of the pancreas were consistent with diffuse hyperinsulinism (islets in multiple areas showed evidence of nucleomegaly in a small percentage of β-cells). A 98% pancreatectomy was performed. At discharge, insulin was briefly needed for hyperglycemia, but the proband was subsequently hypoglycemic and able to tolerate fasts of only 6 h.

Four years later, the proband's brother was born weighing 1,000 g, appropriate for gestational age (AGA), after 27 weeks' gestation. The initial blood glucose was low, 44 mg/dL, and the insulin was elevated, 189 μU/mL. This infant also required a high rate of dextrose infusion to control hypoglycemia (23 mg/kg/day). He was treated with diazoxide (15 mg/kg/day for 5 days) and subsequently with octreotide without improvement of his hypoglycemia. At 10 weeks of age, a near-total pancreatectomy was performed and histology revealed diffuse hyperinsulinism.

The proband's mother presented at 3 months of age with a hypoglycemic seizure and required a 95% pancreatectomy for uncontrolled hyperinsulinism. At 5 years of age, she developed insulin-requiring diabetes. She was 20 years of age at the time of her first delivery, and her pregnancies were complicated by poor diabetes control, edema, hypertension, and diabetic nephropathy with proteinuria.

Direct sequencing of genomic DNA from the proband, his affected mother, and younger brother revealed a single heterozygous A478D mutation in exon 9 of *ABCC8* (SUR1) (Fig. 1).

