A Novel KCNJ2 Nonsense Mutation, S369X, Impedes Trafficking and Causes a Limited Form of Andersen-Tawil Syndrome

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Background—Mutations in KCNJ2, a gene encoding the inward rectifier K⁺ channel Kir2.1, are associated with Andersen-Tawil syndrome (ATS), which is characterized by (1) ventricular tachyarrhythmias associated with QT (QU)-interval prolongation, (2) periodic paralysis, and (3) dysmorphic features.

Methods and Results—We identified a novel KCNJ2 mutation, S369X, in a 13-year-old boy with prominent QU-interval prolongation and mild periodic paralysis. The mutation results in the truncation at the middle of the cytoplasmic C-terminal domain that eliminates the endoplasmic reticulum (ER)-to-Golgi export signal. Current recordings from Chinese hamster ovary cells transfected with KCNJ2-S369X exhibited significantly smaller K⁺ currents compared with KCNJ2 wild type (WT) (1 µg each) (−84±14 versus −542±46 picoamperes per picofarad [pA/pF]; −140 mV; P<0.0001). Coexpression of the WT and S369X subunits did not show a dominant-negative suppression effect but yielded larger currents than those of WT+S369X (−724±98 pA/pF)>−[84+542] pA/pF; 1 µg each; −140 mV). Confocal microscopy analysis showed that the fluorescent protein-tagged S369X subunits were predominantly retained in the ER when expressed alone; however, the expression of S369X subunits to the plasma membrane was partially restored when coexpressed with WT. Fluorescence resonance energy transfer analysis demonstrated direct protein-protein interactions between WT and S369X subunits in the intracellular compartment.

Conclusions—The S369X mutation causes a loss of the ER export motif. However, the trafficking deficiency can be partially rescued by directly assembling with the WT protein, resulting in a limited restoration of plasma membrane localization and channel function. This alleviation may explain why our patient presented with a relatively mild ATS phenotype. (Circ Cardiovasc Genet. 2011;4:253-260.)

Key Words: Long QT Syndrome ▪ tachyarrhythmias ▪ KCNJ2 ▪ ion channels ▪ mutation

Andersen-Tawil syndrome (ATS) (OMIM [Online Mendelian Inheritance in Man] #170390) is a rare disorder inherited in an autosomal-dominant fashion. ATS is characterized by (1) ventricular tachyarrhythmias associated with a prolongation of QT (QU)-interval prolongation, (2) periodic paralysis, and (3) dysmorphic features.1-4 The characteristics of ATS vary greatly among patients, with some presenting with all 3 symptoms and others presenting with only 2.3-7 In 2001, Plaster et al,5 identified heterozygous mutations in the KCNJ2 gene (OMIM *600681) encoding the inward rectifier K⁺ channel Kir2.1 in patients with ATS. The KCNJ2 gene is located at 17q23, and its open reading frame is not interrupted by introns.5 The deduced protein sequence comprises 427 amino acids, with 2 putative membrane-spanning regions connected by a pore-forming domain, and cytoplasmic N- and C-terminal domains.8 Northern blot analysis reveals that KCNJ2 transcripts are present in the human heart, brain, skeletal muscle, placenta, lung, and kidney.9 In the heart, Kir2.1 greatly contributes to the cardiac inward rectifier K⁺ current (I_K1)5,9,10 which determines resting membrane potential and the late phase of action potential repolarization.11

Clinical Perspective on p 260

To date, >40 KCNJ2 mutations have been reported to be responsible for ATS.5,7,12-15 The use of heterologous expression systems has revealed that most of the mutant KCNJ2 channels5,6,12-18 show no whole-cell currents and show dominant-negative effects5,6,12-14,16,18 or haploinsufficiency.15,17 Some KCNJ2 mutations result in intracellular transport failure or a reduced affinity for phosphatidylinositol-4, 5-bisphosphate,
PIP2,7,10 The penetrance and severity of the ATS phenotype are extremely variable, which may be partially due to differences in the extent of the functional outcome induced by the mutations.14 Recently, KCNJ2 mutations were reported not only in patients with ATS, but also in patients with catecholaminergic polymorphic ventricular tachycardia.20

In the present study, we identified a novel nonsense KCNJ2 mutation, S369X, in a 13-year-old boy who had a prominent QU prolongation and mild muscle weakness but lacked dysmorphic features and examined its functional characterization by using electrophysiological and subcellular distribution analyses.

