# *Original Article*

# Functional Effects of Mutations at F35 in the $NH_2$-terminus of Kir6.2 (*KCNJ11*), Causing Neonatal Diabetes, and Response to Sulfonylurea Therapy

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**Heterozygous mutations in the human Kir6.2 gene (*KCNJ11*), the pore-forming subunit of the ATP-sensitive $K^+$ channel ($K_{ATP}$ channel), cause neonatal diabetes. To date, all mutations increase whole-cell $K_{ATP}$ channel currents by reducing channel inhibition by MgATP. Here, we provide functional characterization of two mutations (F35L and F35V) at residue F35 of Kir6.2, which lies within the $NH_2$-terminus. We further show that the F35V patient can be successfully transferred from insulin to sulfonylurea therapy. The patient has been off insulin for 24 months and shows improved metabolic control (mean $HbA_{1c}$ 7.58 before and 6.18% after sulfonylurea treatment; $P$ < 0.007). Wild-type and mutant Kir6.2 were heterologously coexpressed with SUR1 in *Xenopus* oocytes. Whole-cell $K_{ATP}$ channel currents through homomeric and heterozygous F35V and F35L channels were increased due to a reduced sensitivity to inhibition by MgATP. The mutation also increased the open probability ($P_O$) of homomeric F35 mutant channels in the absence of ATP. These effects on $P_O$ and ATP sensitivity were abolished in the absence of SUR1. Our results suggest that mutations at F35 cause permanent neonatal diabetes by affecting $K_{ATP}$ channel gating and thereby, indirectly, ATP inhibition. Heterozygous F35V channels were markedly inhibited by the sulfonylurea tolbutamide, accounting for the efficacy of sulfonylurea therapy in the patient. *Diabetes* 55: 1731–1737, 2006**

Heterozygous mutations in *KCNJ11* have been shown to be a common cause of neonatal diabetes (1,2). These mutations cause pronounced hyperglycemia, which may be either transient or permanent, and is generally diagnosed within the first 3 months of life (3–6). Some mutations can also cause developmental delay (3) or a severe neurological phenotype consisting of marked developmental delay, motor weakness, and epilepsy (DEND syndrome) (2,5).

Kir6.2, the gene product of *KCNJ11*, serves as the pore-forming subunit of the ATP-sensitive $K^+$ channel ($K_{ATP}$ channel) in multiple tissues, including pancreatic β-cells (7,8). Four Kir6.2 subunits form a tetrameric pore, whose opening and closing (gating) is modulated by four regulatory sulfonylurea receptors (SURs) (9). There are two main isoforms of SUR, with that of the β-cell $K_{ATP}$ channel being SUR1 (*ABCC8*) (10). Binding of ATP to Kir6.2 closes the channel, whereas MgADP opens the channel and reverses channel inhibition by ATP by interaction with SUR1 (11–13).

$K_{ATP}$ channels mediate glucose-stimulated insulin secretion from the pancreatic β-cell (11,14). At substimulatory glucose concentrations, $K^+$ efflux through open $K_{ATP}$ channels holds the β-cell membrane at a hyperpolarized potential, preventing electrical activity, calcium influx, and insulin secretion. Increased glucose uptake and metabolism by the β-cell leads to a rise in ATP and an accompanying decrease in MgADP that closes $K_{ATP}$ channels. This produces insulin release by promoting membrane depolarization, opening of voltage-gated $Ca^{2+}$ channels, and $Ca^{2+}$ influx (11,14). Mutations in $K_{ATP}$ channel subunits that reduce channel function lead to congenital hyperinsulinism (15), whereas those that enhance channel function cause diabetes (1,2). $K_{ATP}$ channels are also the target for sulfonylurea drugs, such as tolbutamide and glibenclamide, which are used to treat type 2 diabetes (16). These drugs stimulate insulin secretion by binding to SUR1 and closing $K_{ATP}$ channels directly, thus bypassing β-cell metabolism. They have proved effective in treating neonatal diabetes that results from gain-of-function mutations in Kir6.2 (2,5).

To date, mutations in Kir6.2 associated with neonatal diabetes have been shown to enhance $K_{ATP}$ currents by reducing the ability of ATP to inhibit channel activity (3,4,17–20). They may also increase the sensitivity to Mg-nucleotides (19,20). Almost all of these mutations lie within the ATP-binding site or in regions of the channel that are predicted to be involved in the opening and closing of the channel pore. In this article, we explore the functional effects of two mutations at residue F35 (F35V and F35L), which is not located in either of these regions. These mutations give rise to neonatal diabetes without neurological complications (5,6). We also report the response of the patient carrying the F35V mutation to sulfonylurea therapy.

