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CASE REPORT

Identification of an ethnic-specific variant (V207M) of the KCNQ1 cardiac potassium channel gene in sudden unexplained death and implications from a knock-in mouse model

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Abstract We performed mutation analysis for genes implicated in long QT syndrome (KCNQ1, KCNH2, and SCN5A) in 17 sudden unexplained death autopsy cases. Single-strand conformation polymorphism and subsequent DNA sequencing analyses revealed that in one case, there was a variant, V207M of KCNQ1, a gene encoding a cardiac potassium channel. This case, a 40-year-old African male, was shown to have a heterozygous missense mutation (V207M), which has been previously reported to be ethnic-specific. The heterozygous V207M mutation was found in one case (0.23%) of 444 alleles from African individuals. We developed a knock-in mouse model carrier of the Kcnq1-V206M mutation, the mouse equivalent to the KCNQ1-V207M mutation identified in the victim. Significant prolongation of QT intervals was observed in the Kcnq1$^{V206M/V206M}$ mice. These findings suggest that the KCNQ1-V207M mutation might be pathogenic and might have been associated with the cause of death in the present case.

Keywords Autopsy · ECG · Ethnic-specific · Ion channel · Knock-in mice

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Introduction

Sudden cardiac death is one of the concerns in forensic medicine [1–3]. Forensic pathologists sometimes encounter sudden death cases with no apparent abnormal findings at autopsy. Such cases are usually defined as sudden unexplained death (SUD). Recent progress in postmortem molecular analysis has revealed that congenital long QT syndrome (LQTS) is involved in some cases of SUD [4–8].

LQTS is a genetic disease that is characterized by a prolonged QT interval on an electrocardiogram (ECG), development of ventricular arrhythmia, and sudden death. To date, mutations in at least seven genes encoding cardiac ion channels have been identified in families with LQTS: KCNQ1 (LQT1) [9], KCNH2 (LQT2) [10], SCN5A (LQT3) [11], KCNE1 (LQT5) [12], KCNE2 (LQT6) [13], KCNJ2 (LQT7) [14], and CACNA1C (LQT8) [15]. Most of LQTS in patients are due to LQT1, LQT2, and LQT3 gene mutations [16]. Altered physiological functions of these ion channels can result in a major risk of ventricular arrhythmia, leading to syncope and sudden death. Since no pathological abnormality has been reported in LQTS patients, postmortem molecular analysis is necessary for diagnosis of SUD in these cases.

In this study, we performed mutation analysis for the major genes causing LQTS in 17 SUD cases and found one case with a mutation in the KCNQ1 gene, which encodes

the cardiac potassium channel [9]. We also examined the pathological significance of the mutation.

Materials and methods

Examined cases

We examined 17 SUD cases for the present study: one black African and 16 Japanese subjects. The cases included 13 men and four women with an age range from 12 to 42 years old [17]. In all cases, an autopsy was carried out within 36 h after death. SUD was defined as a sudden unexpected and unexplained death determined after the conclusion of a medico-legal autopsy including microscopic and toxicological examinations. The incidence of the V207M mutation was examined in black African individuals from 121 Ghanaian and 101 Xhosa people.

Mutation analysis

Genomic DNA was prepared from blood lymphocytes using standard procedures. All coding areas of the KCNQ1, KCNH2, and SCN5A genes were amplified by the polymerase chain reaction (PCR) [18–21]. Screening for mutations was performed using single-strand conformational polymorphism, and segments displaying abnormal conformations were analyzed by automated sequencing (ABI PRISM 310 DNA sequencer, Applied Biosystems, Foster City, CA, USA) as described previously [17, 20, 21]. Isolation and analysis of the DNA of the 17 SUD cases and the two African populations were approved by the Ethical Committee for Research of the Human Genome of Osaka Medical College.

Generation of knock-in mouse with the KCNQ1-V207M mutation

The mouse model with the KCNQ1-V207M mutation was commercially developed by Unitech (Chiba, Japan). The mutation was introduced into the mouse *Kcnq1* gene by homologous recombination in mouse ES cells (C57BL/6). A clone found to include the homologous targeted integrand was verified by Southern blot analysis and direct sequencing and injected into blastocysts of Balb/c mice. The targeted *Kcnq1* gene had a LoxP flanked selection marker [a neomycin resistance gene with PGK promoter and poly(A) signal] inserted. The selection marker was removed by crossing with CAG-Cre mice that overexpressed the Cre recombinase. The modified region of the Kcnq1 gene was PCR-amplified and sequenced, confirming the presence of the intended modifications, using the primer pair 5UTR-F (5′-CTGGCCATGGACACGGCCTCGTCCC-3′) and mRNA-R(5′-CTCAGCCAAGTAGACAAAGTAGGAG-3′) internal of the Kcnq1 mRNA extracted from the heart of the knock-in mice. The Kcnq1$^{V206M/+}$ heterozygous mice were backcrossed four times on the C57BL6 background and used for experiments. Generation and experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

ECG recording

ECG recording was performed as described previously [22]. ECG with standard limb leads was recorded under pentobarbital anesthesia (30 mg/kg body weight intraperitoneally). A heat pad was used during the recording to prevent hypothermia.