Methods

Clinical Case

The index patient who reported of muscle weakness and a sense of exhaustion after exercise was referred to our hospital. At the age of 12, he started presenting muscle weakness mainly in his upper limbs and body during hard exercise, which disappeared quickly when he ceased exercise. His ECG showed QU-interval prolongation (QUc=0.50 seconds, QTc=0.48 seconds)21 and body during hard exercise, which disappeared quickly when he ceased exercise. His ECG showed QU-interval prolongation (QUc=0.50 seconds, QTc=0.48 seconds)21 It was sometimes difficult to discriminate the end of the T wave on ECG, but in other recordings of V3, as shown in the inset of Figure 1A, whereas the QT interval was not prolonged clearly (QT=0.36 seconds, QTc=0.48).21

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To investigate the subcellular distribution of KCNJ2 channels with laser-scanning confocal microscopy, EGFP was fused to the N-terminus of WT or the mutant KCNJ2 channel. The wild type (WT) KCNJ2 clone in the pCMS-enhanced green fluorescent protein (EGFP) expression vector (BD Biosciences Clontech; Franklin Lakes, NJ) was provided by Dr Y. Aizawa (Department of Medicine, Niigata University; Niigata, Japan).13 For confonal image experiments, WT-KCNJ2 was subcloned into pIRESC-D819 to coexpress human CD8 as a cell surface marker (WT/CD8). The S369X mutation was generated by site-directed mutagenesis using a QuickChange II XL mutagenesis kit (Stratagene; La Jolla, CA). The primers were 5’-GCAGAAAAAGAA-ATATATCTTCAAAATGCAAATTCATTTTGC-3’ (sense) and 5’-GCAAAATGAATTTGCATTTAGAGGATATATTTCTTTTC-TGC-3’ (antisense). The sequences of these constructs were confirmed by direct sequencing.

To investigate the subcellular distribution of KCNJ2 channels with laser-scanning confocal microscopy, EGFP was fused to the N-terminus of WT or the mutant KCNJ2. The result constructs were designated as EGFP-WT and EGFP-S369X, respectively. Lastly, the constructs were subcloned into pcDNA3.1/Zeocin (Invitrogen; Carlsbad, CA).

Transient Transfection in Mammalian Cell Lines

For electrophysiological experiments, Chinese hamster ovary (CHO) cells were grown in Dulbecco modified eagle medium supplemented with 20% fetal bovine serum (Nacalai Tesque; Kyoto, Japan), 100 IU/mL penicillin, and 100 μg/mL streptomycin in a 5% CO2 incubator for 24 hours at 37°C. The cells were plated at 3.0×10^4 cells/35-mm dish for 24 hours before transfection. They were transfected with WT-KCNJ2 plasmid, S369X-KCNJ2 plasmid, or both using FuGENE6 (Roche Diagnostics; Indianapolis, IN) as directed by the manufacturer. In confocal microscopy experiments, COS7 cells were seeded at 6.0×10^4 cells/35-mm glass-bottomed microwell dish, grown in Opti-MEM (Invitrogen) for 24 hours at 37°C, and transfected with EGFP-fused KCNJ2 using LipofectAMINE 2000 (Invitrogen). The medium was removed 24 hours after transfection, and the cells were then incubated in Dulbecco modified eagle medium supplemented with 20% fetal bovine serum for 24 hours at 37°C. Both functional assays were performed 48 hours after transfection.

Electrophysiological Experiments

At 48 hours after transfection, CHO cells expressing WT and mutant KCNJ2 channels were identified by GFP fluorescence using an
inverted microscope (Diaphot 300; Nikon; Tokyo, Japan). Whole-cell voltage clamp recordings were performed with patch-clamp techniques using an Axopatch 200a amplifier (Axon Instruments; Foster City, CA) at room temperature. Cells were superfused with a bath solution that contained 150 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, and 10 mM HEPES (pH 7.3, adjusted with NaOH). Pipettes of 2.0 to 5.0 MΩ were prepared and heat polished before use and were filled with a solution containing 150 mM KCl, 0.5 mM MgCl2, 5 mM MgCl2, 5 mM EGTA, and 10 mM HEPES (pH 7.3, adjusted with KOH). Data were acquired through a Digidata 1332A digitizer (Axon Instruments) and analyzed with pClamp 9.0 software (Axon Instruments). Currents were elicited by 150-ms pulses applied in 10-mV increments at potentials ranging from −140 to +30 mV from a holding potential of −80 mV.