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$K_{ATP}$ channel, ATP-sensitive $K^+$ channel; SUR, sulfonylurea receptor.

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## RESEARCH DESIGN AND METHODS

**Subject and clinical studies.** A 6-year-old boy from Norway with permanent neonatal diabetes due to a heterozygous F35V mutation in Kir6.2 has been partly described elsewhere (5). Here, we report the results of a sulfonylurea treatment protocol. Assays and treatment protocol were as described (5). Informed consent was obtained from the subject and parents. The studies were performed according to the Declaration of Helsinki and approved by ethical committees.

**Functional studies.** Wild-type or mutant human Kir6.2 (Genbank NM000525 with E23 and I377) and rat SUR1 (Genbank L40624) were coexpressed in *Xenopus laevis* oocytes as previously described (17,21, and online appendix [available at http://diabetes.diabetesjournals.org]). To simulate the heterozygous state, SUR1 was coexpressed with a one-to-one mixture of wild-type and mutant Kir6.2 (19). Whole-cell currents were recorded as previously described (17), at 20–22°C. The external solution contained (in mmol/l) 90 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, and 5 HEPES (pH 7.4 with KOH). Metabolic inhibition was produced by 3 mmol/l Na-azide.

Macroscopic currents were recorded from inside-out patches as previously described (17), at 20–22°C. The pipette solution contained (mmol/l) 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, and 10 HEPES (pH 7.4 with KOH). The Mg-free internal (bath) solution contained (mmol/l) 107 KCl, 1 $K_2SO_4$, 10 EGTA, 10 HEPES (pH 7.2 with KOH), and nucleotides as indicated. The Mg-containing internal solution was the same as the Mg-free solution plus 2 mmol/l $MgCl_2$ and MgATP (instead of ATP) as indicated and without $K_2SO_4$. The slope conductance was measured between −100 and +10 mV. ATP concentration-response curves were fit with the Hill equation:

$$G/G_c = 1/(1 + [\{ATP\}/IC_{50}]^h) \quad (1)$$

where {ATP} is the ATP concentration, $IC_{50}$ is the concentration at which inhibition is half maximal, $h$ is the Hill coefficient, and $G$ is the slope conductance in the presence (or absence; $G_c$) of ATP. $G_c$ was taken as the mean of the conductance in control solution before and after ATP application. In some patches, currents underwent fast rundown with a time course that was well fitted by a single exponential. In these patches, we fit an exponential function to the data in control solution before and after ATP application. The extent of block is then given by the following:

$$\sum_i^n ([G_{ATP}\{i\}/G_{control}\{i\}]/n) \quad (2)$$

where $n$ is the number of ramps during ATP application (usually five) $G_{ATP}\{i\}$ is the conductance obtained from voltage ramp i during ATP application, and $G_{control}\{i\}$ is the conductance in control solution during voltage ramp i extrapolated from the exponential fit.

Single-channel currents were recorded and open probability was determined as previously described (17). Statistical significance was evaluated using an unpaired two-tailed Student's $t$ test.

## RESULTS

**Clinical studies.** Two separate mutations, F35V (5) and F35L (6), at residue 35 of Kir6.2 have been reported to cause permanent neonatal diabetes. Neither patient had any neurological symptoms or dysmorphic features. We now present the response of the patient with the F35V mutation to treatment with sulfonylureas. Basal and stimulated serum C-peptide concentrations are shown in Table 1. Four weeks after introducing sulfonylurea in increasing doses, insulin was discontinued. Frequent capillary glucose measurements showed no deterioration as insulin was removed. The patient has been off insulin for 24 months and is doing well without any notable side effects. Table 1 shows $HbA_{1c}$ (A1C) levels before and after introducing sulfonylurea therapy and insulin requirements before sulfonylureas and with the present sulfonylurea dose. It is evident that the patient is, if anything, better controlled on sulfonylureas.

TABLE 1
Response to sulfonylurea treatment

| | Before sulfonylurea | After sulfonylurea |
|---|---|---|
| Insulin dose (units · $kg^{-1}$ · $day^{-1}$) | 0.6 | 0 |
| Sulfonylurea dose (mg · $kg^{-1}$ · $day^{-1}$) | 0 | 0.23 |
| Basal C-peptide concentrations (nmol/l) | <0.17 | 0.62 |
| Corresponding basal glucose concentrations (mmol/l) | 13.8 | 7.7 |
| Glucagon-stimulated C-peptide concentrations (nmol/l) | 0.19 | 0.86 |
| Corresponding glucagon-stimulated glucose concentrations (mmol/l) | 14.4 | 9.0 |
| A1C (%) | 7.58 ± 0.18 | 6.18 ± 0.3 |
| Muscle and neurological status | Normal | Normal |

Data are means ± SD, unless otherwise indicated. A1C is the mean of five and six measurements before and after treatment, respectively ($P < 0.007$). The intervals between measurements were ~3 months. Basal and glucagon-stimulated C-peptide and glucose concentrations were measured on a single occasion.

