

Severe Congenital Hyperinsulinism Caused by a Mutation in the Kir6.2 Subunit of the Adenosine Triphosphate-Sensitive Potassium Channel Impairing Trafficking and Function

Eric Marthinet, Alain Bloc, Yoshimoto Oka, Yukio Tanizawa, Bernhard Wehrle-Haller, Victor Bancila, Jean-Michel Dubuis, Jacques Philippe, and Valerie M. Schwitzgebel

Pediatric Endocrinology and Diabetology (E.M., J.-M.D., V.M.S.) and Diabetes Unit (E.M., J.P.), University Hospital of Geneva, and Departments of Neuroscience (A.B., V.B.) and Cellular Physiology and Metabolism (B.W.-H.), University of Geneva, CH-1211 Geneva, Switzerland; Biochemical Institute (A.B.), University of Lausanne, CH-1015 Lausanne, Switzerland; Department of Internal Medicine (Y.O.), Tohoku University Graduate School of Medicine Seiryō-machi, Sendai 980-8575, Japan; and Division of Molecular Analysis of Human Disorders (Y.T.), Yamaguchi University Graduate School of Medicine, Ube 755-8505, Japan

Context: The ATP-sensitive potassium (K_{ATP}) channel, assembled from the inwardly rectifying potassium channel Kir6.2 and the sulfonylurea receptor 1, regulates insulin secretion in β -cells. A loss of function of K_{ATP} channels causes depolarization of β -cells and congenital hyperinsulinism (CHI), a disease presenting with severe hypoglycemia in the newborn period.

Objective: Our objective was identification of a novel mutation in Kir6.2 in a patient with CHI and molecular and cell-biological analysis of the impact of this mutation.

Design and Setting: We combined immunohistochemistry, advanced life fluorescence imaging, and electrophysiology in HEK293T cells transiently transfected with mutant Kir6.2.

Patient and Intervention: The patient presented with macrosomia at birth and severe hyperinsulinemic hypoglycemia. Despite medical treatment, the newborn continued to suffer from severe hypoglycemic episodes, and at 4 months of age subtotal pancreatectomy was performed.

Main Outcome Measure: We assessed patch-clamp recordings and confocal microscopy in HEK293T cells.

Results: We have identified a homozygous missense mutation, H259R, in the Kir6.2 subunit of a patient with severe CHI. Coexpression of Kir6.2^{H259R} with sulfonylurea receptor 1 in HEK293T cells completely abolished K_{ATP} currents in electrophysiological recordings. Double immunofluorescence staining revealed that mutant Kir6.2 was partly retained in the endoplasmic reticulum (ER) causing decreased surface expression as observed with total internal reflection fluorescence. Mutation of an ER-retention signal partially rescued the trafficking defect without restoring whole-cell currents.

Conclusion: The H259R mutation of the Kir6.2 subunit results in a channel that is partially retained in the ER and nonfunctional upon arrival at the plasma membrane. (*J Clin Endocrinol Metab* 90: 5401–5406, 2005)

K_{ATP} CHANNELS COUPLE the metabolism of a cell to its electrical activity and are widely expressed. In the heart, these channels are involved in ischemic preconditioning (1), whereas in the brain, they have neuroprotective roles during ischemia (2). In the pancreas, K_{ATP} channels are localized at the plasma membrane of β -cells and to the insulin secretory granule (3), where they sense ATP, which is tightly regulated by glucose levels. Thus, an increase in glucose concentration leads to a higher ATP to ADP ratio and to K_{ATP} closure, β -cell depolarization, and insulin secretion. The functional K_{ATP} channel is an octameric complex formed by four sulfonylurea receptor 1 (SUR1) and four inwardly rectifying Kir6.2 subunits (4). Chronically impaired channel

function causes depolarization of the β -cell with sustained insulin secretion, a condition known as congenital hyperinsulinism (CHI), previously termed as persistent hyperinsulinemic hypoglycemia of infancy. Histologically, two different forms of CHI exist, a diffuse form, where all the islets of the pancreas are altered, and a focal form with a small region of hyperplastic β -cells surrounded by normal pancreatic islets (5). Overall, 40–65% of patients with CHI present a focal form (6–8). A majority of studies have identified mutations in the SUR1 subunit associated with CHI (9, 10). To date, more than 10 Kir6.2 mutations have been identified with CHI (11–16). One Kir6.2 mutation (Y12X) caused the synthesis of a truncated nonfunctional protein (12), whereas another mutation (W91R) showed defective channel assembly with a rapid degradation in the endoplasmic reticulum (ER) (17).

