

## Brief Report

# FAMILIAL PERSISTENT HYPERINSULINEMIC HYPOGLYCEMIA OF INFANCY AND MUTATIONS IN THE SULFONYLUREA RECEPTOR

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**P**ERSISTENT hyperinsulinemic hypoglycemia of infancy is caused by inappropriate and excessive secretion of insulin. Although the disease is rare in outbred communities (approximately 1 case per 50,000 persons), the incidence is approximately 1 per 2500 in inbred Arabic communities in which there is a familial (autosomal recessive) form of the disease. The disease most commonly presents with severe hypoglycemia a few hours after birth, although some cases present after several weeks or months. Some patients have a response to treatment with diazoxide or somatostatin, but others require partial pancreatectomy to control the hyperinsulinism.<sup>1-3</sup>

It has recently been suggested<sup>2,3</sup> that mutations within the sulfonylurea receptor, a subunit of the ATP-sensitive potassium ( $K_{ATP}$ ) channel present in the plasma membrane of pancreatic beta cells, are associated with persistent hyperinsulinemic hypoglycemia of infancy. These channels have a pivotal role in regulating insulin secretion, because their glucose-induced closure initiates the depolarization of the beta-cell membrane and the opening of calcium channels, resulting in an increase in cytosolic calci-

um, which triggers the secretion of insulin (Fig. 1).<sup>3-5</sup> The  $K_{ATP}$  channels of beta cells are formed from two distinct subunit proteins: the high-affinity sulfonylurea receptor SUR1, a member of the ATP-binding cassette superfamily,<sup>6</sup> and  $K_{IR}6.2$ , a member of the inward-rectifier family of potassium channels.<sup>7,8</sup> These proteins are encoded by two adjacent genes on chromosome 11p15.1, the same locus where the gene for persistent hyperinsulinemic hypoglycemia of infancy was mapped.<sup>2,9</sup> To test the hypothesis that a mutation in the gene for the  $K_{ATP}$  channel causes persistent hyperinsulinemic hypoglycemia of infancy, we performed studies in a child with this disorder.

## CASE REPORT

The patient was the 10th child of consanguineous Saudi Arabian parents and the 3rd child of this marriage to be affected with persistent hyperinsulinemic hypoglycemia of infancy. Her two affected siblings had undergone partial pancreatectomy for the disorder. She was born at term after a normal gestation, weighed 4.25 kg, and had macrosomia and plethora, features of in utero hyperinsulinism. Glucose was undetectable (<2 mg per deciliter [0.1 mmol per liter]) in capillary-blood samples obtained after delivery, and simultaneous intravenous infusions of glucose (17 mg per kilogram of body weight per minute) and glucagon (10  $\mu$ g per kilogram per hour) were required to maintain normoglycemia. The presence of recurrent nonketotic hyperinsulinemic hypoglycemia was confirmed during spontaneous episodes of hypoglycemia that occurred when the infusions were stopped. Typical laboratory values were as follows: blood glucose, 38 mg per deciliter (2.1 mmol per liter); serum insulin, 52  $\mu$ U per milliliter (310 pmol per liter; normal value, <4  $\mu$ U per milliliter [20 pmol per liter]); and serum proinsulin, 51 pmol per liter (normal value, <5 pmol per liter). Because of the excessive requirement for glucose, episodes of severe hypoglycemia, and lack of response to medical therapy, 95 percent of the pancreas was removed 17 days after birth (Fig. 2). Histologic examination of the pancreas revealed diffuse nesidioblastosis.<sup>1,2</sup> After surgery, the patient had recurrent hypoglycemia. A second resection of the pancreas (99 percent), performed two weeks later, resulted in hyperglycemia, necessitating insulin-replacement therapy. The child is now 18 months old and continues to require insulin-replacement therapy.

## METHODS

Our studies were approved by an institutional review committee at the Great Ormond Street Hospital for Children and the National Health Service Trust Ethics Committee, and parental consent was obtained.

