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# Cardiac Sodium Channel Dysfunction in Sudden Infant Death Syndrome

Dao W. Wang, MD; Reshma R. Desai, MS; Lia Crotti, MD; Marianne Arnestad, MD, PhD; Roberto Insolia, BSc; Matteo Pedrazzini, BSc; Chiara Ferrandi, BSc; Ashild Vege, MD, PhD; Torleiv Rognum, MD, PhD; Peter J. Schwartz, MD; Alfred L. George, Jr, MD

**Background**—Mutations in genes responsible for the congenital long-QT syndrome, especially *SCN5A*, have been identified in some cases of sudden infant death syndrome. In a large-scale collaborative genetic screen, several *SCN5A* variants were identified in a Norwegian sudden infant death syndrome cohort (n=201). We present functional characterization of 7 missense variants (S216L, R680H, T1304M, F1486L, V1951L, F2004L, and P2006A) and 1 in-frame deletion allele (delAL586-587) identified by these efforts.

**Methods and Results**—Whole-cell sodium currents were measured in tsA201 cells transiently transfected with recombinant wild-type or mutant *SCN5A* cDNA (hH1) coexpressed with the human  $\beta$ 1 subunit. All variants exhibited defects in the kinetics and voltage dependence of inactivation. Five variants (S216L, T1304M, F1486L, F2004L, and P2006A) exhibited significantly increased persistent sodium currents (range, 0.5% to 1.7% of peak current) typical of *SCN5A* mutations associated with long-QT syndrome. These same 5 variants also displayed significant depolarizing shifts in voltage dependence of inactivation (range, 5 to 14 mV) and faster recovery from inactivation, but F1486L uniquely exhibits a depolarizing shift in the conductance-voltage relationship. Three alleles (delAL586-587, R680H, and V1951L) exhibited increased persistent current only under conditions of internal acidosis (R680H) or when expressed in the context of a common splice variant (delQ1077), indicating that they have a latent dysfunctional phenotype.

**Conclusions**—Our present results greatly expand the spectrum of functionally characterized *SCN5A* variants associated with sudden infant death syndrome and provide further biophysical correlates of arrhythmia susceptibility in this syndrome. (*Circulation*. 2007;115:368-376.)

**Key Words:** death, sudden ■ genetics ■ ion channels ■ long-QT syndrome

Sudden infant death syndrome (SIDS) is a leading cause of death during the first year of life in developed countries.<sup>1,2</sup> Cardiac mechanisms, including life-threatening arrhythmias, have been suspected to cause a proportion of SIDS cases,<sup>3</sup> and recent evidence indicates that mutations in genes responsible for the congenital long-QT syndrome (LQTS) are found in as many as 9% of victims.<sup>4,5</sup> Molecular evidence for a link between SIDS and LQTS supports earlier observations that there is an increased risk of SIDS in infants with a QTc >440 ms based on ECG measurements in 34 000 Italian neonates.<sup>6</sup>

## Clinical Perspective p 376

Anecdotal reports originally established an association between SIDS or aborted SIDS with bone fide mutations in LQTS genes. In 2000, Schwartz and colleagues<sup>7</sup> reported a 7-week-old infant found cyanotic, apneic, and pulseless by

his parents. The subject was resuscitated from documented ventricular fibrillation and later demonstrated to have a severely prolonged QTc interval (648 ms). A de novo mutation in *SCN5A* (S941N) associated with abnormal sodium channel inactivation was subsequently found in the infant. One year later, another de novo *SCN5A* mutation (A1330P) with significant biophysical disturbances was reported by Wedekind et al<sup>8</sup> in a 9-week-old SIDS victim. Ackerman et al<sup>9</sup> reported the first systematic survey of autopsied SIDS or unexplained infant death cases for mutations in LQTS genes. This study identified 2 *SCN5A* mutations (A997S, R1826H) in 93 subjects (2.1%). Mutations in *KCNQ1* and *KCNH2* also have been reported in SIDS probands.<sup>4,10,11</sup> Common LQTS gene variants also may increase arrhythmia risk in infants. Plant and colleagues<sup>12</sup> recently suggested that black SIDS victims homozygous for a common *SCN5A* nonsynonymous variant (S1103Y) may

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illustrate this point. Taken together, these previous investigations indicate that a subset of SIDS may be explained by presentations of LQTS or other arrhythmia susceptibility syndromes in early life.

Most recently, the largest survey of SIDS victims involving a series of 201 Norwegian cases, autopsied and classified according to Nordic standards,<sup>13–15</sup> has revealed a prevalence of LQTS gene mutations of nearly 10%.<sup>5</sup> Interestingly, approximately half of the gene variants reported in this series were found in *SCN5A*, and this contrasts the occurrence of *SCN5A* mutations in only  $\approx 10\%$  of congenital LQTS. To validate that rare genetic variants in the major LQTS genes discovered by these efforts are indeed capable of causing LQTS, functional studies of mutant gene products are essential.

In the present study, we report the functional characterization of 8 *SCN5A* variants discovered in the Norwegian SIDS series, more than twice the number of previously published SIDS-associated alleles in this gene. Several variants exhibited overt functional defects that were consistent with pathological alleles. In contrast, dysfunction of 3 variants was dependent on other factors (ie, intracellular pH, splice isoform), suggesting that a latent pathological defect may be present. These findings illustrate the spectrum of mutant cardiac sodium channel dysfunction in SIDS and emphasize the potential for latent arrhythmia susceptibility in *SCN5A* mutation carriers.

## Methods

### Ascertainment and Molecular Genetics

*SCN5A* variants characterized in the present study were identified in a series of 201 SIDS cases ascertained between 1988 to 2004 at the Institute of Forensic Medicine (Oslo, Norway) according to methods reported previously.<sup>14–16</sup> Genomic DNA was isolated from blood or frozen spleen. Sample collection was approved by the Norwegian regional ethics committee. All *SCN5A*-coding exons were amplified by polymerase chain reaction and then screened for polymorphisms using denaturing high-performance liquid chromatography with the Transgenomic Wave instrument (Transgenomic, Inc, Omaha, Neb). Amplicons exhibiting abnormal elution profiles were sequenced with an Applied Biosystems PRISM 310 automated DNA sequencer (Applied Biosystems, Foster City, Calif). Further details of the SIDS cases and the complete results of genetic screening studies are reported separately.<sup>5</sup>

### Mutagenesis and Heterologous Expression of Na Channels

Mutations were engineered in a human heart sodium channel cDNA (hH1)<sup>18</sup> using recombinant polymerase chain reaction. Final constructs were assembled in the mammalian expression plasmid pRc/CMV-hH1 and then sequenced to verify creation of the mutation and to exclude polymerase errors. Certain mutant constructs also were made in the hH1 splice variant (delQ1077) by subcloning a fragment lacking glutamine-1077 derived from hH1c (kindly provided by Dr Jonathan Makielski).<sup>19</sup> A rare variant present in the original hH1 cDNA (R1027Q) was present in all constructs. Cells (tsA201) were transiently transfected with pRc-CMV-hH1 or mutants using FuGene6 (Roche Diagnostics, Indianapolis, Ind) combined with a bicistronic plasmid (pGFP-IRES-h $\beta$ 1) –encoding enhanced green fluorescent protein and the human  $\beta$ 1 subunit (h $\beta$ 1) under the control of the cytomegalovirus immediate early promoter. Only cells exhibiting green fluorescence were selected for patch-clamp recording. Transfections were performed with 0.5  $\mu$ g channel-encoding plasmid DNA and 0.5  $\mu$ g pGFP-IRES-h $\beta$ 1. Transiently transfected cells

were transferred to a chamber (Warner Instrument Corp, Hamden, Conn) 48 hours after transfection for electrophysiological measurements.