**Results**

**De Novo KCNJ2 Mutation in ATS**

Genetic analyses for the aforementioned LQTS-related genes in the proband were all negative except KCNJ2. An analysis for KCNJ2 revealed the presence of a heterozygous C>A substitution at nucleotide 1106 (Figure 1C, arrow). The change was not identified in his family members (Figure 1B) or in 302 unaffected healthy individuals, indicating that it is a novel and de novo mutation. The mutation resulted in the introduction of a premature stop codon at S369 (S369X), which leads to the loss of 59 amino acids at the C-terminus and a truncation of the KCNJ2 channel protein (Figure 2A). The 59 amino acids that follow serine 369 are highly conserved among various species (Figure 2B). This C-terminal segment contains a distinct ER-to-Golgi forward-trafficking signal (FCYENE indicated by boldface letters in Figure 1B) which leads to the loss of 59 amino acids at the C-terminus and a truncation of the KCNJ2 channel protein (Figure 2A). The 59 amino acids that follow serine 369 are highly conserved among various species (Figure 2B). This C-terminal segment contains a distinct ER-to-Golgi forward-trafficking signal (FCYENE indicated by boldface letters in Figure 2B), which is essential for export of the channel protein from the ER to the Golgi. Therefore, the lack of this motif may cause the sequestration of KCNJ2 within the ER.

**Electrophysiological Properties of KCNJ2-WT and KCNJ2-S369X**

To assess functional outcome of the truncated KCNJ2 subunits, we conducted whole-cell voltage-clamp recordings as described in the Methods section. CHO cells transfected with 1 μg of WT exhibited robust inward currents at test potentials below −80 mV and outward currents rectified between −80 and −10 mV (Figure 3A and 3D), which were similar to typical I_K1 as previously reported.9,10 S369X subunits when expressed alone (1 μg), however, showed significantly smaller currents (WT versus S369X, −542±46 versus −83.5±13.5 picoamperes per picofarad [pA/pF]) at −140 mV, P<0.0001, n=19 and n=15, respectively (Figure 3B, 3D, and 3E). BaCl2 (0.5 mM/l) reversibly blocked both WT and S369X channel currents (Figure 3A and 3B). To mimic the genetic condition in the proband, cells were cotransfected with both WT and S369X at an equimolar ratio (0.5 μg each) (Figure 3C through 3E), which displayed ~61% of the current densities of those expressing WT alone.
Figure 3. Functional assay of KCNJ2-S369X in Chinese hamster ovary (CHO) cells. Representative whole-cell currents elicited by 10-mV test pulses ranging from −140 to +30 mV from a holding potential of −80 mV were recorded in cells transfected with WT (1 µg) (A), S369X (1 µg) and blocked with BaCl2 (0.5 mM/mL) (B), and WT (0.5 µg) and S369X (0.5 µg) (C). Extracellular application of 0.5 µM Ba2+ completely blocked the reconstituted I_{v1}^{-}-like currents to the right of A and B. D. Current-voltage relationships of whole-cell currents in CHO cells expressing the S369X mutant (1 µg), WT (0.5 µg)+S369X (0.5 µg), WT alone (1 µg), WT (1 µg)+S369X (1 µg), and WT (1 µg)+S369X (2 µg). Currents were recorded at test potentials ranging from −140 to 30 mV for 150 ms in 10-mV steps from a holding potential of −80 mV. E. Averages of peak current densities were measured at −140 mV in CHO cells transfected with WT and S369X at various ratios. Numbers within the bars indicate the number of observations. The values of the 5 groups were significantly different (P<0.001) by Kruskal-Wallis test. †P<0.001 by Spearman rank correction coefficient. ‡P<0.001 by Wilcoxon test. §P<0.001 by Wilcoxon test. pA/pF indicates picocammperes per picofarad; WT, wild type.

