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Cardiac Ion Channel Gene Mutations in Sudden Infant Death Syndrome

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ABSTRACT: Sudden infant death syndrome (SIDS) is multifactorial and may result from the interaction of a number of environmental, genetic, and developmental factors. We studied three major genes causing long QT syndrome in 42 Japanese SIDS victims and found five mutations, *KCNQ1*-K598R, *KCNH2*-T895M, *SCN5A*-F532C, *SCN5A*-G1084S, and *SCN5A*-F1705S, in four cases; one case had both *KCNH2*-T895M and *SCN5A*-G1084S. All mutations were novel except for *SCN5A*-F532C, which was previously detected in an arrhythmic patient. Heterologous expression study revealed significant changes in channel properties of *KCNH2*-T895M, *SCN5A*-G1084S, and *SCN5A*-F1705S, but did not in *KCNQ1*-K598R and *SCN5A*-F532C. Our data suggests that nearly 10% of SIDS victims in Japan have mutations of the cardiac ion channel genes similar to in other countries. **(*Pediatr Res* 64: 482–487, 2008)**

Sudden infant death syndrome (SIDS) is defined as the sudden unexpected death of an infant <1 y of age, with onset of the fatal episode apparently occurring during sleep, which remains unexplained after thorough investigation (1). Its incidence differs among racial and ethnic groups, being estimated at 0.3–1.0/1000 live births in the United States (2,3) and 0.3–0.5/1000 in Japan.

SIDS is multifactorial and may result from the interaction of a number of environmental, genetic, and developmental factors. The well-known environmental risk factors are prone sleeping, smoking during pregnancy, and nonbreast feeding. Concerning the genetic factors, several genes have been investigated in cases of SIDS (4). Mutations or polymorphic changes associated with SIDS have been reported in the following: complements (*C4A*, *C4B*) (5,6), IL-10 (7), mitochondrial DNA (*MTTL1*, *MTND1*), serotonin transporter (5-HTT) (8,9), a gene associated with sex differentiation (*TSPYL*) (10), and cardiac ion channels (*KCNQ1*, *KCNH2*, *SCN5A*). We have previously investigated the relationship between SIDS and congenital central hypoventilation syndrome (CCHS); however, we failed to find any mutations other than three single nucleotide polymorphisms (SNPs) of *PHOX2B*, a gene responsible for CCHS, in 48 Japanese SIDS victims (11,12).

Recently, cardiac abnormalities causing life-threatening arrhythmias have attracted attention in association with SIDS. Long QT syndrome (LQTS) is a genetic disease characterized by a prolonged QT interval on the electrocardiogram (ECG) and a major cause of malignant ventricular tachyarrhythmias (13,14). Association of SIDS and LQTS has been recognized since the first report in 1976 (15), and recent studies have demonstrated genetic link between SIDS and LQTS, suggesting that 2–10% of SIDS cases are attributable to the mutations in LQTS genes, especially in *SCN5A* (16–24).

Considering the variation in the incidence of SIDS and cardiac channelopathy among racial and ethnic groups, it is possible that the prevalence of the mutations in LQTS genes will differ among racial and ethnic groups. Therefore, we performed a molecular analysis of the three major genes causative of LQTS in Japanese SIDS victims.

METHODS

SIDS cohort. Between 1995 and 2004, a total of 50 cases of SIDS were investigated at four institutes of forensic medicine in Japan. The death certificate was assigned a diagnosis of SIDS if the autopsy, toxicology, and review of the circumstances of death were all negative. Eight cases were eliminated from the study because of DNA degeneration in their specimens. The main characteristics of the 42 cases were: 23 male and 19 female; median (range) age at time of death: 4 mo (5 d to 11 mo). Written informed consent was not always required for the study and the molecular analyses were conducted with the investigators blinded to the identities of the infant victims. The Ethics Committee of the Yamagata University School of Medicine approved this study.

