Phenotypic Variability of ANK2 Mutations in Patients With Inherited Primary Arrhythmia Syndromes

Mari Ichikawa, MD; Takeshi Aiba, MD, PhD; Seiko Ohno, MD, PhD; Daichi Shigemizu, PhD; Junichi Ozawa, MD; Keiko Sonoda, MD; Megumi Fukuyama, MD, PhD; Hideki Itoh, MD, PhD; Yoshihiro Miyamoto, MD, PhD; Tatsuhiro Tsunoda, PhD; Takeru Makiyama, MD, PhD; Toshihiro Tanaka, MD, PhD; Wataru Shimizu, MD, PhD; Minoru Horie, MD, PhD

Background: Mutations in ANK2 have been reported to cause various arrhythmia phenotypes. The prevalence of ANK2 mutation carriers in inherited primary arrhythmia syndrome (IPAS), however, remains unknown in Japanese. Using a next-generation sequencer, we aimed to identify ANK2 mutations in our cohort of IPAS patients, in whom conventional Sanger sequencing failed to identify pathogenic mutations in major causative genes, and to assess the clinical characteristics of ANK2 mutation carriers.

Methods and Results: We screened 535 probands with IPAS and analyzed 46 genes including whole ANK2 exons using a bench-top NGS (MiSeq, Illumina) or performed whole-exome-sequencing using HiSeq2000 (Illumina). As a result, 12 of 535 probands (2.2%, aged 0–61 years, 5 males) were found to carry 7 different heterozygous ANK2 mutations. ANK2-W1535R was identified in 5 LQTS patients and 1 symptomatic BrS and was predicted as damaging by multiple prediction software. In total, as to phenotype, there were 8 LQTS, 2 BrS, 1 IVF, and 1 SSS/AF. Surprisingly, 4/8 LQTS patients had the acquired type of LQTS (aLQTS) and suffered torsades de pointes. A total of 7 of 12 patients had documented malignant ventricular tachyarrhythmias.

Conclusions: Various ANK2 mutations are associated with a wide range of phenotypes, including aLQTS, especially with ventricular fibrillation, representing “ankyrin-B” syndrome. (Circ J 2016; 80: 2435–2442)

Key Words: Acquired long QT syndrome; ANK2; Ankyrin-B syndrome; Bradycardia; Inherited primary arrhythmia syndrome
with the following IPAS: 341 LQTS, 58 Brugada syndrome (BrS), 40 idiopathic ventricular fibrillation (IVF), 27 catecholaminergic polymorphic ventricular tachycardia (CPVT), 22 SSS/AF, 16 SCD (molecular autopsy), 11 short-coupled variant of torsades des pointes (SCTdP), 7 ven- tricular tachycardia (VT), 7 short QT syndrome, and 6 complete atrioventricular block (CAVB) (Table 1). They were suspected to have or diagnosed with IPAS and were referred to the Shiga University of Medical Science (Otsu)/Kyoto University (Kyoto) (n=325) or the National Cerebral and Cardiovascular Center (Suita) (n=210) for genetic evaluation. All subjects gave written informed consent in accordance with the guidelines approved by each institutional review board (SUMS_23-128-2, NCVC_M24-031-4). Their phenotypes were evaluated based on clinical findings and 12-lead ECGs. We defined heart rate ≤60 beats/min as bradycardia for patients aged ≥19 years. For those aged 18 years or younger, we adopted definitions from previous reports.²¹,²²

### Methods

#### Subjects and Genetic Analysis

The cohort consisted of 535 probands (male patients=288) as “ankyrin-B syndrome”.¹⁴⁻¹⁸

However, only a small number of ANK2 mutation carriers, mainly in Caucasians, have been reported, with limited clinical features, and to our best knowledge no Japanese patients with ANK2 mutations have been reported. One reason is that ANK2 is too big (>45 exons) for screening by the conventional Sanger method. Recent advances in genetic screening using a next-generation sequencer (NGS) have enabled us to screen many target genes in a short time. In this study, using a NGS, we tried to elucidate the prevalence of ANK2 mutations in inherited primary arrhythmia syndrome (IPAS) patients with no major causative genes in our previous cohort studies, and the clinical characteristic of patients with ANK2 mutations.