**$K_{ATP}$ channels carrying F35 mutations are not closed by resting ATP levels.** When expressed in *Xenopus* oocytes, wild-type $K_{ATP}$ channels are normally closed, but they are activated by metabolic inhibitors such as azide, which lower intracellular ATP (Fig. 1). In contrast, significant resting whole-cell $K^+$ currents were present in oocytes expressing either homomeric or heterozygous F35V and F35L mutant channels (Fig. 1). This result suggests that cellular metabolism causes less block of mutant $K_{ATP}$ channels than wild-type channels. Both homomeric and heterozygous mutant channel currents were

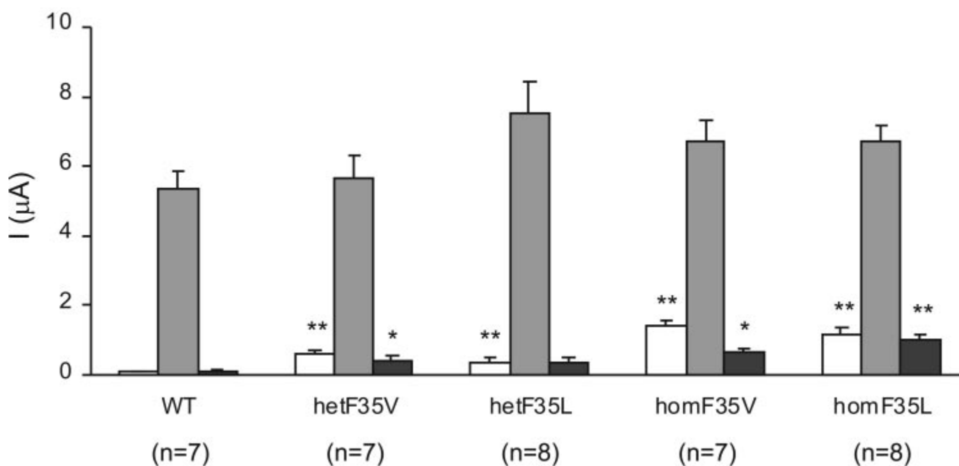


**FIG. 1. Effects of F35 mutations on whole-cell $K_{ATP}$ channel currents. Mean steady-state whole-cell currents evoked by a voltage step from −10 to −30 mV before (□; control) and after (▩) application of 3 mmol/l azide and after application of 0.5 mmol/l tolbutamide plus 3 mmol/l azide (■) for wild-type (WT), heterozygous (hetF35V and hetF35L), and homomeric mutant (homF35V and homF35L) channels. The number of oocytes is indicated below the bars. *$P$ < 0.05; **$P$ < 0.005 against wild-type currents in control solution.**

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increased by 3 mmol/l azide, indicating that they can be further activated by metabolic inhibition. All channels were blocked by 0.5 mmol/l tolbutamide, a concentration that fully saturates the high-affinity binding site for sulfonylureas (16). However, the potency of block was slightly less for mutant channels, being 98 ± 1% ($n$ = 7) for wild-type, 92 ± 3% ($n$ = 17) for hetF35V, and 91 ± 3% ($n$ = 17) for homF35V channels (Fig. 1). The extent of block was similar for hetF35L (96 ± 1%; $n$ = 8) and homF35V (85 ± 2%; $n$ = 8) channels (Fig. 1).

**$K_{ATP}$ channels carrying the F35V mutation have reduced ATP sensitivity.** To explore the molecular basis of the different metabolic sensitivities, we examined the ability of ATP to block wild-type and mutant channels in inside-out patches. We first carried out experiments in the absence of intracellular $Mg^{2+}$, in order to isolate the effects of the mutation on the interaction of ATP with Kir6.2. Mutant channels were substantially less sensitive to ATP than wild-type channels (Fig. 2*A*); the $IC_{50}$ for ATP inhibition increased from 7 ± 1 μmol/l ($n$ = 6; wild-type) to 19 ± 39 μmol/l ($n$ = 9) for hetF35V and to 65 ± 10 μmol/l ($n$ = 6) for homF35V channels. This corresponds to an ~3- and 16-fold increase in $IC_{50}$, respectively. The $IC_{50}$ for ATP inhibition of hetF35L channels was 19 ± 2 μmol/l ($n$ = 6) and for homF35V channels was 47 ± 3 μmol/l ($n$ = 6), corresponding to an approximately three- and sevenfold increase in $IC_{50}$, respectively. Thus, both mutations decrease the channel ATP sensitivity.

