

Clinical and Molecular Characterization of a Dominant Form of Congenital Hyperinsulinism Caused by a Mutation in the High-Affinity Sulfonylurea Receptor

Paul S. Thornton,¹ Courtney MacMullen,¹ Arupa Ganguly,³ Eduardo Ruchelli,² Linda Steinkrauss, Ana Crane,⁴ Lydia Aguilar-Bryan,⁵ and Charles A. Stanley¹

Recessive mutations of sulfonylurea receptor 1 (SUR1) and potassium inward rectifier 6.2 (Kir6.2), the two adjacent genes on chromosome 11p that comprise the β -cell plasma membrane ATP-sensitive K^+ (K_{ATP}) channels, are responsible for the most common form of congenital hyperinsulinism in children. The present study was undertaken to identify the genetic defect in a family with dominantly inherited hyperinsulinism affecting five individuals in three generations. Clinical tests were carried out in three of the patients using acute insulin responses (AIRs) to intravenous stimuli to localize the site of defect in insulin regulation. The affected individuals showed abnormal positive calcium AIR, normal negative leucine AIR, subnormal positive glucose AIR, and impaired tolbutamide AIR. This AIR pattern suggested a K_{ATP} channel defect because it resembled that seen in children with recessive hyperinsulinism due to two common SUR1 mutations, g3992-9a and delPhe1388. Genetic linkage to the K_{ATP} locus was established using intragenic polymorphisms. Mutation analysis identified a novel trinucleotide deletion in SUR1 exon 34 that results in the loss of serine 1387. Studies of delSer1387 in COSm6 cells confirmed that the expressed mutant protein assembles with Kir6.2 and trafficks to the plasma membrane, but it had no ^{86}Rb efflux ion transport activity. These results indicate that hyperinsulinism in this family is caused by a SUR1 mutation that is expressed dominantly rather than recessively. *Diabetes* 52:2403–2410, 2003

From the ¹Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; the ²Division of Endocrinology and Department of Pathology, the Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; the ³Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; the ⁴Department of Molecular and Cellular Biology, Division of Endocrinology, Baylor College of Medicine, Houston, Texas; and the ⁵Department of Medicine, Division of Endocrinology, Baylor College of Medicine, Houston, Texas.

Address correspondence and reprint requests to Charles A. Stanley, MD, Division of Endocrinology, Children's Hospital of Philadelphia, 34th St. & Civic Center Boulevard, Philadelphia, PA 19104. E-mail stanleyca@email.chop.edu.

Received for publication 24 March 2003 and accepted in revised form 22 May 2003.

AIR, acute insulin response; GDH, glutamate dehydrogenase; K_{ATP} , ATP-sensitive K^+ channel; Kir6.2, potassium inward rectifier 6.2; SUR1, sulfonylurea receptor 1.

© 2003 by the American Diabetes Association.

Congenital hyperinsulinism in children is a disorder of persistent hypoglycemia that is caused by genetic mutations in the pathways regulating insulin secretion by pancreatic islets (1). The most common of these disorders is associated with recessive mutations of the two adjacent genes on chromosome 11p that comprise the β -cell ATP-sensitive K^+ (K_{ATP}) channel: the high-affinity sulfonylurea receptor 1 (SUR1 or *ABCC8*) and its regulated ion pore, potassium inward rectifier 6.2 (Kir6.2 or *KCNJ11*) (2–5). Mutations in these channel genes can also cause sporadic focal hyperinsulinism through a two-hit mechanism involving loss of heterozygosity for the maternal 11p, leading to expression of a paternally transmitted K_{ATP} mutation (6,7). In addition, Huopio et al. (8) recently reported a family with a dominantly expressed mutation of SUR1. Children with either the focal or diffuse forms of disease associated with K_{ATP} channel mutations often present with severe hypoglycemia at birth. Because the channel is impaired, they often do not respond to medical therapy with the channel agonist diazoxide and thus frequently require near-total pancreatectomy.

In 1998, we reported three families with congenital hyperinsulinism in whom the disease was transmitted in a dominant, rather than recessive, manner (9). We subsequently identified the genetic defects in two of these families as being dominantly expressed mutations of glutamate dehydrogenase (GDH; *GLUD1* on 10q) (10) and glucokinase (*GCK* on 7p) (11). The genetic locus for the third of these three families with dominant hyperinsulinism was not identified. However, at that time, linkage analysis using tandem repeat markers in the 11p region appeared to exclude SUR1 and Kir6.2 as candidate genes.

The purpose of the present study was to identify the genetic defect in the third of our original three dominant hyperinsulinism families by using pharmacological tests of acute insulin responses (AIRs) to define potential sites of defect in the pathways controlling insulin secretion. Comparison with the AIR patterns found in children with other forms of congenital hyperinsulinism suggested the possibility of a K_{ATP} channel defect in this family. Further investigations, including genetic linkage analysis using intragenic polymorphisms of SUR1, mutation analysis, and in vitro expression studies, confirmed this suspected site of defect.

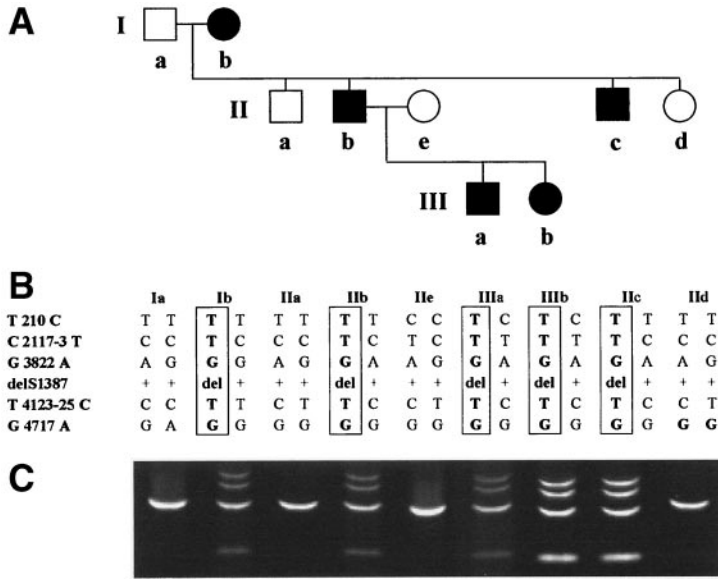


FIG. 1. Family with dominant congenital hyperinsulinism. **A:** The family pedigree with affected members indicated by filled symbols. **B:** The haplotypes of the family members based on single-nucleotide intragenic polymorphisms in SUR1 on chromosome 11p. The delSer1387 notation indicates a novel three-nucleotide deletion found in exon 34 in affected family members only. **C:** Conformation-sensitive gel electrophoresis patterns of exon 34 amplicons subjected to *Xmn* I endonuclease digestion, indicating heterozygosity for the delSer1387 mutation in affected members only.

