A common cardiac sodium channel variant associated with sudden infant death in African Americans, SCN5A S1103Y

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Thousands die each year from sudden infant death syndrome (SIDS). Neither the cause nor basis for varied prevalence in different populations is understood. While 2 cases have been associated with mutations in type Vα, cardiac voltage-gated sodium channels (SCN5A), the “Back to Sleep” campaign has decreased SIDS prevalence, consistent with a role for environmental influences in disease pathogenesis. Here we studied SCN5A in African Americans. Three of 133 SIDS cases were homozygous for the variant S1103Y. Among controls, 120 of 1,056 were carriers of the heterozygous genotype, which was previously associated with increased risk for arrhythmia in adults. This suggests that infants with 2 copies of S1103Y have a 24-fold increased risk for SIDS. Variant Y1103 channels were found to operate normally under baseline conditions in vitro. As risk factors for SIDS include apnea and respiratory acidosis, Y1103 and wild-type channels were subjected to lowered intracellular pH. Only Y1103 channels gained abnormal function, demonstrating late reopenings suppressible by the drug mexiletine. The variant appeared to confer susceptibility to acidosis-induced arrhythmia, a gene-environment interaction. Overall, homozygous and rare heterozygous SCN5A missense variants were found in approximately 5% of cases. If our findings are replicated, prospective genetic testing of SIDS cases and screening with counseling for at-risk families warrant consideration.
A common cardiac sodium channel variant associated with sudden infant death in African Americans, SCN5A S1103Y

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Introduction
Sudden infant death syndrome (SIDS) is the leading cause of infant mortality in the US among all racial and ethnic groups, representing nearly one-third of deaths between 1 month and 1 year of age (1). These losses continue despite dramatic improvement with the 1994 launch of the “Back to Sleep” campaign discouraging prone sleep for infants, a known risk factor for SIDS (2) that indicates a role for environmental stressors in the disorder. SIDS is defined as sudden and unanticipated death in an infant with no recognized lethal disorder after an investigation that includes autopsy, examination of the death scene, and review of the history (3). Suggesting a role for genetic predisposition, there is significant variation in SIDS among racial and ethnic groups; rates for African Americans and Native Americans are 3 times those for whites and 6 times higher than for Hispanics and Asians (1).

While multiple recognized risk factors suggest that SIDS is more than one entity and its pathogenesis multifactorial, the cardiac hypothesis (4, 5) has regained support due to identification in postmortem genetic samples from SIDS cases of mutations in the same ion channel genes that cause inherited and acquired long QT syndrome (LQTS) (6–11), 1 in a potassium channel (12) and 2 in a sodium channel (13). In addition to SIDS and LQTS, mutations in SCN5A cause Brugada syndrome, sudden unexplained nocturnal death syndrome (14), idiopathic ventricular fibrillation (15), congenital sick sinus syndrome (16), and progressive cardiac conduction defect (Lenegre-Lev disease) (17). Of note, many of these disorders are associated with bradycardia, the only consistent pre-morbid arrhythmia seen on cardiopulmonary monitors in SIDS cases immediately prior to death (18). Most SCN5A mutations causing arrhythmia produce channels that continue to operate late in the cardiac cycle when normal channels are silent; such late activity is a substrate for chaotic rhythms and sudden death (19–21). In African Americans, the common missense variant SCN5A S1103Y confers an 8-fold increased risk of arrhythmia without marked change in channel function in vitro (22). Genetic modulation of risk by exposure to extrinsic factors is seen in patients who carry potassium channel variants that are well tolerated until drug exposure produces arrhythmia (10, 11) and is consistent with a multi-hit pathogenesis for cardiac arrhythmia (23). To investigate the impact of changes in cardiac ion channel function on the risk of SIDS, samples were screened for genetic variations in SCN5A, and the attributes of variant channels were studied in the presence and absence of stressors and antiarrhythmic medications.

