# Permanent Neonatal Diabetes Caused by Dominant, Recessive, or Compound Heterozygous SUR1 Mutations with Opposite Functional Effects

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Heterozygous activating mutations in the *KCNJ11* gene encoding the pore-forming Kir6.2 subunit of the pancreatic beta cell $K_{ATP}$ channel are the most common cause of permanent neonatal diabetes (PNDM). Patients with PNDM due to a heterozygous activating mutation in the *ABCC8* gene encoding the SUR1 regulatory subunit of the $K_{ATP}$ channel have recently been reported. We studied a cohort of 59 patients with permanent diabetes who received a diagnosis before 6 mo of age and who did not have a *KCNJ11* mutation. *ABCC8* gene mutations were identified in 16 of 59 patients and included 8 patients with heterozygous de novo mutations. A recessive mode of inheritance was observed in eight patients with homozygous, mosaic, or compound heterozygous mutations. Functional studies of selected mutations showed a reduced response to ATP consistent with an activating mutation that results in reduced insulin secretion. A novel mutational mechanism was observed in which a heterozygous activating mutation resulted in PNDM only when a second, loss-of-function mutation was also present.

The *KCNJ11* (MIM 600937) and *ABCC8* (MIM 600509; GenBank accession number NM_000352.2 incorporating the alternate exon 17 [GenBank L78208 and L78224]) genes encode the Kir6.2 and SUR1 subunits of the hetero-octomeric ATP-sensitive potassium ($K_{ATP}$) channel in the pancreatic beta cell. $K_{ATP}$ channels link cellular metabolism to electrical activity of the plasma membrane and play a key role in regulating insulin secretion. Mutations in the genes encoding Kir6.2 and SUR1 were first reported to cause a recessive form of congenital hyperinsulinism (MIM 601820 and 256450) in patients with loss-of-function mutations that abrogate channel function.[1,2] Heterozygous gain-of-function mutations in the *KCNJ11* gene encoding Kir6.2 impair the ability of ATP to close the channel and are the most common cause of permanent neonatal diabetes (PNDM [MIM 606176]).[3,4] Most of these patients achieve improved glycemic control after transfer from insulin injections to sulphonylurea tablets.[5] PNDM resulting from heterozygous activating SUR1 mutations has been reported in six patients,[6–10] and seven index cases with transient neonatal diabetes have been described.[7] No homozygous or compound heterozygous $K_{ATP}$ channel mutations have been reported in neonatal diabetes.

We studied 84 patients who received a diagnosis of diabetes in the first 6 mo of life who were treated with insulin from the time of diagnosis. Many of these patients were recruited from among the members of the International Society of Paediatric and Adolescent Diabetes (ISPAD). Heterozygous *KCNJ11* mutations were identified in 25 patients by sequencing using methods described elsewhere.[11] Mutations in the *ABCC8* gene were sought in the remaining 59 patients by sequence analysis of the 39 exons and conserved splice sites, with use of the primers listed in table 1.

*ABCC8* mutations were identified in 16 probands (fig. 1 and table 2) and included 17 novel mutations, which were not found in 250 control chromosomes. Of these, 15 were novel missense mutations affecting residues that show evolutionary conservation from human to mouse. Apparent spontaneous mutations were confirmed by testing parental and proband DNA samples with use of a panel of six microsatellite markers on chromosome 11p15.[11]

Heterozygous de novo mutations V86A, V86G, F132L, F132V, D209E, Q211K, and L225P were present in eight patients (table 2). In three further families in which children with PNDM were born to unaffected parents, novel *ABCC8* mutations were identified, but the mode of inheritance was recessive rather than dominant (fig. 1). Two homozygous mutations, E382K and A1185E, were present in probands from pedigrees in which the parents are first