Genetic analyses. Genomic DNA was extracted from frozen tissues or fresh blood from SIDS victims and control subjects. All exons, including the exon–intron boundaries of *KCNQ1*, *KCHH2*, and *SCN5A*, were amplified using the polymerase chain reaction and amplicons were screened for mutations by denaturing HPLC (Transgenomic Inc., Omaha, NE). Fragments showing a heteroduplex were sequenced. An analysis of 150 control subjects verified putative disease-causing mutations.

Molecular biology. cDNAs of *KCNQ1* and *SCN5A* and a cDNA of *KCNH2* were introduced into the expression vectors pcDNA3.1 and pcDNA3 (Invitrogen, Carlsbad, CA), respectively. A cDNA of *KCNE1* (*minK*) β-subunit was subcloned in pCR-Script (Stratagene, La Jolla, CA). Mutations of each gene were introduced using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The presence of the desired mutations and absence of unwanted changes in the clones were verified by DNA sequencing.

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Abbreviation: LQTS, long QT syndrome

Gene-expression studies of KCNQ1 and KCNH2. The plasmids containing mutant and wild-type (WT) *KCNQ1*, *KCNH2*, and *KCNE1* cDNA were linearized with appropriate restriction enzymes and capped cRNAs were synthesized *in vitro* using either T3 RNA polymerase (*KCNE1*) or T7 RNA polymerase (*KCNQ1* and *KCNH2*) with a mMESSAGE mMACHINE kit (Ambion, Austin, TX).

The oocytes (stage V and VI) of *Xenopus laevis* were prepared according to ordinary methods (25). Each of the oocytes was injected with either 9.2 ng of WT or mutant *KCNQ1* cRNA with 1.7 ng of *KCNE1* cRNA, or 9.2 ng of WT or mutant *KCNH2* cRNA and incubated at 18°C for 2 to 4 d before electrophysiological measurements. Whole-cell currents were recorded from oocytes by the two-microelectrode voltage-clamp technique and data acquisition was performed as described previously (26).

Gene-expression studies of SCN5A. The human kidney cell line tsA-201 was transiently transfected with the plasmids encoding WT or mutant *SCN5A* in combination with a bicistronic plasmid (pCD8-IRES-hβ1) encoding CD8 and the human β1 subunit (hβ1) to visually identify cells expressing heterologous hβ1 with Dynabeads M-450 CD8 (Dynal, Oslo, Norway) using the SuperFect Transfection Reagent (Qiagen Inc., Valencia, CA).

Electrophysiological measurements were obtained 24 to 72 h after transfection. Na currents were recorded and analyzed using the whole-cell patch clamp technique as described previously (27).

Data analysis. Data analysis was performed using the Clampfit program (pCLAMP 9.2, Axon Instruments, Union City, CA) and SigmaPlot (SPSS Inc., Chicago, IL). Curve fitting was accomplished using a nonlinear least squares minimization method. The time course of inactivation in I_{Na} and of deactivation in I_{Kr} were fit with the following biexponential function:

$$I/I_{max} = A_0 + A_f \times \exp(-t/\tau_f) + A_s \times \exp(-t/\tau_s)$$

where A_0 refers to a constant value, A_f and A_s to the fractions of fast and slow inactivating components, respectively, and τ_f and τ_s to the time constants of fast and slow inactivating components, respectively. Steady state availability in I_{Na}, and the voltage dependence of channel activation were fit with the Boltzmann equation

$$I/I_{max} = \{1 + \exp[(V - V_{0.5})/\kappa]\}^{-1}$$

to determine the membrane potential for half-maximal inactivation or activation ($V_{0.5}$) and the slope factor κ. Voltage dependence of activation in I_{Na} was estimated by measuring peak sodium current during a variable test potential from a holding potential of −120 mV. Recovery from inactivation in I_{Na} was analyzed by fitting data with the following biexponential function:

$$I/I_{max} = A_f \times [1 - \exp(-t/\tau_f)] + A_s \times [1 - \exp(-t/\tau_s)]$$

Results are presented as the mean± SE. Statistical comparisons were made between WT and mutants using the unpaired *t* test. Statistical significance was assumed for $p < 0.05$ (two-sided).