Results

A KCNQ1 missense mutation was detected in the DNA from the African victim: a change from G to A at nucleotide 619. It was predicted to result in a substitution of valine to methionine at position 207 (V207M) in exon 4 (Fig. 1). This case was heterozygous for the V207M substitution. The mutation has been previously reported as an ethnic-specific variant without examining exact physiological

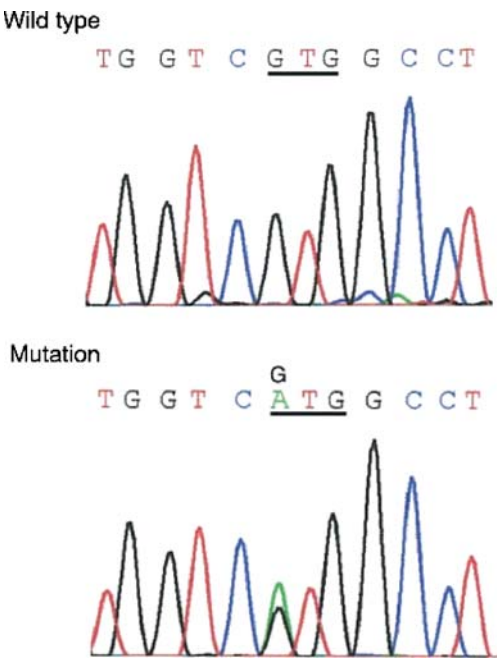

Fig. 1 Identification of the KCNQ1-V207M mutation. DNA sequence of exon 4 amplified from the present case. A heterozygous G to A transition was observed at the first base in codon 207 of the KCNQ1 gene. Sense-strand sequences are shown

functions [23]. The 16 Japanese SUD cases showed no mutations in the gene segments examined.

Since the victim was a black African male, we examined the incidence of the variant in two geographically distant black African populations. The heterozygous mutation was found in one Ghananian case (0.23%) out of 444 alleles in the population.

We developed a knock-in mouse model carrying the V206M mutation, the mouse equivalent to the V207M mutation identified in the victim (Fig. 2). The Kcnq1$^{V206M/V206M}$ mice showed almost normal growth in body weight [Kcnq1$^{V206M/V206M}$; 26.6±0.7 g versus Kcnq1$^{+/+}$; 29.9±1.0 g, P=0.150 (n=6)] at 12 weeks of age. No apparent abnormal findings were observed in their internal organs.

We examined ECGs in Kcnq1$^{V206M/V206M}$ and wild types. Representative ECG traces are shown in Fig. 3. The morphology of the T wave was differed between the two groups. The Kcnq1$^{V206M/V206M}$ mice showed significantly prolonged QT intervals compared with those in the wild types (Table 1). The PR, QRS, and RR intervals did not change significantly between the Kcnq1$^{V206M/V206M}$ and the wild-type mice. The P amplitude was also significantly increased in the Kcnq1$^{V206M/V206M}$ mice.