Here we identified a new homozygous mutation in the Kir6.2 subunit in a patient with severe CHI. Combining immunohistochemistry, advanced life fluorescence imaging, and electrophysiology, we demonstrate that the H259R mutation leads to a nonfunctional K_{ATP} channel and impaired trafficking to the cell membrane.

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Abbreviations: CHI, Congenital hyperinsulinism; EGFP, enhanced green fluorescent protein; ER, endoplasmic reticulum; HBA_{1c}, glycosylated hemoglobin; K_{ATP} , ATP sensitive K^+ channel; SUR1, sulfonylurea receptor 1; TIRF, total internal reflection fluorescence; WT, wild type.

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Patient and Methods

Genetic analysis

Genomic DNA was extracted from peripheral blood using the genomic PrepBlood DNA isolation kit (Amersham Pharmacia Biotech, Piscataway, NJ) after informed consent had been obtained. Individual exons of the *ABCC8* gene coding for SUR1 (39 exons) and the *KCNJ11* gene coding for Kir6.2 (1 exon) (GenBank accession numbers L78207 for *ABCC8* and NM_000525 for *KCNJ11*) were amplified by PCR and screened for mutations by direct nucleotide sequencing (18).

Molecular biology

The plasmid pECE-*hKir6.2* [wild type (WT)] (a generous gift from Dr. J. Bryan, Baylor College of Medicine, Houston, TX) was used to generate the pECE-*hKir6.2* (H259R) using the *in vitro* QuikChange site-directed mutagenesis kit (Stratagene, Amsterdam, The Netherlands) according to the manufacturer's protocol. The following primers were used: forward, 5'-CCGCTGATCATCTACCGTGTTCATTGATGCCAACAGC-3', and reverse, 5'-GCTGTTGGCATCAATGACACGGTAGATGATCAGCGG-3'. The mutation was confirmed by DNA sequencing. WT and mutant *KCNJ11* cDNA were subcloned into the pCDNA3 vector (Invitrogen, Basel, Switzerland). The pCDNA3-*hKir6.2*(WT) and the pCDNA3-*hKir6.2*(H259R) were used to generate pCDNA3-*hKir6.2*_{AAA}(WT) and the pCDNA3-*hKir6.2*_{AAA}(H259R) with the *in vitro* QuikChange site-directed mutagenesis kit by using the following primers: forward, 5'-CCGCGGGCCCTGGCCGCGCCAGCGTGCCCATGG-3', and reverse, 5'-CCATGGGCACGCTGGCCGCGCCAGGGCCCGCGG-3'. All the mutations were confirmed by DNA sequencing. Human *ABCC8* cDNA (pECE-*hSUR1* (a generous gift from Dr. J. Bryan) was subcloned into the pCDNA3 vector.

Cell culture and transfections

The human embryonic kidney (HEK293T) cell line was grown and maintained in RPMI 1640 (Seromed, Basel, Switzerland) supplemented with 5% fetal calf serum, 5% newborn calf serum (Life Technologies, Basel, Switzerland), 100 U/ml penicillin (Seromed), 100 µg/ml streptomycin (Seromed), and 2 mM glutamine (Life Technologies). HEK293T cells were transiently cotransfected using the calcium phosphate precipitation technique with human *ABCC8* and *KCNJ11* cDNA (WT, WT_{AAA}, H259R, and H259R_{AAA}) in a 4:1 ratio (1 µg of *ABCC8* and 0.25 µg of *KCNJ11*) (19). After 48 h, cells were used for the patch clamp technique, for immunohistochemistry or total internal reflection fluorescence (TIRF) microscopy experiments. We transfected HEK293T cells with two enhanced green fluorescent protein (EGFP) fusion constructs [Kitl-EGFP (Mb-EGFP) and KLS-EGFP (ER-EGFP)] to label either the cell membrane or the ER, using the same conditions as described previously, to validate our TIRF experiments (20).