The ATP sensitivity of F35V mutant channels was even more dramatically reduced in the presence of 2 mmol/l $Mg^{2+}$ (Fig. 2*B*). The $IC_{50}$ was 13 ± 2 μmol/l ($n$ = 6) for wild-type channels, 273 ± 38 μmol/l ($n$ = 7) for hetF35V, and 1.1 ± 0.2 mmol/l ($n$ = 6) for homF35V channels, corresponding to an increase of 21- and 85-fold, respectively. There was also a marked increase in the amplitude of the residual currents in the presence of 1 mmol/l MgATP, which was 1 ± 1% ($n$ = 6) for wild-type, 20 ± 3% ($n$ = 7) for hetF35V, and 53 ± 9% ($n$ = 6) for homF35V.

Similar results were found for the F35L mutation in the presence of $Mg^{2+}$. The $IC_{50}$ was 82 ± 11 μmol/l ($n$ = 7) for hetF35L and 1.2 ± 0.5 mmol/l ($n$ = 6) for homF35L channels, an increase of 7- and 85-fold, respectively. The residual current in 1 mmol/l MgATP was 13 ± 3% ($n$ = 7) for hetF35V and 57 ± 4% ($n$ = 6) for homF35L.

These results indicate that both the F35V and F35L mutations impair the ability of ATP to inhibit the $K_{ATP}$ channel via its interaction with Kir6.2. The greater reduction in apparent ATP block in the presence of $Mg^{2+}$ suggests that these mutations may have an additional effect: potentiation of the stimulatory effect of MgATP mediated via SUR1 (19).

**$K_{ATP}$ channels carrying the F35V mutation have increased intrinsic open probability.** Previous studies have shown that point mutations and deletions in the $NH_2$-terminus of Kir6.2 can result in an increase in the intrinsic (unliganded) open probability of the $K_{ATP}$ channel (22,23). As a consequence, the channel ATP sensitivity is reduced (17,24,25). To determine whether the F35 mutations affect the intrinsic open probability [$P_O(0)$], we recorded single-channel currents from inside-out membrane patches in nucleotide-free solution (Fig. 3). The $P_O(0)$ of mutant channels was significantly greater ($P < 0.05$) than that of wild-type channels, being 0.74 ± 0.02 ($n$ = 9) for homF35V and 0.75 ± 0.01 ($n$ = 10) for homF35L compared with 0.45 ± 0.03 ($n$ = 6) for wild-type channels.

Previous studies have shown that some mutations in Kir6.2 (e.g., Y330C) only influence $P_O(0)$ when SUR1 is present (20). To test if this is also true of the F35V mutation, we used a COOH-terminally truncated form of Kir6.2 (Kir6.2ΔC) that expresses in the absence of SUR1 (13). The intrinsic open probability of Kir6.2ΔC-F35V (0.11 ± 0.02; $n$ = 6) was not significantly different from that of wild-type Kir6.2ΔC (0.10 ± 0.02; $n$ = 6). This suggests that residue F35 may affect the mechanism by which SUR1 couples to Kir6.2. There was also no effect of the F35V mutation on inhibition of Kir6.2ΔC by ATP: the $IC_{50}$ was 189 ± 26 μmol/l ($n$ = 5) compared with values ranging from 106 to 190 μmol/l for the wild-type channel (13,20,26,27). This suggests that the mutation does not affect ATP binding to Kir6.2 and is consistent with the effect of the mutation being primarily a result of the altered single-channel kinetics.

## DISCUSSION

Our results demonstrate that both the F35V and F35L mutations cause neonatal diabetes by increasing the $K_{ATP}$ channel current. This results from a reduced ability of MgATP to inhibit the $K_{ATP}$ channel, which is due to two mechanisms. First, there is a decreased ability of ATP to close the $K_{ATP}$ channel, which is mediated via Kir6.2 and is present in the absence of $Mg^{2+}$. Second, the enhanced reduction in ATP sensitivity in the presence of $Mg^{2+}$ argues that the stimulatory actions of MgATP, mediated via SUR1, are enhanced, as is found for other mutations causing neonatal diabetes (18–20). In the heterozygous state, the channels were strongly blocked by 0.5 mmol/l tolbutamide. This is consistent with the fact that the patient with the F35V mutation responded well to glibenclamide and that it was possible to discontinue insulin in favor of the drug. The fact that F35L mutation had similar functional effects to F35V, and was blocked to a similar extent by tolbutamide, suggests that it may also be possible to transfer the patient with this mutation to sulfonylureas.