#### Table 1. Prevalence of ANK2 Mutations in Inherited Primary Arrhythmia Syndrome

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>No. of ANK2 mutations</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-QT syndrome</td>
<td>341</td>
<td>8</td>
<td>2.3</td>
</tr>
<tr>
<td>Brugada syndrome</td>
<td>58</td>
<td>2</td>
<td>3.4</td>
</tr>
<tr>
<td>Idiopathic VF</td>
<td>40</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>SSS/AF</td>
<td>22</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>VT</td>
<td>7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>SCD</td>
<td>16</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>CPVT</td>
<td>27</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>SCTdP</td>
<td>11</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Short-QT syndrome</td>
<td>7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>CAVB</td>
<td>6</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>535</td>
<td>12</td>
<td>2.2</td>
</tr>
</tbody>
</table>

AF, atrial fibrillation; CAVB, complete atrioventricular block; CPVT, catecholaminergic polymorphic ventricular tachycardia; SCD, sudden cardiac death; SCTdP, short-coupled variant of torsades des pointes; SSS, sick sinus syndrome; VF, ventricular fibrillation; VT, ventricular tachycardia.

#### Figure 1. Map of ankyrin-B domain with ANK2 mutations identified in patients with inherited primary arrhythmia syndromes. The novel mutations identified in this study are denoted in red, and those from previous studies in blue. CTD, C-terminal domain; DD, death domain; IP3R, IP3 receptor; MBD, membrane-binding domain; NCX, Na/Ca exchanger; NKA, Na⁺/K⁺ ATPase; SBD, spectrin-binding domain.
Results

Genetic Screening With NGS

We identified 7 different heterozygous ANK2 mutations in 12 probands (2.2%, age 0–61 years, 5 males) (Table 1): p.Y148H (c.442T>C), p.G761S (c.2281G>A), p.T825I (c.2474C>T), p.R895Q (c.2684G>A), p.L1128V (c.3382C>G), p.W1535R (c.4603T>A), and p.I1855R (c.5564T>G) (Figure 1). p.W1535R was the most frequent and was identified in 6 probands, while the other mutations were identified in a single proband (Table 2). These mutations were scattered in various regions of ANK2 (Figure 1) and most were predicted as damaging by multiple prediction software (Table 2).

Genetic screening by NGS detected multiple variants in various genes in the 12 probands. In an effort to identify disease-causing mutations, we excluded genes not expressed in the heart, variants of which had MAF >0.005 in the public databases, or the severity of each variant estimated by the prediction software was minimal. Table S2 shows these remaining variants.

Patient 1: ANK2-Y148H

ANK2-Y148H was identified in a female newborn. She was found to have marked bradycardia with high-grade AVB and QT prolongation (QTc: 532 ms, HR: 73 beats/min) at 2 days old, but no syncope. Based on her ECG at 4 months, she no longer had AVB but still had bradycardia for her age (HR: 43 beats/min, QTc: 442 ms). QT prolongation was not detected during exercise stress test, but epinephrine challenge test prolonged his QT interval had normalized to 433 ms.

Patient 2: ANK2-G761S

ANK2-G761S was identified in a 61-year-old man who had experienced a transient loss of consciousness after dinner. His resting 12-lead ECG (Figure 2A) showed marked bradycardia (HR: 43 beats/min, QTc: 442 ms). QT prolongation was not detected during exercise stress test, but epinephrine challenge test prolonged his QT interval (from 441 ms to 478 ms). His syncope was suspected to be caused by LQTS. His brother had died suddenly at age 59, but other family members remained asymptomatic (Figure 3A). The patient carried the 2281G>A variant in KCNE1, which is known as a causative gene for LQTS type 5 (Table S2), and we could not rule out the possibility that the variant affected his phenotype.

Table 2. Genetic Characteristics of ANK2 Mutations in Inherited Primary Arrhythmia Syndrome

