Phenotype Variability in Patients Carrying KCNJ2 Mutations

Running title: Kimura et al.; Phenotype variability in KCNJ2 mutations

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Abstract:

**Background** - Mutations of *KCNJ2*, the gene encoding the human inward rectifier potassium channel Kir2.1, cause Andersen-Tawil syndrome (ATS), a disease exhibiting ventricular arrhythmia, periodic paralysis and dysmorphic features. However, some *KCNJ2* mutation carriers lack the ATS triad and sometimes share the phenotype of catecholaminergic polymorphic ventricular tachycardia (CPVT). We investigated clinical and biophysical characteristics of *KCNJ2* mutation carriers with “atypical ATS”.

**Methods and Results** - Mutational analyses of *KCNJ2* were performed in 57 unrelated probands showing typical (≥2 ATS features) and atypical (only one of the ATS features or CPVT) ATS. We identified 24 mutation carriers. Mutation-positive rates were: 75% (15/20) in typical ATS, 71% (5/7) in cardiac phenotype alone, 100% (2/2) in periodic paralysis, and 7% (2/28) in CPVT. We divided all carriers (n=45, including family members) into two groups: typical ATS (A) (n=21, 47%) and atypical phenotype (B) (n=24, 53%). Patients in (A) had a longer QUc interval ((A): 695±52 vs. (B): 643±35 msec) and higher U wave amplitude (0.24±0.07 vs. 0.18±0.08 mV). C-terminal mutations were more frequent in (A) (85 vs. 38%, P<0.05). There were no significant differences in incidences of ventricular tachyarrhythmias. Functional analyses of four mutations found in (B) revealed that R82Q, R82W, and G144D exerted strong dominant negative suppression (current reduction by 95, 97, and 96%, respectively, vs. WT at -50mV), and T305S moderate suppression (reduction by 89%).

**Conclusions** - *KCNJ2* gene screening in atypical ATS phenotypes is of clinical importance, because more than half of mutation carriers express atypical phenotypes, despite their arrhythmia severity.

**Key words:** CPVT; ion channels; Andersen-Tawil syndrome; KCNJ2; phenotype
Introduction

Andersen-Tawil syndrome (ATS) represents a disease entity characterized by three features: (1) ventricular arrhythmias with Q(T)U prolongation, (2) periodic paralysis, and (3) dysmorphic features. It is an autosomal-dominant inherited disease resulting from a heterozygous mutation of the KCNJ2 gene. This gene encodes an inward rectifier K channel (Kir2.1), ubiquitously expressed in the myocardium, skeletal muscle, brain, and osteocytes. Since the first discovery of a mutation in this disease in 2001, we have extensively examined KCNJ2 mutations in patients suspected of having ATS. In 2007, we described both the clinical and genetic features of 23 patients (13 probands and 10 family members), and reported that the identification rate of KCNJ2 mutation in this cohort was 100% if the patients satisfied two or more features of ATS. On a closer inspection, however, we noticed that ~30% of the KCNJ2 mutation carriers lacked two of the ATS features: frequent PVC, bidirectional or polymorphic ventricular tachycardias (bVT or pVT), with QT or QU prolongation, without periodic paralysis or dysmorphic features. Recently, Tester and colleagues reported a possible phenotypic overlap between ATS and catecholaminergic polymorphic VT (CPVT). CPVT is a form of inherited cardiac arrhythmia, characterized by exercise- and/or stress-induced p/bVTs with a normal cardiac structure. However, the incidence and clinical characteristics of atypical ATS and KCNJ2-related CPVT remain unknown.

In the present study, we conducted the genetic screening for KCNJ2 mutation-related phenotypes — those fulfilling at least one of the ATS features or CPVT criteria. We compared the clinical and genetic features between typical ATS and atypical phenotype (only one of the ATS features or CPVT) patients. We hypothesized that the mutated channel function found in patients with atypical ATS phenotypes would show different $I_{K1}$ current properties. Using the
patch-clamp technique, we examined the functional features of four mutations found in the atypical phenotype group.

**Methods**

**Study Subjects**

Fifty-seven unrelated probands (65% females, age at diagnosis: 18 ± 12 years old) from 31 institutes in Japan were enrolled in the study. They were clinically diagnosed with either typical ATS (defined as patients with ≥2 ATS features) (n=20), mild ATS (those with one of the ATS features — cardiac arrhythmia alone (n=7), periodic paralysis with an abnormal U wave (n=2)), or CPVT (n=28) (Figure 1, Table 1).

Three features of ATS were clinically determined as follows: (1) cardiac involvement was determined by the presence of ventricular arrhythmias (frequent premature ventricular contractions (PVCs), bigeminy, bVT, pVT, or monomorphic VT), with prolongation of the corrected QU interval and/or a prominent U wave. (2) The presence of periodic paralysis was based on standard criteria 11. (3) Dysmorphic features were defined by the presence of 2 or more of the following: (a) low-set ears, (b) hypertelorism (wide-set eyes), (c) a small mandible, (d) clinodactyly (permanent lateral or medial curve of a finger or toe), and (e) syndactyly (persistent webbing between fingers or toes) 12.