Electrophysiological measurements

As described previously (21), all experiments were performed at room temperature (20–22°C). The pipette solution consisted of 10 mM NaCl, 140 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 1 mM EGTA, 1 mM MgATP (pH 7.2 with KOH), and the extracellular solution used was 145 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, 10 mM HEPES, 10 mM D-glucose (pH 7.2 with NaOH). The equilibrium potential for K⁺ ions (E_K) was -82 mV. Membrane slope conductance values (G_m) were calculated from dI/dV , using ramp voltage-clamp protocol (between -120 and -40 mV, a voltage range symmetrical to E_K). dI was determined from tolbutamide-sensitive currents.

Immunohistochemistry

HEK293T cells were stained with the following antibodies: goat anti-Kir6.2 (sc11228; Santa Cruz Biotechnology, Heidelberg, Germany), guinea pig anti-Kir6.2 (generous gift from Dr. B. Schwappach), and goat anticalreticulin (generous gift from Dr. M. Michalak) to mark the ER and mouse anti-giantin (generous gift from Drs. H. Hauri and M. Spiess) to stain the Golgi apparatus. The following secondary antibodies were used: antigoat fluorescein isothiocyanate, antimouse Alexa568, and antigoat alexa 568 (Molecular Probes, Leiden, The Netherlands). For co-

localization experiments of the protein Kir6.2 with the plasmic membrane marker, toxin-GPI-alexa546 (generous gift from Dr. F. van der Goot) was used. Slides were viewed on a Zeiss LSM 510 confocal microscope (Carl Zeiss AG, Göttingen, Germany).

TIRF microscopy

We used an inverted microscope Axiovert 100M (Carl Zeiss) equipped with a high numerical aperture objective (×100 NA 1.45; Carl Zeiss) and a combined epifluorescence/TIRF adapter (TILL Photonics, Gräfelfing, Germany). Fluorophores were excited at 488 nm with a 150-mW argon-ion laser through a monomode optical fiber (488/568/647 nm) and the fluorescence filter set containing a laser clean-up filter (488/10), dichroic mirror (DCLP500), and band pass emission filter (BP525/50). Images were acquired with a 12-bit CCD camera (Orca 9742-95; Hamamatsu, Hamamatsu City, Japan). The laser shutter, the camera and the microscope set up were controlled by the Openlab software (Improvision, Basel, Switzerland).

Quantification and statistical analysis

The percentage of colocalization of the protein Kir6.2 (WT or H259R) with the different markers used in this study was calculated with the Metamorph software version 6.2r4 (Universal Imaging, Puchheim, Germany). Results are expressed as mean ± SD. Where indicated, the statistical significance of the differences between groups was estimated by the Mann-Whitney *U* test or the *t* test. Statistical significance is indicated as follows: *, $P < 0.01$; **, $P < 0.001$.

Results

Case synopsis

The infant was born at term after an uneventful pregnancy and presented with macrosomia (body weight, 4460 g; body length, 54 cm; both values are above the 90th percentile). Thirty minutes after birth, severe hypoglycemic episodes were observed [glucose level of 31 mg/dl (1.7 mmol/liter) with a simultaneous insulin level of 172 mU/liter] leading to the diagnosis of hyperinsulinism. The newborn was treated with iv glucose at a rate of 20 mg/kg·min, and diazoxide was added at a dose of 17 mg/kg·d. Despite treatment, the newborn continued to suffer from severe hypoglycemic episodes, and octreotide (17 µg/kg·d) and nifedipine (0.25 mg/kg·d) were added successively without therapeutic success. Continuous iv glucagon (1 mg/d) was needed to stabilize the blood glucose levels. The patient developed clinical signs of cardiac insufficiency; cardiac ultrasound showed biventricular hypertrophy. At 4 months of age a pancreatic catheterization with measurements of insulin levels (6) suggested a diffuse form of CHI. At 5 months of age, a subtotal pancreatectomy (95%) was performed, and pancreatic histopathology confirmed the diagnosis. After pancreatectomy, the infant became diabetic and was treated with an insulin pump. At the age of 20 months the total daily insulin dose was 0.54 U/kg with a glycosylated hemoglobin (HbA_{1c}) of 6.9%. Blood glucose measurements showed maximal levels of 181 mg/dl (10 mmol/liter). The insulin dose was gradually tapered and eventually stopped at the age of 23 months. Two months after the insulin treatment was completely stopped, the HbA_{1c} was at 6.4%, and daily blood glucose measurements varied between 74.5 mg/dl (4.1 mmol/liter) and 145 mg/dl (8 mmol/liter). The HbA_{1c} values further decreased to 5.4% at the age of 36 months without any treatment, and no hypoglycemic episodes were encountered.

