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Mutation in KCNE1 associated to early repolarization syndrome by modulation of slowly activating delayed rectifier K^+ current

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ABSTRACT

Background: Recent studies have revealed that *mutation* in KCNE1, β-subunits of cardiac potassium channel, involved in ventricular fibrillation. Whereas its role in early repolarization syndrome (ERS) is less well understood.

Objective: To study whether mutant in KCNE1 is associated with ERS and explore the possible underlying molecular mechanisms.

Methods: Whole genome from four unrelated families with ERS was amplified and sequenced. Wild-type (WT) KCNE1 and/or KCNE1-S38G (S38G) were expressed in HEK293 cells with KCNQ1. Functional studies included whole-cell patch-clamp, western blot and immunofluorescence were performed to reveal the possible underlying mechanisms.

Results: The co-expression of KCNE1-S38G and KCNQ1 decreased tail current density of I_{Ks} but had little effect in modulation channel kinetics of I_{Ks}. Compared with KCNE1-WT, the expression and membrane location of KCNE1-S38G decreased. Co-expression of KCNE1-WT and KCNE1-S38G partially rescued the function of I_{Ks} channel.

Conclusions: The S38G mutation induced a loss-of-function of I_{Ks} due to decreasing of KCNE1 protein expression and defecting in KCNE1 protein membrane trafficking. Our findings suggested that KCNE1 may be one of the possible modulatory genes associated to ERS.

1. Introduction

The early repolarization pattern (ERP) is characterized by an elevation of J-wave > 0.1 mV and sometimes involving an ST-segment elevation in at least 2 contiguous inferolateral leads. Since it was first described in 1936 [1], the ERP had been regard as a benign variant of electrocardiogram (ECG) for decades. It had reported that over 5% of the general population have ERP [2,3]. Male sex, adolescents and black race is more commonly associated with ERP [4]. In recent years, a ground number of clinical studies had indicated that ERP is associated with ventricular fibrillation [2, 5–8]. If a subject with ERP and malignant ventricular arrhythmias, that often known as early repolarization syndrome (ERS). Because of the relatively large number of ERPs, and relatively few people with ERPs accompanied by malignant ventricular arrhythmias, it is significant to distinguish the individuals with a high risk mode of ERPs.

Cardiac ion channels disorder had been implicated in the pathogenesis of ERPs. Gain-of-function mutations in the transient outward potassium current (I_{to}) accelerated the early repolarization velocity and suppressed the voltage of the plateau phase in guinea pig cardiomyocytes [9]. Mutations in L-type calcium current (I_{CaL}) [10] and Adenosine triphosphate (ATP)-sensitive potassium current (I_{KATP}) [11] were also reported to be relative with ERP.

Short QT syndrome (SQT) was defined by a characteristic ECG of QT-interval shorting, and liked ERS, with malignant ventricular arrhythmias. Most cases of short QT syndrome involved in mutations of genes encoding I_{Kr} [12,13] and I_{Ks} [14–16]. Whether mutations in I_{Kr} and I_{Ks} are associated with ERS is largely unclear. In this study, we reported a missense mutation (S38G) in the KCNE1 gene, which encodes the regulatory β-subunits of the slowly activating delayed rectifier K^+ current (I_{Ks}), from four unrelated families with ERS. We studied whether mutant KCNE1 (S38G) was associated with ERS and explore the possible underlying molecular mechanisms. Electrophysiology modification of I_{Ks} by KCNE1-S38G was evaluated using patch-clamp technology.

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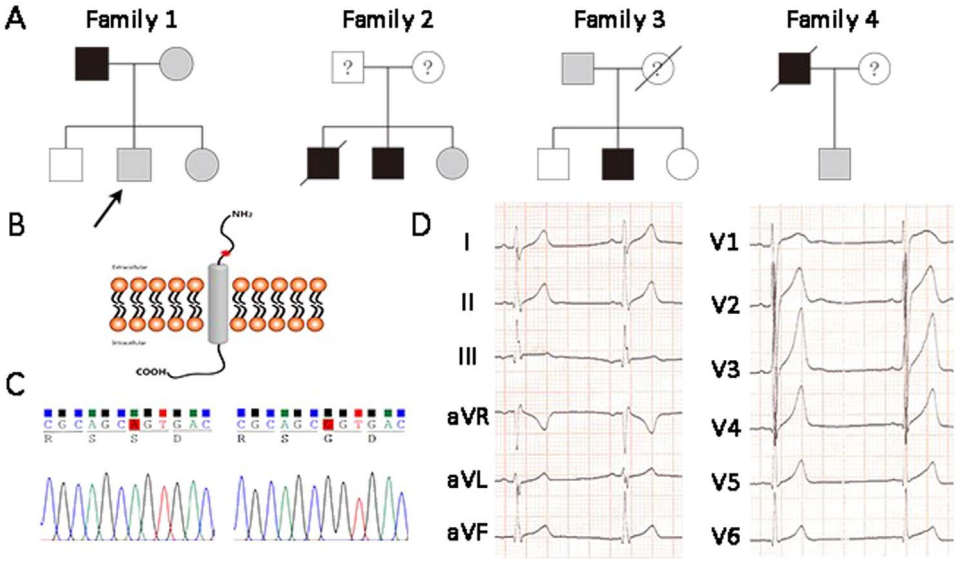