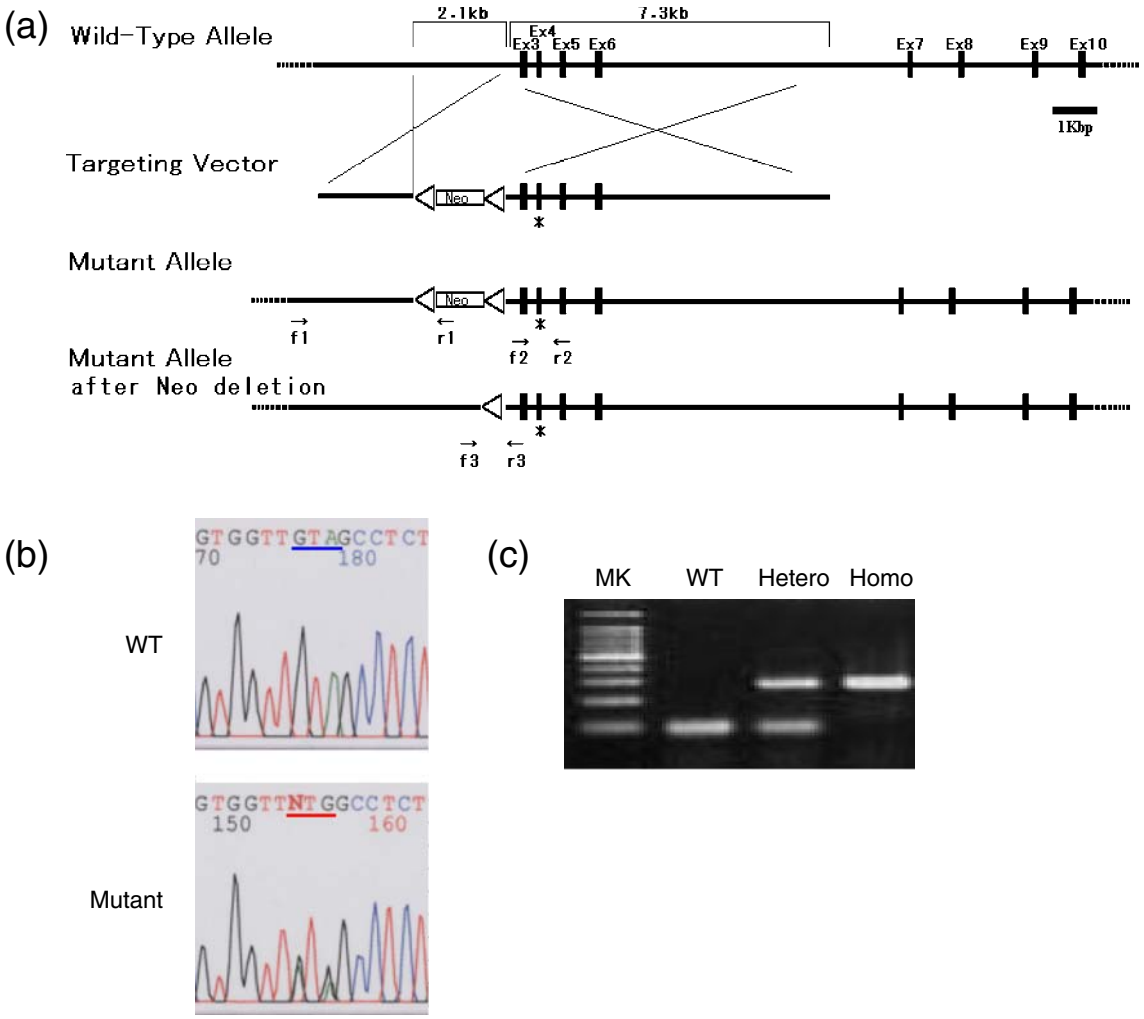

Fig. 2 Targeted engineering of a knock-in mouse model for KCNQ1-V207M mutation. **a** Strategy for generating the mutant. Kcnq1 exon 4 (denoted with an *asterisk*) is replaced with mutant (V206M) containing alternate exon on homologous recombination. In the targeting construct, a 2.1-kb 5′ fragment and a 7.3-kb 3′ fragment containing exon 4 were used as short and long homologous arms, respectively, flanking a floxed Neo selection marker. *Arrows* indicate primers used for the 5′ homologous recombination checking primers (f1, r1), homo/hetero checking primers (f3, r3) flanking the remaining LoxP site, and PCR analysis to genotype the mutation site by using primers (f2, r2) flanking the remaining mutation site. **b** Sequencing of the entire Kcnq1 cDNA from the heart of knock-in heterozygous mice (Neo deletion) confirms that the V206M (GTA to ATG) mutation was successfully recombined. **c** PCR using primers (f3, r3) which amplify a 96-bp fragment from wild-type allele and a 281-bp fragment from the mutant allele in the heterozygous mice