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>ANK2 domain</th>
<th>dbSNP</th>
<th>ExAC Browser</th>
<th>ESP 6500</th>
<th>1000 genomes</th>
<th>HGVD ToMMo</th>
<th>Polyphen2</th>
<th>Provean</th>
<th>SIFT</th>
<th>CADD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>442T&gt;C</td>
<td>Y148H</td>
<td>MBD</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Possibly damaging [0.735]</td>
<td>Deleterious [-4.131]</td>
<td>[0.04]</td>
<td>26.6</td>
</tr>
<tr>
<td>2</td>
<td>2281G&gt;A</td>
<td>G761S</td>
<td>MBD</td>
<td>rs774769455</td>
<td>-</td>
<td>-</td>
<td>0.003</td>
<td>-</td>
<td>Possibly damaging [0.779]</td>
<td>Deleterious [-5.413]</td>
<td>[0.08]</td>
<td>19.99</td>
</tr>
<tr>
<td>3</td>
<td>2474C&gt;T</td>
<td>T825I</td>
<td>MBD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>-</td>
<td>Possibly damaging [0.987]</td>
<td>Deleterious [-3.012]</td>
<td>[0.064]</td>
<td>26.3</td>
</tr>
<tr>
<td>4</td>
<td>2684G&gt;A</td>
<td>R895Q</td>
<td>SBD</td>
<td>rs751513548</td>
<td>0.0002048</td>
<td>-</td>
<td>0.001</td>
<td>-</td>
<td>Benign [0.164]</td>
<td>Deleterious [-3.853]</td>
<td>[0.22]</td>
<td>18.09</td>
</tr>
<tr>
<td>5</td>
<td>3382C&gt;G</td>
<td>L1128V</td>
<td>SBD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Possibly damaging [0.987]</td>
<td>Deleterious [-2.894]</td>
<td>[0.03]</td>
<td>11.56</td>
</tr>
<tr>
<td>6–11</td>
<td>4603T&gt;A</td>
<td>W1535R</td>
<td>DD</td>
<td>rs199473346</td>
<td>0.00003303</td>
<td>-</td>
<td>0.0022</td>
<td>-</td>
<td>Possibly damaging [0.547]</td>
<td>Neutral [-0.691]</td>
<td>[0.37]</td>
<td>16.3</td>
</tr>
<tr>
<td>12</td>
<td>5564T&gt;G</td>
<td>I1855R</td>
<td>CTD</td>
<td>rs201966460</td>
<td>0.00000842</td>
<td>0.0002048</td>
<td>0.003</td>
<td>-</td>
<td>Possibly damaging [0.547]</td>
<td>Neutral [-0.691]</td>
<td>[0.04]</td>
<td>26.6</td>
</tr>
</tbody>
</table>

CADD: Combined Annotation-Dependent Depletion; ExAC: Exome Aggregation Consortium; ESP: Exome Sequencing Project; HGVD: HUMAN Genetic Variation Database; SIFT: Sorting Intolerant From Tolerant; ToMMo: Tohoku Medical Megabank Organization; CTD, C-terminal domain; DD, death domain; MBD, membrane-binding domain; SBD, spectrin-binding domain.
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Patient 4: **ANK2-R895Q**

ANK2-R895Q was identified in a 59-year-old man who had experienced syncope because of VF at work and he had been successfully defibrillated by an ambulance crew. There were no abnormal findings on his resting 12-lead ECG, UCG, and other examinations. In the exercise stress test, neither QT prolongation nor polymorphic VT was observed. He was diagnosed as having IVF, and an implantable cardioverter defibrillator (ICD) was implanted. His mother had died suddenly in

Experienced repeated episodes of loss of consciousness since she was 12 years old, and the events were mostly exercise-related. Her resting 12-lead ECG showed a normal QTc interval (416 ms), and QT prolongation was not detected during exercise stress test; however, epinephrine challenge test prolonged her QT interval (from 436 ms to 508 ms). She had no structural cardiac abnormalities on ultrasound cardiography (UCG) and no notable family history.

**Figure 2.** 12-lead ECGs of the probands with inherited primary arrhythmia syndromes. (A) 12-lead ECG of patient 2 showing marked bradycardia: HR: 43 beats/min, QTc: 442 ms. (B-1) 12-lead ECG of patient 6 at admission shows marked QT prolongation: QTc: 597 ms. (B-2) 12-lead ECG of patient 6 on the following day. QTc shortened to 445 ms. (C) 12-lead ECG of patient 7 showing coved-type ST elevation in the 3rd intercostal position.
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Patient 5: ANK2-L1128V
ANK2-L1128V was identified in a 49-year-old man who, although asymptomatic, showed a saddleback-type ST elevation in V1–2 on ECG recorded at a health checkup. Pilsicainide challenge test unmasked a coved-type ST elevation in V1 at the 3rd intercostal position (BrS type 1), and he was suspected of having BrS. His father and brother had died suddenly at ages 63 and 65, respectively (family tree in Figure 3C); however, no ECGs of his family members were available.