RESEARCH DESIGN AND METHODS

Case synopsis. The clinical characteristics of this family have been previously published and are briefly summarized below (9,12). The index patient (IIb in the pedigree shown in Fig. 1) is currently 40 years old. He first presented with seizures due to hypoglycemia at 5 months of age. He underwent a subtotal pancreatectomy at 3 years old but continued to have persistent hypoglycemia and was treated with diazoxide. Although he was considered to respond to diazoxide, occasional hypoglycemia continued. Diazoxide was discontinued at age 12 years, but hypoglycemia recurred intermittently. At 38 years of age, he was diagnosed with mild diabetes, which has been well controlled by treatment with metformin (HbA_{1c} 5.9%).

The index patient's younger brother (IIc in the pedigree), currently 39 years of age, first presented with hypoglycemia at 8 months of age. He was treated with diazoxide and, like his brother, was considered to have improved blood glucose control, although occasional symptoms of hypoglycemia persisted. As with his brother, at 10 years of age, diazoxide treatment was discontinued in accordance with the expectation at that time of eventual remission of hypoglycemia. He has had occasional seizures that were thought to reflect previous hypoglycemic brain injury, but recently they were found to be caused by symptomatic hypoglycemia. These episodes frequently occur in the early afternoon after protein-containing meals. Because of these episodes of hypoglycemic seizures, he was restarted on diazoxide 1 year ago, with a reduction in the frequency of both seizures and symptomatic episodes of hypoglycemia.

Patient Ib, the mother of the index patient, was not diagnosed with hyperinsulinism until 40 years of age, when she presented with hypoglycemic coma after an overnight fast. In retrospect, she recalled having symptoms of hypoglycemia since before the age of 15. These episodes have continued to occur up to her current age of 64 years, both postprandially and after prolonged fasting. She has not been treated with diazoxide because frequent feeding seems to prevent most episodes of hypoglycemia. Her intellect is normal, and she does not have seizures. She described her father as having unexplained lapses of consciousness that may have been unrecognized hypoglycemia. Patient Ib does not have diabetes.

Patient IIIb, now 8 years old, is the second child of the index patient, and she presented with hypoglycemia at 2 days of age. She was treated with diazoxide and frequent feeds. She has done well but continues to have occasional fasting hypoglycemia, despite being on a moderately high dose of diazoxide (11 mg · kg⁻¹ · day⁻¹). Her brother (patient IIIa), currently 10 years of age, has never had symptoms of hypoglycemia, but he was diagnosed with hyperinsulinism based on an abnormal fasting test performed when he was 5 years of age.

Methods. The diagnosis of hyperinsulinism was based on clinical criteria at times of hypoglycemia, including inadequate suppression of insulin levels, inadequate elevations of β-hydroxybutyrate and free fatty acids, as well as an inappropriate glycemic response to glucagon (13,14).

Tests of AIR to calcium, glucose, leucine, and tolbutamide were performed as previously described (15–17). Briefly, stimuli were given by rapid intravenous infusion at intervals of 1 h. AIR was defined as the mean increment in plasma insulin at 1 and 3 min poststimulation. Results were compared with values previously found in normal adults, in children with recessive hyperin-

sulinism caused by K_{ATP} channel mutations, and in dominant GDH hyperinsulinism patients (15–17). The recessive K_{ATP} hyperinsulinism disease control subjects were either homozygous or compound heterozygous for the two common Ashkenazi Jewish SUR1 mutations (g3992-9a and delPhe1388); most children with these mutations present with severe neonatal hypoglycemia.

The hypoglycemic response to oral protein was determined as previously described using 1 g/kg protein (Resource Instant Protein Powder; Novartis) either after an overnight fast or 4 h after a light breakfast (18). Plasma glucose and insulin responses were compared with values for normal adult control subjects previously reported and also to the responses of a disease control adult male with recessive K_{ATP} hyperinsulinism (SUR1 g3992-9a/g3992-9a) who was known to have persistent fasting hypoglycemia.

Sensitivity to glucose-stimulated insulin secretion was assessed by a graded hyperglycemic stimulation test as previously described (16). Briefly, glucose was infused intravenously in a stepped hyperglycemic ramp over a period of 3 h, and the changes in plasma insulin were measured at 10–20 min intervals. Glucose sensitivity was expressed as the slope of the insulin versus glucose plot (μU · mg⁻¹ · 10⁻²). Results were compared with data previously published from normal adult control subjects and children with recessive hyperinsulinism associated with SUR1 g3992-9a and delPhe1388 mutations (16).

Linkage and genetic analysis. Peripheral blood samples were obtained from family members for isolation of genomic DNA. Amplification of exons 2, 3, 16, 18, 27, 31, 33, 34, and 39 from SUR1 was carried out as previously described (4) to detect the single nucleotide polymorphisms that are listed in Table 4 (19–23). PCR products for the family members were subjected to conformation-sensitive gel electrophoresis, as previously described (24). Products displaying band shifts were further analyzed either by restriction endonuclease digestion for confirmation of common polymorphisms or by sequencing in cases of novel band patterns. SUR1 cDNA nucleotides and amino acids were numbered according to the sequence reported by Nestorowicz et al. (4) that includes the alternatively spliced exon 17 sequence (National Center for Biotechnology Information accession no. L78224).