Twenty-eight of the 57 patients fulfilled the diagnostic criteria of CPVT — exertional syncope plus documentation of bVT or pVT during exercise or exercise tests. Patients with QT prolongation were excluded 10.

**ECG Manifestation**

We measured QU intervals if there was a prominent U wave and QT intervals in cases showing no U wave. The QT interval was defined from the onset of QRS to the end of the T wave. The U
wave was defined as an early diastolic deflection after the end of the T wave. The QU interval was defined from the onset of QRS to the end of the U wave. QT and QU intervals were corrected according to Bazzett’s formula. The end of the T or U wave was the point at which a tangent drawn to the steepest portion of the terminal part of the T or U wave crossed the isoelectric line. Because a prominent U wave is often fused to the next PQ segment in some cases, we defined the isoelectric line as a segment connecting two points preceding consecutive QRS complexes. A diagnosis of QT prolongation was made if the QTc exceeded 440 milliseconds (ms) for males and 460 ms for females, in accordance with the standard criteria.

Abnormal U waves were judged based on the following criteria: (a) wave amplitude ≥ 0.2 mV or (b) amplitude larger than preceding T wave.

bVT was identified as a VT characterized by a beat-to-beat alternation of the QRS axis in most of the documented runs of ventricular tachycardia (more than four consecutive beats).

pVT was defined as the VT with an irregularly variable axis of the QRS.

**DNA Isolation and Mutation Analysis**

The protocol for genetic analysis was approved by the Institutional Ethics Committee and performed under its guidelines. All patients provided informed consent before the genetic analysis was carried out. Genomic DNA was isolated from leukocyte nuclei using a DNA purification kit (Maxwell Blood DNA Purification Kit, Promega, Madison, WI, USA). Genetic screening was first performed using denaturing high-performance liquid chromatography (dHPLC WAVE System; Transgenomic, Omaha, NE, USA). Abnormal conformers were amplified via PCR, and sequencing was performed on an ABI 3130 DNA sequencer (Perkin Elmer, Foster City, CA, USA). The cDNA sequence numbering was based upon the GenBank reference sequence NM_000891.2 for KCNJ2. Regarding suspected CPVT probands, we also...
performed screening involving target mutation analysis for 34 RyR2 gene exons (3, 8-16, 44-49, 83-84, 88-89, 91-97, and 99-105)\textsuperscript{19,20}, and all exons of CASQ2 gene. In addition to these three genes, we examined the entire coding sequence of KCNQ1, KCNH2, SCN5A, and KCNE1-5 to exclude the unexpected presence of compound mutations related to primary electrical diseases\textsuperscript{21,22}. When a mutation was detected, we examined its presence in >200 Japanese control subjects to exclude the possibility of polymorphisms. When mutations were detected in probands, we also screened their family members.

**Genotype-phenotype Correlation**

Baseline clinical characteristics collected were the age at diagnosis, symptomatic episodes, and treatment. As shown in Figure 1, we divided all KCNJ2 mutation carriers into two groups — a typical ATS group (Group A): carriers showing two or more ATS features, and an atypical ATS group (Group B): those showing only one of the ATS features or CPVT. Compound mutation and KCNJ2 double mutation cases were excluded from analysis.

**In Vitro Mutagenesis**

Regarding four KCNJ2 mutations found in Group B (R82W, R82Q, G144D, and T305S), site-directed mutagenesis was employed to construct mutants, as described previously\textsuperscript{23}. Briefly, human KCNJ2 cDNA was subcloned into the pCMS-EGFP plasmid (Clontech, Palo Alto, CA, USA). We engineered KCNJ2 mutants using a site-directed mutagenesis kit, QuickChange II XL (Stratagene, La Jolla, CA, USA). The presence of mutations was confirmed by sequencing.

**Electrophysiological Experiments and Data Analysis**

To assess the functional modulation of KCNJ2 channels, we employed a heterologous expression system with CHO cells\textsuperscript{8,24}. Briefly, the cells were transiently transfected using the Lipofectamine method (Invitrogen, Carlsbad, CA, USA), using a 1.0 μg/35 mm dish of
pCMSEGFP/KCNJ2 (wild-type (WT) and/or mutant). For electrophysiological experiments, GFP-positive cells were selected 24 to 72 hours after transfection. Current measurement was conducted employing the conventional whole-cell configuration of patch-clamp techniques at 37°C, using an EPC-8 patch-clamp amplifier (HEKA Electronik; Lambrecht, Germany). Currents were evoked by 150 ms square pulses applied in 10 mV increments to potentials ranging from −140 mV to +30 mV from a holding potential of −80 mV. Pipettes were filled with a solution containing (in mM): K-aspartate, 60; KCl, 65; KH₂PO₄, 1; MgCl₂, 2; EDTA, 3; ATP (dipotassium salt), 3; and HEPES, 5 (pH adjusted to 7.2 with KOH), and had a resistance of 3.0 to 5.0 MΩ. The bath solution contained (in mM): NaCl, 140; KCl, 5.4; MgCl₂, 0.5; CaCl₂, 1.8; NaH₂PO₄, 0.33; glucose, 5.5; and HEPES, 5 (pH adjusted to 7.4 with NaOH).

