Novel De Novo Mutation in Sulfonylurea Receptor 1 Presenting as Hyperinsulinism in Infancy Followed by Overt Diabetes in Early Adolescence

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OBJECTIVE—Congenital hyperinsulinism, usually associated with severe neonatal hypoglycemia, may progress to diabetes, typically during the 4th decade of life in nonpancreatectomized patients. We aimed to genotype the ATP-sensitive K^+ channel in a 10.5-year-old girl presenting with overt diabetes following hyperinsulinism in infancy.

RESEARCH DESIGN AND METHODS—A female aged 10.5 years presented with new-onset, antibody-negative diabetes (A1C 10.6%). She was born large for gestational age (5 kg) to a nondiabetic mother and developed frequent hypoglycemic episodes, which persisted until age 3 years and responded initially to intravenous glucose and later to oral sweets. Currently, she is fully pubertal and obese (BMI 30.2 kg/m^2), with a partially controlled convulsive disorder (since age 1 year) and poor school performance. Glucose levels were >11.1 mmol/l throughout 72 h of continuous glucose monitoring, with low insulin secretion during intravenous glucose tolerance testing. *KCNJ11* and *ABCC8* mutation analysis was performed, and the mutation identified was characterized in COSm6 cells.

RESULTS—A novel, de novo heterozygous *ABCC8* sulfonylurea receptor (SUR)1 mutation (R370S) was identified in the patient's DNA but not in that of either parent. Cotransfection of Kir6.2 and mutant SUR1 demonstrate that the mutated protein is expressed efficiently at the cell surface but fails to respond to MgADP, resulting in minimal channel activity. Interestingly, the heterozygous channel (WT:R370S) responded well to glibenclamide, a finding that lead to the successful initiation of sulfonylurea therapy.

CONCLUSIONS—This new *ABCC8* mutation is associated with neonatal hyperinsulinism progressing within 10 years to insulinopenic diabetes. Consistent with in vitro findings, the patient responded to sulfonylurea treatment. The mechanism causing the relatively rapid loss in β-cell function is not clear, but it may involve mutation-induced increased β-cell apoptosis related to increased metabolic demand. *Diabetes* 57:1935–1940, 2008

Congenital hyperinsulinism (CHI) is a disorder of glucose metabolism characterized by dysregulated secretion of insulin resulting in hypoglycemia (1). Most cases are attributed to defects in pancreatic β-cell ATP-sensitive K^+ (K_{ATP}) channels, which are critical for coupling glucose metabolism to membrane electrical activity and insulin release (2,3). K_{ATP} channels are activated (opened) by intracellular ADP and inhibited (closed) by ATP, the product of increased glucose metabolism. Closure of the K_{ATP} channel leads to membrane depolarization, which activates voltage-dependent Ca^{2+} channels, leading to an influx of Ca^{2+} and subsequently insulin granule exocytosis (3,4).

Cloning and reconstitution studies demonstrate that the K_{ATP} channel is a hetero-octameric complex of two subunit types: the sulfonylurea receptor (SUR)1 and the inward rectifying K^+ channel Kir6.2 (3,5,6). Given the key role of the K_{ATP} channel in insulin secretion, it is not surprising that inhibiting and activating mutations in the genes encoding the subunits of this channel can result in CHI (7,8) and neonatal diabetes (9,10), respectively. More interestingly, a dominantly inherited SUR1 mutation has been reported in a Finnish family, leading to CHI early in life and impaired insulin secretion or mild diabetes in adulthood (11,12).

Here we report a novel, de novo heterozygous mutation in the SUR1 gene (*ABCC8*) (R370S) causing CHI in early infancy followed by overt diabetes presenting at age 10.5 years, suggesting that this mutation facilitates the development of early-onset nonautoimmune diabetes.

CASE REPORT

A female aged 10.5 years presented with fasting hyperglycemia (12.2 mmol/l), obesity (BMI 30.2 kg/m^2), and aggressive behavior. She was the last born of nonconsanguineous Palestinian parents and has seven older, healthy siblings. Both parents developed type 2 diabetes during their 5th decade of life. Her birth weight was significantly large (4,950 g) despite a normal gestational oral glucose tolerance test. From the 2nd day of life until age 3 years, she had recurrent hypoglycemic convulsions (blood glucose 1.9–2.7 mmol/l) that were initially treated by intravenous glucose infusions and later by administration of sweet drinks at home. Hypoglycemia was not documented after age 3 years, but convulsions were never adequately controlled despite antiepileptic treatment (carbamazepine, hydantoin). Electroencephalogram at age 1 year revealed diffuse epileptic activity, while brain computed tomography scan was normal. Despite nor-

mal developmental milestones, her current school performance is poor, and she behaves aggressively and uses insulting words. Her menarche was at age 10 years. Hyperphagia and obesity have been a concern since early childhood.