Pancreatic histopathology. Paraffin-embedded blocks from the pancreatectomy performed on patient IIb in 1963 were obtained. New sections were cut and examined by hematoxylin and eosin staining and by insulin immunohistochemical labeling. Multiple sections from the head, body, and tail of the pancreas were examined for evidence of endocrine tissue abnormalities (25).

SUR1 mutation expression. Plasmids containing human Kir6.2 and hamster SUR1 cDNA plus and minus a *myc* epitope were used as previously described (26). Point mutation constructs of SUR1 were prepared using a Quick Change site-directed mutagenesis kit (Stratagene). The integrity of the constructs was confirmed through restriction mapping and sequencing.

For transient expression experiments, COSm6 cells were cultured in Dulbecco's modified Eagle's medium containing 4.5 g/l glucose supplemented with 10% fetal bovine serum. At 80% confluence, cells were transfected with 8 μg of SUR1 and 1 μg of Kir6.2 by electroporating 7 × 10⁶ cells at 0.550 V/mm. The following day, whole-cell photolabeling and surface expression were performed. At 48 h after electroporation, ⁸⁶Rb⁺ rubidium efflux assays were performed. Whole-cell photolabeling was determined as previously described (27). Briefly, cells were incubated for 30 min with 1 nmol/l [¹²⁵I]azido-

TABLE 1
Clinical features of affected family members

Patient	Birth weight	Age at diagnosis	Fasting duration at diagnosis (h)	Surgery	Diazoxide treatment
Ib	Normal	40 years	21	—	—
IIb	2.2 kg at 32 weeks	5 months	2	yes	yes
IIc	Normal	8 months	<8	—	yes
IIIa	5.3 kg	Asymptomatic	18	—	—
IIIb	3.9 kg	2 days	4	—	yes

glibenclamide plus and minus 1 $\mu\text{mol/l}$ cold glibenclamide, then cross-linked by exposure to UV light. Cells were washed with PBS and solubilized with loading buffer (30 mmol/l Tris, 1.25% SDS, 1 mmol/l EDTA, 50 mmol/l dithiothreitol, and 10% glycerol), and the extract was run on a 10% SDS acrylamide gel. Surface expression assay measured the relative amount of channel at the plasma membrane as previously described (26). $^{86}\text{Rb}^+$ efflux assays were used to measure specific glibenclamide-inhibitable efflux, as previously described (27,28).

The patient studies were reviewed and approved by the Children's Hospital of Philadelphia institutional review board, and written informed consent was obtained from subjects or their parents.

RESULTS

Table 1 summarizes the clinical features of the five known affected individuals in the family pedigree shown in Fig. 1. The age at diagnosis ranged from 2 days to 40 years, and one child (patient IIIa) was considered to be asymptomatic. The latter was the only one of the patients whose birth weight was large for gestational age. All of the affected family members had documented fasting hypoglycemia, and three of the five (patients Ib, IIc, and IIIb) also had postprandial hypoglycemia secondary to protein sensitivity. Patient IIIb developed protein avoidance during infancy and continues at age 8 years to dislike protein. Only one patient (IIb) had surgery to attempt to control hypoglycemia.

Hypoglycemia was treated with diazoxide in three of the affected individuals; the remaining two were either considered not to have symptomatic hypoglycemia (patient IIIa) or declined therapy (patient Ib). While on diazoxide, all of the three treated patients continued to have abnormal fasting tolerance, indicating that the diazoxide was not completely effective in controlling their hyperinsulinism. In addition, as noted below, protein-sensitive hypoglycemia was not fully prevented by diazoxide (Table 3).

Three of the patients are currently >35 years old (Ib, IIb, and IIc). Only one (patient IIb) has developed diabetes, which has responded adequately to metformin treatment.

TABLE 2
AIR and diazoxide sensitivity in affected family members

Patient	Calcium AIR ($\mu\text{U/ml}$)	Leucine AIR ($\mu\text{U/ml}$)	Glucose AIR ($\mu\text{U/ml}$)	Tolbutamide AIR ($\mu\text{U/ml}$)	Diazoxide responsiveness
Ib	30	0	25	10	Not tested
IIc	37	1	26	2	Partial
IIIa	Not tested	Not tested	44	17	Partial
Control subjects (95% CI)					
Recessive K_{ATP} -HI (SUR1 g3992-9a/g3992-9a and g3992-9a/delP1388) ($n = 9$)	14–34	0–9	5–23	–2 to 3	No
GDH-HI ($n = 5$)	–8 to 4	15–70	63–170	23–36	yes
Normal control subjects (95% I) ($n = 9$)	–1 to 2	–4 to 8	22–62	26–80	yes

HI, hyperinsulinism.

This individual is not obese and had undergone a partial pancreatectomy as a child. The father of patient Ib, who was undiagnosed but may have been affected, never developed diabetes.

Tests of insulin secretion. Table 2 compares the AIRs in three members of the family with those found in children with recessive K_{ATP} channel hyperinsulinism associated with SUR1 mutations, children with hyperinsulinism due to GDH mutations, and normal control adults. Both patients Ib and IIc had abnormal positive AIRs to calcium, normal negative responses to leucine, low-normal responses to glucose, and impaired responses to tolbutamide. Patient IIIa was not tested with calcium or leucine, but he also showed an impaired AIR to tolbutamide, while having a normal AIR to glucose. These abnormalities in AIR responses in the affected family members were different from the pattern seen in hyperinsulinism caused by mutations of GDH. However, they closely resembled the pattern of responses seen in patients with the most common recessive form of hyperinsulinism with mutations in SUR1 (homozygous g3992-9a and compound heterozygous g3992-9a/delPhe1388).

Table 3 shows the responses to oral protein tolerance tests in two of the affected family members. In both cases, plasma glucose fell after the protein meal by more than the normal change of <10 mg/dl. Protein-sensitive hypoglycemia was also noted in the adult patient with recessive K_{ATP} channel hyperinsulinism who was homozygous for the common g3992-9a mutation. As shown in Table 3, diazoxide treatment failed to prevent protein-induced hypoglycemia in patient IIc, consistent with the observation that fasting hypoglycemia was also not fully controlled by diazoxide treatment in this family.