### Electrophysiology

Sodium currents were recorded at room temperature using the whole-cell patch-clamp technique as described previously.<sup>20</sup> At least 2 independent recombinant cDNAs encoding each mutant were tested, and the data were pooled. Specific voltage-clamp protocols are depicted in each figure, and the cycle length was 5 seconds. The bath solution contained the following (in mmol/L): 145 NaCl, 4 KCl, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 HEPES, and 10 glucose, pH 7.35 (adjusted with NaOH). The pipette solution (intracellular solution) contained the following (in mmol/L): 10 NaF, 110 CsF, 20 CsCl, 2 EGTA, and 10 HEPES, pH 7.35 (adjusted with CsOH). Osmolarity was adjusted to 310 mOsm with sucrose for all solutions. For experiments assaying the effects of intracellular acidosis, the pipette solution was titrated to pH 6.7 with CsOH. Data acquisition was carried out with an Axopatch 200B patch-clamp amplifier and pCLAMP 8.0 software. Electrode resistance ranged from 0.8 to 1.5 M $\Omega$ . Junction potential and pipette capacitance were corrected, and whole-cell capacitance and series resistance were 85% to 95% compensated (voltage error <3 mV). Whole-cell currents were acquired at 20 to 50 kHz and filtered at 5 kHz. Recordings from cells exhibiting peak current amplitudes smaller than  $-0.8$  nA were excluded from analysis to avoid potential endogenous channel contamination. Cells exhibiting very large whole-cell currents ( $> -6$  nA) were excluded if voltage control was compromised. Fewer than 7.5% of cells were excluded across all experiments and all variants (range, 3.2% to 7.1%). Leak current was subtracted using a P/4 procedure. Whole-cell capacitance was assessed by integrating the capacitive transient elicited by a 10-mV voltage step from  $-120$  to  $-110$  mV with 10-kHz filtering.

### Data Analysis

All data were analyzed with pCLAMP 8.0 (Axon Instruments, Inc, Sunnyvale, Calif) and plotted using SigmaPlot 2002 (SPSS, Inc, Chicago, Ill). The time course of inactivation was fit with a 2-exponential function:

$$(1) \quad \frac{I(t)}{I_{\max}} = A_1 \times \exp\left(\frac{-t}{\tau_1}\right) + A_2 \times \exp\left(\frac{-t}{\tau_2}\right)$$

where values for  $A$  and  $\tau$  refer to amplitudes and time constants, respectively. Steady-state availability was fit with the Boltzmann equation,

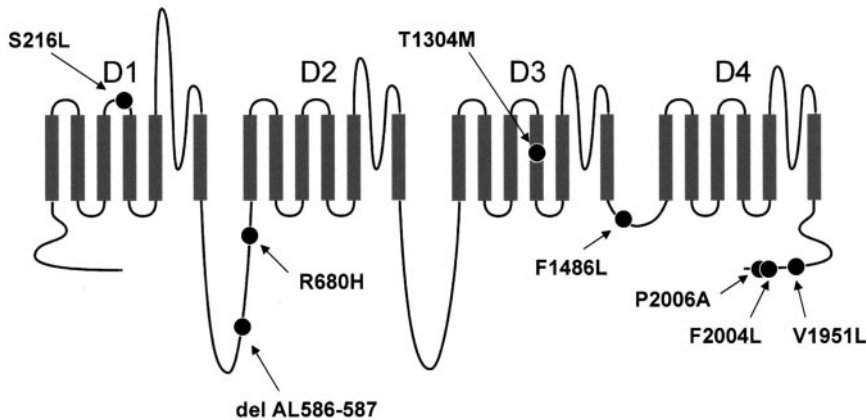
$$(2) \quad \frac{I}{I_{\max}} = \left(1 + \exp\left[\frac{V - V_{1/2}}{k}\right]\right)^{-1},$$

to determine the membrane potential for half-maximal inactivation ( $V_{1/2}$ ) and slope factor ( $k$ ). Recovery from inactivation was analyzed by fitting data with a 2-exponential function:

$$(3) \quad \frac{I(t)}{I_{\max}} = A_r \times \left(1 - \exp\left[\frac{-t}{\tau_r}\right]\right) + A_s \times \left(1 - \exp\left[\frac{-t}{\tau_s}\right]\right).$$

Voltage dependence of channel activation was estimated by measuring peak sodium current during a variable test potential from a holding potential of  $-120$  mV. Current at each membrane potential was divided by the electrochemical driving force for sodium ions and normalized to the maximum sodium conductance. Data were fit using a nonlinear least-squares minimization method (Marquardt-Levenberg algorithm).

The level of TTX-sensitive persistent current was determined with a 100-ms depolarization to  $-10$  mV as the average current recorded between 90 and 100 ms and expressed as a percentage of peak current after digital subtraction of currents recorded in the presence and absence of 10  $\mu$ mol/L tetrodotoxin (TTX; Sigma, St Louis, Mo). Results are presented as mean  $\pm$  SE, and statistical comparisons were made between wild-type (WT)–hH1 and variants using an unpaired Student  $t$  test or 1-way ANOVA followed by a Tukey test. Statistical



**Figure 1.** Predicted transmembrane topology of the cardiac sodium channel  $\alpha$ -subunit illustrating the locations of the 8 variants studied. Homologous domains of are labeled D1 through D4.

significance was assumed for  $P < 0.05$ . In some figures, SE bars are smaller than data symbols.