Genetic analysis

Sequencing of the 39 exons of the *ABCC8* gene, encoding the SUR1 protein, revealed no mutation. In contrast, a homozygous missense mutation, 776A→G, was found in the *KCNJ11* gene, encoding the Kir6.2 protein, leading to a change in the amino acid sequence (H259R). The mutation was located close to the C-terminal end at a highly conserved site, found in 52 proteins related to Kir6.2. This makes DNA sequence polymorphism therefore unlikely (Fig. 1). Both parents were found to be heterozygous for the 776A→G mutation.

Functional analysis of the mutant K_{ATP} channel

To study the functional impact of the mutation in the Kir6.2 protein on the K_{ATP} channel, we used the patch clamp technique in the whole-cell configuration. HEK293T cells were transiently cotransfected with mutant *KCNJ11* cDNA and wild-type *ABCC8* cDNA. GFP cDNA was added to identify successfully transfected cells. As shown in Fig. 2, K_{ATP} currents were absent in cells expressing the mutant K_{ATP} channel (Fig. 2B) but present in the WT (Fig. 2A). Earlier studies have reported that functional recovery of K_{ATP} currents in the case of mutation in the SUR1 subunits can be obtained with diazoxide (22). As shown in Fig. 2B (middle), diazoxide had no effect on the H259R mutant K_{ATP} channel. In fact, the current was completely absent in all cells with the mutated K_{ATP} channel ($n = 8$; $P < 0.001$) (Fig. 2, B and C).

Retention of mutant K_{ATP} channel in the ER

The absence of current could result from several abnormalities such as decreased protein synthesis, defects in assembly and trafficking, increased degradation (17), or impaired function of the channel itself (23). In our case, the H259R mutation did not appear to interfere with protein synthesis, because Kir6.2 protein could be synthesized *in vitro* (data not shown). It has been shown that K_{ATP} channels are subjected to quality control during ER trafficking, whereby the correct assembly of the subunits masks retention signals (24, 25) and allows membrane insertion. To test for trafficking defects of the mutant Kir6.2, we performed immunohistochemical costaining experiments with markers of the ER and the Golgi apparatus. Costaining with antibodies against the calreticulin protein of the ER revealed a 2-fold increase in colocalization of the mutant channel ($42.6 \pm 8.8\%$; $n = 11$) in comparison with WT ($22.3 \pm 7.7\%$; $n = 11$) (Fig. 3). The same results were obtained with the colocalization of

the Kir6.2 protein with the ER-EGFP (data not shown). In contrast, costaining with antisera against the Golgi apparatus showed no difference in comparison with WT ($1.3 \pm 0.1\%$, $n = 4$, *vs.* $1.7 \pm 0.3\%$, $n = 5$) (Fig. 4). We conclude from these experiments that a significant amount of mutant Kir6.2 is retained in the ER, and as a consequence there is a reduced expression of the mutant K_{ATP} channel at the cell membrane.

We therefore tested whether the additional mutation of the ER retention signal RKR to AAA rescued K_{ATP} channels. As expected, this led to a significant decrease of fluorescence that colocalized with the ER marker calreticulin ($13.1 \pm 4.9\%$ *vs.* $42.6 \pm 8.8\%$; $n = 8$ –11 cell; $P < 0.01$; data not shown). However, even after elimination of the ER retention signal, no K_{ATP} currents were recorded (Fig. 5, A and B) ($n = 7$; $P < 0.01$). Furthermore diazoxide applied to cells transfected with H259R_{AAA} still did not restore K_{ATP} currents (Fig. 5C). In contrast, in WT_{AAA}-transfected cells, diazoxide enhanced K_{ATP} currents.