(1 µg) (−330±40 pA/pF at −140 mV, P<0.001 versus WT and P<0.001 versus S369X, n=15) (Figure 3E). These WT/S369X currents also were inhibited by BaCl2 (0.5 mM/mL) (data not shown). These results suggest that there is no dominant-negative suppression by S369X subunits, which sharply contrasts the results from many other mutants.5,7,12,14,17,18,29

Supposing no functional interaction between WT and S369X, the current density of WT/S369X (1 µg each) should increase by ≈16% compared to that of WT (1 µg) alone because cells expressing S369X (1 µg) alone showed ≈16% of the current density produced by WT 0.5 µg (Figure 3E). However, the cells transfected with WT/S369X (1 µg each) (Figure 3E) showed a significantly larger current density, increased by ≈33% to that of WT (1 µg) alone (WT 1 µg+S369X 1 µg, −724±98 pA/pF, n=11; WT 1 µg alone, −542±46 pA/pF, n=19; at −140 mV; P<0.001). Moreover, increasing S369X mutant to 2 µg (Figure 3E) promoted but did not reduce resultant currents (−869±63 pA/pF, n=11, P<0.001 versus WT 1 µg alone). Thus, electrophysiological analyses suggest that WT channel subunits are capable of rescuing KCNJ2-S369X subunit function, presumably through direct association.

Subcellular Distribution of KCNJ2-WT and KCNJ2-S369X

To test whether mutant Kir2.1 subunits are trafficking refractory, confocal microscopic analyses were conducted using EGFP-fused Kir2.1 channels. Fusion of EGFP to the N-terminus of Kir2.1 (EGFP-WT) does not affect the electrophysiological properties of Kir2.1 because EGFP-WT fusions are functional and show an I-V relationship similar to that of WT (data not shown). Current densities of EGFP-WT at −140 mV (−584.9±45 pA/pF, n=12) were not significantly different from those of WT (P=0.50).

Figure 4 shows representative confocal microscopic images obtained from COS7 cells expressing EGFP-WT, EGFP-S369X, and EGFP-S369X+WT. The top 2 rows show phase-contrast microscopic and confocal images from successfully transfected cells. The distribution of GFP signal from WT was consistent with the localization of the channel in the plasma membrane. In contrast, cells expressing EGFP-S369X did not clearly exhibit fluorescence in the membrane, and the signal was mainly localized to the cytoplasm. Because S369X had no dominant-negative effect on WT in electrophysiological experiments, we then examined the interaction between WT and S369X (Figure 4). Cotransfection with non-GFP-tagged WT exhibited a mixed distribution pattern in both the plasma membrane and the cytoplasm. Line intensity histograms are shown in the bottom row of Figure 4. Line intensity (indicated as black [intermitted light] and green [fluorescence]) was detected along the lines illustrated in the third row of magnified images. This analysis confirmed that GFP signals from WT were strongest in the membrane (arrow), and those from EGFP-S369X were retained mainly in the cytoplasm, but cotransfection of non-GFP-tagged WT altered its distribution to the plasma membrane (arrow indicates the GFP signal from the membrane).

Thus, WT-KCNJ2 subunits rescued mutant channel proteins and assisted their transport to the cell membrane.

It is most plausible that the mutant S369X remained in the ER because it lacked the ER export motif. We then examined the location of EGFP-S369X proteins by using an ER-specific marker, DsRed2-ER (Figure 5). EGFP-WT appeared to express on the plasma membrane (Figure 5A-1). It is more evident when this image is merged with that detecting ER-specific marker, DsRed2-ER (Figure 5A-2 and 5A-3). In contrast, EGFP-S369X (Figure 5B-1) mostly colocalized with DsRed2-ER (Figure 5B-2). There was scarce expression of the S369X mutant in the plasma membrane (Figure 5B-3). Finally, cotransfection with nontagged WT resulted in a modest, but evident expression of S369X mutant in the cell membrane (reappearance of GFP signal), strongly suggesting that WT rescued the trafficking of S369X proteins to the membrane (Figure 5C-1 through 5C-3).

To prove a direct interaction between WT and S369X subunits, the FRET imaging technique was used along with the acceptor bleaching method (Figure 6). When CFP-S369X was coexpressed with YFP-WT, both were colocalized as shown in the merged images (Figure 6A). After YFP bleach-
There was a significant and consistent increase in CFP fluorescence intensity in the cytoplasm as shown in Figure 6B. These findings indicate that CFP-S369X physically interacts with the WT subunits in an intracellular compartment. Figure 6C shows the FRET efficiency obtained from 10 independent experiments for a given experimental condition. As shown in Figure 6C, for negative controls, CFP-WT alone and CFP-N1/YFP-N1 were used. The FRET efficiency between WT and mutant was significantly larger than that of the negative controls (P < 0.01 versus WT-CFP, P < 0.01 versus CFP-N1+YFP-N1). The FRET efficiency between CFP-WT and YFP-WT were examined as a positive control and showed a similar FRET efficiency to CFP-S369X/YFP-WT. Exchange of labeled fluorescence for each other (ie, CFP-WT and YFP-S369X) also yielded a large and similar value, indicating the direct interaction between WT and the mutant subunits.