Results

Cohort and genetic analysis. The 224 autopsy-confirmed cases of SIDS in our case-control study reflect the expected distribution of SIDS cases in the US based on sex and ethnicity, with higher numbers of African Americans than whites (African American, 133; white, 86) and males than females (male, 106; female, 79). The control population for the African American SIDS cases consisted of 1,056 African American adults without known health problems. Twenty of the 133 African American SIDS cases had SCN5A variants that changed the channel protein (Supplemental Tables 1 and 2;
supplemental material available online with this article; doi:10.1172/JCI25618DS1). Thirteen cases were heterozygous for a common variant where a C replaces A in the second position of codon 1103 on 1 copy of SCN5A to yield a change from serine to tyrosine in the channel (Figure 1), indicating the genotype S1103/Y1103 (SY). Three cases (2 female, 1 male) were homozygous for the variant genotype Y1103/Y1103 (YY), and so these infants carried only Y1103 channels. Four other cases had rare variants that changed a residue elsewhere in the channel on 1 SCN5A allele (Supplemental Figure 1). Of the 1,056 African American adult controls, the wild-type genotype S1103/S1103 (SS) was observed 935 times, SY was seen 120 times (similar to the previously reported frequency; refs. 22, 24), and 1 individual was homozygous (YY). This last individual could not be relocated to investigate for evidence of family history of SIDS or cardiac rhythm disturbances; he was 40 years of age and healthy at the time of sample collection.

Hardy-Weinberg equilibrium. Observed and expected genotypic frequencies, assuming Hardy-Weinberg equilibrium (HWE), for each genotype in African Americans were as follows. In SIDS cases (n = 133): SS 117, 115 expected; SY 13, 17.6 expected; YY 3, 0.68 expected. In controls (n = 1,056): SS 935, 938 expected; SY 120, 115 expected; YY 1, 3.52 expected. Case genotype frequencies were not in HWE ($\chi^2 = 9.21; P = 0.002$), whereas control frequencies did not significantly deviate from HWE ($\chi^2 = 2.04; P = 0.15$). In SIDS cases, there was a 4-fold excess of YY genotypes over the expected amount. In the controls, there was 1 YY individual, whereas 3 or 4 were expected. Comparing YY frequency in SIDS cases with controls, 3 of 133 (2.3%) versus 1 of 1,056 (0.1%), gives an odds ratio for SIDS of 24.4 (95% confidence interval, 1.9 to 1,279), indicating an approximately 24-fold increase in risk with the YY genotype. Overrepresentation of YY in SIDS cases was statistically significant, with a P value of 0.005 by Fisher’s exact test. To rule out misattribution in SIDS cases identified as African American, all SIDS cases with SY and YY genotypes were evaluated and confirmed to bear the expected genetic ancestral proportions for Americans of African descent and were unrelated (Supplemental Table 1 and Supplemental Figure 2).

$Y1103$ channels inactivate abnormally at low pH. SCN5A encodes a 2016 residue $\alpha$ subunit with 4 similar domains (Figure 1B, DI–DIV) that fold in pseudosymmetric fashion to form a single, central ion permeation pathway lined by 4 pore loops. Position 1103 is in the intracellular linker between DII and DIII. To investigate channel operation, wild-type S1103 or Y1103 channel $\alpha$ subunits were expressed with the $\beta$ subunit of SCN5A, SCN1B, in mammalian tissue culture cells (HEK-293) (25). Channels were first studied using whole-cell patch-clamp mode so currents passed by many channels were assessed simultaneously. At pH 7.4, S1103 and Y1103 channels were found to function indistinguishably, showing the same midpoint for voltage-dependent activation (Figure 2A), midpoint for voltage-dependent steady-state inactivation (Figure 2B), and time course for recovery from inactivation (Figure 2C). Splawski and colleagues (22) observed subtle changes in gating of Y1103 channels under baseline conditions that were not apparent in our system. Hypothesizing that a more significant change in channel function might result from a challenge experienced in vivo, we assessed the effect of acidosis.

