An Andersen-Tawil Syndrome Mutation in Kir2.1 (V302M) Alters the G-loop Cytoplasmic K⁺ Conduction Pathway

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Loss-of-function mutations in the inward rectifier potassium channel, Kir2.1, cause Andersen-Tawil syndrome (ATS-1), an inherited disorder of periodic paralysis and ventricular arrhythmias. Here, we explore the mechanism by which a specific ATS-1 mutation (V302M) alters channel function. Val-302 is located in the G-loop, a structure that is believed to form a flexible barrier for potassium permeation at the apex of the cytoplasmic pore. Consistent with a role in stabilizing the G-loop in an open conformation, we found the V302M mutation specifically renders the channel unable to conduct potassium without altering subunit assembly or attenuating cell surface expression. As predicted by the position of the Val-302 side chain in the crystal structure, amino acid substitution analysis revealed that channel activity and phosphatidylinositol 4,5-bisphosphate (PIP2) sensitivity are profoundly sensitive to alterations in the size, shape, and hydrophobicity of side chains at the Val-302 position. The observations establish that the Val-302 side chain is a critical determinant of potassium conduction through the G-loop. Based on our functional studies and the cytoplasmic domain crystal structure, we suggest that Val-302 may influence PIP2 gating indirectly by translating PIP2 binding to conformational changes in the G-loop pore.

Andersen-Tawil syndrome is characterized by cardiac arrhythmias, periodic paralysis, and dysmorphic features (1, 2). One form, ATS-1, is caused by loss-of-function mutations in a strong inwardly rectifying potassium channel, Kir2.1, encoded by the KCNJ2 gene (3). The Kir2.1 channel normally sets the resting membrane potential and controls the duration of the action potential in many different types of excitable cells (4). In ventricular myocytes Kir2.1 is a major component of IK1, a steep inwardly rectifying potassium current that is responsible for the terminal, phase 3 repolarization of the action potential (5). Consequently, loss of the Kir2.1 repolarizing current lengthens the ventricular action potential, modestly prolongs the QT interval (6), and causes susceptibility to ventricular tachyarrhythmias (7).

Multiple missense mutations in Kir2.1 have been identified in patients with Andersen-Tawil syndrome, affecting channel function by all mechanisms imaginable. Many are believed to disrupt channel regulation by the phospholipid, PIP2 (8, 9). Others have been shown to alter gating (10), protein folding, and membrane trafficking (11). One mutation, V302M, has not been well characterized but was originally categorized with a group of missense mutations that cause defective assembly and trafficking (11). However, the recently resolved atomic resolution structure of the Kir2.1 cytoplasmic domain (12) provides reason to seriously reconsider this idea and rigorously test whether Val-302 might actually play a specific role in potassium conduction and channel gating.

As observed in related channels, Kir3.1 (13) and the bacterial KirBac1.1 (14), the highly conserved cytoplasmic N and C termini of Kir2.1, form a long water-filled cytoplasmic pore that extends ~30 Å from the canonical transmembrane potassium conduction pathway (12). Charged and hydrophobic residues line the inner cavity of the cytoplasmic pore, creating a favorable site for polyamines (15), which is important for blocking outward potassium movement and conferring inward rectification (16, 17). The surface of the cytoplasmic structure also contains docking sites for modulators. For example, a cluster of basic and polar residues at the surface form a binding site for PIP2 (8, 18–20). Like most other inwardly rectifying channels, opening of the Kir2.1 channel requires PIP2 binding to these residues.

The Val-302 residue is located near the apex of the cytoplasmic structure where the four cytoplasmic loops from each subunit in the tetrameric channel form a girdle around the central pore. This structure, called the G-loop, is believed to create a flexible diffusion barrier in the potassium conduction pathway between the cytoplasmic and transmembrane pore (12). In the crystal structures of the Kir2.1 cytoplasmic domain, the Val-302 side chain points away from the pore between a salt bridge (Arg-218:Thr-309) (21) near the putative PIP2 binding site at the cytoplasmic surface. These observations raise the possibility that Val-302 plays an important role in the conformation of the G-loop. In this study, we begin to explore this idea by elucidating the mechanism by which the Andersen-Tawil syndrome mutation, V302M, alters channel function.