Reduced expression of the mutant K_{ATP} channel at the cellular membrane

This conclusion is further supported by confocal images showing a marked reduction in membrane staining of the mutant Kir6.2 K_{ATP} channel compared with WT. Costaining with the plasmic membrane marker, toxin-GPI-alexa546, revealed a reduced colocalization of the mutant channel with the plasma membrane ($9.5 \pm 1.5\%$; $n = 3$) in comparison with WT ($14.5 \pm 1.3\%$; $n = 3$) (Fig. 6). Finally, these results were confirmed by TIRF imaging, which allows selective visualization of fluorescence localized at the cell surface. Again, expressing the mutant *KCNJ11* cDNA led to a significantly lower signal of the protein at the cell surface compared with WT (Fig. 7A). To validate our TIRF experiments and to decrease the likelihood that the fluorescence signal obtained may relate to channels close to the membrane, we transfected HEK293T with two GFP fusion constructs, localizing either to the plasma membrane (Mb-EGFP) or to the ER (ER-EGFP) (20) (Fig. 7B). The fluorescence observed in TIRF with the membrane marker Mb-EGFP is comparable with the fluorescence obtained with Kir6.2 WT. The TIRF fluorescence of the ER-EGFP protein is similar to the one seen with the mutant H259R protein. Taken together, these results thus show that the mutant Kir6.2 is partially retained in the ER, but a fraction still reaches the cell membrane.

FIG. 1. Identification of a new point mutation H259R in the C terminus of the Kir6.2 protein in the index patient. Protein sequence alignment of the region containing the mutation shows the conservation of the mutated amino acid between species.

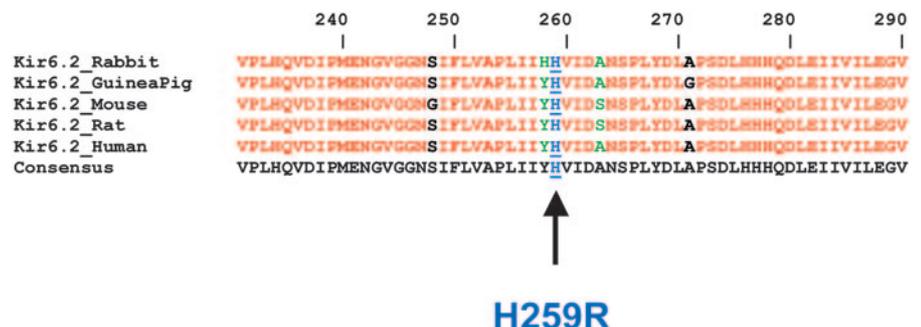
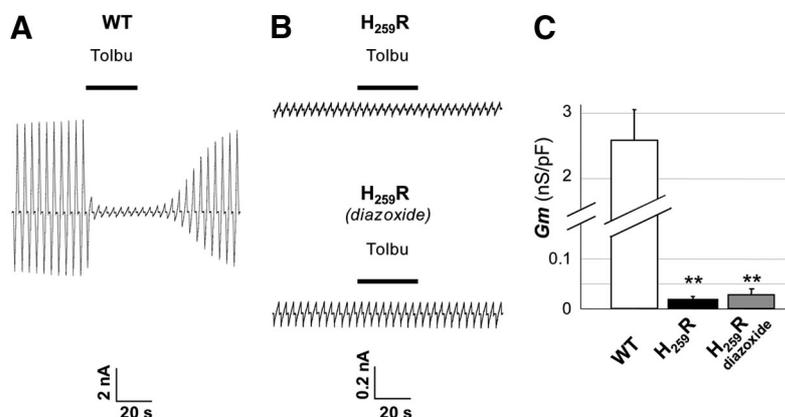


FIG. 2. Functional analysis of mutated K_{ATP} channel. A, Whole-cell currents recorded in response to ramps of voltage from -120 to -40 mV over a 3-sec period from voltage-clamped HEK293T cells (holding potential, -80 mV) coexpressing Kir6.2WT plus SUR1. B, Kir6.2H259R plus SUR1 and Kir6.2H259R plus SUR1 after an incubation with $200 \mu\text{M}$ diazoxide for 48 h. Cells were continuously perfused with extracellular solution (see *Materials and Methods*) during the course of the recording, and $250 \mu\text{M}$ tolbutamide (tolbu) was added when indicated to block recombinant K_{ATP} currents and allow quantification of their amplitude. C, Membrane slope conductance values (G_m as mean \pm SD) calculated for each type of recombinant channel expressed in HEK293T cells: Kir6.2WT plus SUR1 ($n = 9$ cells); Kir6.2H259R plus SUR1 ($n = 8$ cells); Kir6.2H259R plus SUR1 submitted to a 48-h treatment with diazoxide ($n = 8$ cells) ($P < 0.001$).