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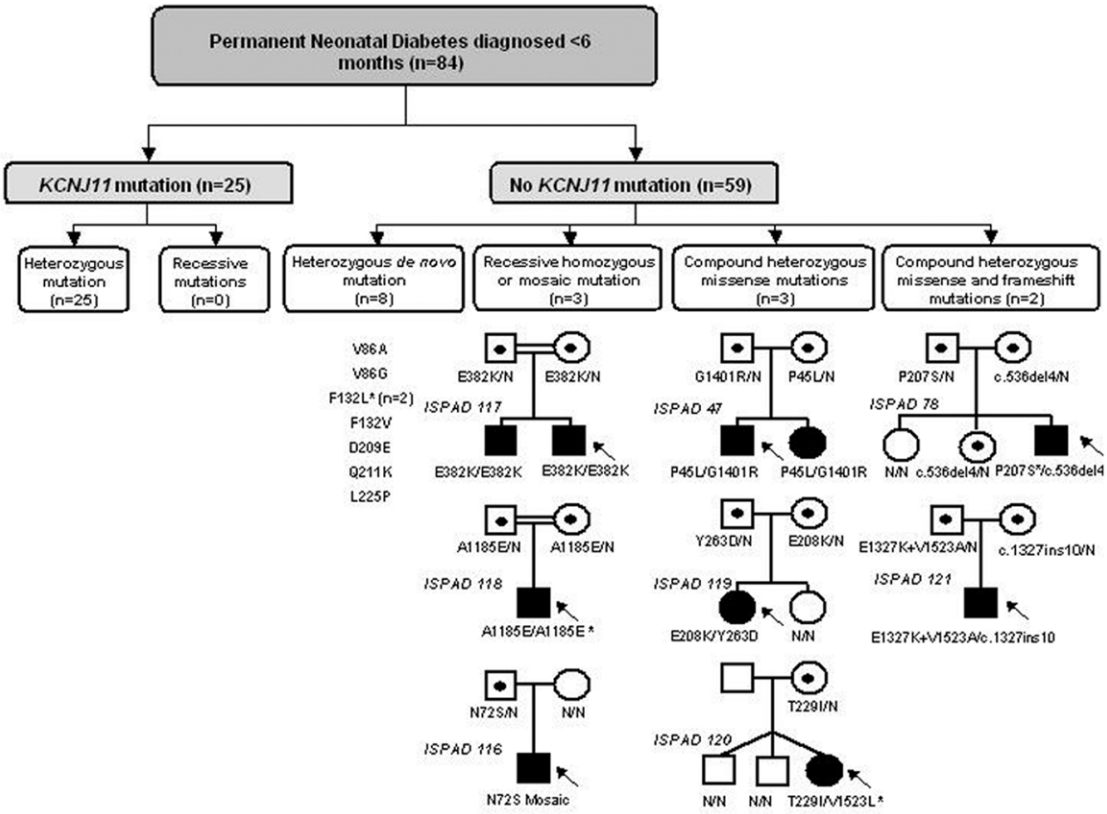


**Figure 1.** *ABCC8* and *KCNJ11* gene mutations in patients with PNDM. Pedigrees are shown for recessively inherited *ABCC8* mutations. Solid symbols denote patients with neonatal diabetes and unaffected heterozygous mutation carriers are indicated by a dot. Asterisks denote those mutations selected for functional analysis.

cousins (ISPAD 117 and 118; see fig. 1). The heterozygous parents were not diabetic. One patient with PNDM (ISPAD 116) had the N72S mutation and mosaic segmental paternal isodisomy for chromosome 11pter to 11p14, demonstrated by microsatellite analysis of markers *D11S2071, D11S922, D11S4177, TH, D11S1318, HBB, D11S4149, D11S1794, D11S904, D11S907, D11S911, D11S4143,* and *D11S1332*. This region includes the *ABCC8* gene, and thus uniparental disomy has unmasked a recessively acting mutation. The father is heterozygous for the mutation but does not have diabetes.

Compound heterozygous mutations were identified in five patients (fig. 1). Three probands were compound heterozygotes for the missense mutations P45L/G1401R (ISPAD 47), E208K/Y263D (ISPAD 119), and T229I/V1523L (ISPAD 120). The parents were heterozygous for a single missense mutation, but none were affected with diabetes (fig. 1). Unexpectedly, two patients were found to be compound heterozygotes for missense and frameshift SUR1 mutations. These heterozygous insertion/deletion mutations in families 78 and 121 (see table 1) result in premature termination codons that are predicted to be loss-of-function mutations typically associated with recessive hyperinsulinism. We showed—in a lymphoblastoid cell line from the unaffected mother of ISPAD 78, who is heterozygous for the c.536del4 mutation—that the mutant mRNA transcript level was reduced to ~25% of the normal transcript (see appendix A for details of methodology and fig. 2 for results). The mutant mRNA transcript is likely to be degraded by nonsense-mediated decay, because incubation with the translation inhibitor cycloheximide increased the expression of this transcript twofold (fig. 2). The second mutation in this family is a novel missense mutation, P207S (see table 2). Three different mutations were detected in proband ISPAD 81; the unaffected mother is heterozygous for the frameshift mutation c.3127ins10 and the unaffected father carries E1327K and V1523A in *cis* (fig. 1). No cell line was available from the heterozygous frameshift-mutation carrier in this family, but this mutation is also likely to result in loss of function, with reduction in mRNA levels and consequently reduced protein levels in the cell membrane. We propose that these loss-of-function mutations lead to a decrease in functional protein and that hence the majority of pancreatic $K_{ATP}$ channels will be homomeric for the missense mutation (see fig. 3).