RESULTS

Direct sequencing of the two K$_{ATP}$ channel genes, *ABCC8* and *KCNJ11*, on patients with diazoxide-unresponsive congenital hyperinsulinism seen at The Children's Hospital of Philadelphia between 1990 and 2010 identified mutations in 232 cases. Most of these were patients with diffuse disease who were biallelic for recessive mutations or were patients with focal hyperinsulinism who were monoallelic for a paternal mutation. However, in 17 children from 15 families, monoallelic missense amino acid substitutions were found that appeared to be responsible for diazoxide-unresponsive hyperinsulinism through an autosomal dominant mode of expression. As shown in Fig. 1 and Table 1, a total of 13 different missense mutations were found in the 15 families. The mutations all affected the *ABCC8* gene, which encodes the SUR1 subunit of the β-cell K$_{ATP}$ channel. These apparently dominant SUR1 mutations accounted for 17% of diffuse cases associated with *ABCC8* mutations and 16% of total diffuse cases with K$_{ATP}$ mutations. Twelve of the mutations were novel; the S1387F mutation has been previously reported in a patient who was heterozygous for this single mutation and had diazoxide-unresponsive diffuse hyperinsulinism (16). We are aware of a third case with the S1387F mutation and diazoxide-unresponsive hyperinsulinism. None of the 13 mutations were identified in 100 normal alleles. SIFT and Polyphen predicted that only the Q1459H substitution would be tolerated. In 7 of the 15 families (families 2, 9–11, and 13–15), copy number variation assays excluded the existence of intragenic insertions or deletions on the other allele that would have been missed by direct sequencing. Mutation analysis of genomic DNA isolated from pancreatic surgical specimens (families 3, 7–8, 12, and 13) confirmed the
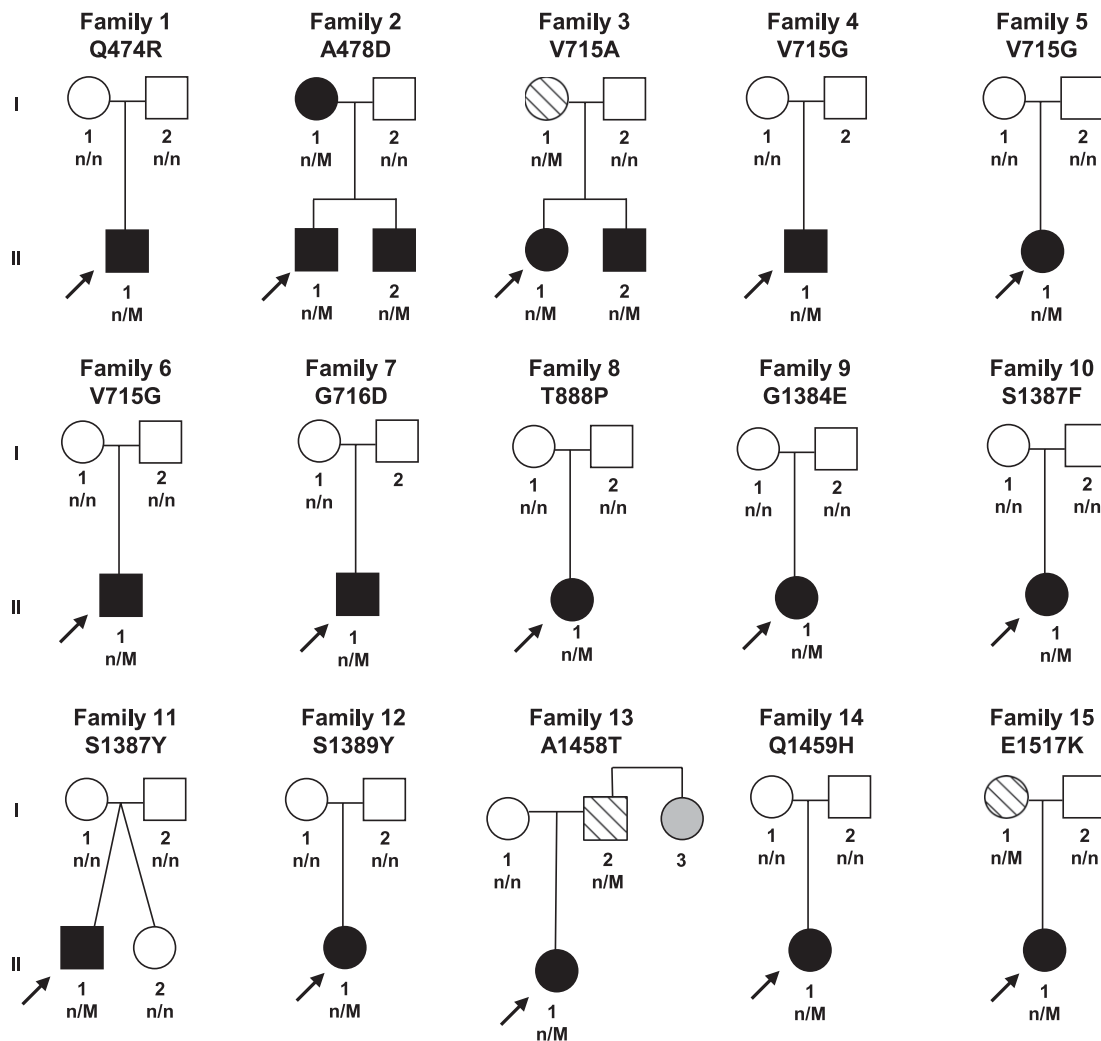
FIG. 1. Pedigrees of families with dominant, diazoxide-unresponsive mutations of SUR1. The pedigrees are listed in ascending order of SUR1 codons. Fraternal twins are indicated by diagonal lines connecting the offspring to the parents. Arrows indicate probands. Black shapes, hypoglycemia diagnosed; diagonal lines, asymptomatic carrier; and filled gray, suspected hypoglycemia. n/M, mutation positive; n/n, mutation negative.

germline mutation, but failed to detect any postzygotic, second K_{ATP} mutations.

Table 1 compares the clinical features of the 17 children with diazoxide-unresponsive, dominant SUR1 mutations with patients with diazoxide-responsive, dominant K_{ATP} mutations, and with patients with typical diffuse disease due to biallelic recessive mutations. The majority of the diazoxide-unresponsive, dominant patients were LGA, which was similar to the other two groups of patients. The median age at presentation was similar to that of the group with recessive mutations but was earlier than that in the diazoxide-responsive dominant group, suggesting a more severe form of hyperinsulinism. Fifteen of the 17 diazoxide-unresponsive, dominant cases required near-total pancreatectomy to control their hypoglycemia; two patients were treated with octreotide and frequent feeds. All of the 15 patients who underwent surgery had histologic evidence of diffuse hyperinsulinism as evidenced by islets containing enlarged nuclei throughout the pancreas.