**Discussion**

In the present study, we report a novel nonsense KCNJ2 mutation, S369X, in a patient with a prominent QU prolongation and mild ATS phenotype. To our knowledge, this report is the first of a nonsense mutation resulting in truncated KCNJ2 channel subunits. Electrophysiological experiments revealed that mutant channels showed significantly smaller currents and did not exhibit dominant-negative effects on WT. Confocal microscopic imaging showed that the EGFP-fused S369X channel is mainly retained inside the cell and that coexpression with KCNJ2-WT partially restored the cell surface expression of the mutant proteins.

Loss-of-function type KCNJ2 mutations are known to be responsible for ATS.5,6,12–14,16–18 The KCNJ2 mutations reported previously in ATS are nonfunctional and have dominant-negative effects (ie, the mutant subunits suppressed the WT subunits’ function5,6,12–14). S369X showed no dominant-negative effects, which may explain why the index patient exhibited a relatively mild form of ATS without ventricular tachyarrhythmia or dysmorphic features. Because the truncation occurs at the very end of the C-terminus, it is suggested that the structure of the pore region would be preserved, and therefore, proteins containing the truncated subunit could partially function as WT (Figure 3B).
As previously reported, abnormal trafficking of mutant proteins (KCNJ2-V302M, H9004314–315) is recognized as 1 of the mechanisms causing ATS.17,18 The trafficking defect was hypothesized to be a result of ER retention, degradation of folding-defective mutant proteins, or mutation of a binding motif essential for trafficking. Defective trafficking in ion channelopathies also was reported in other types of LQTS.30,31 Confocal image analysis was used in the present study to identify KCNJ2-S369X as the trafficking-deficient mutation, which is similar to that of V302 and H9004314–315. Mutant S369X subunits were, however, transported to the plasma membrane after coassembled with WT subunits and formed functional tetramers.

The Kir channel family contains several trafficking motifs at their C-terminus. For example, PDZ motif-binding proteins are important for targeting channels and moving them to specific subcellular locations.32 Notably, Kir2.1 contains an ER-to-Golgi export signal,28 the motif FCYENE (Figure 2), in its C-terminal domain. By using various truncated Kir2.1 channels, Ma and colleagues28 demonstrated that FCYENE consensus at codon 374 to 379 (Figure 2B) played the role of export signal from ER to Golgi and that lack of C-terminus including this motif resulted in reduced expression to the cell surface. Their truncated Kir2.1 (1 to 362) showed cellular phenotypes similar to those displayed by S369X (Kir2.1, 1 to 367). Therefore, S369X is exactly a naturally occurring mutation lacking this motif. In the heterozygous condition mimicking the clinical setting, 2 types of subunits, WT and S369X, can assemble to form heteromeric tetramers, although this leads to the presence of <4 ER-to-Golgi export-signaling

**Figure 5.** ER colocalization with WT and S369X in COS7 cells. Microscopic images of COS7 cells expressing EGFP-WT (A-1 ~ A-3, 1 μg/dish), EGFP-S369X (B-1 ~ B-3, 1 μg/dish), and EGFP-S369X cotransfected with WT-KCNJ2 pIRES/CD8 (C-1 ~ C-3, 0.5 μg each/dish) (bars, 20 μm). Each cell was cotransfected with DsRed2-ER (1 μg/dish). Images are shown for GFP alone, DsRed2-ER, and the merged image. Colocalization between EGFP-KCNJ2 and DsRed2-ER appears as yellow. ER indicates endoplasmic reticulum. Other abbreviations as in Figure 4.