MATERIALS AND METHODS

Molecular Biology—All studies were performed with the modified mouse Kir2.1 channel, containing an external hemag-
glutinin (HA) epitope tag as described before (22). Site-directed mutagenesis was carried out using a PCR-based strategy with PfTurbo DNA polymerase (QuikChange, Stratagene). All constructs for studies in *Xenopus* oocytes were subcloned between the 5′/H11032- and 3′/H11032-untranslated region of the *Xenopus* /H9252-globin gene in the modified pSD64 vector as before (23). The pcDNA3.1 mammalian expression vector (Invitrogen) was used to express the cloned channel genes in the COS7 and HEK293 cells. pEGFP-C1 was used to express the N-terminal enhanced green fluorescent protein (EGFP)-tagged channels in HEK293 and rat neonatal heart cells. All sequences were confirmed by dye termination DNA sequencing (University of Maryland School of Medicine Biopolymer Core). pcDNA-CD8 expressing vector was generously provided by Dr. Min Li from Johns Hopkins Medical Institutions.

Neonatal Rat Ventricular Cardiomyocyte Culture—Isolation and handling of rat neonatal ventricular cardiomyocytes was performed as previously described (24). In brief, hearts were removed from 1–2-day-old Sprague-Dawley rats using a protocol approved by the Institutional Animal Care and Use Committee at the University of Maryland School of Medicine. The ventricles were separated from the atria and then digested in a buffer (116 mm NaCl, 5.4 mm KCl, 20 mm HEPES, 5.5 mm d-glucose, 1.0 mm NaH2PO4, 0.8 mm MgSO4, and 15 μM phenol red, pH 7.35) containing collagenase type II (75 units/ml; Worthington Biochemical) and pancreatin (0.6 mg/ml; Sigma). Samples were digested 30 min at 37 °C, then pelleted and resuspended in heat-inactivated horse serum and plated onto 25-mm coverslips precoated with fibronectin at a density of 350–500 cells/mm². Cells were incubated at 37 °C in humidified air with 5% CO2 (4:1 mixture of Dulbecco’s modified Eagle’s medium-M199 to which 5% fetal calf serum, 10% heat-inactivated horse serum, 1 mm 5-bromo-2′-deoxyuridine, and 2% penicillin-streptomycin was added). After 24 h the cultures were irradiated with γ irradiation (2500 rads) to eliminate fibroblast growth.

Transfection of HEK293 Cells and Rat Neonatal Heart Cells for Patch Clamp Analysis—HEK293 cells were plated onto 25-mm No.1 coverslips 1 day before transfection at 10–20% confluence. A 1.5-g DNA mixture containing channel plasmid DNA and the CD8 gene in 1:6 ratio were used to transfect HEK293 cells with FuGENE 6 reagent (Roche Applied Science). After transfection, the cells were cultured at 37 °C for another 24–36 h. To select transfected cells for patch clamp analysis, cells were incubated with anti-CD8-coated magnetic beads (Dynal, Oslo, Norway) for 30 min. The unbound beads were removed with gentle PBS washes. Only CD8 bead-decorated cells were used for electrophysiological recording. Three days after plating rat neonatal heart cells were transfected using Lipofectamine 2000 as recommended by the manufacturer (Invitrogen). 3 μg of channel plasmid and 0.5 μg of CD8 plasmid were mixed for each transfection. The lipid DNA complexes were removed on the second day after transfection. Transfected cells for electrical recording were identified with CD8 beads and green fluorescence as above.

Oocyte Isolation and Injection—Oocytes from *Xenopus laevis* (Xenopus Express, Homosassa, FL) were isolated and main-
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**Electrophysiology**—Standard methods were used for whole cell voltage clamp studies in HEK cells and neonatal cardiomyocytes. The pipettes were made from thin wall glass (TW150F-3, WPI, Sarasota, FL) and had resistances of 2–3 megohms. The currents were recorded using an Axopatch 200A amplifier at 5 kHz and digitized using an ITC-16 analog-to-digital, digital-to-analogue interface (Instrutech Corp.), filtered at 1 kHz, and digitized at a rate of 1.2 ml min⁻¹ (26).