### Genetic Analysis

DNA was extracted from peripheral blood with the use of the Wizard purification kit (Promega). SUR1 intron primers were used to amplify exon 35 by the polymerase chain reaction (PCR).<sup>6</sup> The reaction products were analyzed by single-strand conformation polymorphisms, digestion with the restriction enzyme *MspI*, and direct sequencing.

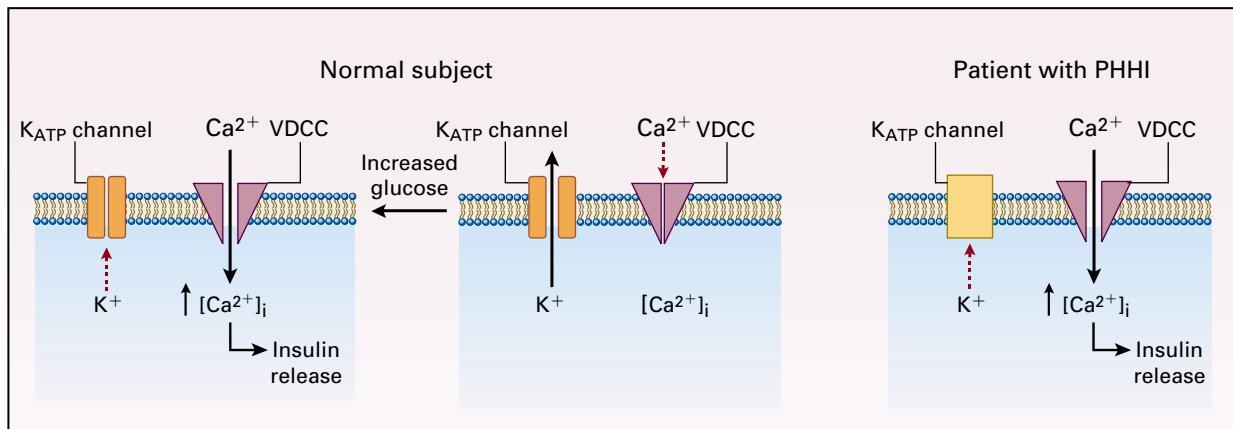
### Construction of SUR1 and $K_{IR}6.2$ Expression Plasmids

The vectors used for the expression of  $K_{ATP}$ -channel subunits were described previously.<sup>7</sup> The patient had a mutation in exon 35 that shifts the SUR1 reading frame after the arginine at codon 1437. This shift results in the addition of 23 extraneous amino acids (R1437Q(23)X) before a stop codon is encountered. We created a parallel mutation in hamster SUR1 — T1381P(20)X —

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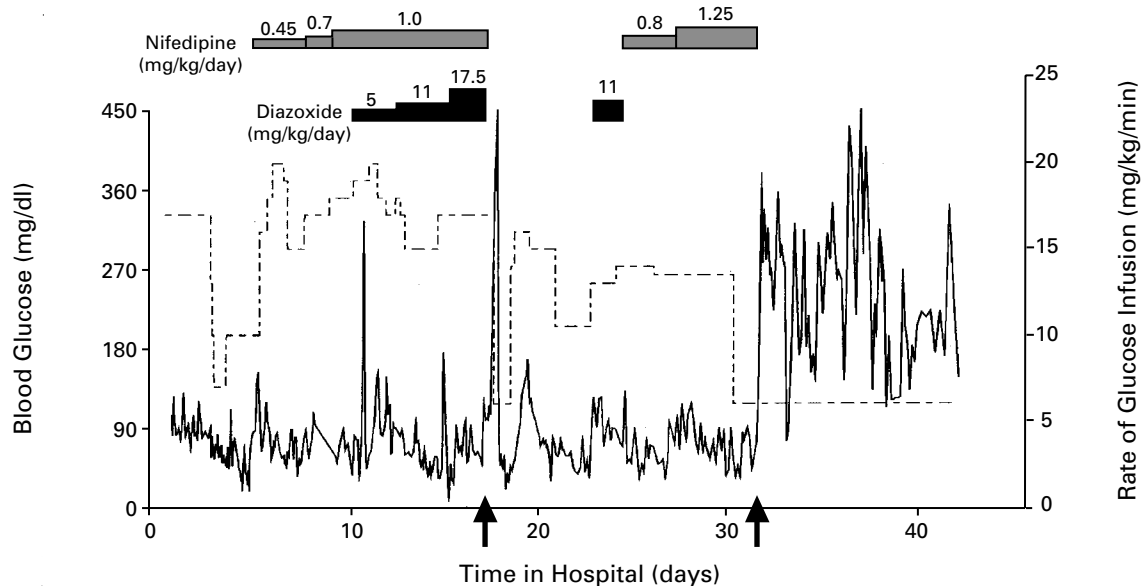
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**Figure 1.** Diagram of a Pancreatic Beta Cell Showing the Role of the ATP-Sensitive Potassium ( $K_{ATP}$ ) Channel and the Voltage-Dependent Calcium Channel (VDCC) in Insulin Secretion.