**Immunocytochemistry**

The hemagglutinin (HA) epitope (YPYDVPDYA) was introduced into the pCMS-EGFP/KCNJ2 (WT and mutants) between Ala-115 and Ser-116 (extracellular lesion between TM1 and TM2), as previously described. CHO cells were transfected with 1.0 μg of plasmid DNA in 35-mm glass-bottom dishes. Forty-eight hours later, the cells were washed twice with phosphate-buffered saline (PBS), followed by incubation with a mouse anti-HA primary antibody (1:400) (Roche Diagnostics GmbH, Mannheim, Germany) overnight at 4°C. The cells were then washed twice with PBS and incubated with an anti-mouse antibody conjugated to Alexa 568 fluor (1:400) (Molecular Probes, Eugene, OR, USA) as a secondary antibody for 120 minutes, at room temperature. Finally, cells were washed with and immersed in PBS, and confocal imagings were obtained with a Nikon C1si (Nikon Instruments, Tokyo, Japan).

**Statistical Analysis**

Clinical data are expressed as the mean ± standard deviation (SD) for continuous variables.
Comparisons were performed using the chi-square test (for counts ≥ 5) and Fisher exact test (for counts < 5) for categorical variables, and Wilcoxon test for continuous variables. All analyses of the 45 KCNJ2 mutation-positive patients and families took into account the relatedness of patients, using a mixed model for continuous data and GEE for categorical data. The electrophysiological current data are shown as the mean ± SEM. A value of P < 0.05 was considered significant.

Results

Incidence and Characteristics of KCNJ2 Mutations in Probands

We identified 16 different KCNJ2 mutations in 24 of 57 probands (42%) (Table 1, Figure 1). The mean QUc intervals became longer in ATS with an increasing number of ATS features. Prevalences of KCNJ2 mutation were: 75% (15/20) in typical ATS, 71% (5/7) in mild ATS with a cardiac phenotype alone, 100% (2/2) in mild ATS with periodic paralysis alone, and 7% (2/28) in CPVT.

Table 2 summarizes the genotype/phenotype of KCNJ2 mutation-positive probands. The mean age at diagnosis of all probands was 18 ± 12 years old. It was significantly younger (14 ± 12 years old) in probands with typical ATS compared to those with mild ATS (21±14 years old) or the CPVT phenotype (34 ± 3.5 years old). In sixteen KCNJ2 mutations, 13 (81%) were missense, 2 (13%) nonsense, and 1 (6%) insertion (Table 2, Figure 2). Six were located in the N terminus, three in the pore region, and 9 in the C terminus. Figure 2 depicts the phenotypes and mutation sites of probands and family members. Open circles indicate mutation carriers with two or more ATS features (Group A), and closed symbols those with atypical ATS (Group B); closed circles show the ATS cardiac phenotype alone, closed triangles show periodic paralysis alone,
and closed squares show the CPVT phenotype. The letter P in each symbol indicates proband. A five-year-old boy (case #15, Table 2) was found to have double KCNJ2 mutations, paternal V302M and maternal R80C. Compound mutations were detected in two probands: KCNJ2 R82W plus KCNH2 P1093L (case #3) and KCNJ2-G144S plus KCNQ1-A341V (case #4). We excluded these three cases from further analyses.

**Characteristics of KCNJ2 Mutation Carriers**

After excluding the compound mutation cases, 45 KCNJ2 mutation carriers (27 females, 21 probands and 24 of their mutation positive family members) were enrolled (Table 3). Their mean age at diagnosis was 23 ± 16 years, and the average QUc was 667 ± 50 msec. Regarding arrhythmias, ECGs detected PVC in 30 (67%), bVT in 15 (33%), and pVT in 5 (11%) carriers. One patient experienced ventricular fibrillation, four patients (9%) syncope, and 11 (24%) received β-blocker therapy.

**Prevalence of Three ATS Features (Figure 3)**

In 45 KCNJ2 mutation carriers, ventricular arrhythmias (A) were found in 67% (n = 30), periodic paralysis (P) in 40% (n = 18), and dysmorphism (D) in 36% (n = 16). Abnormal U wave (U) was positive in 88% (n = 38 of 43) after excluding 2 cases whose U waves were not measured because of the presence of bigeminy. Twenty-one patients (47%) belonged to Group A (open sections in the pie chart of Figure 3). Six of them (13%) had all three features, 7 (16%) both (A) and (P), 7 (16%) both (A) and (D), and 2 (5%) both (D) and (P). On the other hand, 24 patients (53%) with one of the ATS features belonged to the Group B (closed sections of Figure 3): 11 (24%) only (A), 3 (9%) only (P), 2 (4%) only (D). Eight genotype-positive family members (18%) displayed only (U).