Four of the mutations, shown in Fig. 1, were present in multiple affected individuals, consistent with dominant inheritance. These include the mother and her two sons in family 2 and two siblings in family 3. The V715G mutation occurred in three different, unrelated probands; in

two of these, the mutation was de novo. As noted above, the S1387F mutation has also been found in multiple affecteds. Three of the four parents who carried a mutation were asymptomatic; however, one of these parents had a sibling with a history of childhood seizures and retardation consistent with symptomatic hypoglycemia (family 13). Half of the probands (9 of 17) had de novo mutations. Two additional case subjects, with the V715G and G716D mutations, may also be de novo mutations; however, DNA was available on only one of the parents for genetic testing. Overall, a dominant mode of inheritance could be confirmed for 5 of the 13 mutations. The others also appear to be dominant based on functional assays of the mutations. (See below.)

Figure 2 illustrates the predicted location of the dominant, diazoxide-unresponsive mutations in the SUR1 protein (17,18). Most are clustered in the second nucleotide binding fold of SUR1, where many of the known dominant, diazoxide-responsive mutations are also located. Several of the diazoxide-unresponsive mutations affect the same or closely adjacent residues that are also affected by diazoxide-responsive mutations (e.g., S1384, S1387, S1389, and E1517). However, four of the diazoxide-unresponsive mutations cluster around the Walker A and B motifs of the

TABLE 1
Clinical phenotype of patients with dominant, diazoxide-unresponsive SUR1 mutations

	Birth weight	Age of presentation (days)	Diazoxide-responsive	Histology	Mutation (p.)	Parent of origin
Family 1 II-1	LGA	1	No	Diffuse	Q474R	De novo
Family 2 II-1	LGA	1	No	Diffuse	A478D	Maternal
Family 2 II-2	AGA	1	No	Diffuse	A478D	Maternal
Family 3 II-1	AGA	1	No	Diffuse	V715A	Maternal
Family 3 II-2	LGA	1	No	No surgery	V715A	Maternal
Family 4 II-1	LGA	1	No	No surgery	V715G	Not maternal
Family 5 II-1	LGA	1	No	Diffuse	V715G	De novo
Family 6 II-1	LGA	42	No	Diffuse	V715G	De novo
Family 7 II-1	LGA	5	No	Diffuse	G716D	Not maternal
Family 8 II-1	AGA	3	No	Diffuse	T888P	De novo
Family 9 II-1	AGA	2	No	Diffuse	G1384E	De novo
Family 10 II-1	LGA	1	No	Diffuse	S1387F	De novo
Family 11 II-1	LGA	1	No	Diffuse	S1387Y	De novo
Family 12 II-1	LGA	1	No	Diffuse	S1389Y	De novo
Family 13 II-1	SGA	180	No	Diffuse	A1458T	Paternal
Family 14 II-1	LGA	88	No	Diffuse	Q1459H	De novo
Family 15 II-1	AGA	167	No	Diffuse	E1517K	Maternal
Dominant diazoxide-unresponsive ($n = 17$)	65% LGA	median 1 day range (1–180 days)	100% no	Diffuse		5 of 17 maternal 1 of 17 paternal 9 of 17 de novo 2 of 17 not maternal
Dominant diazoxide-responsive ($n = 13$)	77% LGA	median 30 days range (1–1,215 days)	100% yes	Diffuse		5 of 13 maternal 4 of 13 paternal 4 of 13 de novo
Recessive ($n = 48$)	87% LGA	median 1 day range (1–9 days)	100% no	Diffuse		48 of 48 biallelic

LGA, AGA, SGA = large, appropriate, small for gestational age.

first nucleotide binding fold, in which none of the diazoxide-responsive mutations occur. The V715 and S1387 codons are mutated to multiple different amino acids that cause diazoxide-unresponsive disease.