Because neither residue is charged, an amino acid change from S to Y is not expected to produce pH sensitivity a priori. Nonetheless, lowering cytosolic pH from 7.4 to 7.0 and 6.7 was found to induce progressive shifts in the voltage dependence of steady-state inactivation ($V_{1/2}$) for Y1103 channels (Figure 2, bottom row); thus, Y1103 showed $V_{1/2}$ to equal $-88 \pm 1$, $-84 \pm 2$, and $-77 \pm 2$ mV at pH 7.4, 7.0, and 6.7, respectively (n = 4–8). Wild-type S1103 channels did not show a similar change ($V_{1/2} = -87 \pm 2$, $-88 \pm 1$, and $-84 \pm 1$ mV at pH 7.4, 7.0, and 6.7, respectively; n = 7–21) and so significantly differed with Y1103 at pH 6.7 (P = 0.005). In contrast, external acidification is expected to block peak current and shift steady-state inactivation (27), and these effects were observed for both channels to the same degree (Supplemental Table 3). Internal acidification was also found to speed recovery from inactivation of Y1103 but not wild-type SCN5A channels (Figure 2, bottom row). Changing the internal solution to pH 6.7 led to a small decrease in recovery time for Y1103 channels that reached statistical significance (P < 0.05; n = 11 for each condition). Internal acidification also induced a small shift in the current/voltage relationship for Y1103 (Figure 2A, bottom inset) that did not achieve statistical significance.

These findings suggest Y1103 channels exposed to low internal pH might operate at times during the cardiac cycle when wild-type channels remain silent. To assess this hypothesis, whole-cell currents were recorded from groups of cells expressing S1103 channels with internal pH 7.4 or 6.7 (Figure 3, A and B; n = 10
cells for each condition). As expected, currents rapidly activated and inactivated to baseline; a double exponential fit revealed time constants of 1.8 ± 0.04 and 2.0 ± 1.8 ms and 1.9 ± 0.01 and 1.8 ± 0.8 ms at normal and low pH, respectively. Conversely, Y1103 channels produced sustained whole-cell currents at low internal pH that did not return to baseline and showed altered inactivation kinetics (Figure 3, A and B). Late current was 5.23% ± 0.85% of peak, and time constants for recovery from inactivation were the same for S1103 and Y1103 channels at physiological pH but were speeded when Y1103, but not S1103, was exposed to pH 6.7 (n = 11). Curves are exponential fits to the recovery time course, l(t)/l(max) = Af[1 – exp(–t/τf)] + As[1 – exp(–t/τs)], where A is the amplitude of fast (f) and slow (s) components and t is time. Values for f are reported in Supplemental Table 3.

Discussion
The SCN5A Y1103 allele has been associated with arrhythmia and sudden death in 3 situations. African Americans with a single copy of the allele show an 8-fold increase in risk for arrhythmia as they reach middle age (22, 28, 29). Three siblings in a white kindred carrying a single allele have syncope, ventricular tachycardia, ventricular fibrillation, and sudden death (31). Here, homozygous African Americans infants carrying 2 Y1103 alleles showed a 24-fold increased risk for SIDS.

**Figure 2**
Y1103 channels show a change in inactivation gating at low pH. Whole-cell currents in HEK-293 cells expressing SCN5A S1103 (open squares) or SCN5A Y1103 (filled squares). Top: Sample traces with S103 channels. Arrows indicate points of measure. Scale bar: 1 nA. Filter and sampling frequencies were 5 and 20 kHz, respectively. (A) Normalized conductance/voltage (G/G(max)) relationships for S1103 and Y1103 channels show no change with a shift of internal pH (pHi) from pH 7.4 to pH 6.7 (n = 4–13). Curves fitted to a Boltzmann relationship (y = 1/(1 + exp(V - V(1/2))/V)), where V(1/2) is slope factor). Inset: Current/voltage relationships. Values for V(1/2) are reported in Supplemental Table 3. (B) Y1103 channels showed a shift in voltage-dependent steady-state inactivation at pH 6.7, whereas S1103 channels did not. Normalized peak current values (I/I(max)) are plotted against prepulse potential (mV). Curves fitted as in A. At pH 6.7, V(1/2) for S1103 is –84 ± 1 mV (n = 7) and for Y1103 is –77 ± 2 mV (n = 8, P < 0.05; 2-population Student’s t test). Values are mean ± SEM. (C) Time constants for recovery from inactivation were the same for S1103 and Y1103 channels at physiological pH but were speeded when Y1103, but not S1103, was exposed to pH 6.7 (n = 11). Curves are exponential fits to the recovery time course, l(t)/l(max) = Af[1 – exp(–t/τf)] + As[1 – exp(–t/τs)], where A is the amplitude of fast (f) and slow (s) components and t is time. Values for f are reported in Supplemental Table 3.