Discussion

We describe a human mutation located at the C terminus of Kir6.2 that impairs trafficking and abolishes channel function. Immunohistochemical visualization of mutated Kir6.2 revealed a decreased surface pool, whereas fluorescence in the ER was enhanced. In addition, whole-cell currents were abolished, which suggests that the fraction of channels that eventually reaches the surface is not functional. This conclusion is also supported by the nonresponsiveness to diazoxide in the patch clamp experiments.

During biosynthesis, the Kir6.2 protein is exported from the ER only if properly assembled into an octameric K_{ATP} channel (24). In fact, the formation of the Kir6.2 tetramer leads to the masking of an ER-retention signal located in the cytoplasmic tail of the Kir6.2 protein. Mutation analysis indicates that the ER-retention signals contain a -RKR- motif (24). Assembly of functional channels that reach the surface will occur only after a second retention signal located on the SUR subunit is subsequently masked. An alternate assembly model proposes the formation of a SUR-Kir6.2 heterodimer before the octamer formation (17). Our data are compatible with both assembly models. In fact, the H259R mutation may interfere in several ways with this quality control mechanism. First, the H259R mutation may have created a new retention signal, a possibility that, however, is not very likely because the mutation did not create any of the established retention motifs including -RKR- (26). Second, the -RKR-

motif could have been indirectly inactivated by the H259R mutation through a change of the tertiary conformation of the Kir6.2 protein. This would cause an inappropriate trafficking of Kir6.2_{H259R} tetramers to the cell surface without the need to coassemble with SUR just as C-terminally truncated Kir6.2 proteins are inserted into the membrane (24, 27). Again, this scenario seems unlikely because such C-terminally truncated tetramers constitute functional channels, whereas the H259R mutation led to a complete absence of currents. Therefore, we favor a model whereby the H259R mutation would change the conformation of the Kir6.2 protein in a way that would prevent the complete masking of the retention signal that normally occurs during assembly (24, 28). This model could explain partial retention in the ER that can be overcome by mutating the RKR sequence. As a consequence, only a fraction of the mutated channels is expressed at the surface. The mutation may cause structural alteration abolishing function, for example by affecting ATP gating, which may provide an explanation why even in the presence of surface fluorescence, no currents were recorded. In line with this interpretation, mutating the ER retention signal RKR in Kir6.2 to AAA did not rescue K_{ATP} currents.

A similar dual defect was reported for the ΔF1388 mutation in the SUR1 subunit; this mutation caused defective trafficking and lack of surface expression (29). Additional experiments led to the conclusion that even if expressed at the surface, the ΔF1388 mutation interfered with K_{ATP} func-

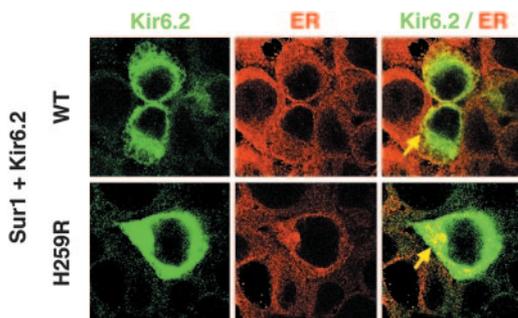


FIG. 3. Coexpression of WT and mutant K_{ATP} channel with markers for the ER. HEK293T cells were cotransfected with SUR1 and Kir6.2WT or SUR1 and Kir6.2H259R. Double immunofluorescence staining showed a 2-fold increase in colocalization of the mutant K_{ATP} channel with the calreticulin protein in the ER (yellow staining, arrow) in comparison with WT. Photomicrographs were imaged confocally.