**Table 1. Primer Sequences for Amplification of *ABCC8***

| Exon | Primer (M13 tailed) Forward[a] | Reverse[b] | Size (bp) |
|---|---|---|---|
| 1 | AGCTGCAAGGGACAGAGG | GAGTGAAGGGATGAGCTGG | 578 |
| 2 | GGGATCCTCATGTGTAGGTG | AGAAGACACTGAGCTGCTGG | 404 |
| 3 | CCCAAATACGCCCATTAAC | GTCTATCCTCCTTCACCAGAC | 442 |
| 4 | AAATGTACACACCCAGGCAC | GGGTAAAACAAGCTGATCCC | 411 |
| 5 | AATTACAGGCGAAAGATGGC | CTCACCAGCCTCAGTTTCC | 501 |
| 6 | CCTATTTGCAGAGGATGTGAC | GATACTGCTATGGGCTTTGC | 454 |
| 7 | GCACAAGCCTTTGCAAGC | AAACATCGTTAATGGGCAAC | 432 |
| 8 | AAGTTGGAACGGTGATACAG | TGTGAAAGGTACAGGCAAGC | 435 |
| 9 | GATAATTTGGAAACCTGGGC | TGAAGTGGCCTACTCAAAGTC | 387 |
| 10 | TCTGGGAAATGGAGTCAATG | GAGTCGGATAATCTCAAGGC | 432 |
| 11 | TCCTGGGCATCTCCATG | GTGGAGAGCCTGTCTTCTGAGG | 260 |
| 12 | ATGAAGGTGTCTCCAACTAAAAGAT | ATCACTCGAGCAAGCCTTG | 399 |
| 13 | GAGCTCTCTATCAGGCTGCGCCCTCT | ATGCTGGGAGTAGCAAGGGGAG | 254 |
| 14 | GCTGTGTCGGACTTCTGCCTTT | GCTCCCTCTGGGAGTTGGTG[c] | 302 |
| 15 | GTGTCTTTTGGCTTTCATGG[c] | CAATAAATGCAGCTTTGTCT[c] | 300 |
| 16 | TCTGGGTAATGGTTGTTCAG[c] | TCCAATAAATGTGTGTGCAT[c] | 293 |
| 17 | ACAGAGGCCATTTGGAAAC | TCTGAAAATATGTAGGCTGCAC | 298 |
| 18 | TCTCTATGCAGCATTTGTGG | AATGGATGCACAGAAACAGC | 366 |
| 19 | AGACCCAGACCTCTCAAACC | GGTGCACCATATGGAGAGG | 439 |
| 20 | GAGGCCTATTAAAGCCATTG | TGTTTGACCTTACTGCAGGC | 348 |
| 21 | TGTCTCCTGAATGGCTGAGG | GGGAGATTGTTGGATGATGG | 194 |
| 22 | TCCAAAGCCACACAGCTAAC | CCAGTGCTGGTCTCTTATGC | 404 |
| 23 | GGTGGCCATTTGTAGTGAGG | AGCAGAACCTTTGCATCCAG | 286 |
| 24 | TGAATGTGTGTCTGTCTGCC | CAGAGGGAAGCCATTTAATC | 370 |
| 25 | GGTGTGGTGCAGTAGTGTGC | AGTGATTTGGAGTTCCAGGG | 473 |
| 26 | CAGGAACTGCTCCCTCAGC | CACTGTCTCCTTGAGTCACCC | 614 |
| 27 | TGAATGACTCCAGAGACACTTA[c] | AGACAGGAGAAGCCCCCAG[c] | 282 |
| 28 | AGTCTGGGCAACAGTGAGAC | TAGGGCGGTGGAATAAGATG | 466 |
| 29 | CACGGGGTAAGAAGCTGAG | GCTTGAGAGAGAACGTGTCC | 349 |
| 30 | GACATTCCAGAGAGGGATAGC | ACACTAGGAGGACCACCAGG | 411 |
| 31 | CCCTTGTGTGTGTCTGGTG | AACCTCCACCTGTCTGGG | 454 |
| 32 | GATGGCAGCAAAAGGAATC | AGTTCTTTGGGATCAGCG | 411 |
| 33 | AGTCCAAGGAGGAGTGTGTC | AGCATTGGGTTGGGCCCG | 307 |
| 34/35 | CCCTGTGACCTCCCACACCT | GATCTGATGGAACTGAGCC | 515 |
| 36 | ACCACCTCGGTGCTTCTC | TAGGACTAAATGGTCCTGCC | 363 |
| 37 | CCATGCACACATTTTCCAAC | ATCCCACTAAACCCTTTCCAA[c] | 325 |
| 38 | GGACTAGGATCGGGGTCAG | TGCTTCAGGGTTCTTTCTTG | 306 |
| 39 | TCATCCTCCTCCTCCAAGC | GTATGGGCAGGGTCCGAAT | 322 |