**Figure 6.** FRET analysis. A, YFP-WT and CFP-S369X were coexpressed in COS7 cells. CFP-S369X is pseudocolored in green (left), and YFP-WT is pseudocolored in red (middle). The merged image (right) shows colocalization of CFP-S369X and YFP-WT (bar, 20 μm). B, Pseudocolor images of CFP-S369X before (CFP prebleach) and after (CFP postbleach) YFP photobleaching. C, Summarized data of FRET efficiency. #P<0.01 versus CFP-WT, ¶P<0.01 versus CFP-N1-YFP-N1, tested by Wilcoxon test. CFP indicates cyan fluorescent protein; FRET, fluorescence resonance energy transfer; YFP, yellow fluorescent protein; WT, wild type.
motifs in 1 functional channel. Along with this idea, cotransfection of the mutant with WT-KCNJ2 indeed promoted the resultant K⁺ current density (Figure 3D).

These results were unexpected because most previously reported KCNJ2 mutations exerted dominant-negative suppressions. Based on the experiments of heterologous expression and FRET analysis, S369X appeared not to act as a dominant-negative mutation (Figures 5 and 6). FRET analysis indicated the direct protein-protein interaction between mutant and WT subunits, suggesting that WT subunits may partially rescue the inappropriate trafficking of the mutant subunits by assembling into heteromeric complexes. Therefore, the truncated mutant proteins, though lacking the intracellular trafficking signal, seem to exert “inverse” dominant-negative effects. Physical interaction of 2 KCNJ2 subunits, fore, the truncated mutant proteins, though lacking the intracellular trafficking signal, seem to exert “inverse” dominant-negative effects. Physical interaction of 2 KCNJ2 subunits, WT and S369X (located to the end of the C-terminus), was shown by using the FRET method (Figure 6), and it would be plausible that incorporation of the mutant subunit eventually increases the number of functional channels and, thereby, produces a partial rescue of currents.

In conclusion, these effects may be the reason why the phenotype of the index patient with KCNJ2-S369X mutation showed milder clinical features. More recently, KCNJ2 mutations have been shown to be a cause not only in ATS, but also in catecholaminergic polymorphic ventricular tachycardia.20 Such subcellular regulation of KCNJ2 protein expression makes the potential extension and severity of the phenotype extremely variable.

The present study had some limitations. In the experiment shown in Figure 3, the results of coexpression with WT and mutant at 0.5 μg each yielded 330 pA/pF and was close to that resulting from the mathematical addition of half of WT and mutant at 1 μg each [(542 + 83.5)/2 = 313 pA/pF]. Increase in current density by coexpression was 5.2%, which was smaller than the case with 1 μg expression (16%). Because we used the liposomal transfection method, which has intrinsic experimental limitations to evaluate the efficiency of optimal cDNA transfection, we should be careful to assess the results quantitatively and await further study to confirm the rescue effect more quantitatively.

We used a heterologous expression system that allowed us to reproduce the I_{K1}-like currents in cells transfected with WT and S369X. However, the electrophysiological experiments were performed with a simplistic model in the absence of cellular heterogeneity. Indeed, Kir2.x channel families may form functional heteromultimers; an additional complication in that heteromultimerization of Kir2.x may alter the biochemical characteristics of channel functions.33 Further studies on the interaction of mutations between Kir2.1 and Kir2.x in ATS may provide further insights into the pathophysiological mechanisms underlying ATS.

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Disclosures

None.

References

Andersen-Tawil syndrome is a rare disorder inherited in an autosomal-dominant fashion. Mutations in KCNJ2, a gene encoding the inward rectifier K\(^+\) channel Kir2.1, are associated with Andersen-Tawil syndrome, which is characterized by ventricular tachyarrhythmias associated with QT (QU)-interval prolongation, periodic paralysis, and dysmorphic features. We identified a novel KCNJ2 mutation, S369X, in a 13-year-old boy with prominent QU-interval prolongation and mild periodic paralysis. The mutation results in the truncation at the middle of the cytoplasmic C-terminal domain that eliminates the endoplasmic reticulum-to-Golgi export signal. KCNJ2-S369X exhibited this deficiency in the present electrophysiological and confocal microscopic analysis, and when coexpressed with KCNJ2 wild type, these abnormalities were partially restored. Fluorescence resonance energy transfer analysis demonstrated direct protein-protein interactions between wild type and S369X subunits in the intracellular compartment. The S369X mutation causes a loss of the endoplasmic reticulum export motif, but the trafficking deficiency can be partially rescued by directly assembling with the wild type protein, resulting in a limited restoration of plasma membrane localization and channel function. This alleviation may explain why our patient presented with a relatively mild Andersen-Tawil syndrome phenotype.
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