To further test pancreatic regulation of insulin secretion, the sensitivity of insulin release to glucose was examined in patient IIc using a graded hyperglycemic

TABLE 3
Responses to oral protein tests in affected family members

Patient	Δ Glucose (mg/dl)	Δ Insulin (μ U/ml)	Nadir glucose (mg/dl)
Ib	-15	11	41
IIC (on diazoxide)	-32	24	53
IIC (off diazoxide)	-46	78	50
Control subjects (95% CI)			
SUR1-HI patient (g3992-9a/g3992-9a)	-49	44	48
GDH-HI patients ($n = 14$)	-14-32	7-21	45-55
Normal adults ($n = 5$)	-9-1	9-19	70-80

HI, hyperinsulinism.

ramp. The result showed a glucose sensitivity of $18 \mu\text{U} \cdot \text{ml}^{-1} \cdot 100 \text{mg/dl}^{-1}$. This value was intermediate between the range of values found in normal adult control subjects (95% CI: $27-87 \mu\text{U} \cdot \text{ml}^{-1} \cdot 100 \text{mg/dl}^{-1}$) and the values previously found in children with hyperinsulinism due to homozygous g3992-9a and compound heterozygous g3992-9a/delPhe1388 SUR1 mutations (-5 to $13 \mu\text{U} \cdot \text{ml}^{-1} \cdot 100 \text{mg/dl}^{-1}$) (16). Together with the abnormal pattern of AIRs and the poor responsiveness to diazoxide, these results suggested an underlying dysfunction of the K_{ATP} channel similar that seen in recessive SUR1 patients with the common Ashkenazi Jewish mutations.

Pancreatic histology. The pancreatic tissue obtained from patient IIB during subtotal pancreatectomy at 3 years of age was originally interpreted as showing "nesidioblastosis" (12). Because the concept of nesidioblastosis as a specific feature of hyperinsulinism in infants has been discarded, sections from the original paraffin blocks were taken for reexamination. No region of focal adenomatosis was found. Nuclear enlargement (diameter $>12 \mu\text{m}$) of a small percentage of islet cells was found throughout the pancreas. These findings are identical to those described by Rahier et al. (25) in infants with diffuse congenital hyperinsulinism associated with recessive mutations of the K_{ATP} genes.

Genetic analysis. Because insulin dysregulation in the affected members of the family appeared to be similar to the pattern associated with recessive homozygous g3992-9a and compound heterozygous g3992-9a/delPhe1388 SUR1 mutations, we reexamined the possibility of linkage to the SUR1/Kir6.2 locus on 11p using the intragenic polymorphisms shown in Table 4. Polymorphisms found in five of the exons were informative for haplotyping members of the family. As shown in Fig. 1, all affected family members shared a common haplotype at the SUR1/Kir6.2 locus, consistent with having a hyperinsulinism mutation located in this region. During the course of the linkage analysis, we discovered a three-nucleotide deletion in exon 34 of SUR1 (4159-4161) that causes an in-frame deletion of a serine at codon 1387 (delSer1387). Screening of all other SUR1 exons failed to reveal any other potential disease-causing mutation. As shown in Fig. 1C, *Xmn* I restriction endonuclease digestion of exon 34 amplicons indicated that the delSer1387 was shared by all affected family members. It was noted that this potential disease-causing delSer1387 mutation is immediately adjacent to the delPhe1388 muta-

TABLE 4
SUR1 polymorphisms used for haplotype analysis

	Polymorphism	Restriction site (gained or lost)
Exon 2	t207c	+ Xma I
Exon 3	c330t	- Nci I
Intron 15	c2117-3t	- Pst I
Exon 18	c2280t	- Taq II
Exon 27	g3348t	
Intron 27	g3402+13a	+ Nla III
Intron 27	c3402 + 45a	
Exon 31	g3822a	
Intron 32	c3992-71a	- Ple I
Exon 33	t4082g	+ Bcn I
Exon 33	t4108g	
Intron 33	t4123-25c	+ Bso FI
Intron 33	c4123-16t	- Ban I
Intron 34	g4201+63a	- Scr FI
Exon 39	g4717a	+ Sfa NI

tion that causes recessive hyperinsulinism in Ashkenazi Jews (4).

Expression studies. To evaluate the effect of the SUR1 delSer1387 mutation on K_{ATP} channel expression, trafficking, and function, mutant SUR1 was reconstituted with wild-type Kir6.2 in COSm6 cells to form mutant K_{ATP} channels (29-31). Photolabeling of cells with the high-affinity sulfonylurea ligand [^{125}I]azido-glibenclamide showed no difference between the labeling patterns of mutant and wild-type SUR1 proteins (Fig. 2A). In contrast, as shown in lane 4, photolabeling of delPhe1388 SUR1, which is unable to traffic normally to the plasma membrane, showed an absence of the 170-kDa band, the location of the mature form of the receptor (32). Surface immunofluorescence studies indicated that, when coexpressed with Kir6.2, the delSer1387 mutant SUR1 forms K_{ATP} channels that traffic to the plasma membrane, similar to wild-type SUR1 (data not shown). Coexpression of an equimolar mixture of delSer1387 and wild-type SUR1 cDNA did not alter surface expression.

Glibenclamide-inhibitable [$^{86}\text{Rb}^+$] efflux was examined to determine whether the mutant K_{ATP} channels observed at the plasma membrane were functional. COSm6 cells coexpressing delSer1387 SUR1 protein with Kir6.2 protein exhibited no rubidium efflux in response to a combination of metabolic inhibition (to lower ATP concentrations) in the presence of diazoxide (Fig. 2B), indicating that K_{ATP} channels containing delSer1387 SUR1 were not functional. Expression studies performed with equimolar mixtures of wild-type and delSer1387 SUR1 yielded COSm6 cells that showed glibenclamide-inhibitable rubidium efflux similar to control. Thus, the mutant delSer1387 SUR1 did not block expression of the wild-type protein. Taken together, these studies confirmed that delSer1387 SUR1 is a disease-causing mutation.