Patients 6–11: ANK2-W1535R
ANK2-W1535R in the death domain (Figure 1) was identified in 6 patients (1 male); 4 had LQTS with documented TdP or VF (patients 6, 8, 9 and 11 in Tables 2 and 3), 1 was asymptomatic but suspected to have LQTS from the family history (patient 10), and the last patient had symptomatic BrS (patient 7).

Patient 6, a 36-year-old woman, was admitted to an emergency hospital with nausea and vomiting. In the emergency room, she repeatedly lost consciousness because of TdPs. Although her 12-lead ECG displayed a marked QT prolongation (QTc: 597 ms) on admission (Figure 2B-1), her QTc had shortened to 445 ms on the following day (Figure 2B-2). She had experienced no syncpe except for this episode and had no notable family history.

Patient 7 was a 39-year-old man who experienced loss of consciousness with seizures after dinner. He had a similar history at age 30 when he had a fever. His 12-lead ECG (Figure 2C) showed a coved-type ST elevation in V1–2 at the 3rd intercostal position and he was diagnosed as having BrS. In the electrophysiological study (EPS), VF was induced by a single extrastimuli (600/240 ms) from the right ventricular outflow tract, and an ICD was then implanted. His mother also had a history of syncope, although the details were uncertain. We could not obtain her consent for genetic analysis.

Patient 8, a 45-year-old woman, had experienced repeated episodes of loss of consciousness with seizures after dinner. He had a similar history at age 30 when he had a fever. His 12-lead ECG (Figure 2C) showed a coved-type ST elevation in V1–2 at the 3rd intercostal position and he was diagnosed as having BrS. In the electrophysiological study (EPS), VF was induced by a single extrastimuli (600/240 ms) from the right ventricular outflow tract, and an ICD was then implanted. His mother also had a history of syncope at around 20 years old, and the same mutation was identified (Figure 3D). Her 12-lead ECG showed no Brugada-type ST changes or QT prolongation, though we could not perform a provocation test with sodium-channel blocker or an exercise stress test.

Patient 9, a 44-year-old woman under artificial hemodialysis...
was stopped, and an ICD was implanted.

**Patient 12: ANK2-I1855R**

ANK2-I1855R was identified in a 49-year-old man who had been diagnosed with SSS at age 44. Although he was asymptomatic, his bradycardia had progressed gradually. Atrial standstill was detected during EPS, but H-V conduction was normal. In addition, VF was induced by triple extrastimuli from the right ventricle (600/300/280/240 ms). Therefore, an ICD was implanted. His sister carrying the same mutation had bradycardia, but his mother, who was not a carrier, showed a normal heart rate of ECG. His grand-uncle on his father’s side had been implanted with a pacemaker (Figure 3F). Both his grand-uncle and father were already deceased, so their genetic data were not available.

**Summary of ANK2 Mutation Carriers (Table 3)**

ANK2 mutations were identified in probands manifesting LQTS (n=8), BrS (n=2), IVF (n=1), and SSS/AF (n=1); 8 of 12 genotyped probands (67%) were symptomatic with syncope, and malignant ventricular tachyarrhythmias were documented in 5 of the 8 symptomatic probands (63%). Bradycardia was present in 8 of the 12 probands (67%; patients 1, 2, 4–7, 10, 12), and patient 1 had CAVB. None of the probands had any structural cardiac abnormalities. All 5 male probands had bradycardia. A patient with BrS and 1 with SSS/AF underwent an EPS, and VF was induced in both cases. In 7 of the 8 LQTS patients carrying ANK2 mutations, the baseline QT