RESULTS

Postmortem genetic analyses of the SIDS cohort. Genetic analysis revealed five mutations, *KCNQ1*-K598R, *KCNH2*-T895M, *SCN5A*-F532C, *SCN5A*-G1084S, and *SCN5A*-F1705S, in four cases; one case had both *KCNH2*-T895M and *SCN5A*-G1084S (Table 1).

Case-62 had a missense mutation, K598R (c.1793A > G), within the C-terminal region of *KCNQ1*. K598R is expected to replace an evolutionally conserved residue that is shared by the KCNQ potassium channel family.

Case-9 had one missense mutation, T895M (c.2684C > T), within the C-terminal region of *KCNH2* and another, G1084S (c.3250G > A), in the IIS6-IIIS1 region of *SCN5A*. T895 of *KCNH2* is conserved among various species, but G1084 of *SCN5A* is conserved only in the rat and mouse.

Case-63 had a missense mutation, F532C (c.1595T > G), at IS6-IIS1 of *SCN5A*. F532C was previously detected in an adult patient with arrhythmia (28).

Table 1. *Compendium of identified mutations*

Case no.	Gene	Exon	Variant	Location
62	*KCNQ1*	15	K598R*	C–terminus
9†	*KCNH2*	11	T895M*	C–terminus
	SCN5A	18	G1084S*	II S6–III S1
40	*SCN5A*	28	F1705S*	IV S5–IV S6
63	*SCN5A*	12	F532C	I S6–II S1

* Novel mutations.

† Compound heterozygosity with *KCNH2*-T895M and *SCN5A*-G1084S.

Case-40 had a missense mutation, F1705S (c.5114T > C), at IVS5-IVS6 of *SCN5A*. F1705 is located in the pore region and is highly conserved among various species.

Four mutations except *SCN5A*-F532C were novel and were not found in 150 healthy Japanese controls. Several registered or nonregistered SNPs were also detected in this cohort.

Electrophysiological characterization of mutant channels. WT or K598R *KCNQ1* channels expressed in oocytes elicited slowly activating and deactivating I_{Ks} currents (29,30). There were no significant differences in the current–voltage relationship, voltage dependence of activation, or activation and deactivation kinetics (data not shown). The K598R mutation did not noticeably alter the gating of *KCNQ1* channels expressed in oocytes.

Figure 1*A* shows typical WT and T895M *KCNH2* currents. The current–voltage relationships at the end of 2s depolarizing voltage steps showed a strong inward rectification at potentials positive to −10 mV in both WT and mutant channels because of fast C-type inactivation (31–33). T895M showed significantly reduced steady-state currents as compared with WT at test potentials between −50 and + 20 mV ($p < 0.001$ at V_m between −50 and 0 mV, $p < 0.05$ at V_m of + 10 and + 20 mV) (Fig. 1*B*). There was no significant difference in peak-tail current between WT and T895M (Fig. 1*B*). The voltage dependence of activation in T895M displayed a 3 mV shift in the positive direction compared with that in WT (Fig. 1*C*). Although both data, $V_{0.5}$ and slope factor, showed statistically significant differences between WT and T895M {WT: $V_{0.5} = -22.6 \pm 0.4$ mV, $\kappa = 9.3 \pm 0.3$; T895M: $V_{0.5} = -19.4 \pm 0.4$ mV ($p < 0.001$), $\kappa = 7.6 \pm 0.1$ ($p < 0.001$)}, the effect on clinical condition may be equivocal because of these small differences. The deactivation time course in T895M was significantly slower than that in WT for both fast and slow components between a V_m of −60 and −40 mV ($p < 0.001$) (Fig. 2).