Whole cell potassium currents in *Xenopus* oocytes were monitored using a two-microelectrode voltage clamp as described previously (23). Briefly, oocytes were bathed in a 45 mM K⁺ solution (45 mM KCl, 45 mM N-methyl-d-glucamine-Cl, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM HEPES, pH 7.4) or a 90 mM KCl solution (90 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM HEPES, pH 7.4). Microelectrodes had resistances of 0.5–1.5 megohms when backfilled with 3 M KCl. Once a stable membrane potential was attained, oocytes were clamped to a holding potential of −40 mV, and currents were recorded during 500-ms voltage steps ranging from −100 mV to +40 mV in 20-mV increments. For assessment of cation selectivity (PK/PNa) reversal potentials were measured as before (27) in 5 mM potassium and 5 mM sodium (85 mM N-methyl-d-glucamine-Cl, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM HEPES, pH 7.4). Data were collected using an ITC16 interface (Instrutech Corp.), filtered at 1 kHz, and digitized on line at 2 kHz using Pulse software (HEKA Electronik). Kir2.1 currents are taken as the barium-sensitive inward current (2 mM barium acetate) as we have done before (23). Electrophysiological data were analyzed with Igor (WaveMetrics, Lake Oswego, OR). Values reported in the text are the means ± SEM. Total channel protein expression was compared by anti-HA immunoblot. RLU/RLUWT, relative light units.

**Immunofluorescent Microscopy**—36 h after plasmid transfection, intact COS-7 cells or rat neonatal heart cells were fixed with 2% paraformaldehyde, blocked with 5% fetal bovine serum in 1× PBS buffer, and then incubated with mouse monoclonal anti-HA antibody (Covance Inc.) for 1 h at room temperature to specifically label exposed HA-channel epitopes on the cell surface membrane. After incubation,
the unbound antibodies were removed with PBS washes. The intracellular recombinant channels were subsequently labeled with rabbit polyclonal anti-HA antibody for 1 h at room temperature after 0.1% Triton X-100 permeabilization. The two channel populations (intracellular and extracellular) were labeled differentially with secondary antibodies (Alexa-488-conjugated goat anti-mouse secondary antibody and Alexa-568-conjugated goat anti-rabbit secondary antibody). After extensive washing with 1× PBS buffer, the coverslips were mounted on the glass slide with Vectashield (Vector Laboratories). The labeled cells were visualized under Zeiss 410 confocal microscopy.

**Quantitative Chemiluminescence Detection of Surface Proteins**—Channel surface expression in oocytes was quantified by chemiluminescence as described previously (25).

To quantify the cell surface expression in mammalian cells, COS7 or HEK293 cells were plated on 6-well tissue culture dishes and transfected with 1 μg of plasmid DNA using FuGENE6 reagent at a 50–60% cell confluence. After 36 h, transfected cells were incubated on ice with blocking buffer (5% fetal bovine serum in 1× PBS, 30 min), incubated with mouse monoclonal anti-HA (Covance) (in blocking buffer, 1 h), washed (with blocking buffer, 3 times for 5 min), incubated with goat anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (1:500 dilution, Jackson) (in blocking buffer, 20 min), and extensively washed (1× PBS for 5 min/time for 4 times in total). The cells were scraped from the plates and resuspended into 500 μl 1× PBS. 10 μl of cell suspension was incubated with 100 μl of mixed Supersignal enzyme-linked immunosorbent assay Femto solution, and then chemiluminescence was measured as above after 3 min of incubation. Reported values represent the average of triplicate transfections.

**Immunoprecipitation and Western Blot Analysis**—Standard molecular biology protocols were followed for immunoprecipitation and Western blot analysis as before (28).

Mouse monoclonal anti-HA was obtained from Covance. Rabbit polyclonal anti-Myc and mouse monoclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. For quantification of subunit immunoprecipitation, densitometry was performed using NIH image. For comparison of multiple blots, the density of immunoprecipitated bands was first normalized to the density of the corresponding input for each blot.
RESULTS

To begin to clarify the mechanisms by which the V302M mutation in Kir2.1 causes channel malfunction in ATS-1, we evaluated the functional properties and surface expression of external epitope-tagged channels bearing the mutation. The HA epitope tag was incorporated into an external site that does not perturb channel activity (22) for quantitative measurements of plasmalemma expression. Biophysical properties of the channels were assessed in HEK cells under whole-cell patch clamp (Fig. 1). For electrophysiological experiments, cells were co-transfected with CD8 and Kir2.1 channels (WT or V302M) and then selected for analysis on CD8 antibody-coated beads. The CD8 selection procedure provides a highly efficient method for detecting Kir2.1-transfected cells; nearly all CD8-selected cells contain immunodetectable Kir2.1 (98.7%) or Kir2.1 V302M (100%) (Fig. 1B). Cells transfected with the wild-type channel exhibited robust potassium-selective, barium-sensitive inward-rectifying currents, typical of Kir2.1 (29). By contrast, barium-sensitive potassium currents were not detected above background in cells transfected with Kir2.1 V302M (Fig. 1, A and C). As measured by HA antibody binding and analytical luminometry, the V302M mutation had no inhibitory effects on surface expression. In fact, the Kir2.1 V302M channels exhibited a somewhat higher level of expression at the plasmalemma than the wild-type channels. Taken together, these observations strongly suggest that the V302M mutation directly alters potassium conduction and/or gating rather than disrupting protein stability or preventing plasma membrane trafficking.