Except for few pink striae on the abdomen, her physical exam was unremarkable for a fully pubertal adolescent. Laboratory investigations showed a blood glucose level of 12.2 mmol/l, moderate glucosuria, and undetectable anti-GAD ($<$1.0 μ/ml) and anti-insulin autoantibodies. Thyroid function tests and prolactin were normal, and a mildly elevated urinary free cortisol (299 nmol/24 h; normal $<$250 nmol/24 h) was easily suppressed by 90% to 37.5 nmol/24 h after 1 day of 0.5 mg dexamethasone every 6 h. Neurological evaluation, electroencephalogram, and brain magnetic resonance imaging were normal. Hydantoin replacement by topiramate achieved better epileptic control.

RESEARCH DESIGN AND METHODS

Clinical studies. Before any antidiabetes treatment, a continuous glucose monitoring system (CGMS; Minimed, Sylmar, CA) was applied for 48 h, during which the patient had a low-carbohydrate diet. An intravenous glucose tolerance test (IVGTT) using 25% dextrose solution (0.5 g/kg) was performed following a 10-h fast. Glucose and insulin levels were determined at $-$5, 0, 1, 3, 5, and 10 min.

Genetic studies. DNA was extracted from leukocytes of the patient, her parents, and one brother. For the patient, all coding exons and exon/intron boundaries of *KCNJ11* and *ABCC8*, coding for Kir6.2 and SUR1 components of K_{ATP} channel, respectively, were PCR amplified and sequenced as previously described (13,14). Genetic markers were used to confirm parenthood, and the relevant DNA segment was sequenced in both parents. DNA from 1 brother and 65 unrelated Jerusalem Palestinians was analyzed by a specifically designed primer mismatch assay, which creates a new Sdu1 restriction site on the mutant allele.

Functional studies (recombinant K_{ATP} channel experiments)
Construction of SUR1 and Kir6.2 plasmids. The R370S point mutation of SUR1 was introduced into hamster SUR1 cDNA in the pECE plasmid using the QuikChange site-directed mutagenesis kit (Stratagene). The mutation was confirmed by DNA sequencing (15). Mutant clones from at least two independent PCRs were analyzed to avoid false results from undesired mutations introduced by PCR. Rat Kir6.2 cDNA is in the pCDNAI vector.
Chemiluminescence assay. Cell surface expression level was determined by a chemiluminescence assay using SUR1 containing a flag epitope (DYKD-DDDK) at the NH_2 terminal (fSUR), as previously described (16). Briefly, COSm6 cells grown on 35-mm dishes were transfected with 0.6 μg fSUR1 and 0.4 μg Kir6.2 cDNA per 3 μg Fugene6 at $\sim$70% confluency. At 48–72 h post-transfection, cells were fixed with 2% paraformaldehyde, incubated in M2 anti-FLAG antibody (10 μg/ml), then horseradish peroxidase–conjugated anti-mouse antibody (1:1,000 dilution; Jackson Immuno Research), followed by luminol. Luminescence signal was quantified using a Turner 20/20 luminometer.
$^{86}Rb^+$ efflux assay. COSm6 transiently expressing K_{ATP} channel subunits were loaded with $^{86}RbCl$ (1 μCi/ml) overnight. Before measurement of $^{86}Rb^+$ efflux, cells were incubated for 30 min at room temperature in Krebs-Ringer solution with metabolic inhibitors (2.5 μg/ml oligomycin and 1 mmol/l 2-deoxy-D-glucose). At selected time points, the solution was aspirated from the cells and replaced with fresh solution. At the end of a 40-min period, cells were lysed. The $^{86}Rb^+$ in the aspirated solution and the cell lysate was counted. The percentage efflux at each time point was calculated as the cumulative counts in the aspirated solution divided by the total counts from the solutions and the cell lysates.
Electrophysiology. Inside-out patch-clamp recording of COSm6 cells transiently expressing channel subunits was performed as previously described (4,16,17) using Axopatch 1D amplifier and Clampex 9.2 software. Recordings were made at $-$50 mV membrane potential in symmetrical K-INT solution containing (in mmol/l) 140 KCl, 10 K-HEPES, and 1 K-EGTA (pH 7.3 with KOH). For measuring MgADP, diazoxide, and glibenclamide responses, $MgCl_2$ was added for a free concentration of $\sim$1 mmol/l. Data were analyzed using pCLAMP software. Statistical analysis was performed with Origin 6.1 using an independent two-population two-tailed Student's t test.
Clinical therapeutic trials. Following the in vitro study results, oral hypoglycemic agents repaglinide (NovoNorm, 1 mg before meals for 1 day), and glibenclamide (5–10 mg b.i.d. for 4 consecutive days) were administered,

without insulin. Fasting and postprandial glucose levels were recorded and compared with pretreatment values.