Functional testing was performed on these dominant, diazoxide-unresponsive mutations in COSm6 cells. First, Western blots and surface immunofluorescence staining were used to determine whether the mutant channels were appropriately glycosylated and transported to the cell surface. Figure 3 shows an example of one of the dominant, diazoxide-unresponsive mutations, S1387Y. The S1387Y mutant channels are correctly glycosylated and transported to the plasma membrane surface like wild-type channels and like the dominant, diazoxide-responsive mutation R1539E (Fig. 3A and B). Next, channel responses to the metabolic regulator MgADP (Fig. 3C) and the channel agonist diazoxide (Fig. 3D) were evaluated using inside-out patch-clamp electrophysiological recordings. As shown in the representative traces and the averaged response from multiple recordings (Fig. 3E), wild-type channels were well stimulated by MgADP or diazoxide in the presence of inhibitory ATP (0.1 mmol/L). By contrast, the diazoxide-responsive mutant R1539E had reduced, although still significant, residual response to both MgADP and diazoxide, whereas the diazoxide-unresponsive mutant S1387Y completely lacked responses to MgADP and diazoxide. Under the simulated heterozygous expression condition, the diazoxide-responsive mutant R1539E also exhibited better response to MgADP and diazoxide than the diazoxide-unresponsive mutant S1387Y (Fig. 3E).

Table 2 summarizes the quantitative chemiluminescence surface expression and functional testing results for the dominant, diazoxide-unresponsive mutations. All

13 missense mutations showed similar retention of ability to traffic to the membrane. However, once at the membrane surface, the responses of the dominant, diazoxide-unresponsive mutations to MgADP and diazoxide were practically obliterated. Figure 4 compares the responses to diazoxide and MgADP of channels expressing the diazoxide-unresponsive and the diazoxide-responsive mutations. While there was overlap between the two groups of mutations, the diazoxide-responsive, dominant mutations retained significantly greater responses to effectors compared with the
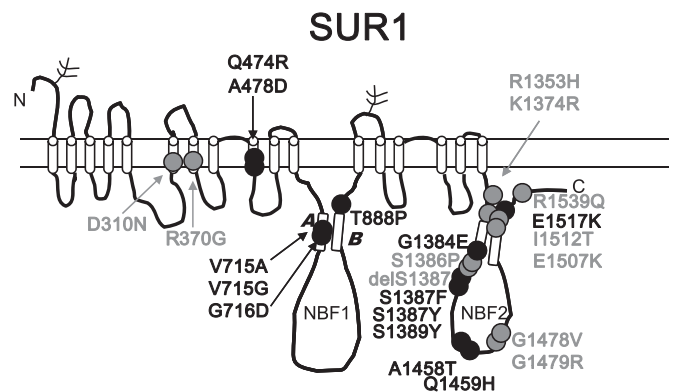