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Sex</th>
<th>Dx</th>
<th>ANK2 mutation</th>
<th>Symptoms</th>
<th>TdP/VF</th>
<th>ECG</th>
<th>QTc (ms) at events, Diagnosis of Acquired LQTS</th>
<th>Other ECG or genetic abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>F</td>
<td>LQTS</td>
<td>Y148H</td>
<td>–</td>
<td>–</td>
<td>107 P 433</td>
<td>QTc 532 ms, HR 73 beats/min</td>
<td>2:1 AVB, PAT</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>M</td>
<td>LQTS</td>
<td>G761S</td>
<td>Syncope</td>
<td>–</td>
<td>43 P 442</td>
<td>QTc 478 ms, epinephrine load test</td>
<td>KCNE1-S28L (rs199473350)</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>F</td>
<td>LQTS</td>
<td>T825I</td>
<td>Syncope</td>
<td>–</td>
<td>80 – 436</td>
<td>QTc 508 ms, epinephrine load test</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>M</td>
<td>IVF</td>
<td>R895Q</td>
<td>Syncope</td>
<td>VF</td>
<td>58 P 384</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>M</td>
<td>BrS</td>
<td>L1128V</td>
<td>–</td>
<td>–</td>
<td>60 P 426</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>F</td>
<td>LQTS</td>
<td>W1535R</td>
<td>Syncope</td>
<td>TdP</td>
<td>60 P 445</td>
<td>QTc 597 ms with nausea and vomiting</td>
<td>Coved ST elevation, EPS-induced VF</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>M</td>
<td>BrS</td>
<td>W1535R</td>
<td>Syncope</td>
<td>–</td>
<td>55 P 353</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>F</td>
<td>LQTS</td>
<td>W1535R</td>
<td>Syncope</td>
<td>TdP</td>
<td>67 – 450</td>
<td>QTc 550 ms, hypokalemia and drug-induced TdP</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>F</td>
<td>LQTS</td>
<td>W1535R</td>
<td>Syncope</td>
<td>VF</td>
<td>94 – –</td>
<td>QTc 622 ms after hemodialysis</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>F</td>
<td>LQTS</td>
<td>W1535R</td>
<td>Syncope</td>
<td>TdP</td>
<td>50 P 446</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>F</td>
<td>LQTS</td>
<td>W1535R</td>
<td>Syncope</td>
<td>TdP</td>
<td>70 – 448</td>
<td>QTc 557 ms, drug-induced TdP</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>49</td>
<td>M</td>
<td>SSS, AF</td>
<td>I1855R</td>
<td>–</td>
<td>–</td>
<td>49 P 507</td>
<td>EPS-induced VF</td>
<td></td>
</tr>
</tbody>
</table>

P, positive; –, negative. AVB, atrioventricular block; BrS, Brugada syndrome; CRBBB, complete right bundle branch block; EPS, electrophysiological study; IVF, idiopathic VF; LP, late potential; LQTS, long QT syndrome; MRI, magnetic resonance imaging; PAT, paroxysmal atrial tachycardia; PM, pace maker; TdP, torsades des pointes; UCG, ultrasound cardiography; VPC, ventricular premature contraction. Other abbreviations as in Table 1.
interval was nearly within the normal range, but was markedly prolonged by epinephrine load test or other QT prolongation factors. In particular, 4 of the LQTS patients showed prominent QT prolongation around episodes of TdP; in other words, they were diagnosed as having an acquired type of LQTS (aLQTS).

In 7 families (58%), other members showed clinical phenotypes: SCD, syncope or bradycardia (Table 3). Among the phenotype-positive family members, 5/6 males and 1/4 females had died suddenly. Except for patient 1 and 10, most of the patients were diagnosed in adulthood.

### Discussion
This comprehensive analysis using NGS firstly demonstrated that the prevalence of ANK2 mutations was 2.2% (12 probands, 7 mutations) in the study cohort of 535 Japanese IPAS probands with no major IPAS-related genes identified. Their expressivity varied considerably: LQTS, BrS, IVF and SSS/AF as previously reported. Furthermore, an ANK2-W1555R variant was identified in 4 patients with aLQTS.

Ankyrin is a multifunctional protein involved in the targeting and stabilization of ion channels and transporters in various tissues. The ankyrin genes, ANK1 (chromosome 8p11), ANK2 (4q25–27), and ANK3 (10q21) encode 3 different ankyrins: canonical ankyrin-R (210 kD), ankyrin-B (220 kD), and ankyrin-G (190 kD), respectively. The ankyrins have 4 domains: (1) membrane-binding domain (MBD), (2) spectrin-binding domain (SBD), (3) death domain (DD) and (4) C-terminal domain (CTD) (Figure 1). The MBD of 24 ankyrin repeats interacts with various membrane proteins, and the DD and CTD comprise the regulatory domain. The known ANK2 variants in abnormal cardiac function are reported to localize around the CTD and DD regulatory domains. Figure 1 summarizes the location of ANK2 mutations identified in the present patients, showing a wide distribution over the gene.