**Mechanism of reduced ATP block.** The lack of effect of the F35V mutation on the ATP sensitivity of Kir6.2ΔC indicates that ATP binding to Kir6.2 is unaffected by the mutation. Instead, the data suggest that the lower ATP inhibition is primarily due to an increase in $P_O(0)$. However, although the F35V mutation increased $P_O(0)$ of SUR1-containing channels, it had no effect on Kir6.2ΔC expressed in the absence of SUR. This indicates the mutation influences the interaction of Kir6.2 with SUR1. This seems plausible given that F35V is predicted to lie on the outer surface of the tetramer in a model of Kir6.2 (Fig. 4) (28), a position that would facilitate interactions with SUR.

**Magnitude of the reduction in ATP inhibition.** Previous studies (3,19) have shown that all Kir6.2 mutations associated with neonatal diabetes reduce the ability of MgATP to inhibit the heterozygous $K_{ATP}$ channel and that the current magnitude at physiological MgATP levels (1–3 mmol/l) is reasonably well correlated with the severity of the clinical phenotype. About 8% of the current through hetF35V channels remained in the presence of 3 mmol/l MgATP (~4% for hetF35L channels), which is comparable with that observed for other mutations causing neonatal diabetes alone (<15%) and significantly less that than

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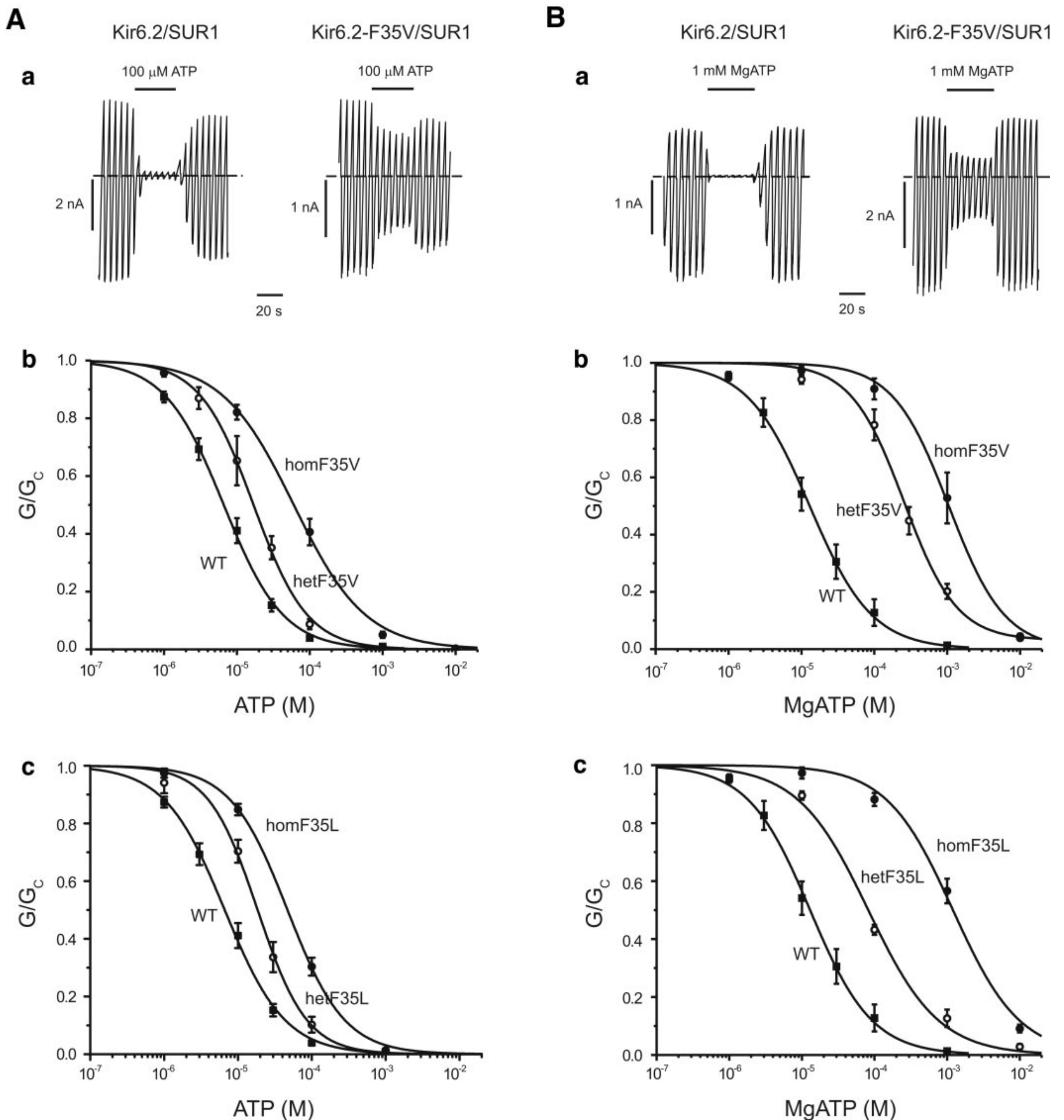