These observations are supported by immunocytochemical analysis of external HA-tagged Kir2.1 channels in COS cells. As shown in Fig. 2, HA-antibodies readily label non-permeabilized COS cells expressing either wild-type Kir2.1 or Kir2.1 V302M channels, indicating that the mutation does not disrupt cell surface expression (Fig. 2A). As a negative control, Kir2.1 mutant channels, lacking an endoplasmic reticulum export signal (ΔFCYENE) (22), are trapped in the endoplasmic reticulum and cannot be detected in intact cells by cell surface antibody binding. Independent assessment of plasma membrane expression by luminometry provided a quantitative corroboration of the immunocytochemistry (Fig. 2B). Again, more Kir2.1 V302M could be detected at the plasmalemma than the wild-type Kir2.1. Protein expression of wild-type and mutant channels, as detected in HA immunoblots, were comparable.

To test whether the mutation affects subunit assembly, immunoprecipitation studies were performed with differently tagged Kir2.1 subunits. In these studies, COS-7 cells were either co-transfected with Myc-tagged Kir2.1 (WT) and HA-tagged Kir2.1 (WT or V302M) at a 1:1 ratio or transfected with HA-tagged Kir2.1 alone. Recovered immunoprecipitates on anti-Myc-bound beads were resolved by SDS-PAGE, and the extent of HA-tagged channel subunit interaction was assessed using anti-HA antibodies in immunoblots. As shown in the representative experiment (Fig. 3), both the wild-type HA-Kir2.1 and the mutant V302M co-immunoprecipitated with the Myc-tagged subunits. Co-immunoprecipitation of HA-tagged subunits with Myc antibodies required co-transfection of the Myc-tagged subunit, verifying specificity. As quantified by densitometry, equal amounts of HA epitope-tagged and wild-type and mutant channel co-purified with wild-type Myc-tagged Kir2.1, indicating the mutation does not alter subunit-subunit interaction (Fig. 3B). In addition, immunocytochemical analysis of co-transfected cells (Fig. 3C) reveal that the Myc-tagged WT Kir2.1 and HA-tagged V302M Kir2.1 subunits are highly co-localized, offering further support that the mutant channel is capable of assembling with the wild-type channel in cells.

As a functional test of subunit assembly, we explored the possibility that the Kir2.1 V302M mutant exhibits a negative effect on the wild-type Kir2.1 channel. In these studies, whole cell potassium currents were measured in Xenopus oocytes co-injected with a constant amount of wild-type Kir2.1 and varying amounts of Kir2.1 V302M cRNA. As shown in Fig. 4, expression of Kir2.1 V302M suppressed wild-type Kir2.1 channel activity in a dose-dependent manner, indicating that V302M subunits can assemble with wild-type subunits, and this diminishes channel function. The effect is specific. In fact, we found that co-injection of a dominant negative Kir1.1 (renal outer medullary potassium (ROMK)) channel, which bears a disrupting alanine sub-
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A substitution mutation within the pore “GYG” sequence (Kir1.1AAA) and does not assemble with Kir2.1, had no suppressive effect on Kir2.1. Likewise, co-injection of cRNA encoding a non-channel membrane protein, CD4, had no effect on Kir2.1 activity. Thus, the general effects of translational interference cannot explain the decrease in current by V302M cRNA.

We also examined whether V302M channels exhibit negative effects on the native IK1 current, encoded by Kir2.1/2.2, in neonatal cardiomyocytes (30, 31). In these studies, cardiomyocytes were transfected with CD8 and either the wild-type Kir2.1 or the V302M Kir2.1 channel (both EGFP-tagged) and then compared with cells transfected with CD8 alone. Cells expressing either channel were selected with a high level of confidence for whole-cell patch clamp analysis using EGFP fluorescence and anti-CD8-coated beads (Fig. 5, A and B); nearly all CD8-selected cells contain EGFP-positive Kir2.1 (96%) or Kir2.1 V302M (96%). As summarized in Fig. 5, C and D, expression of the Kir2.1 V302M mutant channel completely suppressed the endogenous IK1 current. The effect appears to be specific as expression of the mutant channel had no effects on the endogenous sodium current (not shown). Furthermore, wild-type Kir2.1-transfected cells carried inward potassium currents that were ~19-fold greater than the endogenous IK1.