RESULTS

Clinical studies. As CHI was evident in infancy, continuous blood glucose monitoring was performed to exclude occult hypoglycemic episodes. The CGMS revealed 48 h of continuous hyperglycemia (glucose 9.2–20 mmol/l) with no hypoglycemic events (Fig. 1*A*). Following a 12-h fast, while glucose was consistently high (12.2 mmol/l), insulin was 285 pmol/l, cortisol 338 nmol/l, growth hormone 81.4 pmol/l, and ammonia 44 μmol/l (reference range 10–60). β-OH-butyrate, acetoacetate, and free fatty acids were elevated at 232, 101, and 460 μmol/l, respectively. IVGTT revealed some incremental insulin secretion, up to 582 pmol/l, that failed to restore euglycemia (Fig. 1*B*) but suggested a possible role for insulin secretagogues as a treatment modality.

Genetic analysis. The increased birth weight, recurrent hypoglycemia, and consequent neurological damage suggested neonatal hyperinsulinism despite the current reduced insulin secretion. A novel heterozygous missense mutation was detected in exon 7 of *ABCC8* (AGG→AGC at the 1,111 nucleotide position) (Fig. 2*A*), predicting an arginine to serine mutation at the 370th amino acid of the SUR1 protein (R370S). Exon 7 was sequenced in both parents' DNA and was found to be entirely normal with no evidence of mosaicism. One sibling and 130 alleles from control subjects of the same ethnic background were also negative for the mutation (Fig. 2*B*). Parenthood was confirmed by genetic markers.

Expression studies. To confirm the functionality of our patient's mutation, several expression studies were performed. Cotransfection of Kir6.2 and mutant SUR1 (SUR1-R370S) in COSm6 cells demonstrated normal channel expression on the cell membrane (Fig. 3*A*). The effect of metabolic inhibition on different channel states was studied by $^{86}Rb^+$ efflux assays. Upon metabolic inhibition, wild-type (WT) channels were rapidly activated, giving rise to over 90% cumulative efflux at the end of the 40-min incubation period (Fig. 3*B*). On the other hand, the mutant channel only gave rise to $<$10% efflux. Inside-out patch clamp recordings revealed that the homozygote mutant channels completely lacked response to MgADP, in contrast to the stimulatory effect of MgADP on WT channels (Fig. 3*C*). When cells were made heterozygous by expressing both wild-type and mutant (WT:R370S) proteins to imitate our clinical setting, the metabolic inhibition–induced channel activity observed was greatly reduced and MgADP-induced current was reduced by 50% of the WT (Fig. 3*D*). These results corresponded with in vivo membrane depolarization in the fasting state, causing hyperinsulinemic hypoglycemia. Similar responses were seen when applying the K_{ATP} channel opener diazoxide, which could reverse the inhibitory effect of 0.1 mmol/l ATP on WT channels but failed to stimulate the homozygous mutated channels (Fig. 3*E*). In the heterozygous cells (WT:R370S), the channel was partially stimulated by diazoxide (Fig. 3*F*). The sulfonylurea glibenclamide, given at 10 and 100 nmol/l, blocked the WT channels but also had a similar blocking effect on the mutant channels, in homozygous and heterozygous states (Fig. 3*G* and *H*).

