Supplement to:

A Common Cardiac Sodium Channel Variant Associated with Sudden Infant Death Syndrome in African Americans, *SCN5A* S1103Y

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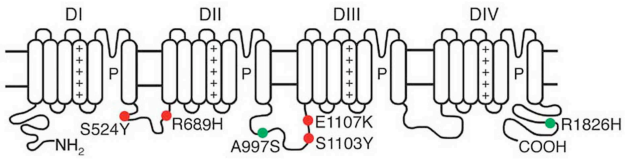
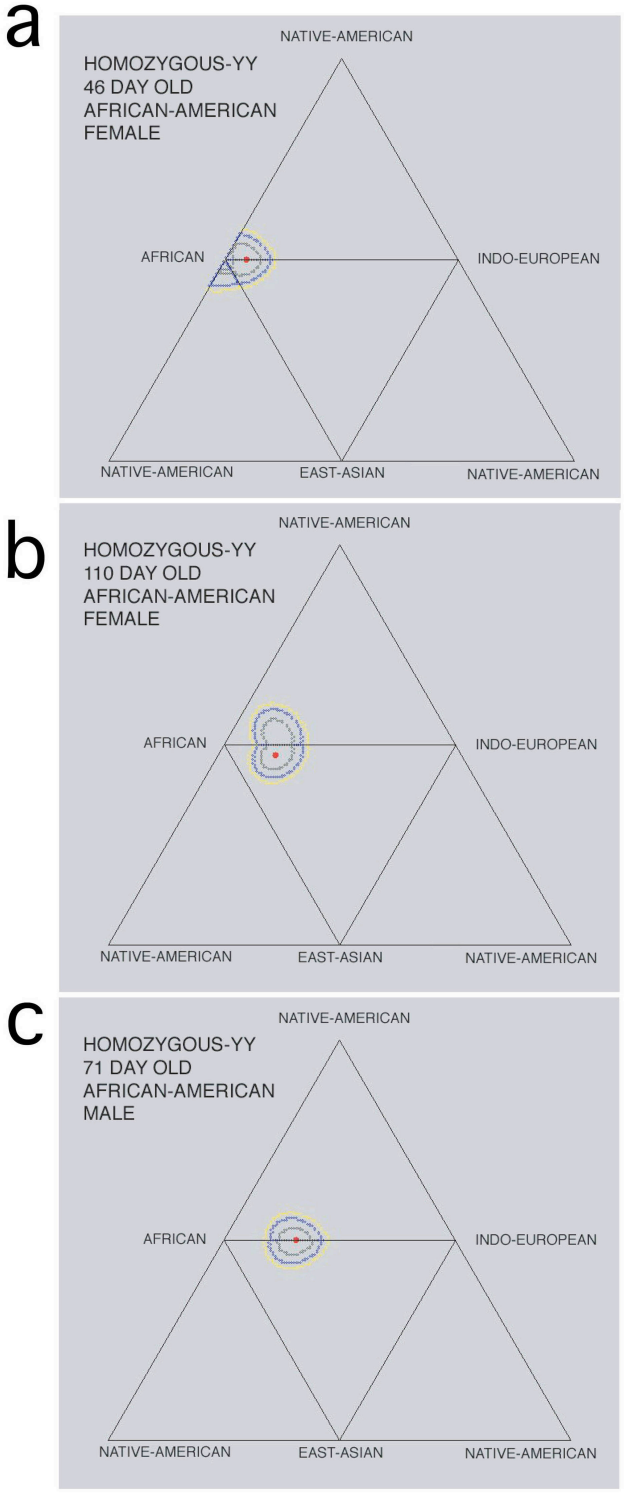
Included:

Supplement
Figure 1



Legend for Figure 1. Topology of SCN5A showing identified missense variants in African-American SIDS cases. The transmembrane domains (DI-DIV), pore-forming domains (P) and voltage sensor domains (+) are indicated as in Figure 1 in the accompanying paper. Identified missense variants from this study are labeled in red and those reported by Ackerman et al (*JAMA* **286**, 2264-9 (2001)) are noted in green. All SIDS missense variants identified in *SCN5A* are intracellular; S524Y and R689H are located in the linkers between the first and second domains and S1103Y and E1107K between the second and third domains.

Figure 2



Legend for Figure 2. Ancestry analysis of SIDS probands homozygous for *SCN5A* S1103Y. For each of the three homozygous African-American SIDS cases the triangle plot shows the maximum likelihood estimate (red dot) and the 2 fold, 5 fold and 10 fold likelihood confidence intervals in black, blue and yellow, respectively. See http://www.ancestrybydna.com for details.