**FIG. 2.** ***A*: Effects of the F35V mutation on $K_{ATP}$ channel ATP-sensitivity in Mg-free solution. *a*: Currents recorded from inside-out patches from oocytes expressing Kir6.2/SUR1 or homKir6.2-F35V/SUR1 channels in response to voltage ramps from −110 to +100 mV from a holding potential of 0 mV. ATP was added as indicated by the bar. The dashed line indicates the zero current level. *b* and *c*: Mean relationship between ATP and $K_{ATP}$ channel conductance ($G$), expressed relative to the conductance in the absence of nucleotide ($G_c$). *b*: Kir6.2/SUR1 channels (■; $n = 6$) and heterozygous (○; $n = 9$) or homomeric (●; $n = 6$) Kir6.2-F35V/SUR1 channels. The smooth curves are the best fit to Eq. 1. For Kir6.2/SUR1 channels, $IC_{50}$ = 6.6 μmol/l, $h$ = 1.1. For hetF35V, $IC_{50}$ = 17 μmol/l, $h$ = 1.2. For homomeric F35V, $IC_{50}$ = 60 μmol/l, $h$ = 0.88. *c*: Kir6.2/SUR1 channels (■; $n = 6$), and heterozygous (○; $n = 6$) or homomeric (●; $n = 6$) Kir6.2-F35L/SUR1 channels. The smooth curves are the best fit to Eq. 1. For Kir6.2/SUR1 channels, $IC_{50}$ = 6.6 μmol/l, $h$ = 1.1. For hetF35L, $IC_{50}$ = 18.8 μmol/l, $h$ = 1.3. For homomeric F35L, $IC_{50}$ = 47 μmol/l, $h$ = 1.1. *B*: Effects of the F35V mutation on $K_{ATP}$ channel ATP sensitivity in the presence of $Mg^{2+}$. *a*: Currents recorded from inside-out patches from oocytes expressing Kir6.2/SUR1 or homKir6.2-F35V/SUR1 channels in response to voltage ramps from −100 to +100 mV from a holding potential of 0 mV. MgATP was added as indicated by the bar. *b* and *c*: Mean relationship between ATP and $K_{ATP}$ conductance ($G$), expressed relative to the conductance in the absence of nucleotide ($G_c$). *b*: Kir6.2/SUR1 channels (■; $n = 6$) and heterozygous (○; $n = 7$) or homomeric (●; $n = 6$) Kir6.2-F35V/SUR1 channels. The smooth curves are the best fit to Eq. 1. For wild-type channels, $IC_{50}$ = 13 μmol/l, $h$ = 1.0. For hetF35V, $IC_{50}$ = 255 μmol/l, $h$ = 1.19. For homF35V, $IC_{50}$ = 1.1 mmol/l, $h$ = 1.13. *c*: Kir6.2/SUR1 channels (■; $n = 6$) and heterozygous (○; $n = 7$) or homomeric (●; $n = 6$) Kir6.2-F35L/SUR1 channels. The smooth curves are the best fit to Eq. 1. For Kir6.2/SUR1 channels, $IC_{50}$ = 13 μmol/l, $h$ = 1.0. For hetF35L, $IC_{50}$ = 82 μmol/l, $h$ = 0.9. For homomeric F35L, $IC_{50}$ = 1.2 mmol/l, $h$ = 0.95.**