Caucasian patients with ANK2 variants present with various dysrhythmia phenotypes, including ventricular tachyarrhythmia and SAN disturbance, a syndrome now known as ankyrin-B syndrome. Le Scouarnec et al demonstrated that a trafficking dysfunction of ankyrin-B caused abnormal SAN electrical activity and sinus node dysfunction, thereby causing bradycardia and heart rate variability. The known ANK2 variants around the regulatory domain (DD and CTD) contribute to the various degrees of ankyrin-B loss-of-function, and proposed that it may reflect the clinically wide spectrum.

Ankyrin-B as a membrane “adapter” binds to various integral membrane proteins, including the sodium channel. Probands carrying ANK2 mutations presented with phenotypes of BrS in our study as well as in previous reports. SCN5A, encoding an α-subunit of the cardiac sodium channel, is the most frequent causative gene responsible for BrS, and its loss-of-function type mutations cause BrS. Ankyrin-B knockout mice have been shown to reduce peak Na while increasing late Na currents, indicating that loss-of-function type ANK2 mutations may present as BrS and long QT phenotypes.

Ankyrin-B also binds to the Na/Ca exchanger (NCX), and several ANK2 mutations are reported to reduce the NCX expression. Bögeholz and colleagues demonstrated that heterozygous NCX-knockout mice had significantly shortened action potential duration (APD). ANK2 mutations may therefore affect the APD through NCX reduction and cause heterogeneity of repolarization, which is potentially arrhythmogenic.

To the best of our knowledge, this is the first report on ANK2 mutations in a large Japanese population. The phenotypes of the mutation carriers were diverse, and 8 of 12 genotyped probands (67%) showed bradycardia. In addition, across the various diagnoses, 7 of 12 probands presented with documented malignant ventricular tachyarrhythmia, and 2 of the remaining 5 patients had a family history of SCD. As shown in the family trees (Figure 3), the genotype appeared to be associated with the phenotype. These findings suggest that ANK2 mutations may be associated with malignant ventricular arrhythmias. Although 7 of 12 probands carried several variants in genes expressed in the heart (Table S2), most of the variants were of genes reported as the cause of structural cardiac diseases, and our probands showed no cardiac structural abnormalities that were explained by these variants. Furthermore, the genes reported had no association with IPAS except for KCNE1 and ANK2.

Surprisingly, 7 of 8 probands showing LQTS phenotype (87.5%) had the concealed type of LQTS. Additional factors such as epinephrine, hypokalemia, sympathetic nerve stimulator, and bradycardia, aggravated their QT prolongation and after removal of these modifiers, the QTc interval nearly normalized (443±37 ms, Table 3). The mean age at first diagnosis (39±17 years) was older than for typical congenital LQTS, which is also consistent with aLQTS.

In particular, W1555R was identified in 6 of 12 probands (50%), and 4 of those 6 (67%) had aLQTS. TdP/VF was documented in these 4 aLQTS probands. Though functional assay of ANK2-W1555R was not available, according to prediction software this variant is thought to be damaging, and may be associated with the occurrence of aLQTS. More recently, we demonstrated in 188 clinically diagnosed aLQTS patients that the prevalence of “concealed” congenital LQTS was higher than expected. We screened for the 5 major LQTS genes: KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2, and identified mutations in these LQTS-related genes in 53 patients (28%). ANK2 may therefore be a candidate gene for aLQTS.

### Study Limitations
We were not able to perform functional analysis of the ANK2 mutations identified in the probands because ankyrin-B can combine with or affect multiple ion channels and exchangers. We were also unable to further analyze family members to show co-segregation. Therefore, this study may have a limitation to elucidating the pathogenicity of the diseases and diverse phenotypes caused by ANK2 mutations. In addition, NGS could detect multiple variants of different genes, which were not completely excluded as responsible for the phenotypes.

### Conclusions
We identified multiple ANK2 mutations in IPAS patients with diverse phenotypes using NGS. Ankyrin-B syndrome has primary importance in the differential diagnosis of IPAS, especially in those with malignant ventricular tachyarrhythmias and/or the aLQTS phenotype.

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Disclosures
None.

References

Supplementary Files
Supplementary File 1
Table S1. Target genes of the bench-top type of next-generation sequencer
Table S2. Rare variants in other causative genes
Please find supplementary file(s) at http://dx.doi.org/10.1253/circj.CJ-16-0486