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Table 1.
Ancestry analysis for SIDS probands with changes at *SCN5A* codon 1103

			Percent of Alleles by Ancestry			
S1103Y	**Ethnicity**	**Age (days) & Sex**	**African**	**Indo-European**	**East Asian**	**Native American**
YY	AA	46 F	91	9	0	0
YY	AA	110 F	75	20	4	1
YY	AA	71 M	69	31	0	0
SY*	Cauc	66 M	11	89	0	0
SY	AA	208 F	90	10	0	0
SY	AA	188 F	87	13	0	0
SY	AA	48 M	86	14	0	0
SY	AA	43 M	86	14	0	0
SY	AA	142 F	82	18	0	0
SY	AA	90 F	81	19	0	0
SY	AA	150 F	81	19	0	0
SY	AA	21 F	75	14	11	0
SY	AA	35 F	73	26	1	0
SY	AA	48 M	73	27	0	0
SY	AA	110 F	73	13	14	0
SY	AA	35 F	69	18	13	0
SY	AA	57 F	64	35	0	1

Legend. Ancestry analysis for SIDS probands with changes at *SCN5A* codon 1103 showing genotype, ethnicity reported by medical examiner, age at death in days, gender, and percent of African, Indo-European, East Asian and Native American ancestral alleles. Abbreviations: YY – homozygous Y1103; SY – heterozygous S1103/Y1103; AA – African-American; Cauc – Caucasian; M – male; F – female.

*One of 86 Caucasian SIDS cases in our study was SY heterozygous at position 1103, a genotype not seen in any of 511 healthy Caucasian adults in one study [1] or 200 in another [2]. Genetic analysis of ancestry confirmed that the SIDS case with SY genotype identified as Caucasian was of European descent, showing less than 11% sub-Saharan African ancestry. Others have reported SY genotype in 3 Caucasian siblings with syncope, ventricular arrhythmia or sudden death in the third to fifth decade of life and suggested heterozygosity to be a risk factor for arrhythmia [2].

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Table 2.

***SCN5A* variants in African-American SIDS probands at codon other than 1103**

Nucleotide Change	Amino Acid Change	Mutation Type	Frequency in African-Americans	
			Cases	Controls
C1571T	S524Y	Missense	2/133	1/150
G2066A	R689H	Missense	1/133	0/150
C3249T	S1083S	Silent	1/133	0/1056
G3319A	E1107K	Missense	1/133	0/1056
G3363A	A1121A	Silent	1/133	29/1056

Legend. Additional *SCN5A* variants in African-American SIDS probands showing nucleotide and amino acid changes (silent indicates no amino acid change) and frequency of heterozygous alleles in African-American SIDS cases and controls.

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Table 3.
Gating parameters for SCN5A S1103 (S) and Y1103 (Y) channels

pH_{out}	PH_{in}		G–V, $V_{1/2}$ mV	Inactivation $V_{1/2}$ mV	Recovery τ (ms) Fast	Slow	Current density pA/pF
7.4	**7.4**	**S**	–44.2 ± 1.2 (13)	–87.3 ± 2 (21)	12.7 ± 2	39.1 ± 3.3 (11)	840 ± 88 (46)
		Y	–43.6 ± 1.4 (4)	–87.7 ± 1.3 (4)	13.4 ± 3.8	42.6 ± 3.1 (11)	934 ± 110 (23)
7.4	**6.7**	**S**	–45.9 ± 1.3 (9)	–83.6 ± 1.3 (7)	12.8 ± 1.3	38.6 ± 2.8 (11)	852 ± 108 (12)
		Y	–46.6 ± 1.3 (7)	–77 ± 1.7 (8)*	19.8 ± 2.3	28.4 ± 1.6 (11)	964 ± 118 (31)
6.7	**7.4**	**S**	–45.2 ± 0.2 (3)	–78.7 ± 1.4 (3)	nd	nd	815 ± 94 (7)
		Y	–42.9 ± 0.9 (4)	–79.7 ± 0.9 (3)	nd	nd	861 ±155 (6)
7.0	**7.0**	**S**	nd	–82.5 ± 1.3 (12)	nd	nd	1024 ± 175 (10)
		Y	nd	–80.3 ± 1.0 (13)	nd	nd	1140 ±166 (7)
7.4	**7.0**	**S**	nd	–87.8 ± 1.2 (9)	nd	nd	978 ± 96 (12)
		Y	nd	–84 ± 2.7 (6)	nd	nd	1181 ± 110(13)

Legend. For conductance-voltage (G-V) analysis, a sodium reversal potential (E_K) of 67 mV was calculated and subtracted from the membrane potential to estimate driving force. Both G-V and steady-state inactivation curves were fitted to a Boltzmann relation. Recovery was determined by a fit to a two exponentials. Number in parenthesis indicates numbers of separate experiments. Values are mean ± S.E.M. **P* = 0.005, compared with S1103 by *two-population student t-test*; nd – not determined.