Therapeutic trials by insulin secretagogues. Based on some retained insulin response to IVGTT and the response of the mutated channel to glibenclamide, we studied our patient's response to insulin secretagogues. Repaglinide (1
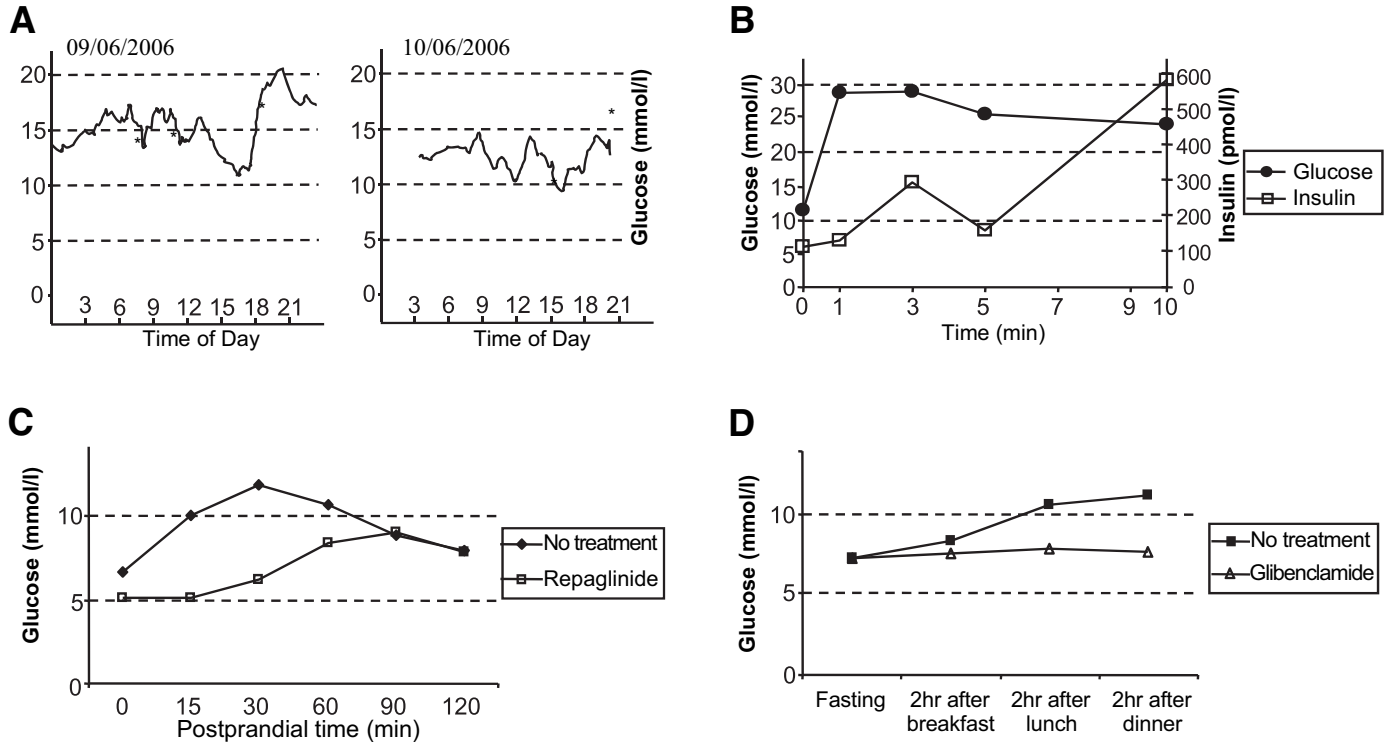
FIG. 1. Clinical studies and trials. *A*: Continuous subcutaneous glucose monitoring using the MiniMed CGMS Sensor. Measurements were made every 10 s and averaged over 5 min. During 48 h, hyperglycemia was constant, with no hypoglycemic events. *B*: IVGTT revealing a retained, although insufficient, insulin secretion capacity and failure to restore the euglycemia. *C*: A therapeutic trial with 1 mg repaglinide before meals. As an example of one out of two consecutive trials, the 2-h postprandial glucose levels following repaglinide administration decreased from 9.7–10.5 mmol/l to 5–8.3 mmol/l. *D*: A therapeutic trial with 5 mg glibenclamide twice daily lowered postprandial glucose levels from 8.3–11 mmol/l to 7.2–7.8 mmol/l.

mg), which closes K_{ATP} channels and binds to a site distinct from sulfonylureas (18), was given before meals; in two consecutive trials, it resulted in a decrease of 60-min postprandial glucose levels from 9.7–10.5 mmol/l to 5–8.3 mmol/l (Fig. 1*C*). Similarly, 5 mg glibenclamide administered twice daily also lowered postprandial glucose levels from 8.3–11 mmol/l to 7.2–7.8 mmol/l (Fig. 1*D*),

and a further increment in the glibenclamide dose seemed even more effective.

DISCUSSION

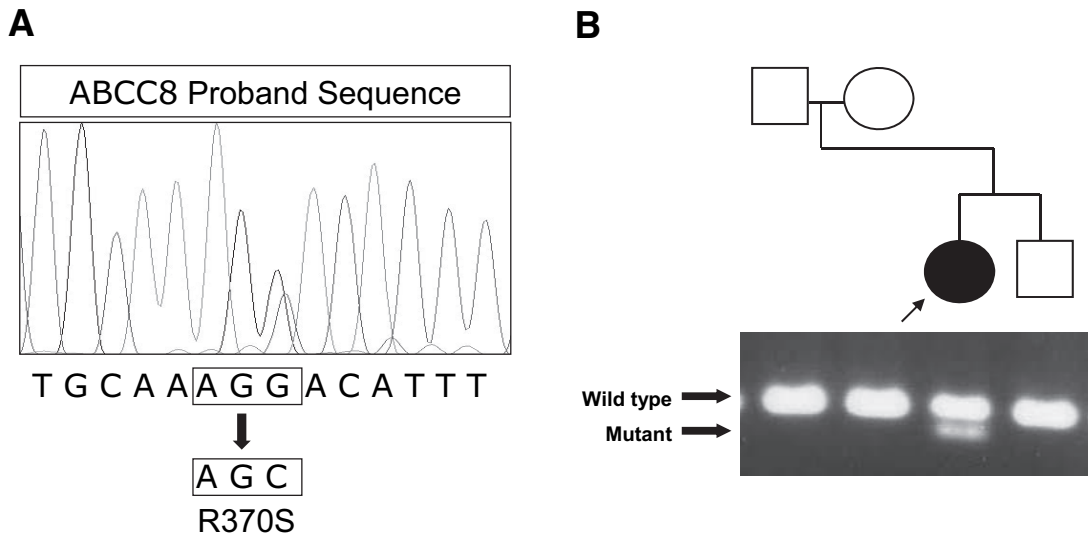Here, we report a patient with a de novo heterozygous mutation in the SUR1 gene (R370S) resulting in congenital