The specific negative effects of the mutant Kir2.1 on the wild-type Kir2.1 channel in oocytes and IK1 activity in cardiomyocytes strongly suggest that V302M mutant channels are appropriately synthesized, are folded correctly, and are capable of assembly. Such behavior is also consistent with the dominant pattern of inheritance in ATS-1.

Together the observations above indicate that the V302M mutation impairs channel function by altering potassium conduction and/or gating rather than disrupting cell surface expression or subunit assembly. Visualization of the recently solved Kir2.1 cytoplasmic domain structure (12) reveals a possible mechanism. As shown in Fig. 6, the hydrophobic side chain of Val-302 projects away from the cytoplasmic pore between residues (Arg-218:Thr-309) that are believed to form a salt bridge and hold the G loop cytoplasmic pore in an open conformation (21). Based on the apparent position of Val-302 relative to the salt bridge, we hypothesized that channel activity would be sensitive to alterations in the size, shape, and hydrophobicity of side chains at the Val-302 position.

To test this idea, macroscopic channel activity and cell surface expression were measured in a panel of Kir2.1 mutants,
bearing different hydrophobic amino acids at position 302 (methionine, alanine, leucine, or isoleucine). The *Xenopus* oocyte expression system was employed in these studies, facilitating a large number of parallel measurements. As observed with the disease-causing V302M mutation, the V302A substitution abolished macroscopic channel activity (Fig. 6B). By contrast, small whole-cell potassium currents were detected in channels containing the V302L mutation, and Kir2.1 V302I channels carried potassium currents that were indistinguishable from wild-type channels. Because the mutations did not disrupt plasmalemma expression (Fig. 6B), the changes in the whole cell current density reflect alterations in gating and/or potassium conductance. In other words, the product of the single channel conductance and channel open probability is profoundly sensitive to subtle changes in the chemistry of amino acid side chains at position 302 (Fig. 6C).

Because Val-302 is in close proximity to residues implicated in PIP2-dependent gating, including Arg-218, we tested the possibility that Val-302 may also be an important determinant of the regulated gating process. In these studies the muscarinic receptor, M1, was co-expressed with the different Kir2.1 channels (WT, V302L, V302I) in *Xenopus* oocytes, and potassium currents were monitored upon acetylcholine-induced receptor activation (Fig. 7). In this system, receptor-activated phospholipase Cβ causes PIP2 degradation. This can affect Kir channel inhibition (32), depending on the strength of PIP2 binding affinity. Channels that have a tight apparent PIP2 binding affinity, like Kir2.1, are not affected by PIP2 hydrolysis, whereas channels such as Kir2.3 that have a weak PIP2 binding affinity are remarkably sensitive to receptor activation of phospholipase C and PIP2 hydrolysis (Ref. 31 and see Fig. 7). In this regard the observations that agonist significantly inhibited the mutant Kir2.1 V302L and Kir2.1 V302I channels (Fig. 7) suggest that mutations decrease the PIP2 binding affinity and/or change the stability of the open state in such a way that alters the allosteric link between PIP2 binding and channel gating. Thus, the data are consistent with our hypothesis that Val-302 participates in the regulated gating mechanism.

**DISCUSSION**

In this study we have elucidated the mechanism by which the V302M missense mutation in Kir2.1 (*KCNJ2*) causes loss of
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channel function in Andersen-Tawil syndrome, highlighting the G-loop structure (12) as an important determinant of the cytoplasmic potassium conduction pathway. The mutation does not impair cell surface localization, alter protein expression, or perturb subunit assembly. Thus, it is highly unlikely that V302M disrupts membrane trafficking or introduces a global-folding defect. Instead, we found that the valine at position 302 plays a specific role to maintain the potassium conduction pathway in an open configuration.