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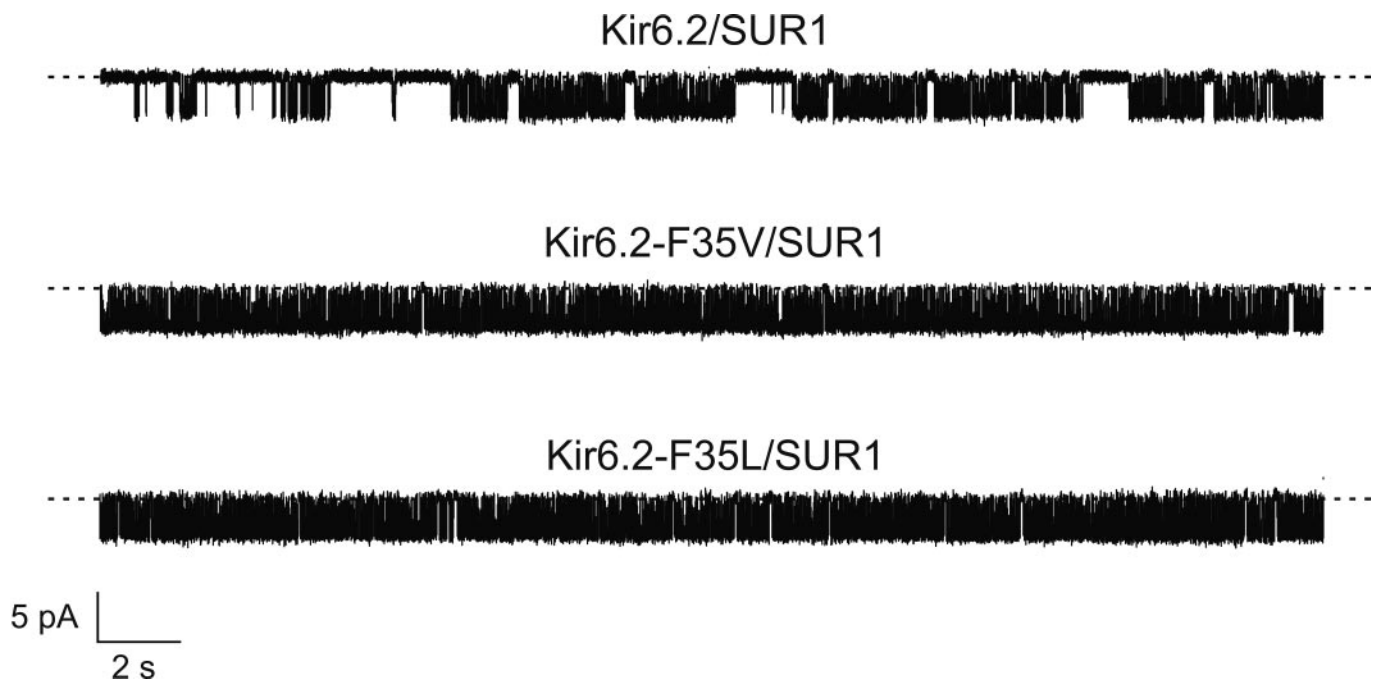


**FIG. 3. Effects of F35 mutations on the single-channel kinetics. Single-channel currents recorded at −60 mV from inside-out membrane patches excised from oocytes expressing Kir6.2/SUR1, Kir6.2-F35V/SUR1, or Kir6.2-F35L/SUR1.**

observed for mutations associated with DEND syndrome (>35%) (19).

The F35V and F35L mutations are associated with an increase in the $P_O(0)$ of homF35V channels. However, unlike those mutations that influence the channel kinetics that have been reported to date, neither mutation is associated with neurological symptoms. This can be attributed to the fact that both mutations only produce a small increase in the heterozygous current at 3 mmol/l MgATP in inside-out patches and a correspondingly small increase in the whole-cell $K_{ATP}$ current. The smaller currents may be due in part to the fact that the increase in the $P_O(0)$ of homomeric channels (to 0.74 for F35V and 0.75 for F35L) is less than that found for mutations associated with DEND syndrome (~0.85) (17,19). It also suggests that the mutation may not affect the gating of heterozygous channels as strongly as homomeric channels. However, this is difficult to assess directly because at least five different types of channel will be present in the heterozygous state. The possibility that the location of the mutant subunit