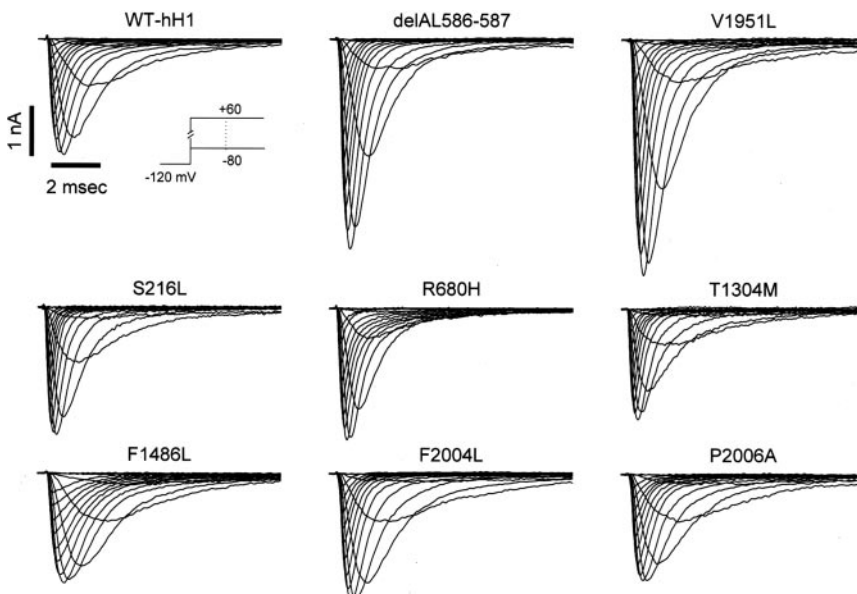
The authors had full access to the data and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## Results

Eight previously uncharacterized *SCN5A* variants were identified in a series of 201 Norwegian SIDS cases.<sup>5</sup> To distinguish between pathogenic mutations and benign polymorphisms, we determined the functional properties of SIDS-associated *SCN5A* variants (Figure 1) by coupling heterologous expression of a recombinant human cardiac voltage-gated sodium channel (hH1) with whole-cell patch-clamp recording. Figure 2 illustrates representative whole-cell current recordings obtained from cells expressing either wild-type (WT-hH1) or variant sodium channels. Cells expressing the engineered channels exhibited rapid activation and inactivation in response to a series of depolarizing test potentials typical of voltage-gated sodium channels. Two variants, delAL586-587 and V1951L, had significantly larger peak current amplitudes than WT-hH1, whereas the other 6 alleles exhibited expression levels similar to WT channels. No variant had an overt loss-of-function phenotype.

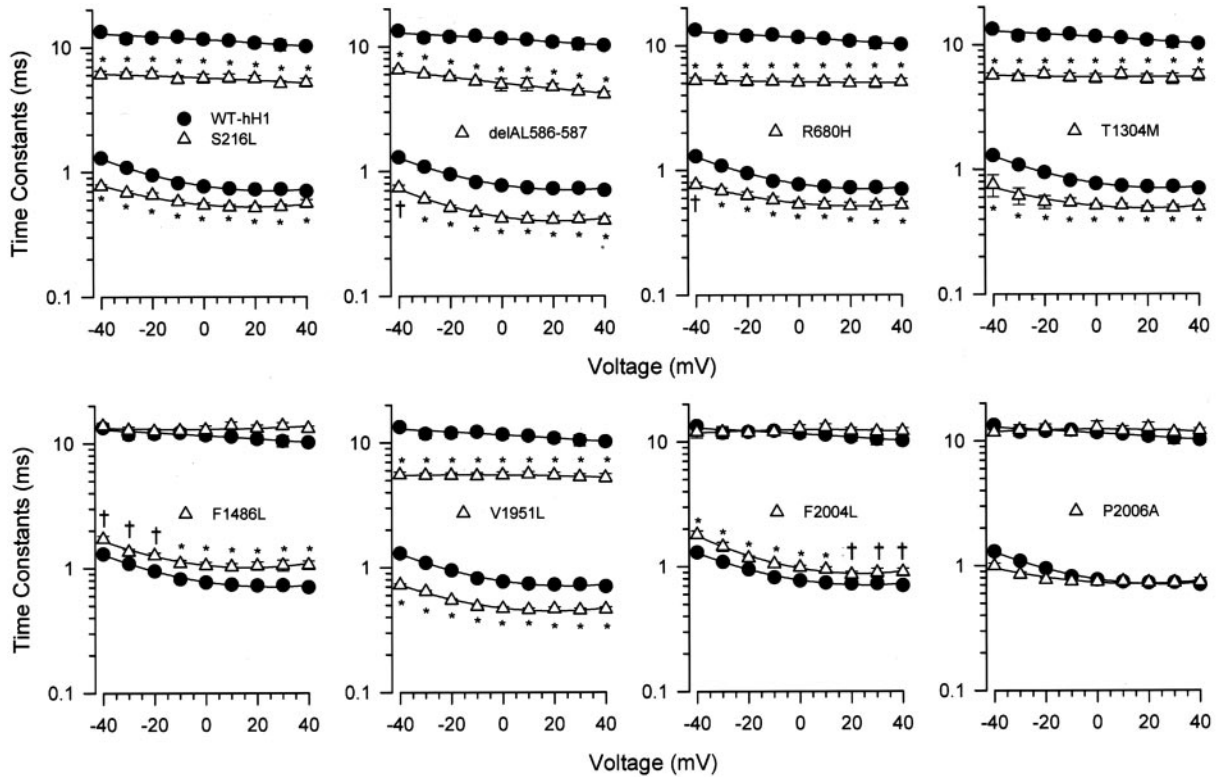
## Biophysical Disturbances Resembling LQTS Mutations

Pathogenic cardiac sodium channel mutations associated with congenital LQTS (LQT3 subtype) typically cause increased persistent whole-cell current and sometimes exhibit abnormal macroscopic inactivation.<sup>21–25</sup> We analyzed fast inactivation properties of all 8 SIDS-associated *SCN5A* variants and compared these data with the WT channel. Time constants ( $\tau_1$ ,  $\tau_2$ ) were derived from 2-exponential fits of the decay phase of macroscopic sodium current measured at various test potentials (Figure 3). Two alleles (F1486L, F2004L) exhibited significantly larger  $\tau_1$  values across a wide range of test potentials, suggesting that fast inactivation may be impaired. In contrast, 5 variants (S216L, delAL586-587, R680H, T1304M, V1951L) exhibited significantly smaller time constants compared with WT channels, indicating that macroscopic inactivation proceeds with a faster time course. There were no differences in time constants for P2006A and no differences in fractional amplitudes of the 2 components observed for any mutant. These results illustrate heterogeneity of effects on macroscopic inactivation kinetics caused by the SIDS-associated variants.



**Figure 2.** Representative whole-cell current traces of WT and variant sodium channels. Currents were recorded at membrane potentials from  $-80$  to  $60$  mV in  $10$ -mV increments from a holding potential of  $-120$  mV (protocol shown as an inset with WT-hH1 current traces).