[a] All forward primers start 5′-TGTAAAACGACGGCCAGT.
[b] All reverse primers start 5′-CAGGAAACAGCTATGACC.
[c] Primers described by Hansen et al.[12]

The location of the SUR1 missense mutations in patients with PNDM is shown in fig. 4. One example of each type of genetic mutation (as shown in fig. 1) was tested in an in vitro functional assay using *Xenopus* oocytes (see appendix A). All mutations caused a significant increase in the ATP concentration required to half-maximally block the channel (figs. 5 and 6). Importantly, the $K_{ATP}$ current at physiological ATP concentrations (3 mM MgATP) was also significantly increased (fig. 5). This is expected to produce an increased $K_{ATP}$ current in the beta cells of affected patients and thus impair insulin secretion. We also examined the ATP sensitivity of the $K_{ATP}$ channels expected to occur in the unaffected parents. Compared with homomeric A1185E or T229I/V1523L channels, the heterozygous A1185E, T229I, and V1523L channels showed greater ATP sensitivity and smaller $K_{ATP}$ currents at 3 mM MgATP (see fig. 6). However, there was some overlap between the functional effects of the mutations present in unaffected heterozygotes and those in patients with PNDM (fig. 5). Although none of the heterozygous mutations had more-severe effects than their homozygous counterpart, a clear distinction between the functional effects of the recessively acting heterozygous mutations and the dominantly acting heterozygous or homozygous mutations was not seen. This is in contrast to Kir6.2 mutations, in which the phenotype, genotype, and functional severity show a clear correlation.[4] One explanation is that the relative expression of wild-type and mutant SUR1 subunits differs between oocytes and human beta cells. An alternative view is that there exists an unidentified cytosolic modulator, which interacts differentially with wild-type and mutant SUR1, that is not present in our excised patch studies.

Our results extend the spectrum of *ABCC8* mutations causing neonatal diabetes from activating mutations with a dominant effect to include recessively acting gain-of-function mutations that result in neonatal diabetes only when homozygous, mosaic due to segmental uniparental isodisomy, compound heterozygous for another activating mutation or if the second allele is inactivated. In contrast, all *KCNJ11* mutations in patients with neonatal diabetes reported to date are heterozygous, dominant mutations. Knowledge of the mode of inheritance is important when counseling the families of patients with *ABCC8* mutations about the risk of further family members being affected. The risk that unaffected parents will have a second affected child is considerably higher for recessively acting mutations than for de novo dominant

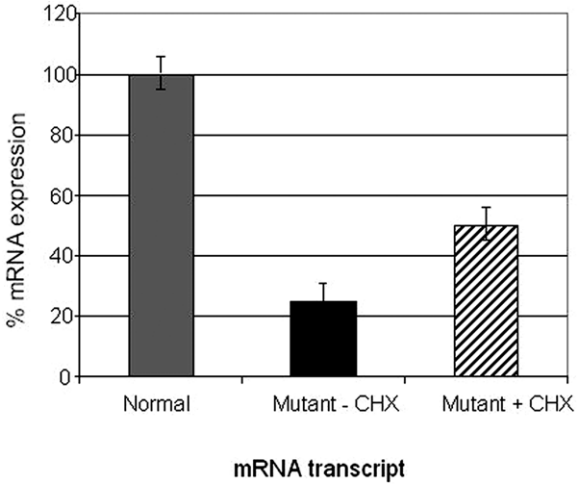


**Figure 2.** Quantification of normal and c.536delATGG mutant transcripts in a heterozygous lymphoblastoid cell line. The relative levels of normal (*gray bar*) and mutant mRNA transcripts were quantified in a lymphoblastoid cell line derived from the proband's mother, who was heterozygous for the c.536delATGG mutation. Error bars show the upper and lower limits of quantification based on three independent measurements. Cells were incubated in the presence (*diagonal stripes*) or absence (*black*) of cycloheximide (CHX) before RNA extraction.