In normal beta cells, increased glucose metabolism raises the ratio of ATP to adenosine diphosphate and closes the  $K_{ATP}$  channels. As a result, the membranes are depolarized, the VDCCs open, and intracellular calcium ( $[Ca^{2+}]_i$ ) is increased, causing the release of insulin. In beta cells from patients with persistent hyperinsulinemic hypoglycemia of infancy (PHHI), the  $K_{ATP}$  channels are inactive, the cell membranes are constitutively depolarized, and the VDCCs are spontaneously active. The increase in cytosolic calcium results in the continuous release of insulin. Sulfonylurea drugs close the  $K_{ATP}$  channels and stimulate insulin secretion, whereas diazoxide opens the channels and inhibits insulin secretion.



**Figure 2.** Blood Glucose Concentrations (Solid Line) and the Rate of Glucose Administration (Broken Line) before and after Two Pancreatectomies (Arrows) in a Patient with Persistent Hyperinsulinemic Hypoglycemia of Infancy.

Nifedipine and diazoxide were administered at various doses, as shown, before both operations. To convert values for blood glucose to millimoles per liter, multiply by 0.05551.

in which the threonine at codon 1381 is followed by 20 extraneous amino acids. This mutation lies between mutations in intron 32 and exon 35 that have been described previously<sup>2,3</sup> and that completely remove the second nucleotide-binding fold from *SUR1*. These two mutations are present in familial cases of persistent hyperinsulinemic hypoglycemia of infancy. For technical reasons, it is not possible to recreate the exact mutation in exon 35. Deletions of more than 12 to 14 amino acids from the C-terminal end of *SUR1* result in inactive  $K_{ATP}$  channels (unpublished data). Thus, the mutation we created accurately reflects the physiologic consequences of the exon 35 truncation.

### Cell Culture and Transfection

After both operations, pancreatic islets were isolated from the resected tissue by collagenase digestion<sup>10</sup> and maintained for short periods of time (less than seven days) under standard tissue-culture conditions.

COS-1 and COSm6 cells were maintained in culture as described previously.<sup>7</sup> Studies were carried out with untransfected COS cells and cells transfected with the gene for  $\beta$ -galactosidase and with *SUR1*,  $K_{IR}6.2$ , and the mutated *SUR1*. Transfections were performed with plasmids pCMV  $K_{IR}6.2$ , pCMV hamster *SUR1*, and pCMV hamster mutated *SUR1* (the T1381P(20)X mutation).<sup>7</sup> The green-fluorescence protein was used to identify transfected cells for electrophysiologic studies.<sup>8</sup>

Electrophysiologic studies were performed with the use of beta cells obtained from the patient during surgery and from 1 normal infant and 12 normal adult cadaveric organ donors. The electrical activity of intact cells and isolated cell-free membranes was assessed with patch-clamp recordings.<sup>11,12</sup>