DISCUSSION

The combination of clinical, genetic, and expression studies demonstrate that congenital hyperinsulinism in this family is caused by a delSer1387 mutation of SUR1 that is transmitted in an autosomal-dominant fashion. This conclusion is supported by the similarities of insulin dysregu-

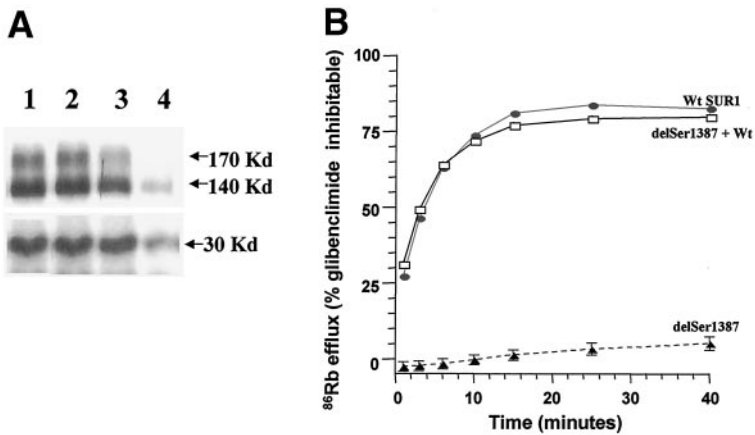


FIG. 2. Expression analysis of mutation delSer1387. **A:** Whole-cell photolabeling of reconstituted K_{ATP} channels with [125 I]iodoazido-glibenclamide. COS cells coexpressing Kir6.2 plus SUR1 wild type (lane 1), equimolar SUR1 delSer1387 and SUR1 wild type (lane 2), SUR1 delSer1387 (lane 3), or a trafficking defective delPhe1388 SUR1 mutation (lane 4). Labeled bands show SUR1 core glycosylated protein (140 kDa), mature or complex glycosylated protein (170 kDa), and Kir6.2 protein (40 kDa). **B:** Relative K_{ATP} channel activity as determined by measurement of glibenclamide-inhibitable efflux of $^{86}\text{Rb}^+$ from COS cells transfected as noted in A. After loading with $^{86}\text{Rb}^+$, cells were incubated with metabolic inhibitors (2-deoxy-glucose plus oligomycin) and diazoxide. Efflux was measured in the presence or absence of 1 $\mu\text{mol/l}$ glibenclamide. Data are expressed as a percentage of the glyburide-inhibitable efflux normalized for the total $^{86}\text{Rb}^+$ loaded in the cells. Wt, wild type.

lation in this family to the abnormalities previously found in the recessive form of hyperinsulinism associated with the two common Ashkenazi Jewish SUR1 mutations g3992-9a and delPhe1388 (15,16). Linkage analysis in the family using polymorphisms contained within the SUR1 gene was consistent with dominant transmission of a defect at this locus. Mutation analysis identified a potential disease-causing mutation, delSer1387 in exon 34 of SUR1. Coexpression of the delSer1387 mutant SUR1 protein with wild-type Kir6.2 confirmed that the mutation encodes a K_{ATP} channel with impaired function.

The features of abnormal islet function in the family are consistent with impairment of the β -cell plasma membrane K_{ATP} channel. As shown in Fig. 3, activity of this channel is inhibited by the increase in the ATP-to-ADP ratio during stimulation of the β -cell by glucose and other fuels; the inhibition of potassium efflux results in membrane depolarization and activation of a voltage-gated calcium channel that ultimately triggers release of insulin from stored granules. A combination of observations in affected family members directed attention to the K_{ATP} channel as the probable site of defect: 1) impaired AIR to the channel inhibitor tolbutamide, 2) abnormal positive AIR to calcium, 3) diminished responsiveness to either acute or graded glucose stimulation, and 4) incomplete suppression of fasting or protein-induced hypoglycemia by

treatment with the channel agonist diazoxide. This pattern of abnormalities mimics that previously noted in children with recessive hyperinsulinism associated with g3992-9a or delPhe1388 SUR1 mutations. In addition, the β -cell histological abnormalities seen in the pancreas of one of the family members were identical to those that have been described in children with recessive K_{ATP} channel hyperinsulinism (25). Unlike the latter patients, most of the family members did not show evidence of fetal overgrowth and did not present with hypoglycemia as neonates. They also appeared to be partially responsive to diazoxide therapy, suggesting that individuals with the delSer1387 mutation do not have a complete loss of function of the channel. Residual K_{ATP} channel function may explain why the mother of the index patient was not diagnosed until adult life and why symptomatic hypoglycemia was not recognized in either the index patient's son or maternal grandfather.

It should be emphasized that individuals with the delSer1387 SUR1 mutation were sensitive to protein-induced hypoglycemia, although, unlike patients with hyperinsulinism caused by GDH mutations, they did not have abnormal AIRs to leucine. Protein sensitivity has not been well studied in children with recessive K_{ATP} channel hyperinsulinism. However, as noted in Table 3, one disease control hyperinsulinism patient who was homozy-