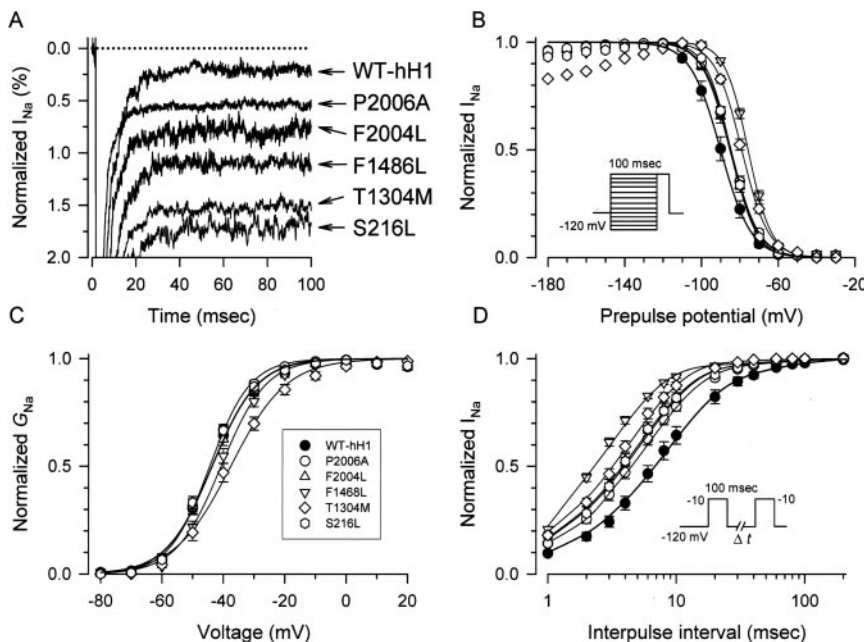




**Figure 3.** Voltage dependence of inactivation time constants. Lower and upper bundles of symbols indicate fast ( $\tau_1$ ) and slow ( $\tau_2$ ) component values, respectively. Data for WT-hH1 are plotted as solid circles in all panels; data for each mutant or variant are plotted as open triangles. Statistically significant differences in time constants comparing mutant channels with WT-hH1 are indicated (\* $P < 0.001$ , † $P < 0.01$ , Student  $t$  test). In some cases, error bars are smaller than the data symbols.

We measured the level of TTX-sensitive persistent current as a percentage of peak sodium current elicited by a 100-ms depolarization to  $-10$  mV and compared values obtained with each variant with that of WT-hH1. These experiments demonstrated significantly increased levels of persistent current under our standard recording conditions for 5 of the 8

alleles (Figure 4A and Table 1), ranging from 2.4-fold (P2006A) to 7.8-fold (S216L) greater than WT channels. Interestingly, the 5 variants with increased persistent current also exhibit significant depolarizing shifts in the voltage dependence of steady-state inactivation, ranging from 4.7 to 14.3 mV (Figure 4B and Table 2). Two of these alleles



**Figure 4.** SIDS-associated variants exhibit an LQT3 phenotype. A, Increased TTX-sensitive persistent sodium currents for P2006A, F2004L, F1486L, T1304M, and S216L. Zero current is indicated by a dotted line. Representative persistent current-voltage relationships for WT-hH1 and S216L are illustrated in Figure I of the online supplement. B, Sodium channel availability for WT-hH1, P2006A, F2004L, F1486L, T1304M, and S216L recorded using the pulse protocol shown in the inset and fitted with Boltzmann distributions (solid lines). The half-maximal voltage for inactivation ( $V_{1/2}$ ) and slope factor ( $k$ ) are given in Table 2. C, Voltage dependence of activation for WT-hH1, P2006A, F2004L, F1486L, T1304M, and S216L. Curves were fit with a Boltzmann distribution, and values determined for  $V_{1/2}$  and  $k$  are given in Table 2. D, Time course of recovery from inactivation was elicited using the 2-pulse protocol shown in the inset. Mutants (P2006A, F2004L, F1486L, T1304M, and S216L) exhibit faster recovery from inactivation than WT-hH1. Time constants and fractional amplitudes are given in Table 2.

**TABLE 1. Level of Persistent Sodium Current for SIDS-Associated *SCN5A* Variants**

	Persistent $I_{Na}$ , %	n	$P^*$
WT-hH1	0.22±0.05	7	...
S216L	1.71±0.24	6	<0.001
delAL586-587	0.12±0.04	8	NS
R680H	0.18±0.04	6	NS
T1304M	1.58±0.24	9	<0.001
F1486L	1.14±0.16	11	<0.001
V1951L	0.37±0.09	8	NS
F2004L	0.85±0.23	10	<0.05
P2006A	0.54±0.09	10	<0.05

$I_{Na}$  indicates persistent sodium current.

\*Comparisons with WT-hH1 using 1-way ANOVA followed by a Tukey test.

(T1304M, F1486L) also have depolarizing shifts in the voltage dependence of activation but to a lesser extent than the corresponding shift in steady-state inactivation (Figure 4C). These shifts in voltage dependence suggest that an increased “window current” approximated by the degree of overlap between activation and inactivation curves may partially explain increased persistent current. Recovery from inactivation also was significantly accelerated in the 5 variants with increased persistent current (Figure 4D and Table 2). There were no significant effects on the kinetics or voltage dependence of slower forms of inactivation (data not shown). The increased persistent current, accelerated recovery from inactivation, and variable effects on the kinetics and voltage dependence of channel gating observed for S216L, T1304M, F1486L, F2004L, and P2006A resemble the biophysical disturbances exhibited by several *SCN5A* mutations associated with congenital LQTS.<sup>21–25</sup> These observations provide a plausible explanation for increased arrhythmia susceptibility possibly leading to SIDS.

### Mutants With Latent Phenotypes

Three variants (R680H, delAL586-587, V1951L) did not exhibit an overt LQT3-like phenotype under standard experimental conditions but did have other significant functional

disturbances. As illustrated in Figure 3, sodium current in cells expressing R680H inactivates significantly faster than WT-hH1 channels. This variant also affects recovery from inactivation (Table 2). The remaining functional properties of this variant were not different from WT channels, however. The 2 remaining variants (delAL586-587, V1951L) exhibited significantly greater peak sodium current densities than WT channels (Figure 5A) and small but significant effects on recovery from inactivation. These biophysical defects were not sufficient to evoke pathological behavior in the Luo-Rudy action potential model<sup>26</sup> (data not shown). Therefore, the impact of these biophysical disturbances on the risk of sudden death is not apparent.

We considered that a latent pathological phenotype may exist in these 3 alleles that might be exposed by other experimental maneuvers or by expressing the mutants in the context of a different *SCN5A* splice isoform. On the basis of recent work by Plant and colleagues<sup>12</sup> demonstrating a dependence of *SCN5A*-S1103Y on internal pH, we reexamined R680H, delAL586-587, and V1951L under conditions of intracellular acidosis (internal pH 6.7). As demonstrated in Table 3, intracellular acidosis increases the proportion of persistent current by a similar degree among WT-hH1 and several SIDS-associated alleles. However, internal acidosis evokes a significantly greater level of increased persistent current in cells expressing R680H compared with WT-hH1. This difference was explained by a 1.8-fold-greater magnitude of persistent current density (WT-hH1:  $-2.9±0.6$  pA/pF,  $n=12$ ; R680H:  $-5.1±0.06$ ,  $n=12$ ;  $P<0.05$ ) rather than a difference in peak current density (WT-hH1:  $-460±39$  pA/pF,  $n=12$ ; R680H:  $-401±51$ ,  $n=12$ ;  $P=NS$ ). These data suggest that R680H may become pathological under certain metabolic conditions.