FIG. 2. The R370S mutation. *A*: Sequence analysis revealed a heterozygous G→C mutation in the SUR1 gene resulting in the substitution of arginine for serine at the 370th amino acid of SUR1 protein. *B*: Detection of the mutation in the proband, but not in either the parents or the healthy sibling, by primer mismatch Sdu1 restriction analysis of PCR-amplified genomic DNA. The mutant is digested, whereas the WT is not. Direct sequencing of DNA from both parents failed to reveal any evidence of mosaicism.
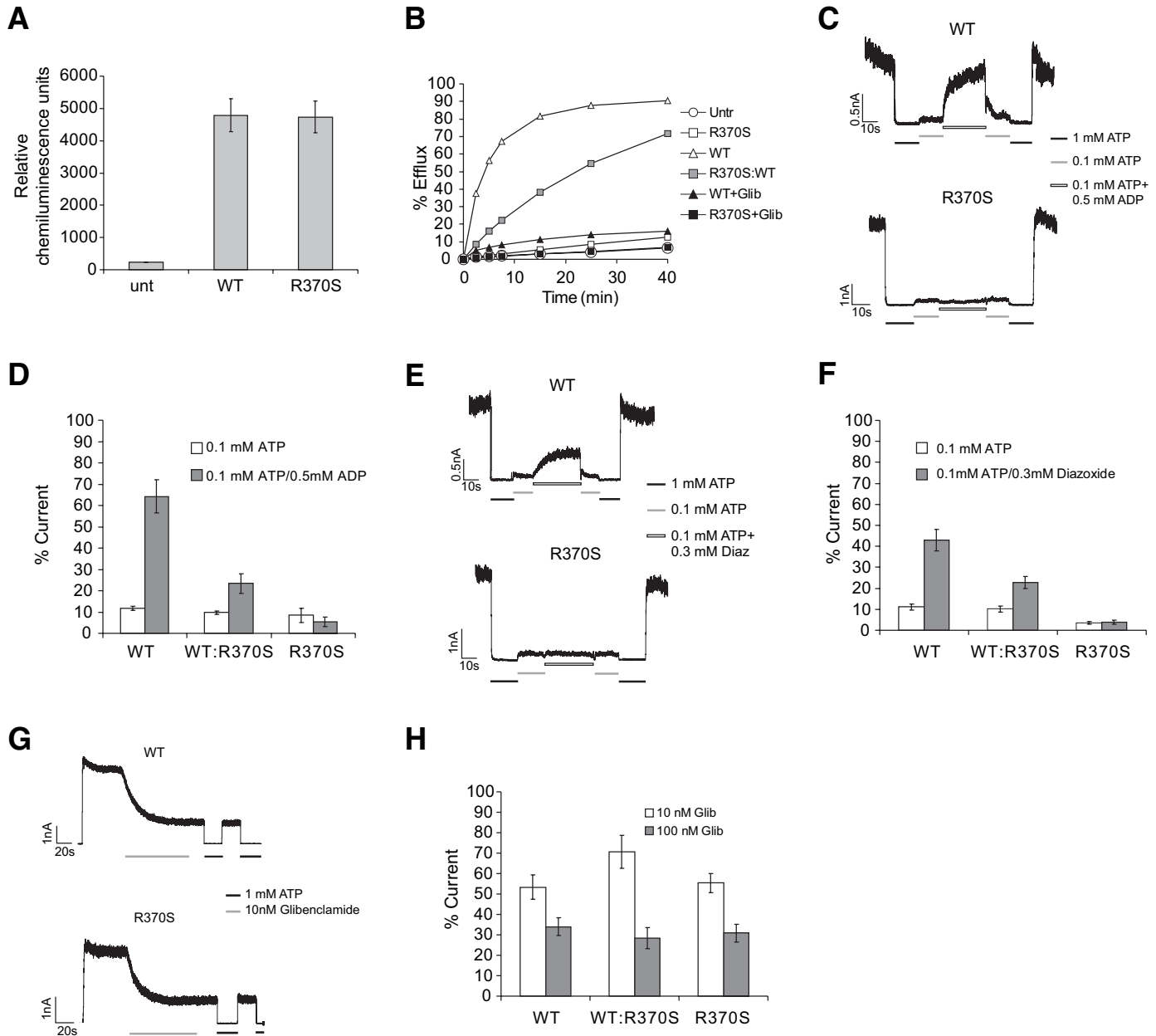
FIG. 3. Expression studies. COS cells were untransfected (unt) or transiently transfected with Kir6.2 and SUR1 of WT, R370S, or WT and R370S at 1:1 equal molar cDNA ratio (WT:R370S) and subjected to various expression and functional analyses. *A*: Surface expression level of K$_{ATP}$ channels assessed by chemiluminescence assays. The relative chemiluminescence units of untransfected, WT, and R370S samples are 234 ± 8 (*n* = 11), 4,788 ± 509 (*n* = 14), and 4,736 ± 485 (*n* = 15), respectively. The WT and R370S expression levels are not significantly different (*P* > 0.5). *B*: Channel response to metabolic inhibition assessed by ^{86}Rb^{+} efflux assays. Untransfected cells exhibited background efflux (<10% in 40 min), whereas cells expressing WT channels exhibited >90% efflux, which was inhibited by 1 μmol/l glibenclamide. Cells expressing the R370S mutant had near background flux levels, and cells expressing a mixture of WT and R370S showed intermediate flux level between WT and the mutant. *C*: Representative current traces of WT and R370S channels in response to MgADP stimulation obtained by inside-out patch clamp recording. In contrast to WT channels, which were activated by ADP, the mutant failed to be stimulated. *D*: Averaged response of channels to MgADP stimulation. The current in 0.1 mmol/l ATP or 0.1 mmol/l ATP/0.5 mmol/l ADP was expressed as percentage of those observed in K-INT solution. In 0.1 mmol/l ATP/0.5 mmol/l ADP, the percent current of WT, WT:R370S, and R370S was 64.3 ± 7.9 (*n* = 10), 23.4 ± 4.7 (*n* = 6), and 5.4 ± 2.3 (*n* = 7), respectively; the values of both WT:R370S and R370S are significantly lower than that of WT (*P* < 0.05 and 0.005, respectively). *E* and *F*: Same as *C* and *D*, except that channel response to diazoxide was examined. The percent current in 0.1 mmol/l ATP/0.3 mmol/l diazoxide for WT, WT:R370S, and R370S was 43.0 ± 5.2, 22.8 ± 3.0, and 3.9 ± 1.0, respectively. Again, both the simulated heterozygous and homozygous expression conditions gave significantly less response than WT (*P* < 0.001). *G*: Representative current traces showing channel response to 10 nmol/l glibenclamide in K-INT. Note: The inhibition was irreversible. *H*: Averaged channel response to 10 or 100 nmol/l glibenclamide. The percent current in 10 nmol/l glibenclamide was 53.3 ± 6.0 (*n* = 8), 70.6 ± 8.1 (*n* = 4), and 55.4 ± 4.8 (*n* = 5) for WT, WT:R370S, and R370S, respectively; these values are not significantly different (*P* > 0.1). In 100 nmol/l glibenclamide, the percent current was 34.0 ± 4.3 (*n* = 10), 28.4 ± 5.3 (*n* = 5), and 30.9 ± 4.3 (*n* = 5), respectively; the values are not significantly different (*P* > 0.5).