FIG. 2. Location of dominant, diazoxide-unresponsive (black circles) and dominant, diazoxide-responsive (gray circles) mutations of SUR1 (7). Mutation locations are approximate based on current SUR1 topology models (17,18). Secondary structures of SUR1 are shown, including the amino (NH$_2$-) and carboxy (COOH-) termini of the protein, glycosylation sites, and nucleotide binding fold 1 (NBF1) and nucleotide binding fold 2 (NBF2), which contain Walker A and B motifs (A and B) associated with regulatory nucleotide binding.
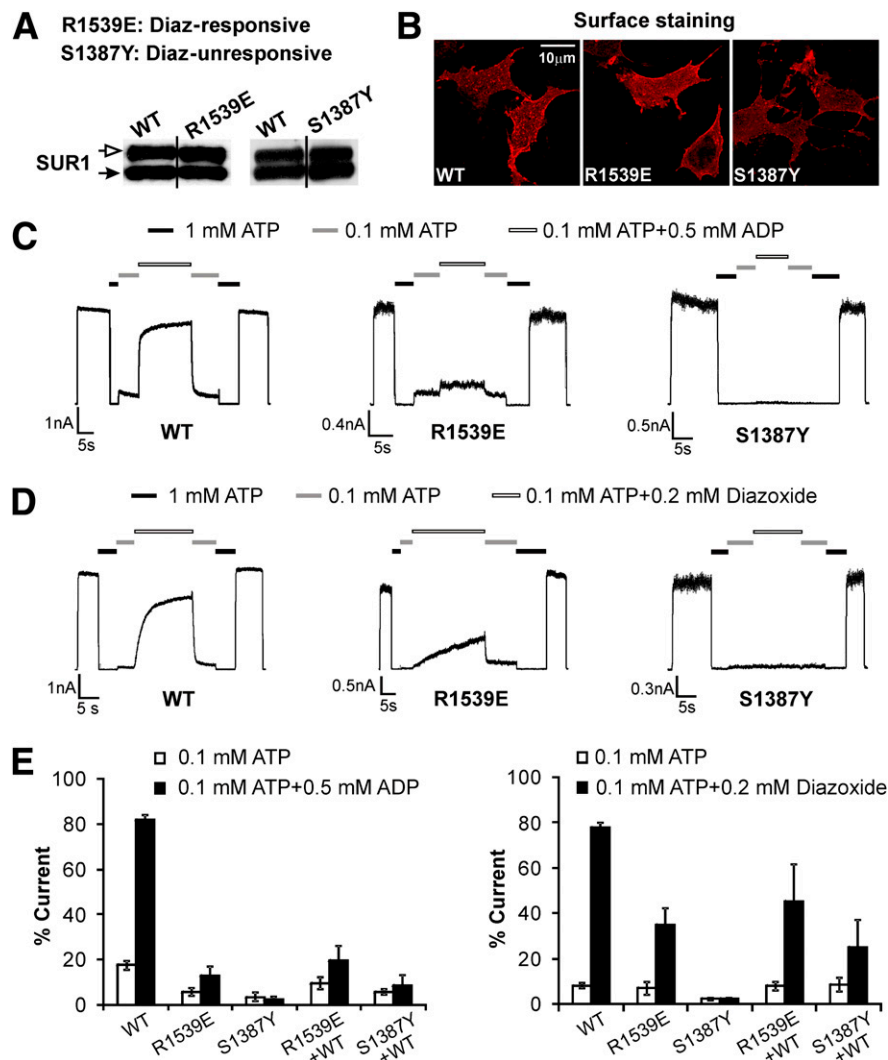
FIG. 3. Functional analysis of mutant SUR1 expressed in COSm6 cells. *A*: Western blot analysis of mutant flag-SUR1 proteins. COSm6 cells were cotransfected with cDNAs for Kir6.2 and wild-type (WT) FLAG-SUR1, the diazoxide (Diaz)-responsive mutant R1539E FLAG-SUR1 or the diazoxide-unresponsive mutant S1387Y FLAG-SUR1. Both mutants exhibit the lower core glycosylated band (solid arrow) and the upper complex glycosylated band (open arrow) as seen in wild-type, indicating that the mutant proteins are processed correctly like the wild-type protein. *B*: Immunofluorescence staining of surface channels in COSm6 cells transfected with wild-type, R1539E, or S1387Y FLAG-SUR1 and wild-type Kir6.2. Both mutants were well expressed at the cell surface like wild-type channels. *C*: Representative inside-out patch-clamp recordings of wild-type, the diazoxide-responsive mutant R1539E, and the diazoxide-unresponsive mutant S1387Y show differences in MgADP response. Currents were measured at –50 mV in symmetrical K-INT solution, and inward currents are shown as upward deflections. Patches were exposed to differing concentrations of ATP and ADP, as indicated by the bars above the records. Free Mg^{2+} concentration was maintained at 1 mmol/L in all ATP-containing solutions. *D*: The same as in *C* except that channel response to diazoxide was compared. *E*: Quantification of MgADP response (*left panel*) and diazoxide response (*right panel*) using recordings shown in *C* and *D*. Currents were normalized to that seen in K-INT and expressed as percentage of currents. Responses in both homozygous and simulated heterozygous expression conditions are shown. In the MgADP response graph, each bar represents means ± SEM of 16 wild-type and 4–6 mutant patches. In the MgADP response graph, each bar represents means ± SEM of 15 wild-type and 4–6 mutant patches. (A high-quality digital representation of this figure is available in the online issue.)

diazoxide-unresponsive mutations (MgADP, $P = 0.0003$; diazoxide, $P = 0.0002$).