**Table 2. Details of *ABCC8* Mutations and Clinical Information**

| ISPAD Number | Mutation (Protein Effect) | Nucleotide Change | Zygosity | Age at Diagnosis (wk) | Birth Weight[a] (Percentile) | Neurological Feature | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Developmental Delay | Muscle Weakness | Epilepsy |
| 123 | V86A[b] | c.257T→C | Heterozygous | 8 | 2,900 (9) | No | No | No |
| 124 | V86G | c.257T→G | Heterozygous | 5 | 2,900 (13) | No | No | No |
| 68 | F132L[b] | c.394T→C | Heterozygous | 13 | 2,200 (<1) | Yes | Yes | Yes |
| 125 | F132L | c.394T→C | Heterozygous | 26 | 2,440 (9) | Yes | Yes | No |
| 82 | F132V | c.394T→G | Heterozygous | 20 | NA | No | No | No |
| 46 | D209E | c.627C→A | Heterozygous | 5 | 2,720 (13) | No | No | No |
| 134 | Q211K[b] | c.631C→A | Heterozygous | 16 | 2,400 (3) | No | No | No |
| 122 | L225P[c] | c.674T→C | Heterozygous | 4 | 2,500 (11) | No | No | No |
| 117 | E382K | c.1144G→A | Homozygous | 8 | 2,700 (4) | No | No | No |
| 118 | A1185E | c.3554C→A | Homozygous | 0 | 4,200 (95) | No | Yes | Yes |
| 116 | N72S | c.215A→G | Mosaic | 5 | 3,870 (74) | No | No | No |
| 47 | P45L + G1401R | [c.134C→T] + [c.4201G→A] | Compound heterozygous | 6 | 2,520 (18) | Yes | Yes | No |
| 119 | E208K + Y263D | [c.622G→A] + [c.787T→G] | Compound heterozygous | 13 | 2,950 (28) | Yes | No | No |
| 120 | T229I + V1523L | [c.686C→T] + [c.4567G→T] | Compound heterozygous | 4 | NA | No | No | No |
| 78 | P207S + Y179X | [c.619C→T] + [c.536_539delATGG] | Compound heterozygous | 8 | 3,290 (29) | No | No | No |
| 121 | [E1327K; V1523A] + T1043QfsX74 | [c.3979G→A; 4568C→T] + [c.3127_3129delACCinsCAGCCAGGACCTG] | Compound heterozygous | 1 | 2,380 (<1) | No | No | No |

[a] NA = not available. Birth weight is first given in grams. Birth weight percentiles were calculated using population-based data,[15] and, when information on gestational age was not available ($n = 3$), a gestational age of 40 wk was assumed.

[b] Patients ISPAD 68, 123, and 134 have been reported by us elsewhere.[9,10,14]

[c] The L225P mutation has been reported in another patient by Masia et al.[8]

mutations (25% vs. the risk of germline mosaicism), but, in the next generation, the offspring of the proband are very unlikely to be affected for a recessively acting mutation, compared with the 50% risk for a dominant heterozygous mutation.

Mutations in the *ABCC8* gene encoding the SUR1 subunit accounted for 27% of non-Kir6.2 PNDM in this cohort and are the most common cause after *KCNJ11* mutations. The phenotype was similar for dominantly acting heterozygous *ABCC8* mutations, recessive *ABCC8* mutations, and heterozygous *KCNJ11* mutations,[11] as shown by age at diagnosis ($P = .14$) and birth weight, a biomarker for insulin secretion in utero ($P = .15$). Median birth weight in this study was 2,710 g (percentile range <1–95), with one macrosomic child reported (ISPAD 118). This child received a diagnosis of diabetes in the 1st wk of life, and his mother was not known to have diabetes during the pregnancy; hence, his macrosomy remains unexplained. In contrast, for loss-of-function *ABCC8* mutations causing hyperinsulinism, the common recessive mutations result in a more severe hypoglycemia than the rare dominant mutations that can often be treated with diazoxide.[16,17] The similarity of the phenotype in gain-of-function beta cell $K_{ATP}$ channelopathies implies that diabetes results from failure of channel closure in response to ATP and that a further reduction in ATP sensitivity does not lead to a more severe beta cell phenotype.

To our knowledge, this is the first disease phenotype reported to be a result of compound heterozygosity for both gain-of-function and loss-of-function mutations. In contrast, there are many examples of genes in addition to *ABCC8* in which mutations of opposite effects can result in different phenotypes in different patients. For example, activating GCK mutations cause hypoglycemia with inactivating mutations resulting in hyperglycemia,[18] gain-of-function *RET* proto-oncogene mutations cause MEN2/FMTC with loss-of-function mutations found in Hirschsprung disease,[19] and mutations in the *SCNN1B* and *SCNN1G* genes encoding the epithelial sodium channel $\beta$ and $\gamma$ subunits can cause hypertension (Liddle syndrome; dominant gain of function) or hypotension (pseudohypoaldosteronism type 1; recessive loss of function).[20] We propose that, in the patients we have described with PNDM resulting from compound heterozygous activating/inactivating mutations, the loss-of-function mutation leads to a decrease in functional protein, and the channels are essentially homomeric for the activating SUR1 mutation. This mutational mechanism is possible for other channelopathies in which the functional channel is a multimeric structure.