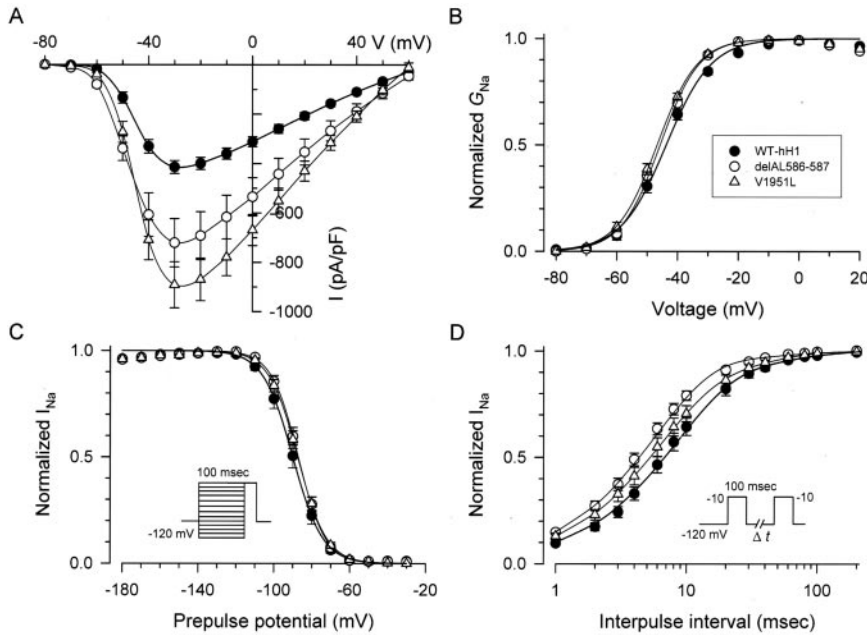
We also examined whether functional differences exist when specific mutants are examined in the common *SCN5A* splice variant, delQ1077.<sup>19</sup> As shown in Table 3, R680H, delAL586-587, and V1951L exhibit significantly increased levels of persistent current when examined in the delQ1077 background. Furthermore, the acidosis-evoked increase in persistent current observed for R680H also was observed in

**TABLE 2. Biophysical Parameters for Activation and Fast Inactivation of SIDS-Associated *SCN5A* Variants**

	Voltage Dependence of Activation			Steady-State Availability (100 ms)			Recovery From Inactivation (100 ms)		
	$V_{1/2}$ , mV	k, mV	n	$V_{1/2}$ , mV	k, mV	n	$\tau_1$ , ms (%)	$\tau_2$ , ms (%)	n
WT-hH1	-44.3±0.6	7.1±0.3	14	-89.3±1.1	-7.0±0.2	16	10.3±1.3 (94.5±1.0)	112.8±13.8 (5.5±1.0)	12
S216L	-44.5±0.8	6.8±0.2	13	-84.6±0.6‡	-6.4±0.1*	15	5.4±0.2§ (94.0±1.0)	47.8±4.5§ (6.0±1.0)	13
delAL586-587	-44.6±0.9	5.8±0.4	10	-87.1±1.3	-6.7±0.1	15	5.9±0.5‡ (94.7±0.9)	59.5±7.0‡ (5.3±0.9)	10
R680H	-46.2±0.9	7.1±0.2	12	-89.2±0.7	-7.0±0.1	12	9.0±0.8 (90.0±2.0)	46.3±8.1§ (10.0±2.0)	12
T1304M	-37.6±1.7‡	8.8±0.4*	8	-78.1±1.0§	-6.7±0.2	7	4.7±0.4‡ (98.0±0.5)‡	50.7±3.8‡ (2.0±0.5)‡	7
F1486L	-40.6±1.1*	6.9±0.2	14	-75.0±0.8§	-5.9±0.2§	13	3.4±0.2§ (96.0±0.6)	68.1±9.3* (4.0±0.6)	13
V1951L	-45.9±1.1	6.3±0.5	12	-87.5±1.3	-7.0±0.2	17	8.0±0.9 (92.0±1.0)	71.9±10.8* (8.0±1.0)	16
F2004L	-43.6±1.1	7.2±0.3	17	-84.6±1.1‡	-6.4±0.1	18	5.1±0.3‡ (98.0±0.5)‡	104.8±14.3 (2.0±0.5)‡	13
P2006A	-44.5±1.0	6.4±0.2	12	-84.6±0.8‡	-6.9±0.2	18	6.3±0.6‡ (94.9±0.5)	59.5±4.9§ (5.1±0.5)	19

% indicates percentage of total recovery represented by the time constant.

\* $P<0.05$ , † $P<0.01$ , ‡ $P<0.005$ , § $P<0.001$  (comparisons with WT-hH1 using 1-way ANOVA followed by a Tukey test).



**Figure 5.** Properties of delAL586-587 and V1951L sodium channels. A, Comparison of current-voltage relationships for WT-hH1, delAL586-587, and V1951L. Current is normalized to cell capacitance to give a measure of sodium current density. There are significant differences ( $P < 0.05$ ) in current density between WT-hH1 and 2 mutants at all tested voltages except  $-80$ ,  $-70$ ,  $-60$ ,  $50$ , and  $60$  mV. B, Voltage dependence of sodium channel activation. The activation curve was constructed as described in the legend of Figure 3; parameters are given in Table 2. C, Voltage dependence of sodium channel availability was obtained using the 2-pulse protocol illustrated by the inset. Values for  $V_{1/2}$  and  $k$  are provided in Table 2. D, Time course of recovery from inactivation at  $-120$  mV. The time constants and fractional amplitudes are provided in Table 2.

the context of delQ1077 (Table 3). These results indicate that all 8 SIDS-associated *SCN5A* variants encode sodium channels with the potential for functional disturbances consistent with LQTS that can be predicted to contribute to increased risk of cardiac arrhythmia and sudden death.

## Discussion

Sudden unexplained death is a vexing clinical problem affecting all age groups. In adults, sudden death often is attributed to cardiac arrhythmias, particularly ventricular fibrillation.<sup>27</sup> Sudden unexplained death in adolescents and young adults is related predominantly to occult cardiac disease<sup>28</sup> but also may herald the presence of an inherited arrhythmia syndrome. In neonates and infants, however, sudden unexplained death is categorized as SIDS when

rigorous efforts to identify the cause of death, including a forensic examination, are unrevealing. SIDS is essentially a diagnosis of exclusion, and established guidelines exist.<sup>29</sup> Identification of factors contributing to sudden unexpected death in infants has great importance for the diagnosis and treatment of preventable causes of this devastating clinical problem.