hyperinsulinism followed by overt diabetes becoming evident before age 10.5 years. *ABCC8* gene mutations have been known to cause autosomal recessive and dominant hyperinsulinism (7,11,19). Although the amino acid location of the R370S mutation could not predict its dominant effect, we demonstrated normal membrane expression, a finding common to previously reported dominant SUR1 gene mutations (16,19). As the K$_{ATP}$ channel is a heterooctamer containing four SUR1 subunits, a normally processed and translocated heterozygous mutant SUR1

subunit such as R370S would be expected to be present in 15 of 16 of the channels on the membrane and thus may alter their function. Conversely, mutated SUR1 proteins that do not reach the membrane will not result in abnormal channel activity in the heterozygous state because all channels reaching the membrane would contain four normal SUR1 subunits.

Activating SUR1 mutations were recently found to cause neonatal diabetes without antecedent hyperinsulinemic hypoglycemia (10). Huopio and coworkers (11,12) reported a dominant inactivating *ABCC8* mutation, E1506K, that caused hyperinsulinemic hypoglycemia followed by hypoinsulinemic diabetes; however, when compared with our patient, nonpancreatectomized E1506K patients in whom overt diabetes presented later, during middle age, had mild neonatal hypoglycemia. Studies of predominantly Ashkenazi Jews with recessive *ABCC8* mutations suggest that the natural history of the disease is one of clinical remission despite pancreas preservation. In some cases, the remission progressed to impairment of glucose tolerance (20).

While the molecular mechanisms causing *ABCC8* mutation-mediated CHI and neonatal diabetes have been clarified, the molecular mechanisms resulting in the progression from hyper- to hypoinsulinism are not known. A slow, progressive loss of β-cell mass due to increased β-cell apoptosis may be responsible for both the remission in CHI and for the progression to diabetes (20–22). Interestingly, the heterozygous *HNF4A* mutation causing maturity-onset diabetes of the young (MODY1) was recently found to cause a phenotype similar to that described here, with transient neonatal hyperinsulinemic hypoglycemia followed by diabetes later in life (23), implying the possibility of similar underlying pathophysiology.