The special requirements for the side-chain chemistry at the Val-302 position along with the atomic structure of the wild-type Kir2.1 cytoplasmic domain provides important clues about the functional role of Val-302 in the G-loop. In crystal structures of the Kir2.1 cytoplasmic domain (12, 21), the side chain of Val-302 points away from the G-loop pore next to residues (Arg-218:Thr-309) that are believed to form a salt bridge. Although the highly conserved arginine residue (Arg-218) has completely different orientations in the crystal structures of Kir3.1 (12, 13) and Kirbac1.1 (14), it has been suggested that formation of the Arg-218:Thr-309 salt bridge in the Kir2.1 channel is important for holding the G-loop pore in an open conformation (21). Significantly, the side chain methyl group of Val-302 appears to engage the side-chain methylene groups of Arg-218 in the Kir2.1 structures. Such an interaction is expected to properly align the Arg-218 side chain with Thr-309 for efficient salt bridge formation. Thus, modifications in the size and shape of residues at the Val-302 position may destabilize the salt bridge and dramatically alter potassium conduction at the G-loop.

The G-loop, comprising a six-amino acid stretch in the cytoplasmic C-terminal domain between the βC and βD strands, has been recently suggested to form a gating structure in Kir2.1 (12, 21). The G-loops form a girdle around the narrowest part of the cytoplasmic potassium conduction pathway. Consistent with an important role, channel function is profoundly sensitive to alterations in specific G-loop residues. For example, mutations in amino acids that have side chains, which face into the central axis of the cytoplasmic pore, alter channel gating and inward rectification (12). Moreover, analysis of Kir2.1-Kir1.1 chimeras revealed that a structure, including the G-loop, contributes to the single channel conductance, consistent with the notion that the G-loop acts as a resistance barrier to ion flow (33). The present findings extend these observations and reveal a possible mechanism for physiologically regulating the G-loop. Because the Val-302 side chain is in close proximity to residues, including Arg-218, that are believed to participate in PIP2 binding and gating (8), our observations that PIP2-dependent regulation of Kir2.1 is sensitive to side-chain chemistry at position 302 suggests that Val-302 may either be an important determinant of the PIP2 binding site or may translate PIP2 interaction at the external surface of the cytoplasmic domain to conformational changes at the G-loop.

Although the precise locus of the PIP2-dependent gate is still a matter of conjecture, there is general agreement that PIP2-dependent channel opening involves conformational changes in the second transmembrane domain (34, 35). This view is supported by numerous observations, demonstrating that second transmembrane domain residues near the bundle crossing are determinants of gating (36–38). Given the physical proximity of the second transmembrane domain (TM2) bundle branch crossing to the G-loop, it seems reasonable to propose that ligand binding (i.e. PIP2) at the surface of the cytoplasmic domain is allosterically coupled to TM2 movement through conformational changes at the G-loop. The Arg-218:Val-302 side-chain interaction, suggested here, is poised to play a critical role. A similar coupling mechanism has been proposed to explain a gating defect in developmental delay, epilepsy, and neonatal diabetes syndrome, involving a mutation in a G-loop residue (39). Obviously, further studies are required to determine whether the Val-302 side chain participates in gating by directly affecting the aperture of the G-loop pore or by allosterically coupling the PIP2 binding site with the transmembrane potassium conduction pathway or by a combination of these processes.

Our observation that the V302M mutation specifically affects channel gating contrasts a result from an initial survey of ATS-1 mutations (11). Studying mutations in Kir2.1 channels fused to the EGFP, Bendahhou et al. (11) reported that they could not detect full-length protein of Kir2.1 V302M channels with EGFP antibodies. By contrast, in the present study using many different expression systems, including cardiomyocytes, and different antibodies, we could find no evidence that V302M
disrupts protein stability or alters plasma membrane trafficking of the channel. Instead, we established that V302M Kir2.1 is able to assemble with wild-type Kir2.1 subunits and exhibits a dominant negative effect on the wild-type channel in oocytes and the IK1 current in cardiomyocytes, indicating that V302M does not introduce a misfolding or protein destabilizing defect. This result is also consistent with the dominant pattern of inheritance in ATS-1, offering further support for our observations. We do not have an obvious explanation for the discrepancy between our results and the earlier report, except that the antisense Kir expression we used in our experiments is a transgene in the mouse and might not be the same expression pattern as the endogenous human Kir. In any case, a dominant negative effect on the wild-type channel in oocytes is not the dominant phenotype we report here, or other Kir channels (23) does not disrupt protein expression or alter biophysical properties. Thus, placement of EGFP rather than EGFP itself could be responsible.

The study of disease-causing mutations can often reveal insightful clues about the function of structures that have previously escaped attention. The present analysis of Kir2.1 of EGFP rather than EGFP itself could be responsible. Thus, placement of EGFP rather than EGFP itself could be responsible.

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