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Table 4.

Concentration of drug giving half-maximal block of S1103 (S) or Y1103 (Y) channels

Channel pH internal	Mexiletine		Propranolol		Amiodarone	
	tonic	phasic	tonic	phasic	tonic	phasic
S1103 pH_{in} = 7.4	45.4 ± 2.1	23.2 ± 1.1	3.0 ± 0.2	1.8 ± 0.2	3.6 ± 0.2	1.3 ± 0.1
Y1103 pH_{in} = 7.4	23.2 ± 2.2	13.7 ± 1.3	9.2 ± 0.7	2.3 ± 0.3	2.78 ± 0.4	1.22 ± 0.2
Y1103 pH_{in} = 6.7	26.4 ± 1.7	16.2 ± 1.1	6.9 ± 0.7	1.1 ± 0.1	3.9 ± 0.4	1.6 ± 0.2

Legend. The use-dependent block of sodium currents was assessed using a train of 10 ms depolarization pulses from –100 mV to –30 mV applied at 2.5 Hz (Methods and Materials). Only cells showing stable current magnitude for 3 min before drug application were studied. Tonic block refers to inhibition of the first pulse after drug exposure; phasic block assesses inhibition at equilibrium during a repetitive pulse protocol (here simulating a heart rate of 150 beats per minute). Half-maximum values were assessed by fitting the percentage block to the Hill equation with a coefficient of 1 (as this was close to the determined value in each case). Values are mean ± S.E.M. of 3-7 experiments.

Supplement Table 5. *SCN5A* Primers, Amplicon Size, PCR and dHPLC Conditions.
The amplicon, primers (5' to 3'), amplicon size and PCR buffer (Epicentre Technologies; Madison, WI), PCR annealing T ^{o}C and denaturation T ^{o}C for dHPLC analysis are shown.