Many hypotheses have been proposed to explain SIDS, and there is consensus that the syndrome is heterogeneous. Some theories have suggested that SIDS may result from an abnormality in either respiratory or cardiac function or the neural control of these physiological events that may be transient but sufficient to evoke lethal consequences.<sup>30–32</sup> A reduction in number of reported SIDS cases followed institution of programs emphasizing avoidance of the prone

**TABLE 3. Level of Persistent Sodium Current Under Different Experimental Conditions**

	Persistent $I_{Na}$ , %	n	P
Intracellular acidosis (pH <sub>i</sub> 6.7)			
WT-hH1	0.62 ± 0.09	12	...
delAL586-587	0.67 ± 0.13	10	NS*
R680H	1.41 ± 0.27	12	<0.05*
V1951L	0.88 ± 0.18	9	NS*
Expression in delQ1077 splice isoform			
WT-hH1 delQ1077	0.32 ± 0.04	15	
delAL586-587 delQ1077	2.09 ± 0.34	9	<0.001†
R680H delQ1077	0.89 ± 0.12	13	<0.001†
V1951L delQ1077	0.83 ± 0.11	15	<0.001†
Expression in delQ1077 splice isoform with intracellular acidosis			
WT-hH1 delQ1077	0.79 ± 0.11	12	...
R680H delQ1077	1.90 ± 0.42	8	<0.005‡

$I_{Na}$  indicates persistent sodium current.

\*Comparisons with WT-hH1 using 1-way ANOVA followed by a Tukey test.

†Comparisons with WT-hH1|delQ1077 using 1-way ANOVA followed by a Tukey test.

‡Comparisons with WT-hH1|delQ1077 using Student *t* test.



position for sleeping infants.<sup>33,34</sup> SIDS remains a leading cause of infant death in the Western world, however, and more work is needed to discern its causes.<sup>16,35</sup>

Evidence for genetic subsets in SIDS has come from work identifying mutations in various genes.<sup>36,37</sup> Deficiencies in fatty acid metabolism caused by mutations in the medium-chain acyl-CoA dehydrogenase gene have been extensively investigated.<sup>38</sup> The most well-studied mutation is A985G; however, <1% of SIDS cases investigated have this mutation.<sup>39</sup> Fatty acid oxidation disorders other than medium-chain acyl-CoA dehydrogenase deficiency have an unclear role in SIDS.<sup>40</sup> Common variants in the interleukin-10, serotonin transporter, and certain regions of mitochondrial DNA have been shown to have genetic association with SIDS.<sup>36</sup> Mutations in genes associated with LQTS appear to represent the largest genetic subset delineated so far. Because LQTS mutations may explain the increased risk of sudden death, one could consider removing these cases from the strict definition of SIDS, whereas polymorphisms in other genes (ie, cytokines), combined with triggering events such as prone sleeping, may predispose to fatality during vulnerable periods of development.<sup>36,41</sup>

### Spectrum of *SCN5A* Dysfunction in SIDS

In our present study, we characterized the biophysical properties of recombinant cardiac sodium channels engineered with 8 genetic variants identified in a large Norwegian SIDS series.<sup>5</sup> We observed different levels of defective sodium channel inactivation among the 8 SIDS-associated variants that can be grouped into 3 categories. In 1 subgroup including S216L, T1304M and F1486L, we observed an overt pathological phenotype characterized by a substantial increased persistent current (>1% of peak current) that closely resembles the defect shared among most congenital LQT3 mutations.<sup>21–25</sup> Indeed, T1304M was previously identified in a family-segregating congenital LQTS, but the mutant allele was never characterized.<sup>42</sup> The largest level of persistent current was observed in cells expressing S216L, a variant found at low frequency in the general white population (minor allele frequency <0.4%).<sup>43</sup> The pathological significance of increased persistent current in heart has been well documented using advanced computational modeling of cardiac action potentials<sup>44</sup> and an *in vivo* mouse model.<sup>45</sup> Therefore, we contend that this subset of *SCN5A* variants has dysfunctional properties sufficient to elevate the risk of sudden death.

A second subset of *SCN5A* variants includes 2 alleles (F2004L, P2006A) affecting residues in the extreme carboxyl terminus. Both variants exhibited smaller, yet statistically significant, levels of increased persistent current combined with depolarizing shifts in voltage-dependence of inactivation and faster recovery from inactivation. Both F2004L and P2006A also occur at low frequency in the general population (minor allele frequencies <0.4%).<sup>43</sup> We speculate that the level of increased persistent current observed for these 2 variants alone is not sufficient to evoke arrhythmia susceptibility, and other factors are probably needed for full pathophysiological expression. For example, it is conceivable that the presence of other common polymorphisms such as

H558R on the same or opposite allele may influence the functional phenotype.<sup>46,47</sup> In the Norwegian SIDS series, heterozygosity for H558R was observed in subjects carrying the *SCN5A* variants delAL586-587, V1951L, F2004L, and P2006A, but no subjects were homozygous. It was not possible to determine on which parental allele the polymorphism resided except for the subject with delAL586-587 who carried the variant and H558R on separate chromosomes (data not shown). Therefore, we did not characterize V1951L, F2004L, and P2006A in the context of the R558 allele because of uncertainty with respect to the relevance to the actual genomes of the victims, but further work on this issue may be revealing.

The third subgroup of *SCN5A* variants (R680H, delAL586-587, V1951L) exhibits latent functional defects that depend on intracellular pH or the absence of Q1077. Interestingly, one of these variants, V1951L, is a common polymorphism in Hispanics<sup>43</sup> but was not observed in Norwegian white control subjects, the ethnic background represented in the Norwegian SIDS series.<sup>5</sup> When expressed in the context of the delQ1077 splice isoform, V1951L exhibited significantly increased persistent current. However, a prior study reported no functional abnormality associated with this allele in either the presence or absence of Q1077.<sup>48</sup> The experimental conditions that differ between our study and that of Tan et al<sup>48</sup> are relatively minor and do not explain the discordance in observed channel behavior. We did not study all variants in the delQ1077 context because of experimental limitations (difficulties in assembly of correct cDNA constructs) but also because the magnitude of increased persistent current observed for several alleles in the Q1077 background appears sufficient to explain an arrhythmia-prone condition.

Brugada syndrome also may predispose to premature death in young children and infants.<sup>49</sup> Sodium channel mutations in this disorder generally cause loss-of-function phenotypes.<sup>50</sup> We did not observe sodium channel dysfunction consistent with Brugada syndrome. However, one of the first reported *SCN5A* mutations associated with Brugada syndrome exhibited more rapid inactivation, and this was predicted to be an important pathophysiological feature.<sup>51</sup> Several of the variants we studied also exhibited more rapid inactivation (S216L, delAL586-587, R680H, T1304M, V1951L), although the clinical significance of these findings is not clear, especially when combined with an overt increased persistent current.

### Conclusions

A significant subset of SIDS may be explainable by mutations in LQTS genes, with many of the known SIDS-associated variants occurring in *SCN5A*. Our work triples the number of functionally characterized *SCN5A* variants associated with SIDS and illustrates that a spectrum of biophysical defects, ranging from overt to latent pathological phenotypes, occur. Our present study is limited by the lack of clinical data on probands and related mutation carriers and the lack of data demonstrating cosegregation of LQTS phenotypes in families with SIDS victims. Nonetheless, these data are helpful in validating the association of SIDS with occult LQTS gene mutations and provide additional functional information that



could translate into new treatment strategies for preventing this disorder, provided that early diagnosis in at-risk infants can be made feasible.