SCN5A-WT, *SCN5A*-F532C, *SCN5A*-G1084S, and *SCN5A*-F1705S were transiently expressed in the human kidney cell line tsA-201. Heterologously expressed three mutant Na channels showed robust Na currents (Fig. 3). The macroscopic current decay of WT and mutants was comparable, and a persistent Na^+ current, characteristic for the most LQT3 mutant Na channels, was not evident in those channels. The *SCN5A*-F532C channel did not show any significant difference in gating properties in the expression experiments (data not shown). The voltage dependence of steady-state inactivation of G1084S showed a small but significant hyperpolarizing shift by −5.5 mV {WT: $V_{0.5} = -88.3 \pm 0.8$ mV, $\kappa = 7.5 \pm 0.1$; G1084S: $V_{0.5} = -93.8 \pm 1.3$ mV ($p < 0.05$), $\kappa = 7.0 \pm$

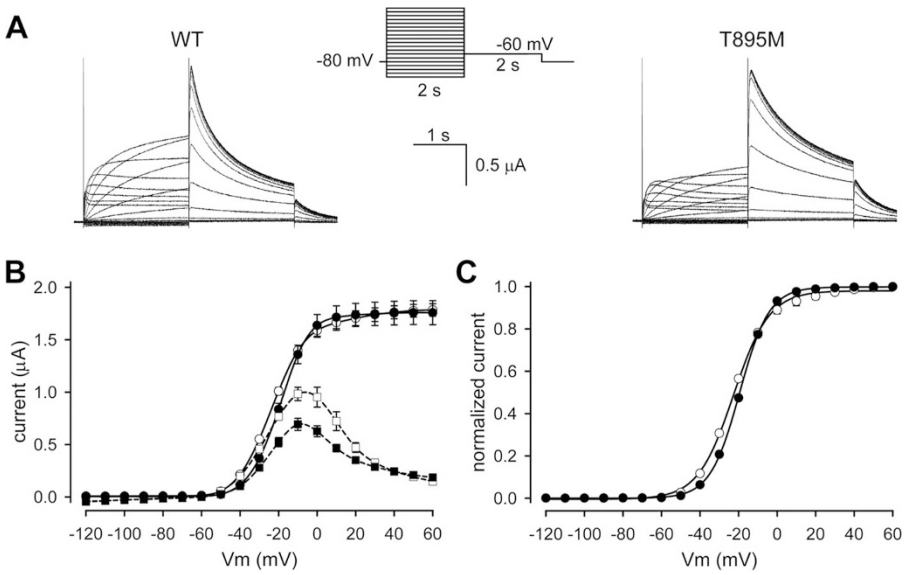

Figure 1. Characteristics of WT and T895M *KCNH2* currents. (*A*) Representative current traces of WT (*left*) and T895M (right) elicited by a two-step voltage clamp protocol (*inset*). Oocytes were clamped at a holding potential of −80 mV, and 2s pulses of voltage from −120 to + 60 mV were imposed in steps of 10 mV. Tail currents were recorded by stepping to −60 mV for 2s. (*B*) The average amplitudes of steady-state (squares) and peak-tail (circles) currents. (*C*) Voltage dependence of activation: Activation curves were obtained by plotting the normalized tail currents as a function of the prepulse potential. The voltages for half-maximal activation were obtained by fitting with the Boltzmann equation (solid lines). Some error bars are smaller than symbols. In each panel of (*B*) and (*C*), open circles or squares correspond to WT ($n = 10$), and closed circles or squares correspond to T895M ($n = 10$).