**CPVT Phenotype in KCNJ2 Mutation Carriers**
We identified two KCNJ2 mutations, G144D (Table 2, case #23) and T305S (case #24), in two of 28 probands with CPVT phenotypes (Table 1), without dysmorphic features or periodic paralysis. These two cases experienced first syncope after the age of 30 (G144D, 36 years old; T305S, 32 years old). Furthermore, these probands’ ECGs showed bidirectional VT at rest as well, and, as in CPVT, exercise-aggravated ventricular arrhythmia. In the G144D case, ECG always showed a PVC bigeminy even at rest, and the exercise stress test increased PVC and produced polymorphic VTs. Flecainide (150 mg per day) reduced her VTs. In the T305S case, exercise also increased numbers of PVC. She experienced pVT and VF while nursing her son. Her baseline ECG showed no abnormal U waves (QUc = 644 msec, Tpeak-Upeak interval = 185 msec).

**Comparison of Patients with Typical vs. Atypical Phenotype**

Table 3 summarizes the clinical characteristics of patients and compares them between Groups A and B. There were no significant differences in the number of probands, age at diagnosis, and gender. Group A had a significantly longer QUc interval (Group A, 695 ± 52 msec vs. Group B, 643 ± 35 msec, P = 0.004), higher U wave (0.24 vs. 0.18 mV, P = 0.024). PVCs were significantly more frequent in Group A (pt. n = 19 vs. pt. n = 11, P = 0.032); however, the incidences of bVT, pVT, and ventricular fibrillation were not different between the two groups. There were also no significant differences regarding the incidence of cardiac events and content of treatment.

Concerning the mutation site, C-terminal mutation carriers were more frequent in Group A (n = 18 vs. n = 9, P = 0.002). In contrast, N-terminal mutations were more frequent in Group B (n = 2 vs. n = 13, P = 0.001). As described above, we excluded one case with KCNJ2 double mutations (Table 2, case#15) from this analysis. However his father, carrying a C-terminal
mutation alone (V302M), belonged to Group A and showed a full set of ATS features. In contrast, his mother, carrying an N-terminal mutation (R80C), only displayed an abnormal U wave.

**Functional Assay of Four Mutants Found in Atypical Phenotype Group B**

We conducted electrophysiological functional assays for four mutations in Group B — R82W, R82Q, G144D, and T305S (see Table 2, case # 16, 17, 23, and 24). Figure 4 A-a shows a family of current traces recorded from a CHO cell transfected with WT-KCNJ2 (1 μg). The lower inset in panel A-a indicates the test pulse protocol. WT-KCNJ2 expressed ample and time-independent currents showing a strong inward rectification, as depicted in the voltage-current relation in Figure 4B (closed squares). In contrast, all mutants (1 μg) were non-functional when expressed alone (Figure 4A-b). In order to simulate the allelic heterozygosity, WT and each of the mutant-KCNJ2 clones were co-transfected at an equimolar ratio (0.5 μg each). Representative results are shown in Figure 4 A-e. Outward KCNJ2 channel currents were dominantly suppressed. In contrast, inward currents were variously reduced when co-expressed with WT.

From the results of multiple experiments, the mean current densities were measured at their respective test potentials. In panel B, they are plotted as a function of the potentials, and in panel C, those at -140 and -50 mV are presented as dot plots. Outward current densities at -50 mV when co-expressed with WT-KCNJ2 were 3.5±1.7 pA/pF in R82Q, 2.3±2.4 pA/pF in R82W, 2.6±0.9 pA/pF in G144D and 8.1±2.4 pA/pF in T305S. Compared to the current densities obtained with the WT clone alone (1 μg, left plot in panel C), the percent reductions were 95, 97, 96, and 89% respectively. In contrast, inward current densities at -140 mV were -162±20 pA/pF in R82Q, -152±22 pA/pF R82W, -43±13 pA/pF in G144D, and -199±20 pA/pF in T305S. Percent reductions were 58, 39, 89 and 49%, respectively. Thus, G144D mutation exerted dominant negative suppression effects on both out- and inward currents. The other three
mutations, however, had such effects only on outward currents.

**Immunocytochemistry of Mutant Channels (R82Q, R82W, G144D, T305S)**

Regarding several KCNJ2 mutations, impaired intracellular transport has been reported to cause loss of function. We therefore examined the trafficking of these four mutants and WT channels using an HA-tagging method. Figure 5 depicts typical results of confocal imaging. WT and HA-R82Q, HA-R82W, and HA-T305S mutants showed normal trafficking, while the HA-G144D mutant displayed no rim of red fluorescence, suggesting the presence of a trafficking defect.