DISCUSSION

In this group of children with congenital hyperinsulinism who failed to respond to treatment with diazoxide, we identified 13 mutations of the K_{ATP} channel that appear to cause diffuse hyperinsulinism in an autosomal dominant fashion. All of the mutations involved the *ABCC8* gene that encodes the SUR1 subunit of the channel. Evidence for autosomal dominant expression included one example of transmission from affected parent to affected child and several examples of hyperinsulinism occurring in multiple individuals sharing the same mutation. The clinical phenotype of hyperinsulinism in this disorder is indistinguishable from that of children with the more common form of diffuse hyperinsulinism due to two recessive K_{ATP} mutations, including onset of symptomatic hypoglycemia at birth and need for surgical pancreatectomy to control hypoglycemia. However, the phenotype appeared to be more severe than that seen in children with dominantly expressed K_{ATP} mutations that are responsive to diazoxide, as reflected by an earlier median age of onset. In vitro expression studies showed that the mutations associated with diazoxide-unresponsive hyperinsulinism produce SUR1 subunits that can form channels with Kir6.2 that traffic normally to the plasma membrane—similar to mutations associated

TABLE 2
Mutant protein biogenesis and activity

Mutation	Surface expression (% of wild-type channel level)	MgADP stimulation (% current in 0.1 mmol/L ATP plus 0.5 mmol/L ADP)	Diazoxide stimulation (% current in 0.1 mmol/L ATP plus 0.2 mmol/L diazoxide)
1) Q474R	119.12 ± 13.87	3.16 ± 1.34 (n = 3)	2.03 ± 0.77 (n = 3)
2) A478D	83.67 ± 5.33	0.69 ± 0.27 (n = 6)	3.14 ± 1.37 (n = 3)
3) V715A	83.00 ± 5.29	0.53 ± 0.28 (n = 3)	1.73 ± 0.53 (n = 4)
4) V715G	96.33 ± 3.18	0.28 ± 0.16 (n = 3)	2.35 ± 1.99 (n = 3)
5) G716D	66.67 ± 5.24	2.39 ± 1.90 (n = 6)	2.37 ± 1.32 (n = 4)
6) T888P	54.33 ± 5.33	0.38 ± 0.30 (n = 3)	1.30 ± 0.44 (n = 3)
7) G1384E	101.80 ± 8.50	0.17 ± 0.09 (n = 4)	1.64 ± 0.49 (n = 4)
8) S1387F	83.91 ± 5.30	0.84 ± 0.17 (n = 4)	4.05 ± 1.34 (n = 4)
9) S1387Y	83.13 ± 5.38	2.46 ± 0.96 (n = 4)	2.44 ± 0.42 (n = 4)
10) S1389Y	70.00 ± 7.81	1.26 ± 0.73 (n = 4)	1.65 + 0.63 (n = 3)
11) A1458T	94.33 ± 10.48	0.52 ± 0.21 (n = 3)	1.06 ± 0.37 (n = 3)
12) Q1459H	91.00 ± 2.31	0.69 ± 0.39 (n = 3)	1.32 ± 0.29 (n = 3)
13) E1517K	72.88 ± 5.85	1.73 ± 0.39 (n = 3)	1.96 ± 0.55 (n = 4)
Mean of dominant diazoxide-unresponsive SUR1 mutations	84.6 ± 4.64 (n = 13)	1.42 ± 0.35 (n = 13)*	1.83 ± 0.18 (n = 13)†
Mean of dominant diazoxide-responsive SUR1 mutations	91.05 ± 5.67 (n = 10)	15.26 ± 5.06 (n = 10)	19.96 ± 6.29 (n = 10)
Wild type	100	61.19 ± 5.53 (n = 11)	77.8 ± 2.2 (n = 9)

Data are means ± SE. *Compared with the dominant diazoxide-responsive group, $P = 0.0002$. †Compared with the dominant diazoxide-responsive group, $P = 0.0003$.

with diazoxide-responsive dominant disease. However, the diazoxide-unresponsive mutations produced a more severe impairment in the expressed channel responses to activation by diazoxide and MgADP.