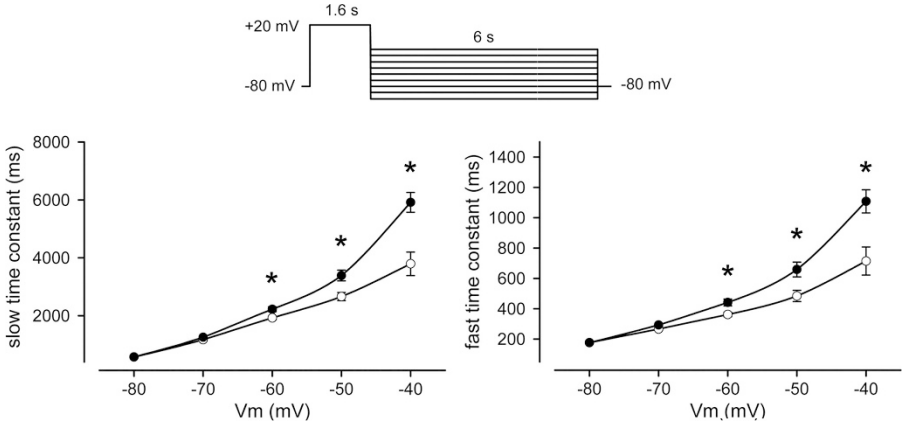

Figure 2. Deactivation kinetics of WT and T895M *KCNH2* channels: Deactivation time constants were obtained by fitting a biexponential function (slow and fast time constant) of the decaying currents elicited by a 6s test pulse between −100 and −20 mV preceded by a 1.6s prepulse to + 20 mV (inset). The curves that failed to fit the biexponential function were not included {$*p <$ 0.001 *vs* control, (WT: open circles, $n = 10$, T895M: closed circles, $n = 10$)}.

0.2 ($p < 0.05$)}, whereas that of F1705S showed a large hyperpolarizing shift by −17 mV {F1705S: $V_{0.5} = -105.3 \pm 1.2$ mV ($p < 0.001$), $\kappa = 7.7 \pm 0.2$ (P = NS)} (Fig. 3*B*, left). The hyperpolarizing shifts of the inactivation curve were showing that the voltage dependence of inactivation was increased in G1084S and F1705S. The $V_{0.5}$ values of activation in G1084S and F1705S were comparable with WT, but the slope factors were significantly different {WT: $V_{0.5} = -44.4 \pm 0.7$ mV, $\kappa = -6.2 \pm 0.2$; G1084S: $V_{0.5} = -42.9 \pm 1.1$ mV (P = NS), $\kappa = -7.3 \pm 0.2$ ($p < 0.05$); F1705S: $V_{0.5} = -45.4 \pm 1.0$ mV (p = NS), $\kappa = -8.0 \pm 0.1$ ($p < 0.001$)}, showing small but significant differences in the voltage-dependence of activation (Fig. 3*B*, right). The time constants of fast (τ_f) or slow (τ_s) recovery components were significantly larger in F1705S than WT, whereas G1084S only slightly affected the time constants {WT: $\tau_f = 14.7 \pm 1.8$ ms, $\tau_s = 120.4 \pm 19.0$ ms; G1084S: $\tau_f = 15.2 \pm 1.2$ ms (P = NS), $\tau_s = 361.2 \pm 165.3$ ms ($p < 0.05$); F1705S: $\tau_f = 29.6 \pm 2.8$ ms ($p < 0.001$), $\tau_s = 455.2 \pm 104.7$ ms ($p < 0.001$)}, showing significantly delayed recovery kinetics in F1705S (Fig. 3*C*).

DISCUSSION

The present study provides a possible causal association between SIDS and major cardiac channelopathy with a detailed functional assessment. We detected five mutations, *KCNQ1*-K598R, *KCNH2*-T895M, *SCN5A*-F532C, *SCN5A*-G1084S, and *SCN5A*-F1705S, in four cases.

We did not find significant gating changes in *KCNQ1*-K598R and we cannot deny the possibility that the *KCNQ1*-K598R is a rare polymorphism. However, *KCNQ1*-K598 is an evolutionally conserved amino acid residue and the K598R (c.1793A > G) possibly changes the splicing site based on a computer splice prediction program (SpliceView, http://bioinfo.itb.cnr.it/oriel/splice-view.html) (data not shown). Our expression experiments using oocytes and the *KCNQ1*-K598R cRNA may not reflect *in vivo* gating properties of the channel encoded by *KCNQ1*-K598R (c.1793A > G).