**Discussion**

**Prevalence of KCNJ2 Mutation and ATS Features in Typical ATS**

In the present study, we could identify heterozygous KCNJ2 mutations in 24 (42%) of 57 probands of different cohorts showing typical ATS, and atypical phenotypes (one of the ATS features or CPVT). Regarding 20 typical ATS (≥ 2 of three ATS features) probands, KCNJ2 mutations were positive in 15 (75%). This value was comparable to those of previous reports: Plaster and colleagues identified 13 KCNJ2 positives in 16 unrelated ATS kindreds (81%). Tristani-Firouzi and colleagues identified 17 KCNJ2 positives in 25 kindreds (68%), and Donaldson and colleagues reported 9 positives in 17 kindreds (53%). The prevalence of KCNJ2-positive probands was, however, lower when screened in the total patients, compatible with long QT syndrome (LQTS): Eckhardt and colleagues reported 4 KCNJ2 mutation positives from 541 LQTS probands (0.74%). Fodstad and colleagues reported 2 carriers in 188 LQTS patients (1%) .

**Atypical ATS Phenotype**
After excluding the compound mutation cases, there were 45 mutation carriers, and 24 (53%) had atypical ATS phenotypes and 8 had none of them. They only showed abnormal U waves. Accordingly, probands in this group received a genetic diagnosis at a significantly older age. Among the three features of ATS, dysmorphic features could be seen from the infant period; in contrast, ventricular arrhythmia appeared later, presumably because the $I_{K1}$ current could be reduced in females by gonadal steroids. The ATS-related phenotype was reported to be dependent on gender — female subjects with KCNJ2 R67W from a Caucasian family displayed ventricular arrhythmias after the age of 10 years, and arrhythmias were reduced during pregnancy and after age 55 years, coinciding with menopause. In contrast, male mutation-positive subjects from the same family showed no ventricular arrhythmias, but periodic paralysis. Interestingly, a case with R67W in our cohort was a male and complained of only periodic paralysis, supporting their conclusion, although there is a conflicting report by Donaldson and colleagues. They reported that the R67W mutation is capable of causing all phenotypes of ATS, and the pattern observed in the sex-specific kindred is not universal. It appears that other genetic or environmental factors contribute to a family’s susceptibility to disease symptoms.

The topological location of KCNJ2 mutations may influence the expression of ATS features. In the present study, C-terminal mutations were more frequent in the typical ATS group (Table 3). Zhang and colleagues also reported that dysmorphism and periodic paralysis were more frequently observed in C-terminal mutation carriers. The Kir 2.1 C-terminus relates to various types of loss of function in $I_{K1}$ currents. Lopes and colleagues identified 12 basic residues in Kir2.1 that changed channel-PIP$_2$ interactions — 10 of them were located in the C-terminus. The C-terminus also contains the endoplasmic reticulum (ER) export sequence,
FCYENE and the trafficking-related acidic cluster EDDSE at positions 374-379 and 386-391, respectively \(^3^2, ^3^3\). More recently, we reported an S369X mutation located close to this ER export signal that impedes ER-Golgi transport \(^7\).

We tested the trafficking function of four mutations, and only G144D mutation showed a trafficking defect (Figure 5). Our results suggest that the phenotype expression variability of \(KCNJ2\) mutations may be influenced by the topological location of mutations; however, the other possibilities e.g. environmental factors, modifier genes, or SNPs \(^3^4\), remain unstudied.

**Phenotypic Overlap between CPVT and ATS**

The prevalence of \(KCNJ2\) mutation carriers in the CPVT phenotype was lower than in the other phenotypes (Figure 1, Table 1). Our two CPVT probands with \(KCNJ2\) mutation (G144D, T305S) experienced first syncope after the age of 30 years, and their ECGs showed bidirectional VT or PVCs at rest as well. In contrast, the age at first syncope of \(RyR2\)-related CPVT patients was reportedly younger age (mean age of 8 years) \(^1^0\), and their syncope occurred mainly during exercise, but not while resting. These findings, i.e. late onset of symptoms and ventricular arrhythmia at rest, may be clues to distinguish between \(KCNJ2\)-related and \(RyR2\)- or \(CASQ2\)-related CPVT. Functional assays revealed that both G144D and T305S exerted dominant negative suppression effects on outward currents when co-expressed with WT Kir2.1 subunits. Apparently, therefore, baseline functional modulation by these mutations was not related to the phenotypic expression of ATS or CPVT.