### Recombinant Expression System

The ability to reconstitute  $K_{ATP}$  channels<sup>7</sup> provided the opportunity to study the expression of the wild-type and mutated subunits in vitro. This investigation was undertaken with isolated cells and groups of cells by using patch-clamp recordings and rubidium-efflux techniques, respectively. Photolabeling was performed with the use of a radioactive derivative of glyburide to evaluate the integrity of the receptor as well as its ability to bind sulfonylurea drugs. Patch-clamp recordings were made at 20° to 22°C with cells identified by fluorescence with the use of whole-cell and inside-out patches, as previously reported.<sup>7</sup> Rubidium-86 efflux was measured as previously described.<sup>7</sup> Photolabeling of the native and truncated *SUR1* was carried out as previously described.<sup>6</sup>

## RESULTS

### Clinical Findings

This infant had severe hyperinsulinism; stable normoglycemia and fasting for more than four hours could not be achieved, despite increases in the infant's carbohydrate intake and treatment with several hyperglycemic drugs (glucagon, diazoxide, and nifedipine) (Fig. 2). The severity of the disease was reflected by the failure of subtotal (approximately 95 percent) pancreatectomy to restore normoglycemia and the need to perform a near-total (99 percent) pancreatectomy.

### Genetic Analysis

Analysis of single-strand conformation polymorphisms in the patient's DNA revealed a variation in the electrophoretic mobility of the exon 35 PCR product. Digestion with *MspI* confirmed the loss of

a restriction site as a result of the mutation<sup>2,3</sup> and demonstrated allelic homozygosity in the patient and heterozygosity in the parents. Direct sequencing confirmed the substitution of adenine for the terminal guanine in exon 35 of the *SUR1* gene in both the patient and the parents.

### Functional Studies of Isolated Beta Cells

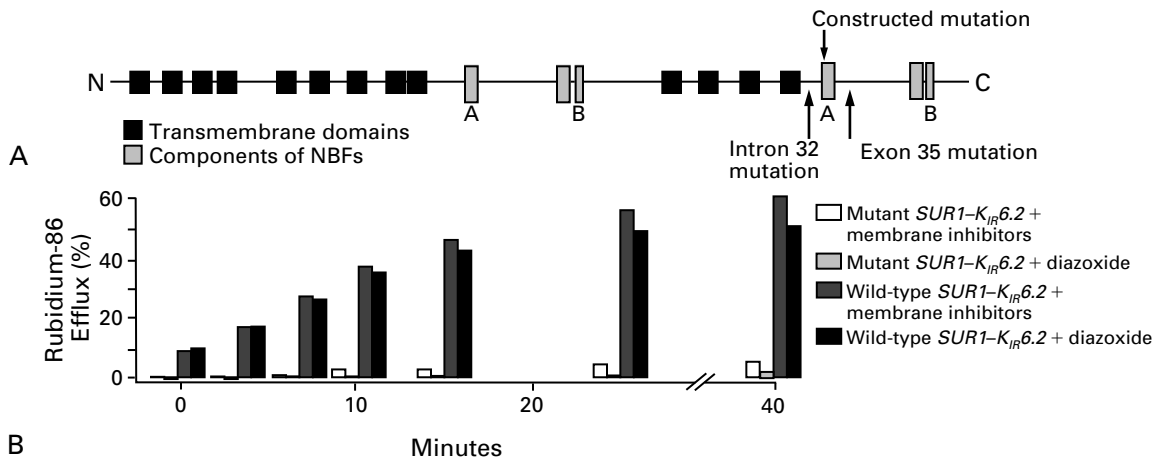
In normal beta cells,  $K_{ATP}$  channels are open so that the resting membrane potential is close to the equilibrium potential for potassium ions (approximately -70 mV). In contrast, in the beta cells from the patient, the opening of  $K_{ATP}$  channels was not detected, and the absence of activity in the  $K_{ATP}$  channels was associated with spontaneous electrical activity in the form of action potentials. The dysfunction of the  $K_{ATP}$  channels was confirmed in beta cells obtained from the patient under ATP-free conditions and by recordings made in the presence of diazoxide, a specific agonist (opener) of  $K_{ATP}$  channels in normal beta cells.<sup>13</sup> We believe that the spontaneous nature of the electrical events in the patient's beta cells indicates that the loss of  $K_{ATP}$ -channel function removed the intrinsic control of the membrane potential, leading to the persistent activation of voltage-dependent calcium channels and unregulated secretion of insulin.