The thirteen dominant, diazoxide-unresponsive mutations identified in this study are all missense mutations and were primarily, but not exclusively, located in the first and second nucleotide-binding folds of the SUR1 subunit of the K_{ATP} channel (Fig. 2). These mutation sites involve the same regions or, in some cases, the same amino acid residues as many of the dominant, diazoxide-responsive mutations of SUR1, indicating that the two types of dominant mutations cannot be distinguished by location alone. They also affect the same regions as many of the known recessively expressed mutations. It is interesting to note that involvement of the Walker A and B sites of nucleotide binding fold 1 appears to be limited to the diazoxide-unresponsive dominant mutations, whereas both types of dominant mutations can affect nucleotide binding fold 2. Flanagan et al. (9) have also described three novel mutations of SUR1 associated with diazoxide-unresponsive hyperinsulinism that appear to act in an autosomal dominant fashion. One of these mutations, D1506E, occurred in an affected mother and child, confirming dominant expression. The other two (G1485E and M1514K) occurred de novo. These three mutations were located in the nucleotide binding fold 2 of SUR1. In the current study, seven of the thirteen diazoxide-unresponsive, dominant mutations were also located in the nucleotide binding fold 2; however, this was not the only region of SUR1 affected by these types of defects (see Fig. 2). It is also interesting to note that, although three of the fourteen diazoxide-responsive dominant mutations that we previously reported involved the Kir6.2 subunit (7), to date diazoxide-unresponsive defects have exclusively involved SUR1.

The important distinction between dominant and recessive missense mutations of the *ABCC8* gene that cause hyperinsulinism appears to be whether they produce SUR1 subunits that can combine with Kir6.2 and then be trafficked to the
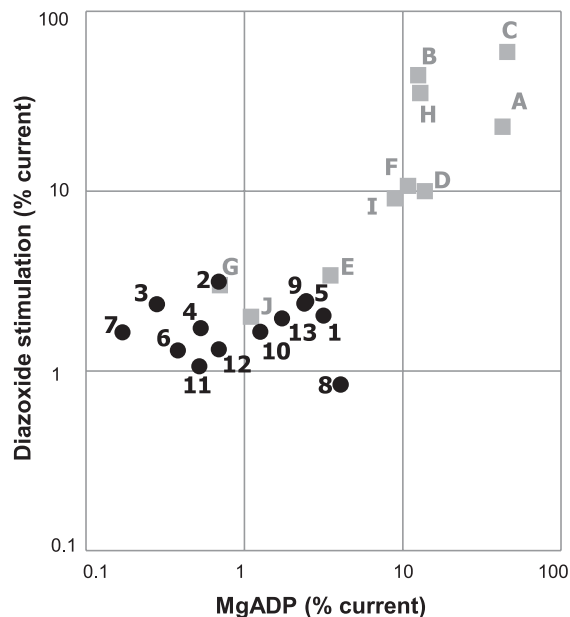

FIG. 4. Responsiveness of dominant diazoxide-unresponsive and dominant diazoxide-responsive SUR1 mutations. Responses to diazoxide and MgADP of expressed K_{ATP} channels containing diazoxide-unresponsive SUR1 mutations (black circles) and diazoxide-responsive SUR1 mutations (gray squares) are compared. Diazoxide-unresponsive mutations are labeled according to numbers in Table 2. Diazoxide-responsive mutations are as follows: A, D310N; B, R370G; C, R1353H; D, K1374R; E, G1478V; F, G1479R; G, S1386P; H, R1539Q; I, I1512T; and J, E1507K.