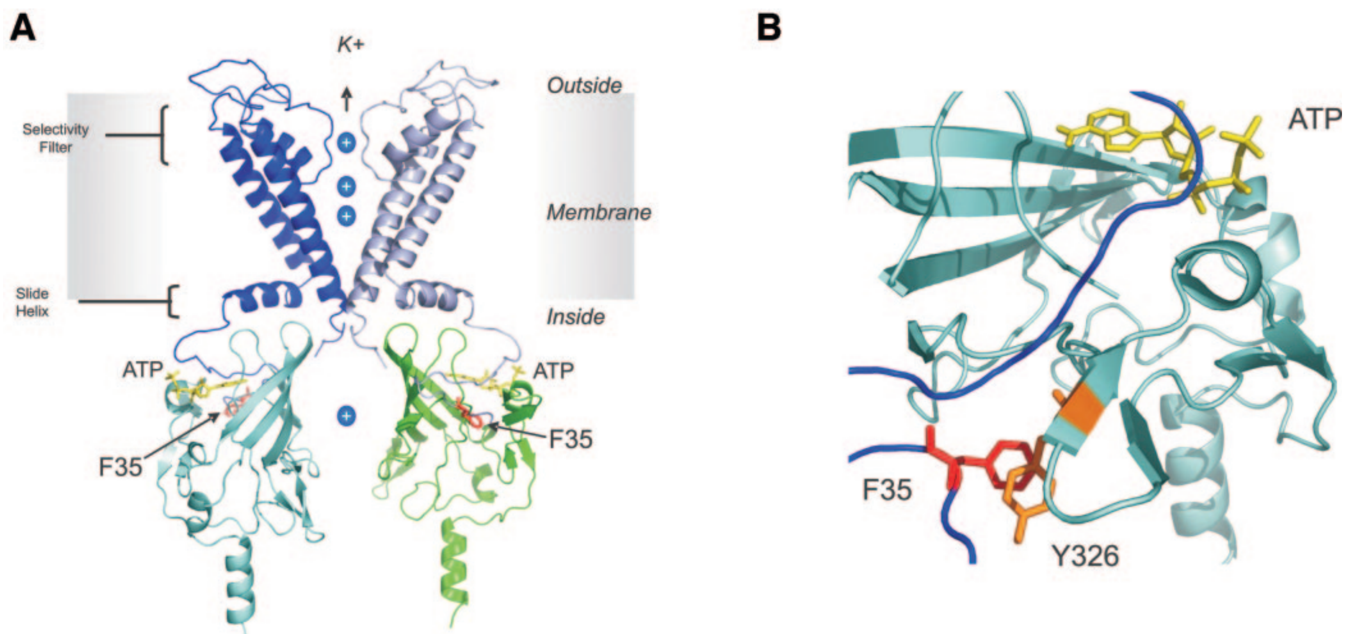


**FIG. 4. Location of F35 in Kir6.2. *A*: Molecular model of Kir6.2 (28), viewed from the side. For clarity, each subunit is shown in a different color, and only two transmembrane domains and two cytosolic domains are illustrated. Residue F35 is shown in ball-and-stick format and ATP in yellow. *B*: Close-up of the $NH_2$-terminus of one subunit (dark blue) and the COOH-terminus of the adjacent subunit (cyan) showing the position of F35 (red), the π-stacking interaction with Y326 (orange) in the adjacent subunit and ATP in its binding site.**

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within the tetramer also influences the gating behavior makes the analysis even more complex.

**Structural considerations.** Phenylalanine 35 lies within the $NH_2$-terminus of Kir6.2 (Fig. 4). Most Kir channels, with the exception of Kir1.1 channels, have an aromatic residue at this position, which suggests that it may play some functionally important role. In a model of the three-dimensional structure (28), F35 makes a π-stacking interaction with Y326 in the COOH-terminus of the adjacent Kir6.2 subunit (Fig. 4*B*). This interaction may help stabilize the tetrameric structure of the channel. Mutation to a nonaromatic residue such as valine or leucine will abolish the π-stacking interaction and might destabilize the interaction between the $NH_2$- and COOH-termini. The loop on which F35 sits resembles a bent arm whose shoulder forms part of the ATP-binding site of one subunit (via residue R50) and whose elbow contributes to the ATP-binding site of the adjacent subunit (residues K38 and K39). Mutation of F35 may therefore also lead to structural changes that influence two ATP-binding sites. The location of F35 on the outer surface of the Kir6.2 tetramer (Fig. 2*C*) is consistent with the possibility that this residue interacts with SUR1 and that mutations at this position enhance channel gating by disrupting this interaction. The ability of the F35V mutation to influence Mg-nucleotide stimulation of channel activity also points to an altered interaction between Kir6.2 and SUR1.

**Physiological and clinical implications.** The reduction in ATP sensitivity produced by the F35V and F35L mutations is associated with a small but significant increase in the resting $K_{ATP}$ channel current when expressed in *Xenopus* oocytes. An increase in $K_{ATP}$ channel current may be expected to hyperpolarize the β-cell membrane and reduce or abolish the membrane depolarization evoked by glucose. This will prevent electrical activity, calcium influx, and insulin secretion and could thereby account for the diabetes phenotype of the patients.

The ability of tolbutamide to block the whole-cell hetKir6.2-F35V/SUR1 current was 92% compared with 98% for wild-type channels. This is consistent with the fact it is possible to control the diabetes of the patient carrying the F35V mutation with sulfonylureas. Not only were we able to switch from insulin to sulfonylurea, a very important practical issue for the patient and parents, but multiple measurements of A1C revealed that the metabolic control was significantly improved. These results should be interpreted with caution, as the follow-up time was limited; moreover, the daily oscillations of glucose concentration were not measured. It is, however, of interest that similar results have also been successfully achieved for patients with Kir6.2 mutations that cause a similar degree of reduction in $K_{ATP}$ channel ATP sensitivity (3,5). Our patient has now been treated for 24 months with sulfonylureas, which is the longest period off insulin published so far. Our data further suggest that the patient carrying the F35L mutation may also respond to sulfonylureas.

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