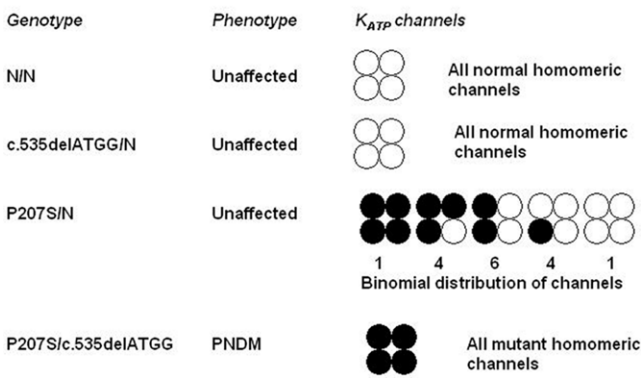


**Figure 3.** Schematic representation of $K_{ATP}$ channels in family ISPAD 78. The unaffected parents are heterozygous for the c.536del4 frameshift or P207S missense mutation. Lymphoblastoid cells showed a decrease in c.536del4 mRNA (see fig. 2), and the majority of $K_{ATP}$ channels in the proband are predicted to be homomeric for the P207S mutation.

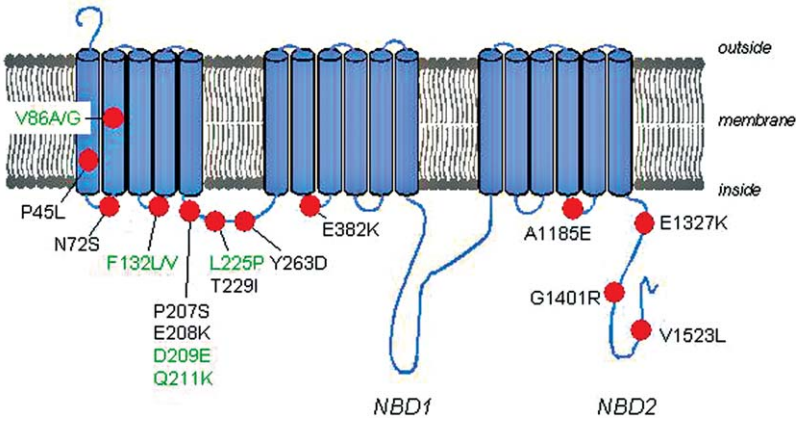


**Figure 4.** Schematic of the transmembrane topology of SUR1 showing the missense mutations identified. Recessive mutations are shown in black and dominant mutations in green. NBD, nucleotide binding domain.

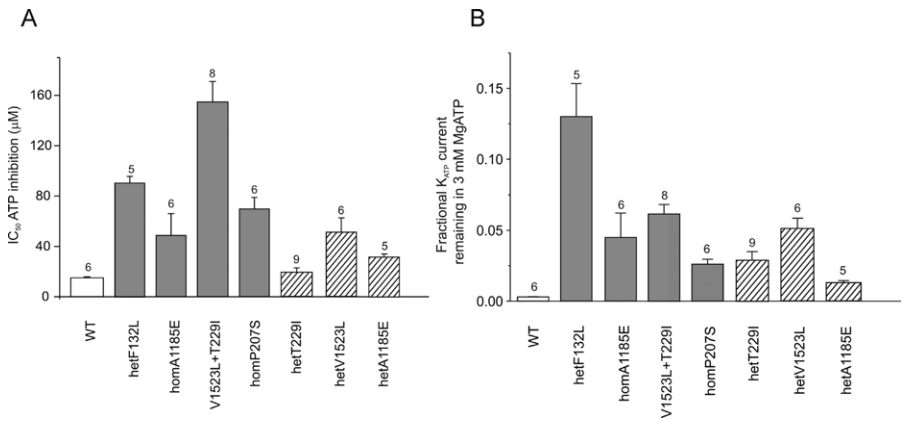


**Figure 5.** *A,* Mean ($\pm$ SEM) concentration of ATP required to half-maximally inhibit the $K_{ATP}$ current ($IC_{50}$) for the wild-type $K_{ATP}$channel (WT), channels carrying the indicated SUR1 mutations which cause neonatal diabetes (grey bars) and channels carrying the SUR1 mutations found in the unaffected parents (hatched bars). Numbers above the bars indicate the number of patches (oocytes) tested. To simulate the patient's genotype, we coinjected Kir6.2 mRNA with hetF132L (1:1 mix of WT and F132L SUR1 mRNAs); homA1185E or P207S (mutant SUR1 only); or V1523L+T229I (1:1 mix of V1523L and T229I SUR1). B, Mean ($\pm$SEM) $K_{ATP}$ current remaining in the presence of 3mM MgATP in the inside-out patch for the wild-type $K_{ATP}$ channel (WT; *white bars*) channels carrying the indicated SUR1 mutations which cause neonatal diabetes (grey bars) and channels carrying the SUR1 mutations found in the unaffected parents (hatched bars). Numbers above the bars indicate the number of patches (oocytes) tested.