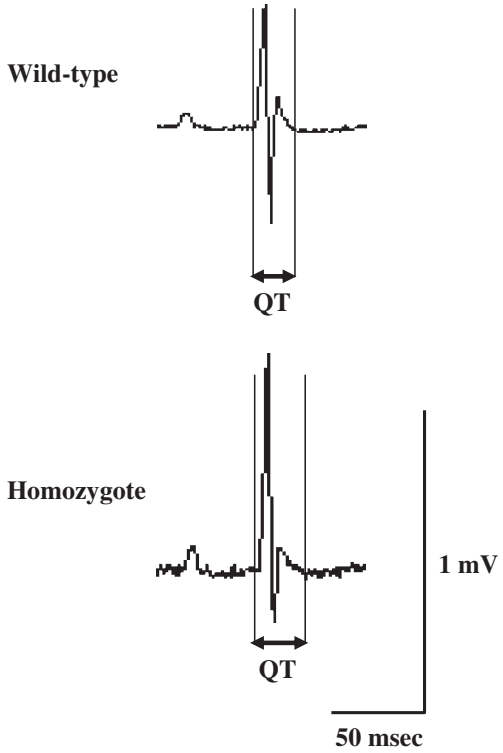

Fig. 3 Representative ECG traces (lead II) recorded in wild-type and homozygous mice

Discussion

The KCNQ1 mutation has been shown to be the most common factor involved in LQTS [16]. The KCNQ1 V207M mutation is located in the third transmembrane domain (S3) of the channel, which constitutes the ion pore.

DNA analysis (after receiving written informed consent from living family members of the victim) revealed that two other members of the family possessed the same heterozygous mutation (data not shown). Another member of the family died suddenly at the age of 21, and the cause of death was not determined. Unfortunately, the exact cause of that death and genotype were not determined, and ECG profiles of the mutation carriers were not available. Thus, we studied the pathological significance of the V207M mutation by population studies and by a mouse knock-in model.

Table 1 Comparison of ECG parameters in $Kcnq1^{+/+}$ and $Kcnq1^{V206M/V206M}$ mice

Parameters	$Kcnq1^{+/+}$	$Kcnq1^{V206M/V206M}$	p value
RR (ms)	116.2±11.9	92.3±1.8	0.129
P (1/100 mV)	3.8±0.3	6.3±0.4*	0.008
P duration (ms)	7.7±0.3	7.8±0.4	0.983
PR (ms)	40.5±0.8	39.3±1.9	0.818
QRS (ms)	8.8±0.2	9.0±0.4	0.939
R (1/100 mV)	53.3±5.8	63.0±7.6	0.584
S (1/100 mV)	31.2±3.1	34.7±4.6	0.827
QT (ms)	22.2±0.5	26.5±0.9*	0.004

The population study revealed that V207M was not considered a polymorphism but a rare mutation. Since the QRS intervals did not change significantly between the $Kcnq1^{V206M/V206M}$ and wild types, the prolonged QT interval in the $Kcnq1^{V206M/V206M}$ mice must have been caused by an increase in the T wave duration when the Kcnq1-affected outwardly rectifying current is generated. Thus, it suggests a dysfunction of the Kcnq1-encoded channel in the $Kcnq1^{V206M/V206M}$ mice.

Several model animals of the Kcnq1 mutation have been developed and displayed prolonged QT intervals on ECG [22, 24–26]. The present model showed some differences in ECG profiles compared with those previously reported. The Kcnq1 mutant, vtg-2J, mice displayed T wave elevation [22], which is one of the characteristics for LQT1 patients [27]. Mutant mice lacking exon 2 of the Kcnq1 gene showed prolonged P wave and QRS duration along with prolonged QT intervals [24]. These findings were not observed in the present model.

Kcnq1 is known to be essential for gastric acid secretion, and gastric hyperplasia has been reported in Kcnq1 mutant animals [22, 25, 26]. The present model did not show gastric hyperplasia (data not shown); therefore, the effect of the mutation on the channel function may not be as severe compared with the previous models.

Recently, the V205M mutation, which is located only two residues from V207, has been identified in LQTS patients in a Canadian aboriginal community [28]. Electrophysiological studies revealed that the outward ionic charge through the channel expressing the V205M mutation was reduced by more than 75% compared with the wild type, which suggests that the V205M mutation is most likely to be pathogenic for LQTS [28]. It was also suggested that the V205M mutation would show increased susceptibility to the initiation of arrhythmias, especially at high heart rates [28].

The findings in this study and the previous report [28] strongly suggest that the KCNQ1-V207M mutation might be pathogenic for LQTS and might have been associated with the cause of the death in the present case of sudden death during exercise. Judging from the incidence (0.3%) of V207M [23], about 90,000 of the total black population of over 30 million people in the USA would be expected to carry the V207M mutation, which might be pathogenic for LQTS.

It might now be necessary to perform mutation analysis of the arrhythmogenic diseases of LQTS for the postmortem diagnosis of SUD cases [29, 30]. Such an approach is useful not only for the diagnosis of the deceased but also

for detection of the mutation carriers in living family members. Appropriate counseling, examinations, and medications, if available, should be performed for prevention of possible cardiac events.

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