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cases out of HWE. We hypothesize that the allele is a substrate for cardiac QT interval prolongation and ventricular arrhythmia in the setting of a secondary challenge that is tolerated by infants with wild-type channels. We previously proposed a similar mechanism to mediate some cases of drug-induced arrhythmia where variant ion channel activity or was markedly increased or did not come to light until the stress of drug action (10, 11). In the case of Y1103 sodium channels, acidosis was seen to gain-of-function late reopenings, abnormal behavior associated with dangerous arrhythmias in patients with familial LQTS due to SCN5A mutations. The operation of Y1103 channels under baseline conditions was normal; this suggests that homozygous S1103Y infants will have a normal electrocardiogram in the absence of stressors and that this test would therefore not identify these genetically at-risk infants. Lastly, while the present report focuses on SCN5A S1103Y, the multifactorial nature of SIDS argues for mutation screening of other cardiac and noncardiac genes.

In this context, it is notable that many of the known risk factors for SIDS are stressors that can lead to prolonged apnea and respiratory acidosis — a common event in normal infants that may be exacerbated by diminished respiratory drive in situations including sleep, respiratory infection, multiple siblings (increasing infectious exposures), prematurity, birth, and exposure to secondhand cigarette smoke. We speculate that increased risk for arrhythmia in aging African Americans with the Y1103 allele (22) may result from a similar mechanism, that is, low intracellular pH due to metabolic acidosis in poorly perfused cardiac muscle fed by an atherosclerotic coronary bed. Importantly, the internal pH levels of 7.0 and 6.7 used here to challenge Y1103 channels in vitro are relevant to cardiac physiology. While normal arterial pH is approximately 7.4, intracellular pH of cardiac myocytes is much lower. Cardiomyocytes in anesthetized, ventilated rats, rabbits, dogs, sheep, and ferrets have internal pH levels between 6.9 and 7.1 at baseline (32, 33). Internal pH decreases to 6.6–6.8 in poorly perfused cardiac muscle due to metabolic acidosis in poorly perfused cardiac muscle fed by an atherosclerotic coronary bed. Importantly, the internal pH levels of 7.0 and 6.7 used here to challenge Y1103 channels in vitro are relevant to cardiac physiology. While normal arterial pH is approximately 7.4, intracellular pH of cardiac myocytes is much lower. Cardiomyocytes in anesthetized, ventilated rats, rabbits, dogs, sheep, and ferrets have internal pH levels between 6.9 and 7.1 at baseline (32, 33). Internal pH decreases to 6.6–6.8 with respiratory acidosis and/or hypoxia (34, 35). Furthermore, acid loads dissipate slowly in myocardium, suggesting that basal microheterogeneity in intracellular pH will be pronounced under stress (32, 36).

It should be noted explicitly that suppression of abnormal function of Y1103 channels in vitro does not indicate in vivo effectiveness and that therapy with antiarrhythmic medication carries risk; conversely, mexiletine shortens the QT interval in animal models and adults with LQTS type 3 (37), and 2 infants with LQTS type 3 have been treated with mexiletine and shown both tolerance of therapy and shortened QT (38, 39). These studies and our results suggest a strategy for prophylactic therapy, that is, use of state-dependent blockers that target late reopenings. The results do not indicate that mexiletine should be administered to YY infants to prevent SIDS at the present time. Should future work support our conclusion that YY infants are at significant risk, a study of prophylactic therapy is first required to assess benefits and risks. Moreover, it is important to note that the composition and environment of the channels we studied in cultured cells only approximate cardiac cells from SIDS patients; indeed, the identity of accessory gene products that may influence the behavior of the channels we studied in cultured cells must be determined.