plasma membrane surface. In vitro expression studies of missense mutations of SUR1 that act in a recessive fashion show that they interfere with normal surface expression of channels by preventing trafficking of channels from the endoplasmic reticulum to the plasma membrane (5,14). In contrast, both types of dominant SUR1 mutations, when expressed in vitro, permit the normal assembly of channels and subsequent trafficking to the plasma membrane. Once at the surface, the difference between diazoxide-unresponsive dominant mutations and diazoxide-responsive mutations appears to be their degree of residual responsiveness to activation by MgADP and diazoxide. Stimulated potassium channel currents were reduced to only 1–2% of normal in the diazoxide-unresponsive mutations such as S1387Y, whereas the responsive mutations such as R1539E retain about 20% of normal channel activities. Under simulated heterozygous expression conditions, the diazoxide-unresponsive mutation S1387Y also exhibited decreased responses to MgADP and diazoxide when compared with the diazoxide-responsive mutant R1539E, although the difference was not as pronounced (Fig. 3E). As shown in Fig. 4, however, there was a small overlap between the two types of mutations, making it difficult to differentiate them completely by in vitro expression studies.

An interesting feature of the dominant, diazoxide-responsive K_{ATP} mutations we previously reported was the high frequency of asymptomatic carriers (7). This was also true of the diazoxide-unresponsive group, despite the fact that the affected children appeared to be more severely affected, as reflected by their younger age of onset (Fig. 1 and Table 1). The diazoxide-unresponsive, dominant mutation case subjects closely resemble patients with recessive K_{ATP} defects, in which patients with biallelic mutations almost always present in the first days of life. We have encountered one patient with a homozygous, recessive SUR1 mutation who escaped detection until 18 months of age, but such examples are extremely rare. Because K_{ATP} channels are hetero-octamers containing four subunits of SUR1 and four of Kir6.2, it is attractive to speculate that dominant K_{ATP} defects act as dominant negatives to impair function of channels containing one or more mutant subunits. Using concatemers to express channels containing from zero to four subunits of a mutant SUR1 associated with neonatal diabetes, Babenko (19) recently reported that the activation of channel activity increased with increasing numbers of mutant subunits. We previously speculated that the small percentage of channels formed entirely of wild-type subunits might account for diazoxide responsiveness of dominant K_{ATP} mutations (7). However, the present series of diazoxide-unresponsive dominant defects demonstrate that this explanation of "spare channels" is not sufficient. The reasons for the lack of symptoms in some carriers with dominant SUR1 mutations might include nongenetic factors, such as chance exposure to fasting stress or protein meals, or possibly epigenetic modifiers. It should, however, be noted that the $SUR1^{-/-}$ mouse model has only a mild hypoglycemia phenotype (20,21), suggesting that genetic deficiency of K_{ATP} function alone may not be sufficient to always cause readily appreciated symptomatic hypoglycemia.

The data strongly support the idea that the single mutations found in the patients in this report all act in dominant fashion. Evidence of dominant transmission from affected parent to affected child occurred only once, and the series by Flanagan et al. (9) also included a similar case. The present series also included several instances in which de novo mutations were associated with disease in multiple affected individuals, consistent with a dominant mode of action. For some of the de novo mutations in our series, there was no additional clinical genetic evidence to support a dominant mode of action. The in vitro mutation expression data, however, provide strong evidence in favor of dominant action for all of the mutations studied; when expressed with wild-type Kir6.2, the mutations produced channels that trafficked to the plasma membrane but had markedly reduced responses to Mg-ADP or diazoxide.

In summary, these observations expand the range of hyperinsulinism disorders that can be associated with inactivating missense mutations of the β-cell K_{ATP} channel. These missense mutations may be 1) recessive, 2) dominant and diazoxide-responsive, or 3) dominant and diazoxide unresponsive. In our series, the third group made up 17% of the children who required surgery for diffuse hyperinsulinism associated with SUR1 mutations. In vitro expression studies may often be necessary for determining which category a mutation fits. Because many K_{ATP} mutations associated with hyperinsulinism are novel, information about the specific functional effect of a mutation is increasingly necessary for accurate genetic diagnosis. The present results suggest that dominant mutations of SUR1 may be a relatively common cause of hyperinsulinism in children whose hypoglycemia cannot be controlled by usual medical management.

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