Our data in COS cells suggest equal biogenesis efficiency (data not shown) and surface expression of the WT and mutant R370S proteins at the plasma membrane. Thus, it is unlikely that the decrease in functional β-cell mass is related to apoptosis induced by misfolding of the mutant SUR1 protein, as has been suggested for neonatal diabetes–associated insulin gene mutations (24). Kassem et al. (21) have previously shown increased β-cell apoptosis in patients with recessive *ABCC8* mutations. This apoptosis could be precipitated by increased metabolic stress due to continuous stimulation of insulin secretion, resulting in oxidative stress–mediated apoptosis. Alternatively, apoptosis could be initiated by chronically elevated cytoplasmic calcium levels, as have been seen in β-cells of patients with CHI (25). Studies of Kir6.2 and SUR1 knockout mouse models yielded variable phenotypes of glucose tolerance ranging from normoglycemia to diabetes, but this experimental model also appears to be associated with increased β-cell apoptosis and progression from hypoglycemia to diabetes, albeit at a different rate (26–29).

Regardless of the precise mechanism, enhanced apoptosis appears be a generic response to inactive K_{ATP} channels and not related to the specific mutation. Why then did our patient develop diabetes at such a young age (10.5 years)? It is likely that the obesity and the early puberty present in our case played an important role. Both factors contribute to insulin resistance that may have unmasked an insulin secretion defect caused by the *ABCC8* mutation. Her constant intake of sweet drinks during childhood may have caused β-cell exhaustion. Furthermore, both parents developed type 2 diabetes during their 5th decade, suggesting the presence of a relatively heavy genetic burden

for this disease. However, none of her seven older siblings developed adolescent or early adult diabetes, and adolescent-onset type 2 diabetes is rare in Palestinians despite a high prevalence of severe obesity, making it likely that the *ABCC8* mutation contributed significantly to both the obesity and the diabetic phenotype.

Our in vitro findings had significant implications for clinical management. Initially, our patient was treated with insulin. However, the surprising response of the mutated R370S K_{ATP} channel to sulfonylureas in vitro suggested the use of these medications for glycemic control. The good response to glibenclamide enabled our patient to be treated with oral medications rather than insulin injections, a significant advantage given her poor compliance. In addition, the partial response of the WT:R370S heterozygous channel to diazoxide suggests that this treatment modality might have been effective in our patient, perhaps preventing hypoglycemia and improving the neurological outcome. In retrospect, adequate treatment with diazoxide might have also prevented the need for constant glucose loading and decreased the risk of obesity, perhaps preventing or delaying the onset of diabetes.

In conclusion, we describe a new de novo mutation in the *ABCC8* gene in a patient with CHI in infancy followed by overt diabetes in childhood. This clinical phenotype gives another perspective to the crucial role of the SUR1 protein in β-cell function. The expression studies performed on the mutated SUR1 confirmed channel dysfunction and indicated a possible mechanism for dominant CHI. The clinical consideration of atypical diabetes at this young age and the in vitro studies had an important impact on patient management, suggesting the use of oral sulfonylurea treatment which resulted in improved glycemic control. The similarity between the phenotype described here and the phenotype related to *HNF4A* mutations (MODY1) emphasizes the complex interrelationship between hyperinsulinemic hypoglycemia and early-onset hypoinsulinemic diabetes. Our findings suggest a critical role for the ABCC8 R370S mutation in the early presentation of diabetes in this patient.

REFERENCES

1. Stanley CA: Hyperinsulinism in infants and children. *Pediatr Clin North Am* 44:363–374, 1997
2. Giurgea I, Bellanne-Chantelot C, Ribeiro M, Hubert L, Sempoux C, Robert JJ, Blankenstein O, Hussain K, Brunelle F, Nihoul-Fékété C, Rahier J, Jaubert F, de Lonlay P: Molecular mechanisms of neonatal hyperinsulinism. *Horm Res* 66:289–296, 2006
3. Aguilar-Bryan L, Bryan J: Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocr Rev* 20:101–135, 1999
4. Nichols CG, Shyng SL, Nestorowicz A, Glaser B, Clement JP 4th, Gonzalez G, Aguilar-Bryan L, Permutt MA, Bryan J: Adenosine diphosphate as an intracellular regulator of insulin secretion. *Science* 272:1785–1787, 1996
5. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP 4th, Boyd AE 3rd, González G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA: Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 268:423–426, 1995
6. Inagaki N, Gonoi T, Clement JP 4th, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J: Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 270:1166–1170, 1995
7. Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, Bryan J: Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 268:426–429, 1995
8. Thomas P, Ye Y, Lightner E: Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet* 5:1809–1812, 1996
9. Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS,

Howard N, Srinivasan S, Silva JM, Molnes J, Edghill EL, Frayling TM, Temple IK, Mackay D, Shield JP, Sumnik Z, van Rhijn A, Wales JK, Clark P, Gorman S, Aisenberg J, Ellard S, Njølstad PR, Ashcroft FM, Hattersley AT: Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med* 350:1838–1849, 2004

10. Babenko AP, Polak M, Cavé H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Froguel P: Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. *N Engl J Med* 355:456–466, 2006