Figure 3

Cells expressing Y1103 channels show sustained current at a low pH. Whole-cell currents were recorded in HEK-293 cells expressing S1103 or Y1103 channels as indicated with pipette and bath solutions (see Methods) with cells held at –100 mV and stepped to –50 mV for 100 ms at a rate of 0.5 Hz. Scale bars represent 300 pA and 20 ms. (A) Representative currents recorded from cells at the indicated pH. (B) Ensemble averages of 500 sweeps recorded from 10 cells with the indicated channel and pH. Late current was 5.23% ± 0.85% of peak (n = 10 cells). Peak currents were not significantly different and were −1311 ± 143 pA and 1252 ± 139 pA, for S1103 at normal and low pH, respectively, and 1267 ± 173 pA and 1195 ± 245 pA for Y1103 at normal and low pH, respectively (n = 10 cells per condition).

Figure 4

Single Y1103 channels show abnormal late reopenings. Single channels studied in inside-out, off-cell patches excised from HEK-293 cells. Currents were stimulated every 2.5 seconds by a 50-ms depolarizing pulse to –30 mV from a holding potential of –120 mV. Data were recorded at filter and sampling frequencies of 5 and 50 kHz, respectively. Pipettes were filled with bath solution described in Methods. Cells were perfused with the pipette solution described in Methods. For each cell, null sweeps (with no channel activity) were identified, averaged offline, and subtracted from data sweeps before analysis. For display purposes, data were refiltered offline using a 2-kHz Bessel filter. (A) With depolarization, single S1103 channels opened, inactivated rapidly, and did not reopen. This behavior was unaltered when internal pH was lowered from 7.4 to 6.7. Null traces at pH 7.4 and 6.7 were 50% ± 2.7% (n = 15 patches, 1,367 sweeps) and 51% ± 3.4% (n = 12 patches, 1,028 sweeps), respectively. In contrast, Y1103 channels behaved like S1103 channels at pH 7.4 but showed late reopenings at pH 6.7. Null traces were 51% ± 4.5% at pH 7.4 (n = 8 patches, 742 sweeps) and 51.7 ± 2.5% (n = 10 patches, 942 sweeps) when pH was reduced to 6.7. (B) Ensemble average traces (n = 100–150 sweeps) for the indicated channels and conditions. Y1103 channels failed to remain in the inactivated state when exposed to internal pH 6.7. Scale bars: 0.5 pA; 10 ms.
subunits in human sodium channels are still a subject requiring investigation (see ref. 40).

As with any association study, our findings will be need to be replicated in a separate cohort of African Americans. Even in relatively homogeneous genetic isolates, population stratification remains a potential explanation for false positive associations (41). However, the allele has been associated with cardiac arrhythmia in adults (22), and changes in function we observed here with low pH provide support for a plausible disease mechanism. The frequency of SIDS associated with changes in SCN5A channels in African Americans in our cohort was approximately 5%. Thus, 7 of 133 SIDS cases were homozygous for YY or heterozygous for other residue changes in SCN5A channels. Changes at sites other than S1103 altered channel function at normal pH (SS24Y, 2 cases; R689H and E1107K, 1 case each; Q. Liu and S.A.N. Goldstein, unpublished observations). Like individuals with inherited LQTS type 3, these infants had SCN5A gain in function in a single mutant allele. In contrast, African Americans homozygous for Y1103 were at increased risk for SIDS despite channels that appeared to be normal at baseline. The apparent etiology is arrhythmia due to a secondary challenge that is tolerated by others, perhaps respiratory acidosis due to hypoventilation. Genetic modulation of risk on exposure to common, extrinsic factors is recognized in patients who develop drug-induced arrhythmia due to mutations in potassium channels that are revealed upon drug exposure (10, 11) and is consistent with a multi-hit pathogenesis for cardiac arrhythmia (23).