As for *KCNH2*, we found the mutation *KCNH2*-T895M in one subject who also carried an *SCN5A*-G1084S mutation. T895 of *KCNH2* is located in the C-terminus that extends from residue Y667 to S1159 (34), and little information was

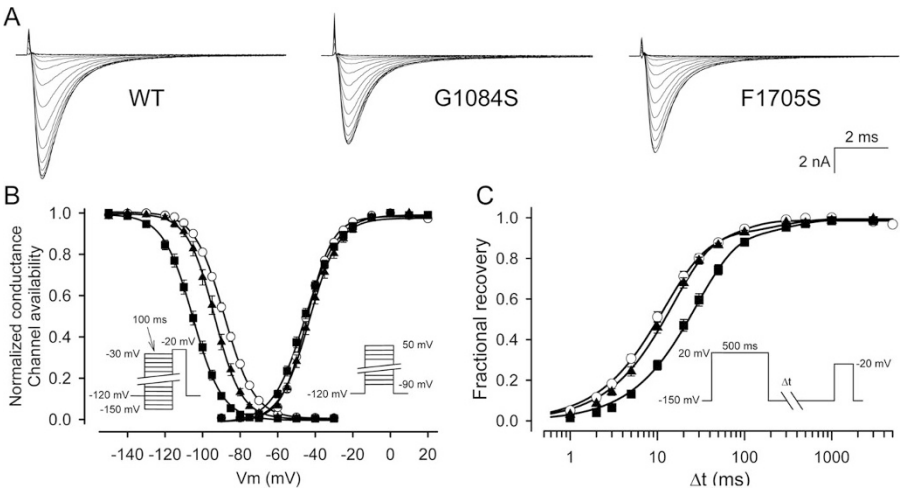

Figure 3. Characteristics of WT, G1084S and F1705S *SCN5A* currents. (*A*) Representative whole-cell current traces of WT (*left*), G1084S (*center*), and F1705S (*right*) *SCN5A* channels expressed in tsA-201 cells. Currents were elicited by a −20 mV test potential after a series of 100ms prepulses from −150 to −30 mV as shown in (*B*) inset (*left*). (*B*) Conductance-voltage relationship and steady-state availability for inactivation. Currents for measuring conductance were recorded at membrane potentials from −90 to 50 mV from a holding potential of −120 mV (*right inset*). The conductance was estimated with the equation $G = I/(V_m - E_{rev})$, where G is conductance, I represents the peak test-pulse current, V_m is the test-pulse potential, and E_{rev} is the measured reversal potential. Normalized peak conductance was plotted as a function of membrane potential. The data from the test potentials higher than 20 mV were omitted. Steady-state availability for inactivation was assessed by the protocol shown in the inset (*left*). Normalized peak current and conductance were fit with the Boltzmann equation (WT: open circles, $n = 37$, G1084S: closed triangles, $n = 8$, F1705S: closed squares, $n = 10$). (*C*) Recovery from inactivation. Recovery from inactivation was measured by a double pulse protocol as shown in the inset. Fractional recovery was determined as the ratio of peak currents measured at −20 mV after a given test interval (Δt) to the maximum peak current (WT: open circles, $n = 30$, G1084S: closed triangles, $n = 8$, F1705S: closed squares, $n = 10$).

available about the mechanisms for *KCNH2* channel dysfunction caused by the mutations in the C-terminus except for their involvement in trafficking (35). *KCNH2*-S818L was reported to partly alter channel gating (36) and polymorphic *KCNH2*-K897T was reported to produce a modest abbreviation of QTc by shifting the voltage of activation of I_{Kr} (37). Biophysical properties of the *KCNH2*-T895M potassium channels included a decrease in amplitude of the steady state current and a delay in deactivation. Since these two gating abnormalities seem to result in opposite functional effects, it is difficult to predict the overall influence of the T895M mutation. The subject with *KCNH2*-T895M also carried *SCN5A*-G1084S. *SCN5A*-G1084S is located in the cytoplasmic II-III interdomain linker, where several mutations have been reported in cases associated with LQTS (38,39). A997S, located in the II–III linker, has been reported in one case of SIDS and an electrophysiological study showed a gain-of-function phenotype characterized by persistent and increased inward sodium current (20). *SCN5A*-G1084S caused a significant hyperpolarizing shift of steady-state inactivation, however, the change was not so remarkable and G1084S is predicted to change a nonconserved amino acid residue, suggesting that *SCN5A*-G1084S may be a rare polymorphism. Although the pathogenetic role of *KCNH2*-T895M in SIDS remains unclear, one possibility is that a subtle disturbance of the *KCNH2* current due to the balance of opposite gating abnormalities increases arrhythmogenecity under the influence of additional factors such as *SCN5A*-G1084S. Furthermore, another possibility is that LQTS coexisted with Brugada syndrome in a single patient. One mutation (*KCNH2*-T895M) is responsible for LQT2, the other (*SCN5A*-G1084S) for Brugada syndrome. Since these two mutations had different gating properties, they might have an equal chance to cause fatal arrhythmias in the patient.