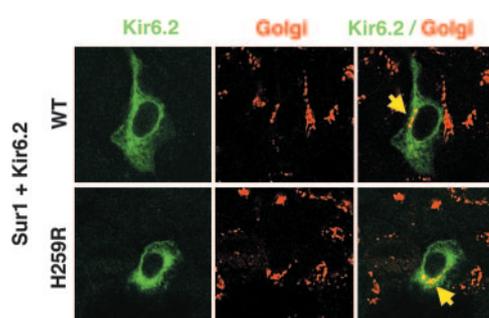
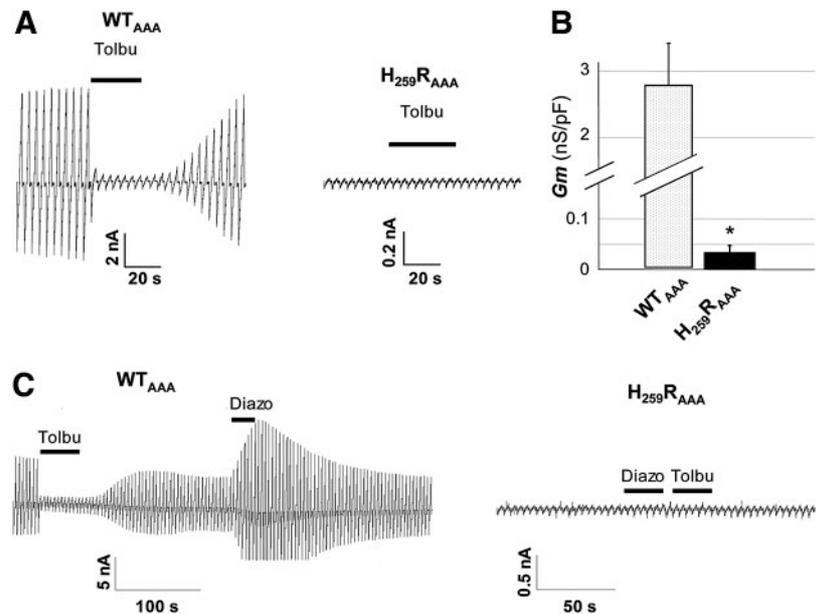


FIG. 4. Coexpression of WT and mutant K_{ATP} channel with markers for the Golgi apparatus. HEK293T cells were cotransfected with SUR1 and Kir6.2WT or SUR1 and Kir6.2H259R. Double immunofluorescence staining of the mutant K_{ATP} channel with the giantin protein of the Golgi apparatus (yellow staining, arrow) showed no difference in comparison with WT. Photomicrographs were imaged confocally.

FIG. 5. Functional analysis of mutated K_{ATP} channel devoid of the RKR retention signal. **A**, Whole-cell currents recorded in response to ramps of voltage from -120 to -40 mV over a 3-sec period from voltage-clamped HEK293T cells (holding potential, -80 mV) coexpressing either Kir6.2 WT_{AAA} plus SUR1 or Kir6.2H259R $_{AAA}$ plus SUR1. **B**, Membrane slope conductance values (G_m as mean \pm SD) calculated for each type of recombinant channel expressed in HEK293T cells: Kir6.2 WT_{AAA} plus SUR1 ($n = 8$ cells); Kir6.2H259R $_{AAA}$ plus SUR1 ($n = 7$ cells) ($P < 0.01$). **C**, Bars indicate application of diazoxide (diazox; $100 \mu M$) and tolbutamide (tolbu; $250 \mu M$). Diazoxide applied to cells transfected with H259R $_{AAA}$ did not restore K_{ATP} currents. In contrast, in WT_{AAA} -transfected cells, diazoxide enhanced K_{ATP} currents.



tion. The severity and the early onset of hypoglycemia in the case described here may reflect the complete loss of channel function revealed in this study. Moreover, the patient was resistant to diazoxide, an observation mirrored by the absence of an effect of diazoxide in the electrophysiological recordings (22).

The observation that diabetes resolved 18 months after subtotal pancreatectomy is unusual and could potentially be explained by a partial regeneration of the pancreas as previously reported (30, 31). We assume that the number of β -cells has increased during pancreas regeneration and that the amount of secreted insulin is sufficient to avoid overt hyperglycemia. The child is still on a strict diet with three main meals and three snacks, including one at bedtime, which could help to avoid severe hypoglycemic episodes, secondary to inappropriate insulin secretion. It is possible that our index case experiences unrecognized hypoglycemic episodes.

