Novel KCNJ2 Mutation in Familial Periodic Paralysis With Ventricular Dysrhythmia

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Background—Mutations in the KCNJ2 gene, which codes cardiac and skeletal inward rectifying K⁺ channels (Kir2.1), produce Andersen’s syndrome, which is characterized by periodic paralysis, cardiac arrhythmia, and dysmorphic features.

Methods and Results—In 3 Japanese family members with periodic paralysis, ventricular arrhythmias, and marked QT prolongation, polymerase chain reaction/single-strand conformation polymorphism/DNA sequencing identified a novel, heterozygous, missense mutation in KCNJ2, Thr192Ala (T192A), which was located in the putative cytoplasmic chain after the second transmembrane region M2. Using the Xenopus oocyte expression system, we found that the T192A mutant was nonfunctional in the homomeric condition. Coinjection with the wild-type gene reduced the current amplitude, showing a weak dominant-negative effect.

Conclusions—T192, which is located in the phosphatidylinositol-4,5-bisphosphate binding site and also the region necessary for Kir.1 multimerization, is a highly conserved amino acid residue among inward-rectifier channels. We suggest that the T192A mutation resulted in the observed electrical phenotype. (Circulation. 2002;105:2592-2594.)

Key Words: arrhythmia ■ paralysis ■ ion channels ■ genes

Some forms of periodic paralysis are associated with ventricular arrhythmias, often with QT prolongation.1-4 Although these features are inherited as an autosomal-dominant trait,5 linkage analysis failed to demonstrate that periodic paralysis and long-QT interval are related to other forms of potassium-sensitive periodic paralysis or long-QT syndrome. Plaster et al6 found 9 novel mutations in the KCNJ2 gene encoding Kir2.1 channels in patients with Andersen’s syndrome, which comprises periodic paralysis, cardiac arrhythmias, and dysmorphic features.7 The expression of 2 of these mutants in Xenopus oocytes revealed loss-of-function and a strong dominant-negative effect on the wild-type (WT) Kir2.1 channel current. Inward rectifier K⁺ channels, including Kir2.1, play a primary role in controlling cell excitability by maintaining the deep resting potential.

The structure and electrophysiological properties of a cDNA-encoding mouse IRK1 (Kir2.1) have been reported.8 The human inward rectifier K⁺ channel is a homologue of mouse Kir1 and is expressed in the heart, skeletal muscle, and brain.9 We report a novel missense mutation of KCNJ2, T192A, in a Japanese family manifesting periodic paralysis and cardiac dysrhythmia. Its electrophysiological properties were examined in the Xenopus oocyte expression system using the 2-electrode voltage-clamp method.

Methods

A 13-year-old boy and his 11-year-old sister were referred because of abnormal ECGs and frequent premature ventricular contractions (Figure 1A). Their paternal grandfather and father had suffered from periodic paralysis since their early teens (Figure 1B). The grandfather had died of liver disease in his 40s. The grandfather’s 4 siblings and their 6 offspring did not manifest periodic paralysis. The 4 affected family members lacked dysmorphic features. There was no family history of sudden cardiac death.

The boy was born after a normal pregnancy and delivery. His developmental milestones were normal. He had afebrile seizures at 3 years; subsequently, he suffered 2 to 3 episodes a year of muscle weakness. They were of varying severity and lasted up to a few days; there were no precipitating factors. His serum potassium level was within normal limits during the paralytic attacks. At 12 years, he began to have weekly attacks, and oral acetazolamide was started. His baseline ECG showed QT prolongation (680 ms) and premature ventricular contraction bigeminy (Figure 1A). A 24-hour ambulatory ECG documented frequent premature ventricular contractions (>10 000 beats/day). He experienced no palpitations or syncope. Electromyograms revealed myogenic patterns but no myotonic discharges. Nerve conduction was normal. Muscle biopsy specimens examined by electron microscopy contained tubular aggregates.

His sister first experienced episodes of muscle weakness at 10 years. They lasted for several days and occurred without precipitating triggers. Oral acetazolamide was started. Her 12-lead ECG showed marked QT prolongation (610 ms; Figure 1A); a 24-hour ambulatory ECG documented frequent premature ventricular contractions (>10 000 beats/day) and unsustained ventricular...
DNA Isolation and Mutation Analysis
The protocol for genetic analysis was approved by the institutional ethics committee and performed under its guidelines. All subjects gave informed consent before gene analysis.

Genomic DNA was isolated from leukocyte nuclei by conventional methods. Screening for mutations of KCNJ2, KCNQ1, HERG, and SCN5A was performed by polymerase chain reaction/single-strand conformation polymorphism (PCR-SSCP) analysis. PCR products were heat-denatured with formamide and applied to a 13% polyacrylamide gel stained with SYBR Green II (Molecular Probes). Sequencing was on ABI sequencers (PRISM 310, PE Applied Biosystems).

In Vitro Mutagenesis
Human Kir2.1 cDNA was subcloned into pGEMHE, a high-expression vector for oocytes. The point mutant was made using a Quick Change Kit (Stratagene). The introduction of a mutation was confirmed by sequencing the mutation primer and the surrounding sequences of the inwardly rectifying K+ channel family around T192 of Kir2.1.

