Novel Cardiocerebral Channelopathy Associated with a KCND3 V392I Mutation

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Summary

While a KCND3 V392I mutation uniquely displays a mixed electrophysiological phenotype of Kv4.3, only limited clinical information on the mutation carriers is available. We report two teenage siblings exhibiting both cardiac (early repolarization syndrome and paroxysmal atrial fibrillation) and cerebral phenotypes (epilepsy and intellectual disability), in whom we identified the KCND3 V392I mutation. We propose a link between the KCND3 mutation with a mixed electrophysiological phenotype and cardiocerebral phenotypes, which may be defined as a novel cardiocerebral channelopathy.

Key words: Atrial fibrillation, Early repolarization syndrome, Epilepsy

Inherited defects in ion channels that regulate both cardiac and neuronal excitability may generate both cardiac and cerebral phenotypes, so-called cardiocerebral channelopathies.1,2 Some mutations in ion channel-encoding genes expressed in both the heart and brain have been reported to be responsible for cardiocerebral channelopathies.3

KCND3, which encodes Kv4.3 composing transient outward potassium currents (Ito or IA), is expressed in both the heart and brain. KCND3 mutations that cause an increase of Kv4.3 have been reported to be responsible for the cardiac phenotypes including Brugada syndrome (BrS),2 early-onset atrial fibrillation (AF),4 and early repolarization syndrome (ERS).5 In addition, a KCND3 V392I mutation has been reported to be identified in one deceased individual with autopsy-negative sudden unexplained death syndrome (SUDS).5,6 In contrast, KCND3 mutations that cause a decrease of Kv4.3 have been reported to be responsible for the cerebral phenotype of spinocerebellar ataxia (SCA) type 19/22.5,7 Moreover, the KCND3 V392I mutation has recently been reported to be identified in one proband with unexplained epilepsy.8 Although the KCND3 V392I mutation appears to be associated with both cardiac and cerebral phenotypes, only limited clinical information on KCND3 V392I carriers is available.

We recently encountered two teenage siblings (Figure 1A) who had exhibited both cardiac and cerebral phenotypes, in whom we identified the KCND3 V392I mutation (Figures 1B, 1C). Here we report the clinical features of a family with this mutation.

Case Report

Written informed consent of the subjects was obtained for their participation in the study. The procedures employed were reviewed and approved by the institutional ethics review board (approval number: 2017-15).

The proband (III-1) (Figure 1A) was a 19-year-old female, whose 12-lead electrocardiogram (ECG) during sinus rhythm (heart rate [HR]: 66 bpm) showed J-point elevations (> 0.1 mV)/J-waves in the infero-lateral leads with a QTc interval of 409 ms (Figure 2A, upper panel). She had been suffering from palpitations due to paroxysmal atrial fibrillation (PAF), which occurred at night and early morning, for two months (Figure 2B). Physical examinations and blood tests revealed no abnormalities. Echocardiography and cardiac magnetic resonance imaging (MRI) demonstrated no sign of structural heart disease. Pilsicainide provocation (50 mg) did not induce coved-type
ST-segment elevation in the right precordial leads. A 12-lead Holter ECG showed that the amplitudes of the J-waves were augmented and the QTc interval was shortened to 359 ms at a lower heart rate (48 bpm) (Figure 2C, left), while the amplitudes of the J-waves were decreased and the QTc interval was prolonged to 499 ms at a higher heart rate (98 bpm) (Figure 2C, right). After the oral administration of 300 mg quinidine sulfate daily, the amplitudes of the J-waves were decreased (Figure 2A, lower) and the frequency of palpitations was markedly reduced. In addition to the cardiac phenotypes, she had had several episodes of focal impaired awareness seizures, which occurred from 3 to 9 years of age, with intellectual disability (ID) (WISC-III, FIQ: 64 at 13 years of age), but without any focal signs or the SCA phenotype. Electroencephalography recorded at 13 years of age displayed focal spikes in the bilateral occipital region (Figure 2D). She was diagnosed with focal epilepsy of unknown etiology. Brain MRI showed no structural abnormalities.