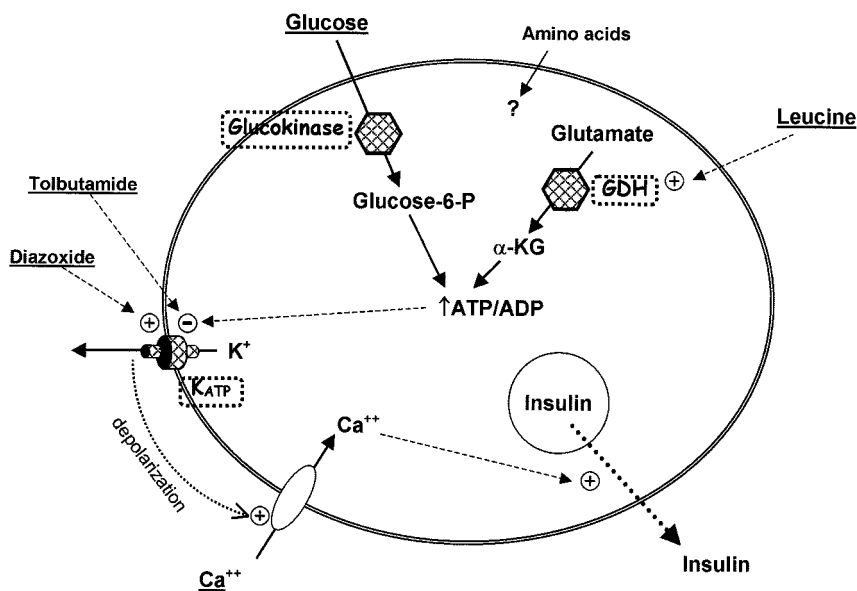


FIG. 3. Pathways of stimulation of insulin release by pancreatic β -cells. Glucose and leucine stimulate insulin secretion via an increase in the ATP-to-ADP ratio, which leads to inhibition of the K_{ATP} channel, depolarization of the plasma membrane, opening of a voltage-gated calcium channel, and elevation of cytosolic calcium, which triggers release of insulin from stored granules. Modulators of insulin secretion used to evaluate family members in the present study are underlined. Tolbutamide stimulates insulin release by inhibiting K_{ATP} channel activity. Diazoxide suppresses insulin release by activating the K_{ATP} channel. Sites of genetic defects associated with hyperinsulinism are outlined: glucokinase, GDH, and K_{ATP} (composed of the sulfonylurea receptor SUR1 and its regulated potassium pore, Kir6.2). The pathways by which amino acids, other than leucine, stimulate insulin secretion remain unclear.

gous for the g3992-9a SUR1 mutation also demonstrated marked susceptibility to protein-induced hypoglycemia (Table 2). Thus, protein sensitivity, without leucine sensitivity, might be a more common feature of K_{ATP} channel forms of hyperinsulinism than has been recognized. Unlike children with the hyperinsulinism/hyperammonemia syndrome, patients with K_{ATP} channel hyperinsulinism do not have abnormalities of GDH. The mechanism by which protein stimulates insulin release in the K_{ATP} channel form of hyperinsulinism requires further investigation.

Previous linkage studies in the present family using tandem-repeat haplotype markers on 11p had appeared to exclude the SUR1/Kir6.2 region (9). These older studies showed a transversion between the proband (IIb) and his brother (IIc) in a region that was believed, at the time, to contain the SUR1 locus. However, reexamination of the data using current improved map assignments indicates that microsatellite markers D11S902 and D11S921 were transposed in the original analysis. Thus, with correct map assignments, linkage to the SUR1/Kir6.2 region could not be excluded. In the present study, we used intragenic polymorphisms within the SUR1 gene for haplotyping. These data provided a clear demonstration that linkage of the hyperinsulinism to SUR1 in this family was possible (Fig. 1), and they led to the successful search for the disease-causing mutation.

Huopio et al. (8) described an extended Finnish family with congenital hyperinsulinism caused by a different dominantly expressed SUR1 missense mutation, Glu1507Lys. In contrast to patients with recessive hyperinsulinism associated with the two common Ashkenazi Jewish SUR1 mutations, g3992-9a and delPhe1388, hypoglycemia in the family Huopio et al. studied appeared to be responsive to diazoxide therapy. It is not clear whether Huopio et al.'s patients were completely responsive to diazoxide or, like the members of our family, hypoglycemia was only partially controlled by diazoxide. As in the present family, pancreatic tissue from one of Huopio et al.'s case subjects showed the same abnormalities of islet nuclear enlargement seen in children with recessive K_{ATP} channel hyperinsulinism.

The obligate carriers in Huopio et al.'s (8) dominant SUR1 Glu1507Lys family had histories consistent with symptomatic hypoglycemia in early childhood. However, many developed glucose intolerance or type 2 diabetes as adults. Huopio et al. speculated that susceptibility to diabetes with increasing age might reflect progressive β -cell failure caused by the channel defect because evidence of increased β -cell apoptosis has been reported in the pancreas of infants with recessive K_{ATP} channel hyperinsulinism (33). Recently, Huopio et al. (34) reported decreased insulin responses to oral and intravenous glucose tolerance tests in the nondiabetic adult carriers of the Glu1507Lys mutation, which they interpret as evidence of progressive β -cell failure. In our family with the delSer1387 mutation, however, diabetes was not a feature. Instead, hypoglycemia persisted into adult life beyond age 60 in one of the affected members, as well as in her father, who was probably affected. Type 2 diabetes developed after age 30 in the index patient in the present family. This may have been partly due to previous partial pancreatectomy; however, his diabetes may also reflect impaired islet

sensitivity to glucose, as seen with the glucose ramp study in his brother. Blunted insulin responses to bolus intravenous glucose were also common in the present family and in children with the recessive form of hyperinsulinism associated with the SUR1 mutations g3992-9a and delPhe1388. Thus, the poor insulin responses to glucose noted in adults with the Glu1507Lys SUR1 mutation by Huopio et al. may reflect the "glucose blindness" that is directly attributable to impaired K_{ATP} channel function, rather than progressive β -cell apoptosis.

In the present family, it is noteworthy that the serine deletion at amino acid 1387 of SUR1 causes dominant hyperinsulinism, whereas a deletion of the adjacent phenylalanine at residue 1388 causes recessive hyperinsulinism. Unlike patients with the dominant delSer1387 mutation, heterozygote carriers with the recessive delPhe1388 defect do not have hypoglycemia or evidence of abnormal insulin regulation, including normal AIRs to calcium, glucose, and tolbutamide (15,16). In addition, hyperinsulinism has been associated with a missense mutation at codon 1387 that replaces serine with phenylalanine (35). In this case, and in a second child we have identified with the Ser1387Phe mutation, surgery revealed diffuse disease, but no second mutation was found. Thus, it is possible that both deletion and substitution of codon 1387 may be expressed dominantly.