Recently, a V227F mutation was identified in a patient with the typical CPVT phenotype but without dysmorphism or periodic paralysis \(^3^5\). A biophysical assay showed that heterozygous WT/V227F channels were identical to WT channels in function, but stimulation by cAMP-dependent protein kinase A (PKA) significantly down-regulated the heterozygous mutant
but not WT Kir2.1 currents. This particular type of loss-of-function may explain why the proband displayed the CPVT phenotype. More recently, Barajas-Martinez and colleagues demonstrated the characteristics of their patient with R260P mutation. She showed typical phenotypes of both ATS and CPVT. The β-blocker nadolol was first used but ineffective. Her symptoms subsided after treatment with flecainide. She had dysmorphic features and bidirectional VT both at rest and during exercise testing. Functional analysis revealed that R260P mutation had strong dominant negative suppression effects, like in our G144D case. Regarding our mutations, their modulation by PKA was not examined because they showed a significant loss-of-function at the baseline (Figure 4).

**Phenotype and Channel Function**

Although R82W, R82Q, G144D, and T305S were found in patients with an atypical phenotype of ATS (Group B), these mutations showed dominant negative suppression effects on outward currents (Figure 4). Therefore, the results obtained for a heterologous expression system did not necessarily correlate with the clinical ATS severity. In this regard, Eckhardt and colleagues identified four KCNJ2 mutations — R67Q, R82W, T75M, and T305A — in probands lacking the ATS triad and a family history of ATS. Surprisingly, we also identified R67W, R82W/Q, and T305S in Group B. Therefore, residues of R67, R82, and T305 may be associated with atypical ATS phenotypes.

**Study Limitation**

Regarding CPVT probands, we screened 34 hot spot exons of the RyR2 gene. We could not exclude some variants in the remaining exons of RyR2. In conclusion, KCNJ2 gene screening in patients with atypical ATS (only one of the ATS features or CPVT) phenotypes is of clinical importance, because 53% of mutation carriers were found to express atypical phenotypes, despite
their severity of arrhythmia.

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Conflict of Interest Disclosures: None

References:


Table 1. Demographic Characteristics of Different Patient Cohorts: KCNJ2 Mutation Incidence Rates (57 Probands)

<table>
<thead>
<tr>
<th></th>
<th>Typical ATS (Group A)</th>
<th>Atypical ATS (Group B)</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ATS cardiac alone</td>
<td>ATS periodic paralysis alone</td>
<td>CPVT</td>
</tr>
<tr>
<td>Probands, n</td>
<td>20</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>13 (65)</td>
<td>5 (71)</td>
<td>0</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>17±11</td>
<td>21±13</td>
<td>19±6</td>
</tr>
<tr>
<td>QT(U)c *, msec</td>
<td>660±90</td>
<td>600±106</td>
<td>548±77</td>
</tr>
<tr>
<td>KCNJ2 positives, n (%)</td>
<td>15 (75)</td>
<td>5 (71)</td>
<td>2 (100)</td>
</tr>
</tbody>
</table>

*QUc interval was employed in Typical ATS, ATS cardiac phenotype alone, and ATS periodic paralysis alone. QTc interval was employed in CPVT.
Table 2. *KCNJ2* Mutation-carrying Probands (n=24)