11. 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

12. 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

13. Tornovsky S, Crane A, Cosgrove KE, Hussain K, Lavie J, Heyman M, Nesher Y, Kuchinski N, Ben-Shushan E, Shatz O, Nahari E, Potikha T, Zangen D, Tenenbaum-Rakover Y, de Vries L, Argente J, Gracia R, Landau H, Eliakim A, Lindley K, Dunne MJ, Aguilar-Bryan L, Glaser B: Hyperinsulinism of infancy: novel ABCC8 and KCNJ11 mutations and evidence for additional locus heterogeneity. *J Clin Endocrinol Metab* 89:6224–6234, 2004

14. Ashfield R, Ashcroft SJ: Cloning of the promoters for the beta-cell ATP-sensitive K-channel subunits Kir6.2 and SUR1. *Diabetes* 47:1274–1280, 1998

15. Taschenberger G, Mougey A, Shen S, Lester LB, LaFranchi S, and Shyng SL: Identification of a familial hyperinsulinism-causing mutation in the sulfonylurea receptor 1 that prevents normal trafficking and function of KATP channels. *J Biol Chem* 277:17139–17146, 2002

16. Shyng SL, Ferrigni T, Shepard JB, Nestorowicz A, Glaser B, Permutt MA, Nichols CG: Functional analyses of novel mutations in the sulfonylurea receptor 1 associated with persistent hyperinsulinemic hypoglycemia of infancy. *Diabetes* 47:1145–1151, 1998

17. Magge SN, Shyng SL, MacMullen C, Steinkrauss L, Ganguly A, Katz LE, Stanley CA: Familial leucine-sensitive hypoglycemia of infancy due to a dominant mutation of the beta-cell sulfonylurea receptor. *J Clin Endocrinol Metab* 89:4450–4456, 2004

18. Fuhlendorff J, Rorsman P, Kofod H, Brand CL, Rolin B, MacKay P, Shymko R, Carr RD: Stimulation of insulin release by repaglinide and glibenclamide involves both common and distinct processes. *Diabetes* 47:345–351, 1998

19. Thornton PS, MacMullen C, Ganguly A, Ruchelli E, Steinkrauss L, Crane A, Aguilar-Bryan L, Stanley CA: Clinical and molecular characterization of a dominant form of congenital hyperinsulinism caused by a mutation in the high-affinity sulfonylurea receptor. *Diabetes* 52:2403–2410, 2003

20. Leibowitz G, Glaser B, Higazi AA, Salameh M, Cerasi E, Landau H: Hyperinsulinemic hypoglycemia of infancy (nesidioblastosis) in clinical remission: high incidence of diabetes mellitus and persistent beta-cell dysfunction at long-term follow-up. *J Clin Endocrinol Metab* 80:386–392, 1995

21. Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B: Beta-cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes* 49:1325–1333, 2000

22. 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

23. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, Ellard S, Ferrer J, Hattersley AT: Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med* 4:e118, 2007

24. Støy J, Edghill EL, Flanagan SE, Ye H, Paz VP, Pluzhnikov A, Below JE, Hayes MG, Cox NJ, Lipkind GM, Lipton RB, Greeley SA, Patch AM, Ellard S, Steiner DF, Hattersley AT, Philipson LH, Bell GI, the Neonatal Diabetes International Collaborative Group: Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci* 104:15040–15044, 2007

25. Straub SG, Cosgrove KE, Ámmälä C, Shepherd RM, O'Brien RE, Barnes PD, Kuchinski N, Chapman JC, Schaeppi M, Glaser B, Lindley KJ, Sharp GWG, Aynsley-Green A, and Dunne MJ: Hyperinsulinism of infancy: the regulated release of insulin by K_{ATP} channel–independent pathways. *Diabetes* 50:329–339, 2001

26. Seghers V, Nakazaki M, DeMayo F, Aguilar-Bryan L, Bryan J: Sur1 knockout mice. A model for K(ATP) channel-independent regulation of insulin secretion. *J Biol Chem* 275:9270–9277, 2000

27. Shiota C, Larsson O, Shelton KD, Shiota M, Efanov AM, Hoy M, Lindner J, Kooptiwut S, Juntti-Berggren L, Gromada J, Berggren PO, Magnuson MA: Sulfonylurea receptor type 1 knock-out mice have intact feeding-stimulated insulin secretion despite marked impairment in their response to glucose. *J Biol Chem* 277:37176–37183, 2002

28. Nakazaki M, Crane A, Hu M, Seghers V, Ullrich S, Aguilar-Bryan L, Bryan J: cAMP-activated protein kinase-independent potentiation of insulin secretion by cAMP is impaired in SUR1 null islets. *Diabetes* 51:3440–3449, 2002

29. Remedi MS, Rocheleau JV, Tong A, Patton BL, McDaniel ML, Piston DW, Koster JC, Nichols CG: Hyperinsulinism in mice with heterozygous loss of K(ATP) channels. *Diabetologia* 49:2368–2378, 2006