The reason for the dominant effect of the delSer1387 mutation may relate to the fact that the channel is a hetero-octameric complex composed of four SUR1 and four Kir6.2 subunits. Another plasma membrane ion channel in which dominant and recessive mutations of the same gene have been noted is the KVLQT1 gene, associated with the long QT syndrome (36). In this case, the functional channel protein is also a multimer containing four KVLQT1 subunits. Expression studies showed that the mutant SUR1 delSer1387 is expressed as a stable protein, retains its binding site for glibenclamide, and associates with Kir6.2 protein to form channel complexes that traffic normally to the plasma membrane. This contrasts with the delPhe1388 mutant SUR1, which fails to be trafficked to the plasma membrane and is expressed in recessive fashion. The activity of the channels formed with delSer1387 SUR1 was impaired as assessed by [$^{86}\text{Rb}^+$] efflux. Under the conditions used in the present studies, mixing experiments were unable to demonstrate a dominant-negative effect of the SUR1 delSer1387 mutant. This is similar to the studies of the dominant Glu1507Lys mutation reported by Huopio et al. (8), which also failed to show a dominant-negative effect when coexpressed with wild-type SUR1. In both cases, this may reflect methodological limitations. Preliminary electrophysiological studies of the delSer1387 show evidence of residual channel activity similar to that found with the Glu1507Lys mutation.

The mechanism of hyperinsulinism associated with heterozygosity for the delSer1387 mutation remains speculative. However, it might be explained by a reduction in the number of active K_{ATP} channels to a point that the plasma membrane is partly, but not completely, depolarized. Assuming that channels containing one or more delSer1387 SUR1 subunits are not able to function, patients would be predicted to have only 1/16 the normal number of

channels. By comparison, a 50% reduction in the channel number would be expected with defects, such as delPhe1388 and other recessive mutations, which do not interfere with expression of the wild-type SUR1. In the patients with the delSer1387 mutation, partial depolarization of the β -cell plasma membrane could explain their inability to adequately suppress insulin release during fasting, similar to a normal subject given a submaximal dose of a long-acting sulfonylurea. The partial responsiveness of the patients to diazoxide could reflect the activating action of the drug on the limited number of normal channels, which might be sufficient to increase potassium current and partly restore membrane hyperpolarization.

In summary, the present studies characterize a form of congenital hyperinsulinism with the unusual feature of being caused by a delSer1387 mutation of SUR1, which is dominantly expressed, rather than the commonly described recessively expressed mutations of this gene. The clinical phenotype of this delSer1387 form of hyperinsulinism and of a previously described dominant SUR1 mutation is slightly milder than is typical of many children with recessive mutations of the SUR1/Kir6.2 complex. However, these dominant SUR1 mutations clearly can have severe consequences, including hypoglycemic seizures or permanent brain damage. Pharmacological stimulation tests of insulin release proved to be useful in identifying the site of defect in insulin secretion and the likely candidate genes in this family. These clinical diagnostic methods may provide an important alternative to genetic diagnosis for discovering the underlying site of defect in other forms of congenital hyperinsulinism.

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health Grants MO1-RR 00240 and RO1-DK56268 (to C.A.S.) and RO1-DK57671 (to L.A.-B.).

The authors thank the nursing and laboratory staff of the General Clinical Research Center for Children at the Children's Hospital of Philadelphia for their help in caring for this family and their expertise in carrying out studies. This manuscript is dedicated to the memory of Dr. Lester Baker, who initiated and stimulated these investigations.