The proband’s 16-year-old younger sister (III-2) (Figure 1A), whose 12-lead ECG during sinus rhythm (HR: 56 bpm) showed the J-waves in the infero-lateral leads with a QTc interval of 394 ms (Figure 3A), had been suffering from PAF for several months (Figure 3B). Physical

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Figure 1. Identification of a KCND3 V392I mutation. A: The pedigree of a family with the KCND3 V392I mutation. Arrow indicates the proband. Filled symbols indicate the carriers of the KCND3 V392I mutation. B: An electropherogram of part of KCND3 in the proband. C: An electropherogram of part of KCND3 in the proband’s younger sister. Nucleotide and amino acid substitutions are indicated.

Figure 2. Cardiac and cerebral phenotypes of the proband. A: 12-lead ECG at baseline (upper), and that during oral administration of 300 mg quinidine sulfate daily (lower). Arrows indicate J-waves. B: II-lead ECG during atrial fibrillation. C: 12-lead Holter ECGs at a lower heart rate (left) and at a higher heart rate (right). Arrows indicate J-waves. D: Electroencephalography at baseline. Arrow heads indicate focal spikes.
examinations and blood tests revealed no abnormalities. Echocardiography demonstrated no sign of structural heart disease. Unfortunately, she suddenly lost consciousness at around midnight at home and died at 16 years of age. Thus, she was diagnosed with ERS. In addition to the cardiac phenotypes, she had had several episodes of focal impaired awareness seizures, which occurred from 2 to 7 years of age, with ID (WISC-III, FIQ: 41 at 12 years of age), but without any focal signs or the SCA phenotype. Electroencephalography recorded at 14 years of age displayed focal spikes in the bilateral occipital region alternately (Figure 3C). She was diagnosed with focal epilepsy of unknown etiology. Brain MRI showed no structural abnormalities.

Figure 3. Cardiac and cerebral phenotypes of the proband’s younger sister. A: 12-lead ECG at baseline. Arrows indicate J-waves. B: II-lead ECG during atrial fibrillation. C: Electroencephalography at baseline. Arrow heads indicate focal sharp waves.
Figure 4. Cardiac phenotypes of the proband’s mother. A: 12-lead ECG at baseline. Arrows indicate J-waves. B: 12-lead Holter ECGs at a lower heart rate (left) and at a higher heart rate (right). Arrows indicate J-waves. C: II-lead Holter ECG showing atrial fibrillation.

Their father (II-1) (Figure 1A) had experienced no syncopal episodes or palpitations, and his 12-lead ECG was unremarkable. On the other hand, their mother (II-2) (Figure 1A) had experienced syncopal episodes twice: one occurred during swimming at 7 years of age and the other occurred while doing stand-up work in her 40s. Her 12-lead ECG during sinus rhythm (HR: 60 bpm) showed the J-waves in the lateral leads with a QTc interval of 421 ms (Figure 4A). Her 12-lead Holter ECG showed that the amplitudes of the J-waves were augmented and the QTc interval was shortened to 393 ms at a lower heart rate (50 bpm) (Figure 4B, left), while the amplitudes of the J-waves were decreased and the QTc interval was prolonged to 441 ms at a higher heart rate (98 bpm) (Figure 4B, right), and a short duration of PAF (Figure 4C) was recorded without any symptoms.

Genomic DNA was extracted from peripheral blood lymphocytes of the subjects using a QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany). We first performed an exome sequencing of the proband (III-1) (Figure 1A). Two hundred nanograms of DNA was fragmented using a Covaris M220 (Covaris, Woburn, MA, USA). Library preparation was performed with an Agilent SureSelectXT Reagent Kit, HSQ (Agilent Technologies, Santa Clara, CA, USA), and exome capture was performed using an Agilent SureSelect Human All Exon v5 Kit (Agilent Technologies). The resulting library was subjected to the paired end sequencing of 76-bp reads on an Illumina NextSeq 500 (Illumina, San Diego, CA, USA), and subsequently aligned against hg19 using BWA-MEM in SureCall v4.0 (Agilent Technologies) with default settings. Mutation calling was performed using an SNPPET in SureCall v4.0 (Agilent Technologies). Annotation for variations was performed using the ANNOVAR database (http://annovar.openbioinformatics.org/en/latest/). Among 47,356 variants detected, we focused on exons and splice sites of genes that are or may be associated with inherited arrhythmia syndromes or inherited epilepsy (Table).9-11) Moreover, we sought to identify variants for which the allele frequency was < 0.0001 in gnomAD (https://gnomad.broadinstitute.org/), and for which the CADD score was > 20 in CADD (https://cadd.gs.washington.edu/). As a result, only one variant, c.1174 G>A, p.V392I in KCND3 (NM_004980.4), which has already been reported,5,8) remained. We then validated the KCND3 V392I mutation by Sanger sequencing (Figure 1B), using the following primers for the PCR reaction: forward primer, TCCCTGCACATAAGGATCTTG; reverse primer, CAGGAAGGGACTGACTACC. The PCR products were purified and directly sequenced using an ABI PRISM 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