Among five mutations found in this study, three are mutations of *SCN5A*, namely F532C, G1084S, and F1705S. Mutations of *SCN5A* account for approximately 5–10% of cases of LQTS (38,39), and increasing attention has focused on *SCN5A* mutations as the cause of SIDS because of the high risk of a cardiac event during sleep in individuals carrying these mutations. Schwartz *et al.* (17) first reported an *SCN5A* mutation in a SIDS victim and Ackerman *et al.* (20) detected *SCN5A* mutations in two of 93 SIDS victims in a postmortem molecular analysis. Among the mutations in LQTS genes, *SCN5A* mutation may be most frequently associated with SIDS.

The *SCN5A*-F532C channel did not show any significant difference in properties in the expression experiments. However, this mutation was previously reported in an adult with paroxysmal atrial fibrillation and atrial tachycardia, and not in 232 healthy controls (28), indicating that it is probably associated with SIDS. The heterologously expressed *SCN5A*-F1705S channel exhibited profound gating abnormalities. This mutation was detected in a 27-d-old girl, the youngest of the subjects in which a mutation was found in the present study. The hyperpolarizing shift of steady-state inactivation and delayed recovery from inactivation would reduce the availability of Na channels and delay the conduction of cardiac impulses. These kinetic properties could result in a decrease of net Na current and this loss-of-function feature supports a proposed linkage between Brugada syndrome and SIDS (40,41).

This study revealed that 9.5% of Japanese SIDS victims carried mutations of the cardiac ion channel genes, *KCNQ1*, *KCNH2*, and *SCN5A*. Arnestad *et al.* (24) recently studied seven genes associated with LQTS in 182 SIDS infants in Norway and reported that 9.5% of cases carried functionally significant variants in LQTS genes. Considering the significant variation in the incidence of SIDS and in cardiac chan-

nelopathy among racial and ethnic groups, we assumed that the prevalence of ion channel mutations in Japanese SIDS victims might be different. However, the present result suggests that nearly 10% of SIDS cases in Japan are associated with LQTS gene mutations, which is similar to the rate in Norway. Even though a relatively small cohort was investigated, this study clearly detected LQTS gene mutations in Japanese SIDS victims. Ethnic disparities in the prevalence of SIDS are probably due to other genetic or socioenvironmental factors. Some investigators have recommended widespread ECG-based screening of infants to prevent SIDS (42,43). However, it requires overcoming several problems including the cost of testing, accuracy of interpretation, managing of false-positive ECGs, and efficacy of treatment for those positive with LQTS (44). Little information is available on whether the mutation carriers exhibit ECG abnormalities such as QT prolongation or Brugada-type changes. As pointed out by Tester *et al.* (45), many individuals may be falsely diagnosed as LQTS, which may raise socioeconomic and psychosocial problems.

Genetic screening is sensitive and possibly detects many mutations, however, it would be sometimes difficult to identify SIDS-associated mutations. Although victims mainly caused by genetic factors would not decrease in the number by the "back to sleep campaign," some of their deaths would be preventable if the primary lesions are unmasked. Establishing a genetic database of SIDS cases and future development of expedited technology, like DNA-microarray analysis, to screen infants may hopefully prevent the tragedy of SIDS.

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