REFERENCES

- Stanley CA: Advances in the diagnosis and treatment of hyperinsulinism in infants and children. *J Clin Endocrinol Metab* 87:4857–4859, 2002
- Thomas PM, Cote GJ, Wohlk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gage RF, Bryan J: Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 268:426–429, 1995
- Thomas P, Ye YY, Lightner E: Mutations of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 5:1809–1812, 1996
- Nestorowicz A, Wilson BA, Schoor KP, Inoue H, Glaser B, Landau H, Stanley CA, Thornton PS, Clement JP, Bryan J, Aguilar-Bryan L, Permutt MA: Mutations in the sulfonylurea receptor gene are associated with familial hyperinsulinism in Ashkenazi Jews. *Hum Mol Genet* 5:1813–1822, 1996
- Nestorowicz A, Inagaki N, Gono T, Schoor KP, Wilson BA, Glaser B, Landau H, Stanley CA, Thornton PS, Seino S, Permutt MA: A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. *Diabetes* 46:1743–1748, 1997
- de Lonlay P, Fournet JC, Rahier J, Gross-Morand MS, Poggi-Travert F, Foussier V, Bonnefont JP, Brunet MC, Brunelle F, Robert JJ, Nihoul-Fekete C, Saudubray JM, Junien C: Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic hypoglycemia of infancy is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. *J Clin Invest* 100:802–807, 1997
- Glaser B, Ryan F, Donath M, Landau H, Stanley CA, Baker L, Barton DE, Thornton PS: Hyperinsulinism caused by paternal-specific inheritance of a recessive mutation in the sulfonylurea-receptor gene. *Diabetes* 48:1652–1657, 1999
- Huopio H, Reimann F, Ashfield R, Komulainen J, Lenko HL, Rahier J, Vauhkonen I, Kere J, Laakso M, Ashcroft F, Otonkoski T: Dominantly inherited hyperinsulinism caused by a mutation in the sulfonylurea receptor type 1. *J Clin Invest* 106:897–906, 2000
- Thornton PS, Satin-Smith MS, Herold K, Glaser B, Chiu KC, Nestorowicz A, Permutt MA, Baker L, Stanley CA: Familial hyperinsulinism with apparent autosomal dominant inheritance: clinical and genetic differences from the autosomal recessive variant. *J Pediatr* 132:9–14, 1998
- Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, Perlman K, Rich BH, Zammarchi E, Poncz M: Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med* 338:1352–1357, 1998
- Glaser B, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, Stanley CA, Thornton PS, Permutt MA, Matschinsky FM, Herold KC: Familial hyperinsulinism caused by an activating glucokinase mutation. *N Engl J Med* 338:226–230, 1998
- Stanley CA, Baker L: Hyperinsulinism in infants and children: diagnosis and therapy. *Adv Pediatr* 23:315–355, 1976
- Stanley CA, Baker L: Hyperinsulinism in infancy: diagnosis by demonstration of abnormal response to fasting hypoglycemia. *Pediatrics* 57:702–711, 1976
- Finegold DN, Stanley CA, Baker L: Glycemic response to glucagon during fasting hypoglycemia: an aid in the diagnosis of hyperinsulinism. *J Pediatr* 96:257–259, 1980
- Ferry RJ Jr, Kelly A, Grimberg A, Koo-McCoy S, Shapiro MJ, Fellows KE, Glaser B, Aguilar-Bryan L, Stafford DE, Stanley CA: Calcium-stimulated insulin secretion in diffuse and focal forms of congenital hyperinsulinism. *J Pediatr* 137:239–246, 2000
- Grimberg A, Ferry RJ Jr, Kelly A, Koo-McCoy S, Polonsky K, Glaser B, Permutt MA, Aguilar-Bryan L, Stafford D, Thornton PS, Baker L, Stanley CA: Dysregulation of insulin secretion in children with congenital hyperinsulinism due to sulfonylurea receptor mutations. *Diabetes* 50:322–328, 2001
- Kelly A, Ng D, Ferry RJ Jr, Grimberg A, Koo-McCoy S, Thornton PS, Stanley CA: Acute insulin responses to leucine in children with the hyperinsulinism/hyperammonemia syndrome. *J Clin Endocrinol Metab* 86:3724–3728, 2001
- Hsu BY, Kelly A, Thornton PS, Greenberg CR, Dilling LA, Stanley CA: Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr* 138:383–389, 2001
- Nestorowicz A, Glaser B, Wilson BA, Shyng SL, Nichols CG, Stanley CA, Thornton PS, Permutt MA: Genetic heterogeneity in familial hyperinsulinism [published erratum in *Hum Mol Genet* 7:1527, 1998]. *Hum Mol Genet* 7:1119–1128, 1998
- Ohta Y, Tanizawa Y, Inoue H, Hosaka T, Ueda K, Matsutani A, Repunte VP, Yamada M, Kurachi Y, Bryan J, Aguilar-Bryan L, Permutt MA, Oka Y: Identification and functional analysis of sulfonylurea receptor 1 variants in Japanese patients with NIDDM. *Diabetes* 47:476–481, 1998
- Glaser B, Furth J, Stanley CA, Baker L, Thornton PS, Landau H, Permutt MA: Intragenic single nucleotide polymorphism haplotype analysis of SUR1 mutations in familial hyperinsulinism. *Hum Mutat* 14:23–29, 1999
- Tanizawa Y, Matsuda K, Matsuo M, Ohta Y, Ochi N, Adachi M, Koga M, Mizuno S, Kajita M, Tanaka Y, Tachibana K, Inoue H, Furukawa S, Amachi T, Ueda K, Oka Y: Genetic analysis of Japanese patients with persistent hyperinsulinemic hypoglycemia of infancy: nucleotide-binding fold-2 mutation impairs cooperative binding of adenine nucleotides to sulfonylurea receptor 1. *Diabetes* 49:114–120, 2000
- Goksel DL, Fischbach K, Duggirala R, Mitchell BD, Aguilar-Bryan L, Blangero J, Stern MP, O'Connell P: Variant in sulfonylurea receptor-1 gene is associated with high insulin concentrations in non-diabetic Mexican Americans: SUR-1 gene variant and hyperinsulinemia. *Hum Genet* 103:280–285, 1998
- MacMullen C, Fang J, Hsu BY, Kelly A, deLonlay-Debeney P, Saudubray JM, Ganguly A, Smith TJ, Stanley CA: Hyperinsulinism/hyperammonemia syndrome in children with regulatory mutations in the inhibitory GTP binding domain of glutamate dehydrogenase. *J Clin Endocrinol Metab* 86:1782–1787, 2001
- Rahier J, Guiot Y, Sempoux C: Persistent hyperinsulinaemic hypoglycaemia

- mia of infancy: a heterogeneous syndrome unrelated to nesidioblastosis. *Arch Dis Child Fetal Neonatal Ed* 82:F108–F112, 2000
26. Sharma N, Crane A, Clement JP 4th, Gonzalez G, Babenko AP, Bryan J, Aguilar-Bryan L: The C terminus of SUR1 is required for trafficking of KATP channels. *J Biol Chem* 274:20628–20632, 1999
 27. Aguilar-Bryan L, Clement JP 4th, Nelson DA: Sulfonylurea receptors and ATP-sensitive potassium ion channels. *Methods Enzymol* 292:732–744, 1998
 28. Clement JP 4th, Kunjilwar K, Gonzalez G, Schwanstecher M, Panten U, Aguilar-Bryan L, Bryan J: Association and stoichiometry of K(ATP) channel subunits. *Neuron* 18:827–838, 1997
 29. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP, Boyd AE, Gonzalez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA: Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 268:423–426, 1995
 30. Aguilar-Bryan L, Clement JP 4th, Gonzalez G, Kunjilwar K, Babenko A, Bryan J: Toward understanding the assembly and structure of KATP channels. *Physiol Rev* 78:227–245, 1998
 31. Sharma N, Crane A, Gonzalez G, Bryan J, Aguilar-Bryan L: Familial hyperinsulinism and pancreatic beta-cell ATP-sensitive potassium channels. *Kidney Int* 57:803–808, 2000
 32. Cartier EA, Conti LR, Vandenberg CA, Shyng SL: Defective trafficking and function of KATP channels caused by a sulfonylurea receptor 1 mutation associated with persistent hyperinsulinemic hypoglycemia of infancy. *Proc Natl Acad Sci U S A* 98:2882–2887, 2001
 33. Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B: β -Cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes* 49:1325–1333, 2000
 34. Huopio H, Otonkoski T, Vauhkonen I, Reimann F, Ashcroft FM, Laakso M: A new subtype of autosomal dominant diabetes attributable to a mutation in the gene for sulfonylurea receptor 1. *Lancet* 361:301–307, 2003
 35. Aguilar-Bryan L, Bryan J: Molecular biology of adenosine triphosphate-sensitive potassium channels (Review). *Endocr Rev* 20:101–135, 1999
 36. Neyroud N, Tesson F, Denjoy I, Leibovici M, Donger C, Barhanin J, Faure S, Gary F, Coumel P, Petit C, Schwartz K, Guicheney P: A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. *Nat Genet* 15:186–189, 1997