K_{ATP} channels containing the Kir6.2 subunit are present in the pancreatic β -cell, the brain, the cardiomyocyte, and the

smooth muscle (32–36); the targeted disruption of Kir6.2 in mice showed exercise-induced arrhythmia and sudden cardiac death (1). We therefore recorded an electrocardiogram over a 24-h period, which included a period of exercise; however, no arrhythmia was noted and the QT interval was normal. Despite severe hypoglycemic episodes during infancy, the child is developing normally at 4 yr of age and has a normal statural growth. Loss of function of Kir6.2 in the

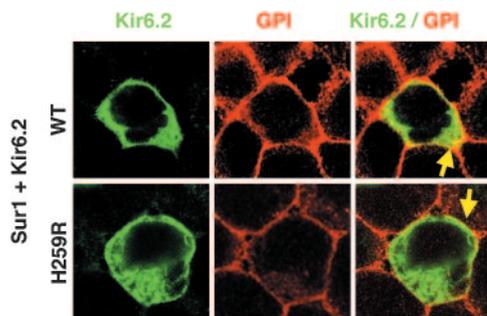


FIG. 6. Reduced surface expression of mutant H259R- K_{ATP} channel. HEK293T cells were cotransfected with SUR1 and Kir6.2 WT or SUR1 and Kir6.2H259R. Colocalization of the wild-type or mutant K_{ATP} channel and the membrane marker, toxin-GPI-alexa546, was visualized by confocal imaging. Double immunofluorescence staining revealed decreased membrane expression (yellow staining, arrow) for the mutant K_{ATP} channel in comparison with WT .

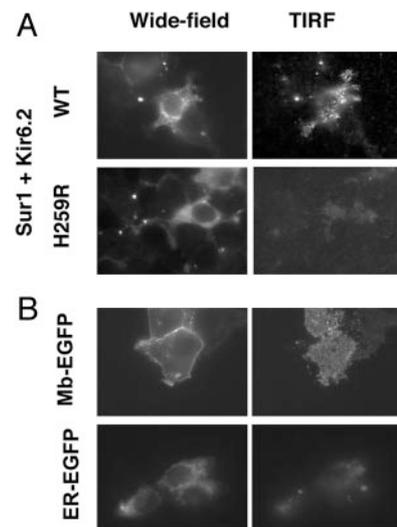


FIG. 7. Wide-field fluorescence and TIRF fluorescence were visualized for the WT K_{ATP} and the mutant K_{ATP} channel. **A**, The same cell is shown with the wide-field and with TIRF technique (WT in the upper panel and H259R in the lower panel). The TIRF technique showed expression of the WT K_{ATP} channel at the surface (white arrows), whereas the mutant K_{ATP} expression was markedly reduced. **B**, Wide-field fluorescence and TIRF fluorescence were visualized for the Mb-EGFP and the ER-EGFP. The same cell is shown with the wide-field and with TIRF technique (Mb-EGFP in the upper panel and ER-EGFP in the lower panel). The TIRF technique showed expression of the Mb-EGFP at the surface, whereas the ER-EGFP expression at the surface was very weak.

human brain seems not to interfere with normal development. It is possible that other inwardly rectifying potassium channels substitute for the neuronal loss of Kir6.2. Kir6.1, however, seems an unlikely candidate because it is mainly expressed in astrocytes (37). However, the child could be at risk for hypoxia-induced seizures, as described in Kir6.2^{-/-} mice (2). The parents, both heterozygous for the H259R mutation, never experienced hypoglycemic episodes, excluding a dominant negative effect of H259R.

In conclusion, we identify histidine 259 as an important residue in the C terminus of human Kir6.2 protein that affects trafficking and is required for channel function.

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Address all correspondence and requests for reprints to: Valérie M. Schwitzgebel, Pediatric Endocrinology and Diabetology, Children's Hospital, 6, rue Willy Donzé, CH-1211 Geneva, Switzerland. E-mail: valerie.schwitzgebel@hcuge.ch.

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