<table>
<thead>
<tr>
<th>Case No.</th>
<th><em>KCNJ2</em>-DNA</th>
<th>Protein</th>
<th>Age/Sex</th>
<th>ECG†</th>
<th>QTc/QUc, ms</th>
<th>Dysmorphism</th>
<th>Paralysis</th>
<th>Mutated channel function‡</th>
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<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118C&gt;T</td>
<td>R40X*</td>
<td>15/F</td>
<td>1,2</td>
<td>451/618</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>BB</td>
</tr>
<tr>
<td>2</td>
<td>200G&gt;A</td>
<td>R67Q</td>
<td>14/F</td>
<td>1,2</td>
<td>360/626</td>
<td>+</td>
<td>+</td>
<td>d.n.</td>
<td>-</td>
<td>Ib, BB</td>
</tr>
<tr>
<td>3</td>
<td>244C&gt;T</td>
<td>R82W</td>
<td>11/M</td>
<td>2</td>
<td>NA/NA</td>
<td>+</td>
<td>-</td>
<td>d.n.</td>
<td>-</td>
<td>BB</td>
</tr>
<tr>
<td>4</td>
<td>430G&gt;A</td>
<td>G144S</td>
<td>32/F</td>
<td>1,2</td>
<td>483/716</td>
<td>+</td>
<td>-</td>
<td>d.n.</td>
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<tr>
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<td>G146S</td>
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<tr>
<td>6</td>
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<td>R82Q</td>
<td>12/F</td>
<td>2</td>
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<td>+</td>
<td>-</td>
<td>d.n.</td>
<td>-</td>
<td>Az</td>
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<tr>
<td>7</td>
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<td>R218W</td>
<td>16/M</td>
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<td>420/700</td>
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<tr>
<td>8</td>
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<td>R218W</td>
<td>27/F</td>
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<td>408/681</td>
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<td>9</td>
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<tr>
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<td>19/F</td>
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<td>-</td>
<td>BB</td>
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<tr>
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<td>2,3</td>
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<td>d.n.</td>
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<td>904G&gt;A</td>
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<td>V302M: d.n.</td>
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<td>R218W</td>
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<td>20</td>
<td>683ins††</td>
<td>R228ins*‡‡</td>
<td>24/M</td>
<td>1,4</td>
<td>498/-</td>
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<td>+</td>
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<td>21</td>
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<td>R67W</td>
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<td>22</td>
<td>1106C&gt;A</td>
<td>S369X</td>
<td>13/M</td>
<td>-</td>
<td>472/600</td>
<td>+</td>
<td>-</td>
<td>tr†</td>
<td>-</td>
<td>-</td>
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<tr>
<td>23</td>
<td>431G&gt;A</td>
<td>G144D</td>
<td>32/F</td>
<td>3</td>
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<td>BB, FL</td>
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<tr>
<td>24</td>
<td>914C&gt;G</td>
<td>T305S*</td>
<td>36/F</td>
<td>1,4,5</td>
<td>443/664</td>
<td>-</td>
<td>-</td>
<td>Figures 4,5</td>
<td>+/+</td>
<td>BB, ICD</td>
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†PVC=1, bVT=2, pVT=3, VT=4, ventricular fibrillation=5, long QT=6; ‡d.n.=dominant negative; tr = trafficking defect; ††683insGAAAAGCCTTGTTGGAAGCTCATGTTTG; ‡‡R228insKSHLVEAHVR; *novel mutation; NA, not available; Age, age at diagnosis; ACA, aborted cardiac arrest; ICD, implantable cardioverter defibrillator; BB, beta-blocker; Ib, mexiletine; IV, verapamil; FL, flecainide; Pil, pilscainide; Az, acetazolamide.
Table 3. Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total, n = 45</th>
<th>Group A, n=21</th>
<th>Group B, n=24</th>
<th>P-value</th>
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<tr>
<td>Proband, n, (%)</td>
<td>21</td>
<td>12 (57)</td>
<td>9 (38)</td>
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<tr>
<td>Age at diagnosis, ave year±SD</td>
<td>23±16</td>
<td>20±14</td>
<td>26±16</td>
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<td>Female, n, (%)</td>
<td>27 (60)</td>
<td>12 (57)</td>
<td>15 (63)</td>
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<tr>
<td>Baseline ECG</td>
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<tr>
<td>HR, ave msec±SD</td>
<td>76±22</td>
<td>75±19</td>
<td>77±25</td>
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<tr>
<td>QTc, ave msec±SD</td>
<td>430±42</td>
<td>418±42</td>
<td>440±40</td>
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<tr>
<td>QTc, ave msec±SD</td>
<td>667±50</td>
<td>695±52</td>
<td>643±35</td>
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<tr>
<td>T amp, ave mV±SD</td>
<td>0.51±0.27</td>
<td>0.56±0.28</td>
<td>0.46±0.26</td>
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<tr>
<td>U amp, ave mV±SD</td>
<td>0.21±0.08</td>
<td>0.24±0.07</td>
<td>0.18±0.08</td>
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<tr>
<td>Tp-Up, ave msec±SD</td>
<td>214±31</td>
<td>224±32</td>
<td>204±28</td>
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<tr>
<td>U/T ratio, ave±SD</td>
<td>0.57±0.5</td>
<td>0.66±0.66</td>
<td>0.48±0.3</td>
<td>0.601</td>
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<tr>
<td>Arrhythmias, pt n (%)</td>
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<tr>
<td>PVC</td>
<td>30 (67)</td>
<td>19 (90)</td>
<td>11 (46)</td>
<td>0.032*</td>
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<td>Bidirectional VT</td>
<td>15 (33)</td>
<td>10 (48)</td>
<td>5 (11)</td>
<td>0.195</td>
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<tr>
<td>Polymorphic VT</td>
<td>5 (11)</td>
<td>3 (14)</td>
<td>2 (8)</td>
<td>0.368</td>
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<tr>
<td>VF</td>
<td>1 (2)</td>
<td>0</td>
<td>1 (4)</td>
<td>0.571</td>
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<tr>
<td>Cardiac events</td>
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<tr>
<td>Syncope, pt n (%)</td>
<td>4 (9)</td>
<td>2 (10)</td>
<td>2 (8)</td>
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<td>ACA, pt n (%)</td>
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<td>Treatment</td>
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<td>β-blockers</td>
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<td>6 (29)</td>
<td>5 (21)</td>
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<tr>
<td>Flecaainde</td>
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<td>1 (5)</td>
<td>1 (4)</td>
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<td>Verapamil</td>
<td>2 (4)</td>
<td>1 (5)</td>
<td>1 (4)</td>
<td>0.925</td>
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<td>ICD implantation</td>
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<td>0</td>
<td>1 (4)</td>
<td>0.533</td>
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<td>Mutation site</td>
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<td></td>
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<tr>
<td>C-terminal, pt n (%)</td>
<td>27 (60)</td>
<td>18 (85)</td>
<td>9 (38)</td>
<td>0.002*</td>
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<tr>
<td>Pore, pt n (%)</td>
<td>2 (4)</td>
<td>1 (5)</td>
<td>1 (4)</td>
<td>0.925</td>
</tr>
<tr>
<td>N-terminal, pt n (%)</td>
<td>16 (36)</td>
<td>2 (10)</td>
<td>14 (58)</td>
<td>0.001*</td>
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</tbody>
</table>

Group A: KCNJ2 mutation carriers showing ≥ ATS features, Group B: those showing only one of the ATS features or CPVT, *P<0.05 compared to Group A. ACA, aborted cardiac arrest; ICD, implantable cardioverter defibrillator; VF, ventricular fibrillation.