Voltage-Clamp Experiments
These were performed as described previously. Briefly, Xenopus oocytes, treated with collagenase (2 mg/mL, type 1, Sigma), were injected with ~50 nL of cRNA solution and incubated at 17°C for 2 to 3 days in frog Ringer solution supplemented with 20 mmol/L KCl. The macroscopic current was recorded under a 2-electrode voltage clamp using an OC-725C amplifier (Warner) and a Digidata 1200A digitizer running pCLAMP software (Axon Instruments). The resis-
tance of the microelectrodes, which were filled with 3 mol/L K acetate containing 10 mmol/L KCl (pH 7.2), ranged from 0.1 to 0.4 MOhm. The bath solution contained 10 mmol/L KCl, 80 mmol/L N-methylglucamine, 70 mmol/L HCl, 3 mmol/L MgCl2, and 10 mmol/L HEPES (pH 7.4). All recordings were at room temperature (23±2°C).

Data Analysis
Student’s unpaired t test (Figure 2C) and one-way ANOVA (Figure 2D) were used. P<0.05 was judged statistically significant.

Results

Mutation Analysis
As shown in Figure 1, PCR-SSCP, using the primer set for KCNJ2, showed extra bands in the 3 affected family members but not the controls or the unaffected mother. PCR-SSCP was negative for other long-QT–related genes (KCNQ1, HERG, and SCN5A). Sequencing of the PCR fragment of the abnormal conformer identified a heterozygous, single base-pair substitution (A to G), corresponding to a heterozygous T192A mutation, was found. D, Alignment of the amino acid sequences of the inwardly rectifying K+ channel family around T192 of Kir2.1.

Functional Assay
The electrophysiological properties of the mutant Kir2.1 subunit were assayed by comparing oocytes injected with WT or mutant cRNA (10 ng/oocyte; Figure 2). The injection of WT cRNA induced K+ currents with strong inward rectification. The hommeric T192A mutant did not manifest measurable K+ currents. The coinjection of WT and mutant cRNA (5 ng of each per oocyte) resulted in a current approximately half of that induced by WT cRNA alone (10 ng/oocyte). The inward rectifying property remained unchanged, as indicated by the current-voltage relationships. Experiments using identical protocols were performed in multiple oocytes; pooled data are summarized in Figure 2C. Increases in the amount of T192A cRNA (0, 5, and 15 ng)
resulted in a slight dose-dependent suppression of expressed current (WT cRNA, 5 ng/oocyte), which was statistically significant by one-way ANOVA (Figure 2D).

**Discussion**

We demonstrated that a novel KCNJ2 mutation, T192A, produced familial periodic paralysis with ventricular dysrhythmia. Our family lacked the disfiguring features characteristic of Andersen’s syndrome. The location of the T192A mutation differed from that of 9 previously reported mutations; it was located immediately after the M2 region in the middle of the PIP2-binding domain (residues 175 to 206). T192, which is conserved in all inward rectifier K+ channels with the exception of Kir7.1, may play a crucial role in arranging the location of the PIP2 binding motif (PKKR, 186 to 189).

As expected, homomeric T192A channels were completely nonfunctional. In the study by Plaster et al, D71V and R218W mutant subunits also failed to form functional homomeric channels. The coexpression of WT and D71V subunits induced an inwardly rectifying K+ current with severely reduced amplitude, demonstrating that D71V has a strong dominant-negative effect. The coexpression of R218W and WT also induced a significant K+ current, suggesting that D71V or R218W.6

This observation can be explained by assuming that even a tetrameric combination of only one WT and 3 T192A subunits forms a functional channel. Only one intact PIP2 binding site may suffice for channel function. Alternatively, T192A may not be able to assemble with WT subunits; M2 and subsequent cytoplasmic regions including T192 are involved in Kir2.1 multimerization.

Our patients did not manifest the dysmorphic features characteristic of Andersen’s syndrome. This suggests that the functional modulation induced by T192A may be different from that induced by D71V or R218W.6

**Figure 2.** Macroscopic current recordings under 2-electrode voltage clamping of WT Kir2.1 and T192A mutant expressed in Xenopus oocytes. A, Representative current recordings in 10 mmol/L K+. The holding potential was −50 mV; step pulses from 50 mV to −150 mV were applied in 10 mV decrements. cRNA was injected as follows: 10 ng of WT (left), 5 ng of WT and 5 ng of T192A (middle; Mix), and 10 ng of T192A (right). B, Plots of current-voltage relationships of the data in A. C, Comparison of current amplitudes at −120 mV from the experiments in A and B. The mean and SD values (oocytes, n=16 for each group) were plotted. The values were significantly different (P<0.01) by Student’s unpaired t test. D, Suppression effect of T192A cRNA on the WT current. cRNA was injected as follows: 5 ng of WT (left), 5 ng of WT and 5 ng of T192A (middle), and 5 ng of WT and 15 ng of T192A (right). The mean and SD values (oocytes, n=20 for each group) of the current amplitudes at −120 mV are shown. The values of the 3 groups were significantly different (P<0.05) by one-way ANOVA.

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**References**

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