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### Disclosures

None.

### References

- Byard RW, Krous HF. Sudden infant death syndrome: overview and update. *Pediatr Dev Pathol*. 2003;6:112–127.
- Ponsonby AL, Dwyer T, Cochran J. Population trends in sudden infant death syndrome. *Semin Perinatol*. 2002;26:296–305.
- Schwartz PJ. Cardiac sympathetic innervation and the sudden infant death syndrome: a possible pathogenetic link. *Am J Med*. 1976;60:167–172.
- Tester DJ, Ackerman MJ. Sudden infant death syndrome: how significant are the cardiac channelopathies? *Cardiovasc Res*. 2005;67:388–396.
- Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Wang DW, Rhodes TE, George AL Jr, Schwartz PJ. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. *Circulation*. 2007;115:361–367.
- Schwartz PJ, Stramba-Badiale M, Segantini A, Austoni P, Bosi G, Giorgetti R, Grancini F, Marni ED, Perticone F, Rosti D, Salice P. Prolongation of the QT interval and the sudden infant death syndrome. *N Engl J Med*. 1998;338:1709–1714.
- Schwartz PJ, Priori SG, Dumaine R, Napolitano C, Antzelevitch C, Stramba-Badiale M, Richard TA, Berti MR, Bloise R. A molecular link between the sudden infant death syndrome and the long-QT syndrome. *N Engl J Med*. 2000;343:262–267.
- Wedekind H, Smits JP, Schulze-Bahr E, Arnold R, Veldkamp MW, Bajanowski T, Borggreffe M, Brinkmann B, Warnecke I, Funke H, Bhuiyan ZA, Wilde AA, Breithardt G, Haverkamp W. De novo mutation in the SCN5A gene associated with early onset of sudden infant death. *Circulation*. 2001;104:1158–1164.
- Ackerman MJ, Siu BL, Sturner WQ, Tester DJ, Valdivia CR, Makielski JC, Towbin JA. Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. *JAMA*. 2001;286:2264–2269.
- Schwartz PJ, Priori SG, Bloise R, Napolitano C, Ronchetti E, Piccinini A, Goj C, Breithardt G, Schulze-Bahr E, Wedekind H, Nastoli J. Molecular diagnosis in a child with sudden infant death syndrome. *Lancet*. 2001;358:1342–1343.
- Christiansen M, Tonder N, Larsen LA, Andersen PS, Simonsen H, Oyen N, Kanters JK, Jacobsen JR, Fosdal I, Wettrell G, Kjeldsen K. Mutations in the HERG K<sup>+</sup>-ion channel: a novel link between long QT syndrome and sudden infant death syndrome. *Am J Cardiol*. 2005;95:433–434.
- Plant LD, Bowers PN, Liu Q, Morgan T, Zhang T, State MW, Chen W, Kittles RA, Goldstein SA. A common cardiac sodium channel variant associated with sudden infant death in African Americans, SCN5A S1103Y. *J Clin Invest*. 2006;116:430–435.
- Gregersen M, Rajs J, Laursen H, Baandrup U, Frederiksen P, Gidlund E, Helweg-Larsen K, Hirvonen J, Isaksen CV, Kock K, Lundemose JB, Loberg EM, Rognum TO, Vege A. Pathologic criteria for the Nordic study of SIDS. In: Rognum TO, ed. *Sudden Infant Death Syndrome: New Trends in the Nineties*. Oslo, Norway: Scandinavian University Press; 1995:50–58.
- Vege A, Rognum TO. Use of new Nordic criteria for classification of SIDS to re-evaluate diagnoses of sudden unexpected infant death in the Nordic countries. *Acta Paediatr*. 1997;86:391–396.
- Arnestad M, Vege A, Rognum TO. Evaluation of diagnostic tools applied in the examination of sudden unexpected deaths in infancy and early childhood. *Forensic Sci Int*. 2002;125:262–268.
- Vege A, Rognum TO, Opdal SH. SIDS—changes in the epidemiological pattern in eastern Norway 1984–1996. *Forensic Sci Int*. 1998;93:155–166.
- Deleted in proof.
- Gellens ME, George AL, Chen L, Chahine M, Horn R, Barchi RL, Kallen RG. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. *Proc Natl Acad Sci U S A*. 1992;89:554–558.
- Makielski JC, Ye B, Valdivia CR, Pagel MD, Pu J, Tester DJ, Ackerman MJ. A ubiquitous splice variant and a common polymorphism affect heterologous expression of recombinant human SCN5A heart sodium channels. *Circ Res*. 2003;93:821–828.
- Benson DW, Wang DW, Dymont M, Knilans TK, Fish FA, Strieper MJ, Rhodes TH, George AL Jr. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). *J Clin Invest*. 2003;112:1019–1028.
- Bennett PB, Yazawa K, Makita N, George AL Jr. Molecular mechanism for an inherited cardiac arrhythmia. *Nature*. 1995;376:683–685.
- Dumaine R, Wang Q, Keating MT, Hartmann HA, Schwartz PJ, Brown AM, Kirsch GE. Multiple mechanisms of Na<sup>+</sup> channel-linked long-QT syndrome. *Circ Res*. 1996;78:916–924.
- Wang DW, Yazawa K, George AL Jr, Bennett PB. Characterization of human cardiac Na<sup>+</sup> channel mutations in the congenital long QT syndrome. *Proc Natl Acad Sci U S A*. 1996;93:13200–13205.
- Wei J, Wang DW, Alings M, Fish F, Wathen M, Roden DM, George AL Jr. Congenital long-QT syndrome caused by a novel mutation in a conserved acidic domain of the cardiac Na<sup>+</sup> channel. *Circulation*. 1999;99:3165–3171.
- Keller DI, Acharfi S, Delacretaz E, Benammar N, Rotter M, Pfammatter JP, Fressart V, Guicheney P, Chahine M. A novel mutation in SCN5A, delQKP 1507–1509, causing long QT syndrome: role of Q1507 residue in sodium channel inactivation. *J Mol Cell Cardiol*. 2003;35:1513–1521.
- Luo CH, Rudy Y. A dynamic model of the cardiac ventricular action potential, I: simulations of ionic currents and concentration changes. *Circ Res*. 1994;74:1071–1096.
- Spooner PM, Albert C, Benjamin EJ, Boineau R, Elston RC, George AL, Jr., Jouven X, Kuller LH, MacCluer JW, Marban E, Muller JE, Schwartz PJ, Siscovick DS, Tracy RP, Zareba W, Zipes DP. Sudden cardiac death, genes, and arrhythmogenesis: consideration of new population and mechanistic approaches from a National Heart, Lung, and Blood Institute workshop, part I. *Circulation*. 2001;103:2361–2364.
- Tester DJ, Ackerman MJ. The role of molecular autopsy in unexplained sudden cardiac death. *Curr Opin Cardiol*. 2006;21:166–172.
- Krous HF, Beckwith JB, Byard RW, Rognum TO, Bajanowski T, Corey T, Cutz E, Hanzlick R, Keens TG, Mitchell EA. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. *Pediatrics*. 2004;114:234–238.
- Schwartz PJ, Southall DP, Valdes-Dapena M, eds. The sudden infant death syndrome: cardio-respiratory mechanisms and interventions. New York: New York Academy of Sciences; 1988. *Annals of the New York Academy of Sciences*, vol 533.
- Schwartz PJ, Segantini A. Cardiac innervation, neonatal electrocardiography, and SIDS: a key for a novel preventive strategy? In: Schwartz PJ, Southall DP, Valdes-Dapena M, eds. The sudden infant death syndrome: cardio-respiratory mechanisms and interventions. New York: New York Academy of Sciences; 1988. *Annals of the New York Academy of Sciences*, vol 533: 210–220.
- Kahn A, Sawaguchi T, Sawaguchi A, Groswasser J, Franco P, Scaillet S, Kelmanson I, Dan B. Sudden infant deaths: from epidemiology to physiology. *Forensic Sci Int*. 2002;130:S8–S20.
- Changing concepts of sudden infant death syndrome: implications for infant sleeping environment and sleep position: American Academy of Pediatrics: Task Force on Infant Sleep Position and Sudden Infant Death Syndrome. *Pediatrics*. 2000;105:650–656.
- Galland BC, Taylor BJ, Bolton DP. Prone versus supine sleep position: a review of the physiological studies in SIDS research. *J Paediatr Child Health*. 2002;38:332–338.
- Li L, Fowler D, Liu L, Ripple MG, Lambros Z, Smialek JE. Investigation of sudden infant deaths in the state of Maryland (1990–2000). *Forensic Sci Int*. 2005;148:85–92.
- Opdal SH, Rognum TO. The sudden infant death syndrome gene: does it exist? *Pediatrics*. 2004;114:e506–e512.
- Hunt CE. Gene-environment interactions: implications for sudden unexpected deaths in infancy. *Arch Dis Child*. 2005;90:48–53.
- Andresen BS, Bross P, Jensen TG, Knudsen I, Winter V, Kolvraa S, Bolund L, Gregersen N. Molecular diagnosis and characterization of medium-chain acyl-CoA dehydrogenase deficiency. *Scand J Clin Lab Invest Suppl*. 1995;220:9–25.