Figure Legends:

**Figure 1:** A Flow Chart of 57 Probands Suspected as Carriers of KCNJ2 Mutations
ATS=Andersen-Tawil syndrome; CPVT=catecholaminergic polymorphic ventricular tachycardia.

**Figure 2:** Topology of Kir2.1 Channel Showing ATS-related Mutation Sites. Open circles indicate probands showing two or more ATS features (Group A). Closed symbols indicate the probands in Group B. Closed circles indicate those showing ATS with the cardiac phenotype.
alone, closed triangles are ATS with periodic paralysis alone, and closed squares represent a clinical diagnosis of CPVT. “P” in each symbol indicates proband.

**Figure 3:** Prevalence of ATS’ Three Features Among 45 KCNJ2 Mutation Carriers (21 probands and 24 family members). (A): ventricular arrhythmia, (P): periodic paralysis, (D): dysmorphic features, (U): abnormal U wave. Open sections indicate Group A: patients who had ≥ 2 ATS features (n=21, 47%). Closed sections indicate Group B (n=24, 53%): those who had only one of the ATS features or an abnormal U wave alone.

**Figure 4:** Functional Analyses of Mutated Kir2.1 Channels Found in Group B. A: representative Kir2.1 currents expressed in CHO cells: (a) wild-type (WT) cDNA 1 μg, (b) R82Q (1 μg), R82W (1 μg), G144D (1 μg), and T305S (1 μg). Cells were held at -80 mV. (c) co-transfection with WT (0.5 μg) and each mutant, R82Q (0.5 μg), R82W (0.5 μg), G144D (0.5 μg), and T305S (0.5 μg). Square pulses of 150-ms duration were applied to the potentials between -140 and +30 mV with 10-mV increments. Scale bars indicate 50 ms and 2 nA. B: Plots for current-voltage relationships obtained by multiple experiments of the same protocol as shown in A. Current densities were calculated by dividing with cell capacitance. C: Dotplots showing mean current densities in WT (1 μg, n=15), 1/2WT (0.5 μg, n=15), co-transfection with WT (0.5 μg) and R82Q (0.5 μg) (n=24), WT (0.5 μg) and R82W (0.5 μg) (n=25), WT (0.5 μg) and G144D (0.5 μg) (n=15), and WT (0.5 μg) and T305S (0.5 μg) (n=20). Upper panel: those at -140 mV, and lower panel: those at -50 mV.

**Figure 5:** Cellular Localization of WT and Four Mutant (R82Q, R82W, G144D, T305S) Kir2.1 channels. In the figure, HA-KCNJ2 indicates HA-tagged KCNJ2 (positive control). The upper panel shows the green fluorescence of GFP, middle panel: red fluorescence of secondary anti-HA antibody, lower panel: merging of green and red fluorescences; white bars in the merged panel indicate 10 μm.
**KCNJ2 suspected probands, n=57**

- **ATS typical phenotype, n=20**
  - **ATS atypical phenotype, n=37**
    - **ATS cardiac alone, n=7**
    - **Periodic paralysis, n=2**
    - **CPVT, n=28**

**<KCNJ2 positives>**

- n=15 (75%)
  - 2 compound mutations
  - 1 double mutation

- n=5 (71%)
  - n=2 (100%)

- n=2 (7%)

**Typical probands, n=12**

**Atypical probands, n=9**

**<KCNJ2 testing for family members>**

- **Typical family members, n=9**
  - **Group A, n=21**

- **Atypical family members, n=15**
  - **Group B, n=24**
Group A: ≥2 ATS features
Group B: Only one of the ATS features or U wave alone

(A) Ventricular arrhythmia
(D) Dysmorphism
(P) Periodic paralysis
(U) Abnormal U wave
Phenotype Variability in Patients Carrying KCNJ2 Mutations

Hiromi Kimura, Jun Zhou, Mihoko Kawamura, Hideki Itoh, Yuka Mizusawa, Wei-Guang Ding, Jie Wu, Seiko Ohno, Takeru Makiyama, Akashi Miyamoto, Nobu Naiki, Qi Wang, Yu Xie, Tsugutoshi Suzuki, Shigeru Tateno, Yoshihide Nakamura, Wei-Jin Zang, Makoto Ito, Hiroshi Matsuura and Minoru Horie

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