We also identified the KCND3 V392I mutation in the proband’s mother (II-2) (Figure 1A) by Sanger sequencing, but not in her father (II-1) (Figure 1A). Notably, the
In the proband’s younger sister (III-2) (Figure 1A), we identified the V392I mutation by Giudicessi, et al. 

The mutation carrier (12-year-old boy) suffered from ventricular fibrillation storm due to ERS. Notably, he also had had refractory epilepsy, ID, and PAF from two years of age.

The KCND3 V392I mutation has already been reported. However, information on the clinical features of this mutation carriers is limited. Giudicessi, et al. recently reported that a de novo KCND3 mutation, G306A, which caused a mixed electrophysiological phenotype: an increase of Kv4.3 (due to an increased current density and delayed inactivation) and a decrease of the increased Kv4.3 (due to a delayed recovery from inactivation), was associated with ERS.6) The mutation carrier (12-year-old boy) suffered from ventricular fibrillation storm due to ERS. Notably, he also had had refractory epilepsy, ID, and PAF from two years of age.

Table. Inherited Arrhythmia Syndromes and Epilepsy-Related Genes

| Inherited arrhythmia syndromes-related genes | Epilepsy-related genes |
|---------------------------------------------|------------------------|
| ABCC9 | CAV3 | HEY2 | KCNJ5 | NPPA | SEMA3A |
| AKAP9 | CEP68 | JPH2 | KCNJ8 | PITX2 | SH3PXD2A |
| ANK2 | CUX2 | KCNA5 | KCNK3 | PKP2 | SHOX2 |
| C9orf3 | FGF12 | KCNAB2 | KCNN2 | PPF1A4 | SLMAP |
| CACNA1C | GATA4 | KCND2 | KCNN3 | PRRX1 | SNTA1 |
| CACNA2D1 | GATA5 | KCND3 | KCNQ1 | RANGRF | SOX5 |
| CACNB2b | GATA6 | KCNE1 | LMNA | RYR2 | SYNE2 |
| CALM1 | GJA1 | KCNE2 | MYH6 | SCN10A | SYNY02L |
| CALM2 | GJA5 | KCNE3 | MYL4 | SCN1B | TBC5 |
| CALM3 | GPD1L | KCNE4 | NELB | SCN2B | TECLR |
| CAND2 | GREM2 | KCNE5 | NEURL | SCN3B | TRDN |
| CASQ2 | HAND2 | KCNH2 | NXX2-5 | SCN4B | TRPM4 |
| CAV1 | HCN4 | KCNJ2 | NXX2-6 | SCN5A | ZFHX3 |

Discussion

Epilepsy is associated with an increased risk of morbidity and mortality, and sudden unexpected death in epilepsy (SUDEP) is the main cause of death in patients with epilepsy.1,2,3) Although the etiologies of SUDEP may be multifactorial, the notion that inherited defects in ion channels that regulate both cardiac and neuronal excitability may generate both arrhythmia and epilepsy phenotype (cardiocerebral channelopathy) is generally accepted as one of the causes of SUDEP.1,2,3) Genetic studies of SUDEP have been extensively performed, and several mutations, including those in long QT syndrome-related genes and HCN1-4 genes, have been identified.2,4) However, there are still some questions as to whether these mutations are indeed responsible for both epilepsy and sudden death. We herein reported a familial case with the KCND3 V392I mutation who had had both cardiac (ERS, PAF) and cerebral (epilepsy and ID) phenotypes.

KCND3, which encodes Kv4.3 composing I\textsubscript{v}, or I\textsubscript{A}, has been associated with several cardiac and neurological disorders. Until recently, an increase of Kv4.3 was reported to be typically associated with BrS and early-onset AF,2,3) while a decrease of Kv4.3 due to trafficking defects was reported to be typically associated with SCA19/22.6,7) In addition, Takayama, et al. recently reported that a de novo KCND3 mutation, G306A, which caused a mixed electrophysiological phenotype: an increase of Kv4.3 (due to an increased current density and delayed inactivation) and a decrease of the increased Kv4.3 (due to a delayed recovery from inactivation), was associated with ERS.6) The mutation carrier (12-year-old boy) suffered from ventricular fibrillation storm due to ERS. Notably, he also had had refractory epilepsy, ID, and PAF from two years of age.