39. Lecoq I, Mallet E, Bonte JB, Laroche D, Travert G. [Screening of A985 to G mutation of medium-chain acyl-CoA dehydrogenase (MCAD) gene in Normandy: evaluation of the role of MCAD deficiency in sudden infant death]. *C R Seances Soc Biol Fil.* 1995;189:295–301.
40. Rinaldo P, Yoon HR, Yu C, Raymond K, Tiozzo C, Giordano G. Sudden and unexpected neonatal death: a protocol for the postmortem diagnosis of fatty acid oxidation disorders. *Semin Perinatol.* 1999;23:204–210.
41. Rognum TO, Saugstad OD. Biochemical and immunological studies in SIDS victims: clues to understanding the death mechanism. *Acta Paediatr Suppl.* 1993;82(suppl 389):82–85.
42. Wattanasirichaigoon D, Vesely MR, Duggal P, Levine JC, Blume ED, Wolff GS, Edwards SB, Beggs AH. Sodium channel abnormalities are infrequent in patients with long QT syndrome: identification of two novel *SCN5A* mutations. *Am J Med Genet.* 1999;86:470–476.
43. Ackerman MJ, Splawski I, Makielski JC, Tester DJ, Will ML, Timothy KW, Keating MT, Jones G, Chadha M, Burrow CR, Stephens JC, Xu C, Judson R, Curran ME. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. *Heart Rhythm.* 2004;1:600–607.
44. Clancy CE, Rudy Y. Linking a genetic defect to its cellular phenotype in a cardiac arrhythmia. *Nature.* 1999;400:566–569.
45. Nuyens D, Stengl M, Dugarmaa S, Rossenbacker T, Compernelle V, Rudy Y, Smits JF, Flameng W, Clancy CE, Moons L, Vos MA, Dewerchin M, Benndorf K, Collen D, Carmeliet E, Carmeliet P. Abrupt rate accelerations or premature beats cause life-threatening arrhythmias in mice with long-QT3 syndrome. *Nat Med.* 2001;7:1021–1027.
46. Ye B, Valdivia CR, Ackerman MJ, Makielski JC. A common human *SCN5A* polymorphism modifies expression of an arrhythmia causing mutation. *Physiol Genomics.* 2003;12:187–193.
47. Viswanathan PC, Benson DW, Balsler JR. A common *SCN5A* polymorphism modulates the biophysical effects of an *SCN5A* mutation. *J Clin Invest.* 2003;111:341–346.
48. Tan BH, Valdivia CR, Rok BA, Ye B, Ruwaltdt KM, Tester DJ, Ackerman MJ, Makielski JC. Common human *SCN5A* polymorphisms have altered electrophysiology when expressed in Q1077 splice variants. *Heart Rhythm.* 2005;2:741–747.
49. Priori SG, Napolitano C, Giordano U, Collisani G, Memmi M. Brugada syndrome and sudden cardiac death in children. *Lancet.* 2000;355:808–809. Letter.
50. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, LeMarec H, Nademanee K, Perez Riera AR, Shimizu W, Schulze-Bahr E, Tan H, Wilde A. Brugada syndrome: report of the Second Consensus Conference. *Heart Rhythm.* 2005;2:429–440.
51. Dumaine R, Towbin JA, Brugada P, Vatta M, Nesterenko DV, Nesterenko VV, Brugada J, Brugada R, Antzelevitch C. Ionic mechanisms responsible for the electrocardiographic phenotype of the Brugada syndrome are temperature dependent. *Circ Res.* 1999;85:803–809.

### CLINICAL PERSPECTIVE

Sudden infant death syndrome is a leading cause of death during the first year of life in developed countries. Cardiac mechanisms, including life-threatening arrhythmias, have been suspected to cause a proportion of sudden infant death syndrome cases, and mutations in genes responsible for the congenital long-QT syndrome are found in some victims. In the present study, functional properties were determined for 8 cardiac sodium channel (*SCN5A* gene) variants identified in a large series of Norwegian sudden infant death syndrome victims. A spectrum of sodium channel dysfunction was observed, ranging from overt to latent pathological phenotypes. Five variants exhibited significantly increased levels of persistent sodium current, a pathophysiological outcome typical of *SCN5A* mutations associated with congenital long-QT syndrome. Three other variants exhibited increased persistent current only under conditions of internal acidosis or when expressed in the context of a common *SCN5A* splice variant, indicating that they have latent dysfunction. These observations validate the association of sudden infant death syndrome with occult long-QT syndrome gene mutations and provide additional functional information that could translate into new treatment strategies.