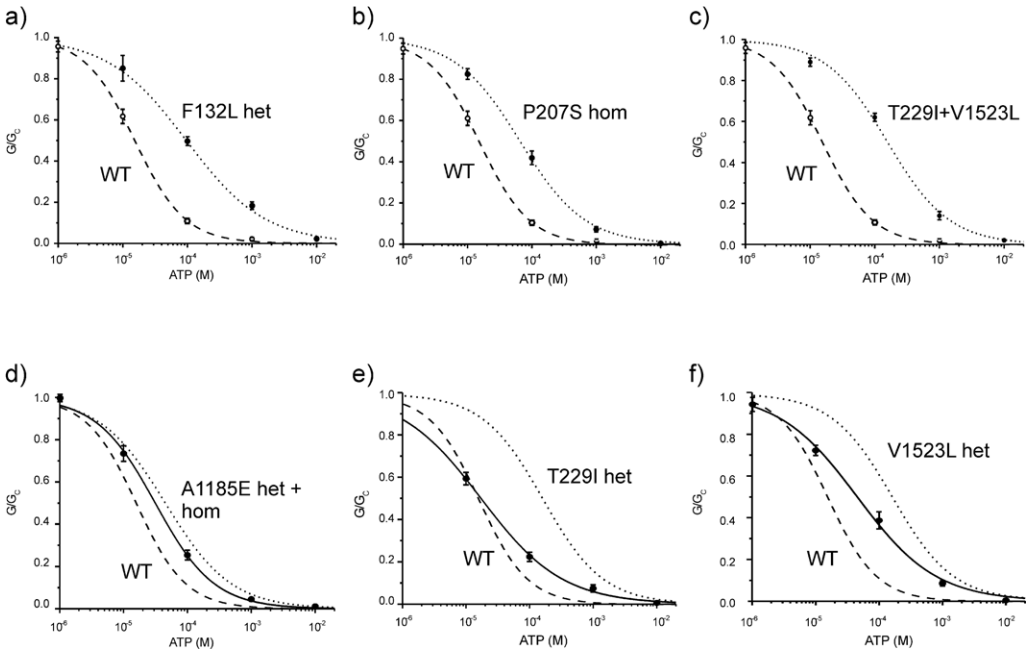


**Figure 6.** ATP sensitivity of $K_{ATP}$ channels corresponding to those found in children with neonatal diabetes and their unaffected parents. Mean relationship between [ATP] and the macroscopic $K_{ATP}$ conductance (G), expressed relative to the conductance in the absence of nucleotide ($G_C$) for Kir6.2/SUR1 channels (WT, *dashed lines*), probands (*dotted lines*) or unaffected parents (*solid lines*). The smooth curves are the best fit of the Hill equation to the mean data. For wild-type channels, $IC_{50} = 15$ $\mu$M, $h = 1.11$, $n = 6$. For hetSUR1-F132L (*a*), $IC_{50} = 105$ $\mu$M, $h = 0.72$, and $n = 5$. For homSUR1-P207S (*b*), $IC_{50} = 65$ $\mu$M, $h = 0.87$, and $n = 6$. For SUR1-T229I+SUR1V1523L (*c*), $IC_{50} = 157$ $\mu$M, $h = 0.91$, and $n = 8$. For homSUR1-A1185E (*d*), $IC_{50} = 35$ $\mu$M, $h = 0.69$, and $n = 6$. For hetA1185E channels (*d*), $IC_{50} = 32$ $\mu$M, $h = 1.0$, and $n = 5$. For hetT229I channels (*e*), $IC_{50} = 17$ $\mu$M, $h = 1.11$, and $n = 9$. For hetV1523L channels (*f*), $IC_{50} = 45$ $\mu$M, $h = 0.7$, and $n = 6$.

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## Appendix A
### *Material and Methods*

***mRNA Analysis.***—A custom real-time PCR assay for the mutant and wild-type c.536delATGG sequences was obtained from the Assays-by-Design service (Applied Biosystems). Mutant and normal sequences were amplified with a common primer set but differentiated by the binding of real-time probes labeled with different fluorochromes. Probe and primer sequences were as follows: c.536 (forward primer), 5′-GCTACGCTTCTGCCTCACA-3′; c.536 (reverse primer), 5′-CTCCTCACCCTGATGACATTGAC-3′; c536WT (wild-type probe), 5′-CATCCCATAGAGGATC-3′; and c.536delATGG (mutant probe), 5′-CAGCATCAGAGGATC-3′. Probes were labelled 5′ with 6-FAM (6-fluoroscein; mutant) and VIC (normal) and 3′ with a minor groove-binding protein (MGB) to enhance probing specificity. Assays were designed to amplify both mRNA and genomic sequences to allow the use of DNA for validation purposes. Probes and primers were validated using standard curve analysis of triplicate serial 1:2 dilutions of heterozygous cDNA to determine assay efficiency and linear range. Reactions contained 5 $\mu$l TaqMan Fast Universal Master Mix no AmpErase (Applied Biosystems), and 0.5 $\mu$l Assays-by Design probe and primer mix (corresponding to 8 $\mu$M each primer and 36 $\mu$M each probe) in a total volume of 10 $\mu$l. Amplification conditions were a single cycle of 95°C for 20 s followed by 40 cycles of 95°C for 1 s and 60°C for 20 s.