This study leads us to consider genetic screening in African Americans for SCN5A S1103Y in at least 3 situations: infants with acute life-threatening events, siblings of SIDS victims, and couples that experience infertility or fetal demise (42). Given the apparent increased risk, a comprehensive study of postmortem molecular analysis of approximately 2,000 SIDS cases (the number reported each year in the US) is recommended. If replicated, prospective screening in a large cohort of African American infants should be considered. Finally, it is imperative to continue to support effective public health efforts to decrease known environmental risk factors (e.g., prone sleeping position). SIDS is argued by this work to result, like many common disorders, from a genetic predisposition that yields poor tolerance of common challenges to physiological homeostasis.

**Methods**

**SIDS probands.** Approval for human study protocols was obtained from the Human Investigations Committee of Yale University. Postmortem tissue was collected from 224 unrelated SIDS cases from 1985 to 1992. Medical information was limited to sex, ethnicity, age at death, time of autopsy, and a brief history. Neither parental DNA nor family history were available. While cases were diagnosed as SIDS, it is not certain that all infants in our cohort died of SIDS because it is a diagnosis of exclusion with no definitive test. Apparently healthy adult African Americans served as controls; they were not screened for a family history of sudden death or SIDS.

**Genomic DNA preparation, amplification, and mutational analysis.** Genomic DNA was extracted from frozen heart, brain, or kidney blocks (~1 cm). Tissue was macerated on ice, and DNA was harvested by phenol/chloroform extraction, ethanol precipitation, and suspension in Tris-EDTA. The coding regions of SCN5A were amplified using primers and PCR conditions as shown in Supplemental Table 5. Denaturing HPLC heteroduplex analysis (WAVE; Transgenomic Corp.) was performed in the presence or absence of wild-type DNA, and variant samples were directly sequenced. For SCN5A S1103Y, all African American SIDS cases and controls were both screened by denaturing HPLC and directly sequenced. S1103Y was previously called S1102Y by Splawski et al. (22), whereas current mutation nomenclature for SCN5A now uses the long isoform NM_198056.1 (43).

**Electrophysiology.** Y1103 was introduced into SCN5A (NM_198056.1), the less abundant and longer isoform (43) carried in pcDNA1, using Pfu (Stratagene). Constructs were transiently cotransfected with GFP-tagged SCN1B (the β subunit of cardiac SCN5A channel, isoform b; NM_001037) in HEK-293 cells using Lipofectamine (Invitrogen Corp.). After 24–60 hours, whole-cell recording was performed using a 200B amplifier and pCLAMP software (version 8.0; Axon Instruments). Voltage errors were minimized using 80% series resistance compensation. Whole-cell recording was performed using internal (pipette) and bath solutions, as previously described (22): internal, 60 mM CsCl, 80 mM CsF, 10 mM EGTA, 1 mM CaCl2, 1 mM MgCl2, 5 mM Na2ATP, and 10 mM HEPES (pH adjusted to indicate levels with CsOH); bath, 130 mM NaCl, 5 mM CsCl, 2 mM CaCl2, 1.2 mM MgCl2, 10 mM HEPES, and 5 mM glucose (pH 7.4 with NaOH). Inside-out patch recording was performed using the same solutions, with the internal solution in the bath and the bath solution in the pipette. Mexiletine, propranolol, and amiadione were from Sigma-Aldrich. Stock solutions were made in bath solution (mexiletine) or dimethyl sulfoxide (propranolol and amiadione). Single channels were recorded in inside-out excised patches using borosilicate pipettes (Clark) coated with Sylgard (Dow Corning). All experiments were performed between 20–22°C.

**Statistics.** In addition to estimation of odds ratios and exact 95% confidence limits, P values were computed using Fisher’s exact test, with a
P value less than 0.05 being considered statistically significant. HWE predictions were reported as expected versus observed for SIDS cases and controls using EpInfo 2000 software (version 1.1.2; http://www.cdc.gov/epinfo/). Electrophysiology data are reported as mean ± SEM with ANOVA unless otherwise noted.

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