Fig. 1. Genetic analyses and ECG in 4 unrelated families with early repolarization syndrome. **A**: Four family pedigrees with ERS. Black symbol: early repolarization pattern with ventricular fibrillation events. Gray symbol: early repolarization pattern without ventricular fibrillation events. **B**: Predicted location of the KCNE1-S38G mutation in the extracellular segment of KCNE1. **C**: Proband DNA sequence of KCNE1 exon showing A > G substitution generating a serine (S) to glycine (G) at residue 38 of KCNE1. **D**: Twelve-lead ECG in a 14-year-old boy in Family 1 (arrow). ERS: Early repolarization syndrome.

Table 1
The features of the ECG for members of the 4 families.

ID	Sex	Age (y)	HR (bpm)	J-wave location	J-wave amplitude (mV)	ST elevation (mV)	R (mV)	QRS (ms)	QTc (ms)
1	M	25	66	V_1-V_4	0.35	0.25	0.85	100	432
2	M	37	76	II, III, AVF, V_1-V_3	0.1	0	1.5	80	360
3	M	32	72	I, aVL	0.1	0.1	0.9	60	394
4	M	71	75	II, III, AVF	0.1	0	0.8	70	391

2. Methods

2.1. Clinical data and genetic analysis

Four unrelated families with ERS and malignant ventricular arrhythmias were studied. Genomic DNA from these index cases was extracted from peripheral venous blood. Whole genome from these four families with ERS was amplified and sequenced to look for mutations

2.2. Heterologous expression of cDNA in HEK293 cells

Full-length cDNA encoding human wild-type (WT) KCNE1 (NM_000219.5) and the KCNE1 cDNA carrying the p. S38G mutation were subcloned into a pEGFP-N1 expression vector. Full-length cDNA encoding human wild-type KCNQ1 was subcloned into a pcDNA3.1 (+) expression vector. KCNE1 and/or its mutants and KCNQ1 plasmids were co-transfected (1:1 M ratio) into human embryonic kidney 293 (HEK293) cells using Lipofectamine 2000 transfection reagent (Invitrogen Life Technologies Inc., Carlsbad, California) according to the manufacturer's instructions.

2.3. Cellular electrophysiology

Forty-eight hours after transfection, cells were attached to a glass coverslip. Patch-clamp experiences were performed in Green Fluorescent Protein (GFP)-positive cells. Whole-cell patch clamps were recorded with an EPC-10 patch-clamp amplifier (HEKA, Lambrecht, Germany). The pipette solution contained (in mmol/L) 110 K-aspartate, 20 KCl, 2 MgCl2, 1 CaCl2, 10 EGTA, 10 HEPES, 40 glucose, 5 Na2 adenosine 5'-triphosphate (PH adjusted to 7.2 with KOH). The extracellular solution contained (in mmol/L) 140 NaCl, 5 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 10 glucose (PH adjusted to 7.4 with NaOH). The extracellular solution was maintained at 25 ℃ during experience. Pipette resistance ranged from 3 to 5 MΩ. I_{Ks} were obtained by depolarizing voltage step from a holding potential of −80 mV to various test potentials. The activating current was elicited from −80 mV to +100 mV at 20 mV increments for 2 s, and the amplitudes of tail current were recorded on return to −40 mV for 5 s. Pulses were delivered every 10 s.

The current-voltage (I-V) relation of step I_{Ks} and tail currents was evaluated by fitting to the Boltzmann equation: $I = 1/[1+\exp((V_{1/2}-V)/k)]$, where $V_{1/2}$ is the voltage at half-maximal activation, V is the test potential, and k is the slope factor.

2.4. Western blot analysis

After 48 h of transfection, HEK293 cells were washed twice with ice-cold PBS, and then cells were homogenized in ice-cold RIPA buffer (Beyotime Biotechnology, Shanghai, China) for 5 min. After homogenized and centrifugation (4 ℃, 12,000 g, 20 min), the supernatant was collected and protein content was determined with the BCA (ThermoFish Scientific, Waltham, MA USA) method according to the manufacturer's instruments.

Total protein samples (50 μg) were denatured and separated on a 12% SDS-PAGE and then transferred to PVDF membranes (Roche, Basel, Switzerland). Membranes were blocked with 5% non-fat milk for 1 h at room temperature and incubated with the anti-KCNE1 antibody (Abcam, Cambridge, UK) (1:1000) and anti-GAPDH antibody (Cell Signaling Technology, Danvers, MA USA) (1:10,000) overnight at 4 ℃. The bound primary antibody was detected with HRP-conjugated goat anti-rabbit secondary antibody (Cell Signaling Technology, Danvers, MA USA) (1:5000) at room temperature for 1 h. Blots were detected with Super Signal West Pico chemiluminescent substrate (ThermoFish Scientific, Waltham, MA USA) and developed on X-ray films. Protein bands were analyzed using ImageJ software (NIH). KCNE1 bands signals were normalized to corresponding GAPDH band in each sample.