Total RNA was extracted from $2 \times 10^6$ lymphoblastoid cells derived from the heterozygous father of the proband for c.536delATGG and from two normal controls using the Eppendorf Perfect RNA mini kit. Before reverse transcription, normal and mutation-bearing mRNA samples were pretreated with TURBO-DNAse (Ambion), according to the manufacturers' instructions, to remove contaminating genomic DNA. cDNA was reverse transcribed from 5 $\mu$g total RNA by use of the Thermoscript RT-PCR system (Invitrogen). Mutant and normal sequences were then amplified from ectopic mRNA transcripts with a single-tube TaqMan approach on the ABI Prism 7900 platform (Applied Biosystems). Triplicate single-round reactions were performed for each cell line with use of 2 $\mu$l cDNA. Reaction conditions are described above. Relative quantification of the two transcripts was performed with use of the equation $2^{-\delta\delta Ct}$ described by Applied Biosystems, which refers to the efficiency of the PCR and the difference between the PCR crossing points (the point at which the signal becomes visible over the background) for each allele normalized to a DNA control (representing a 1:1 ratio of mutant and wild-type targets). This is an accurate measurement of relative abundance, which is independent of amplification and detection efficiency.

To establish whether differences in normal and mutant transcripts were due to the action of the nonsense-mediated decay pathway on abnormal transcripts containing premature-termination codons (PTCs), cultures were split into two fractions and were treated with either the translation inhibitor cycloheximide (100 $\mu$g/ml) or the inert carrier DMSO alone for a period of 4 h. After the incubation, cells were washed once with PBS (Invitrogen), were harvested, and were analyzed as described above.

***Functional Studies.***—Human Kir6.2 ([GenBank] NM_000525 with E23 and I337) and SUR1 ([GenBank] NM_000352 incorporating the alternate exon 17; also L78208 and L78224) were used in this study. Both wild-type cDNAs were cloned in the pBF vector. SUR1 mutants were then generated by site-directed mutagenesis using the Quick-change XL system (Stratagene). After plasmid linearization and purification, capped mRNA was prepared using the mMESSAGE mMACHINE large-scale in vitro transcription kit (Ambion) as described elsewhere.[13]

Currents were recorded from *Xenopus laevis* oocytes 1–3 d after injection with 0.8 ng of wild-type Kir6.2 mRNA and ~4 ng of wild-type or mutant SUR1 mRNA (a 1:5 ratio). To simulate the heterozygous state (e.g., F132L), Kir6.2 was coexpressed with a 1:1 mixture of wild-type and mutant SUR1 mRNA. To simulate the compound heterozygous state of hetSUR1-T229I+hetSUR1-V1523L, Kir6.2 was coexpressed with a 1:1 mixture of SUR1-T229I and SUR1-V1523L mRNAs.

Macroscopic currents were recorded from giant inside-out patches with use of the patch-clamp technique in response to 3-s voltage ramps from −110 mV to +100 mV (holding potential 0 mV) at 20°C–22°C. Currents were filtered at 0.15 kHz and digitized at 0.5 kHz. The pipette solution contained (mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4 with KOH). The internal (bath) solution contained (mM): 107 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with KOH) and Mg nucleotides (as indicated). The macroscopic slope conductance was measured between −100mV and +10mV. ATP concentration-response curves in the absence of $Mg^{2+}$ were fitted with the Hill equation. To control for possible rundown of channel activity, $G_c$ was taken as the mean of the conductance in control solution before and after ATP application. Concentration-response curves were fitted individually for each patch, and the mean ± SEM of the individual parameters are given in the text.

## Web Resources

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *ABCC8* [accession number NM_000352.2 incorporating the alternate exon 17: L78208, L78224], Kir6.2 [accession number NM_000525 with E23 and I337], and SUR1 [accession number NM_000352 incorporating the alternate exon 17; also L78208 and L78224])

International Society for Pediatric and Adolescent Diabetes, http://www.ispad.org/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *KCNJ11, ABCC8,* congenital hyperinsulinism, and PNDM)

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