The KCND3 V392I mutation has already been reported. However, information on the clinical features of this mutation carriers is limited. Giudicessi, et al. firstly reported that the KCND3 V392I mutation was identified in a deceased patient (20-year-old male) who died of SUDS.5) His premortem ECGs were unavailable and his past medical history was unremarkable, with the exception of two syncopal episodes as a teenager. Later, Wang, et al. reported that among a cohort of Chinese children with unexplained epilepsy, the same mutation was identified in a 5-year-old boy with refractory epilepsy from 1.5 years of age.5) Intriguingly, a functional study of the KCND3 V392I mutation by Giudicessi, et al. revealed that this mutation caused a mixed electrophysiological phenotype: an increase of Kv4.3 (due to an increased current density and delayed inactivation) and a decrease of the increased Kv4.3 (due to a delayed recovery from inactivation), although the mutation may cause only an increase of Kv4.3 in the heart because Kv4.3 is expected to recover suffi-
ciently at physiologically normal heart rates.\textsuperscript{31} It is noteworthy that this electrophysiological phenotype resembled that of the KCND3 G306A mutation,\textsuperscript{23} and that carriers of a KCND3 mutation (G306A or V392I) with a mixed electrophysiological phenotype presented with not only cardiac phenotypes (ERS and PAF) but also cerebral phenotypes (epilepsy and ID).

Computer simulations demonstrated that an increase of Kv4.3 in ventricle underlies the creation of J-waves on ECG,\textsuperscript{24} and that an increase of Kv4.3 in atrium results in a shortening of the atrial action potential, and therefore may be the pathogenesis of AF.\textsuperscript{25} Regarding the cardiac phenotypes of our cases, ERS (increased J-wave amplitudes at a lower heart rate) and PAF (which predominantly occurs at a higher heart rate) can be explained by an increase of Kv4.3 in the ventricle and atrium, respectively, due to an increased current density and delayed inactivation.\textsuperscript{3,4,14} In contrast, decreased J-wave amplitudes at a higher heart rate can be explained by a decrease of the increased Kv4.3 due to a delayed recovery from inactivation in the ventricle. In addition, maladaptation of the QT interval to the heart rate can be explained by a decrease of the increased Kv4.3 at a lower heart rate and a decrease of the increased Kv4.3 at a higher heart rate in the ventricle.

On the other hand, regarding the cerebral phenotypes, the mechanisms whereby the KCND3 V392I mutation causes epilepsy are still speculative. Mutations in KCND2 (a paralog of KCND3) that cause either an increase or a decrease of Kv4.2 have been reported to be associated with epilepsy.\textsuperscript{14-17} In contrast, SCA19/22-related KCND3 mutations have recently been reported to be associated with epilepsy.\textsuperscript{15-17} However, it has not been clarified whether an increase of Kv4.3 caused by KCND3 mutations is associated with epilepsy. In case of the KCND3 V392I mutation, an increase of Kv4.3 may be associated with epilepsy. Otherwise, it is also conceivable that a mixed electrophysiological phenotype of Kv4.3 may be associated with epilepsy because cerebral cells may be able to excite frequently enough to decrease Kv4.3 due to a delayed recovery from inactivation. Further studies, using transgenic mice or induced pluripotent stem cells from this mutation carriers, may be necessary to elucidate the mechanisms whereby the KCND3 V392I mutation causes epilepsy.

Conclusions

We have reported for the first time, to the best of our knowledge, a familial case with cardiac (ERS, PAF) and cerebral (epilepsy and ID) phenotypes associated with a KCND3 V392I mutation that caused a mixed electrophysiological phenotype of Kv4.3. We propose a link between KCND3 mutation(s) with a mixed electrophysiological phenotype and cardiocerebral phenotypes, which may be defined as a novel cardiocerebral channelopathy. In some patients with epilepsy, identification of a KCND3 mutation with a mixed electrophysiological phenotype and optimal therapy for cardiac channelopathy will help prevent SUDEP.

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Disclosure

Conflicts of interest: The authors have no conflicts of interest to declare.

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