### Functional Studies of Reconstituted $K_{ATP}$ Channels

Figure 3A shows the constructed mutation, with the mutations in intron 32 and exon 35 on either side. Photolabeling of the native receptor with an analogue of glyburide<sup>3</sup> resulted in a band at 140 kd, whereas the band for the truncated receptor was at 120 kd. In COSm6 cells expressing wild-type  $K_{IR}6.2$  and truncated *SUR1*, the efflux of rubidium-86 was less than 5 percent of that in cells with wild-type *SUR1*, after the addition of metabolic inhibitors (which reduce the ratio of ATP to adenosine diphosphate)<sup>6</sup> or diazoxide (Fig. 3B), a finding consistent with the loss of  $K_{ATP}$ -channel activity in the reconstituted system. Finally, the cells transfected with the mutated *SUR1* construct and  $K_{IR}6.2$  had no  $K_{ATP}$ -channel activity and did not respond to diazoxide or metabolic inhibitors, whereas the cells transfected with wild-type *SUR1* and  $K_{IR}6.2$  had normal  $K_{ATP}$ -channel activity and responded to both diazoxide and metabolic inhibitors.

## DISCUSSION

$K_{ATP}$  in beta cells is composed of two subunits: the high-affinity sulfonylurea receptor *SUR1* and  $K_{IR}6.2$ , a subunit of the inward-rectifier potassium-channel family.<sup>3,7,8</sup> We found that beta cells from a patient with familial hyperinsulinemic hypoglycemia of infancy had a mutation in exon 35 of the *SUR1* gene that results in the absence of  $K_{ATP}$ -channel activity in these cells. The mutation truncates the second nu-



**Figure 3.** Predicted *SUR1* Gene Product and Rubidium-86 Efflux in COSm6 Cells Transfected with *SUR1* and *K<sub>IR</sub>6.2*.

Panel A shows the predicted gene product and the corresponding truncation in a patient with persistent hyperinsulinemic hypoglycemia of infancy, as well as the constructed mutation (T1381P(20)X). NBF denotes nucleotide-binding fold, and A and B denote the Walker-motif characteristics of the nucleotide-binding fold. Panel B shows rubidium-86 efflux in COSm6 cells transfected with wild-type *SUR1* or the constructed mutation in *SUR1* and *K<sub>IR</sub>6.2* and treated with metabolic inhibitors (which should lower the ratio of ATP to adenosine diphosphate and open the *K<sub>ATP</sub>* channels, thus increasing the efflux of rubidium-86) or diazoxide (300  $\mu$ M), a *K<sub>ATP</sub>*-channel opener. Each bar represents the mean ( $\pm$ SE) value from three experiments.

cleotide-binding fold of *SUR1*. Coexpression of a similarly truncated *SUR1* with wild-type *K<sub>IR</sub>6.2* in COS cells also resulted in the absence of *K<sub>ATP</sub>*-channel activity. The loss of channel activity resulting from the truncation of *SUR1* was confirmed by patch-clamp recordings with beta cells from the patient and transfected COSm6 cell membranes that carried a parallel mutation, both in the presence of pharmacologic modulators of *K<sub>ATP</sub>* channels and in the absence of ATP.

These results indicate that *K<sub>ATP</sub>* channels are critical for the regulation of insulin secretion. In a patient with persistent hyperinsulinemic hypoglycemia of infancy, dysfunctional *K<sub>ATP</sub>* channels leave beta cells incapable of regulating their membrane potential, and as a result, when blood glucose concentrations are low, the beta cells remain active because of continuous depolarization of the membrane and the influx of calcium. We conclude that familial persistent hyperinsulinemic hypoglycemia of infancy is a potassium-channel disorder that results from an alteration in the function of the *SUR1* receptor in beta cells.

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