Exon	Forward Primer / Reverse Primer (5'- 3')	Size (bp) / Buffer	Annealing temp (˚C)	dHPLC temp (˚C)
SCN5A-2	GGTCTGCCCACCCTGCTCTCT/ AATGGAGCAGAGGGGGAAGAGG	464 / D	55	60.5/61.5/62.5/64.5
SCN5A-3	AGTCCAAGGGCTCTGAGCCAA/ TTGCAGTTAATACCTGCTGAGTACC	228 / C	65	60.6/61.6
SCN5A-4	GGTAGCACTGTCCTGGCAGTGAT/ GAAGGGGGACTTGAGTCCAGG	293 / I	55	62/7/63.2
SCN5A-5	TCACTCCACGTAAGGAACCTG/ GCTTCCTCCCTGCAGTCCACAT	326 / I	55	60.7/61.7
SCN5A-6	TGGTGTGTTGTCATTGTCTCGTG/ CTGGAAAGGCCCAGGCATATCCCTCTAG	257 / C	58.7	59.1/61.1
SCN5A-7	CCACCTCTGGTTGCCTACACTG/ GAAGACTTTGTGAGACCGCAGAC	342 / D	58.7	63.4
SCN5A-8	CGAGTGCCCCTCACCAGCATG/ TTGTCCTGCCAGGGGAGTCTCC	151 / D	55	59.6/60.9/62.9
SCN5A-9	GGGAGACAAGTCCAGCCCAGCAA/ CAAGGGACAGCAAGTGTGGGCT	262 / C	59.3	63.6
SCN5A-10	TGGCACAACTAGACTAGGTGACTTG/ ACGCCCACAGGCACCAGCTTTAACTG	327 / I	58.7	61.2/61.7
SCN5A-11	AAACGTCCGTTCCTCCACTCT/ AATGGTAATCCCAGCTGTGGGTT	227 / C	58.7	61.6
SCN5A-12	GCCAGTGGCTCAAAAGACAGGCT/ TGCGCCGGACCAGTGCCCAGG	280 / D	55	62.3/64.3
SCN5A-13	CACCACACATCACTGCTGGTGC/ TCTCCCAAACTGATCAGCAGTTCC	286 / C	58.7	64.3.65.3
SCN5A-14	CAGGTCCAGCAGGACAGGAGCTG/ CTGCAGGTACCCTCATCTTTATC	283 / C	65	62.2/65.2
SCN5A-15	CAGGAAGGTATTCCAGTTACATATGA/ ACAGCTGGCACAGCTTCATGGGT	389 / C	55	61.8
SCN5A-16	CTGCCACAGCAAGAGTCAAGAGGCAG/ AGATTGGGTTGTGCCGAGCCTTCCAC	323 / D	58.7	60.3/60.8/61.3/62.8
SCN5A-17	CATGGGCAGCAGGAGCCAGAGCCCTTCAC/ GTGTGTGTGGCCCTTGGCCAAC	413 / C	58.7	61.4
SCN5A-18	TGGCATGGTGCAGTGCCTTGGTG/ GTGGGAGGCACCTTCTCCGTCTCTG	306 / I	65	62.9
SCN5A-19	TTCTGCTGTGGTCTCCTGCGGCAG/ CTCTGGGCCCTGGAGATGTAGGTG	283 / D	58.7	63.4/64.9
SCN5A-20	AGGGCCCTGGAGACCCTCTGGCTG/ CCAAGCAGCGGCGCCCAGCTGGCTTC	280 / I	55	63.8/64.8
SCN5A-21	TAGCCCTAAGCTCCTGCAGACTCTG/ TGGGCTCCAAGGAGCTGCTGGTCCTC	300 / I	58.7	63.4
SCN5A-22	CATTAGATGTGGGCATTCACAG/ CTCCAGCCGTCCCTGCCACAACCCTG	270 / I	55	62.9/63.9
SCN5A-23	GAATCGGCAGTGGTCCAGGCTTC/ CACCGGCAATGGGTTTCTCCTTC	276 / L	65	61.6/62.6
SCN5A-24	TAGTGGGACCAGAAGGCCTACTGTCTG/ TCCTGGGTGGCATGTGGCTGGGCCTG	279 / D	58.7	62.6/63.6
SCN5A-25	CTGGGCACCCCCAGGAACTGAGCCAC/ TGGGAGATGGCCATGCTGCTCAACAG	390 / I	65	61.2/71.7
SCN5A-26	CTCAAGCGAGGTACAGAATTAAATGA/ ATCAGTGTCTGCATCTGAAAGCCC	202 / C	65	58.4/60.4/62.4
SCN5A-27	CACAGAAATGGACACCCCTAGACAG/ GTGAGATGGGACCTGGAGCCTGAGTG	271 / C	65	58.4/60.4/62.4
SCN5A-28	CCATGCTGGGGCCTCTGAGAAC/ CACATGGCCAGCCATCAGAGCC	248 / D	58.7	60.9/61.4/61.9/63.4
SCN5A-29	CCCAGCGAGCACTTTCCATTTG/ TACAAGTCAGCTGGACGGAGAAGC	338 / C	55	59.5/60.5
SCN5A-30	TGCACAGTGATGCTGGCTGGAA/ CCAACAGCATGCTGTGCCTCTTC	369 / C	65	61.6/62.6
SCN5A-31	AAGTGGGAGGCTGGCATCGAC/ GCGTGGCCACGGAGGAGAGCAC	302 / D	65	62.4/64.4
SCN5A-32	GAGCCCAGCCGTGGGCATCCT/ CTGCCCATGGTGAGTGGGGAC	310 / D	58.7	60
SCN5A-33	CCAACCAGATAAGCCTCATCAACA/ TCCTCTTCCGTCAGCAGGCGG	309 / D	55	61.3/61.8/62.3
SCN5A-34	TGCTGCAACGCTCTTTGAAGCAT/ AGGACACACTGAAAAGCAGCCTTT	347 / D	65	62.8/63.3

Supplement Text on METHODS

SIDS cases. Postmortem solid organ tissue was collected from 224 unrelated cases of autopsy confirmed SIDS from 1985 to 1992 by the NIH-sponsored Brain and Tissue Banks for Developmental Disorders unit at the University of Maryland. Medical information was limited to sex, ethnicity, age at death, time of autopsy, and for most cases a brief clinical history. Both parental DNA and family history are unavailable. Investigators were blinded to the identities of the affected individuals and their families. This study was approved by the Human Investigations Committee of Yale University, HIC# 13174. Adult African-Americans were used as controls without specifically questioning for a family history of sudden cardiac death or SIDS and include 100 previously described samples from Coriel (Camden, NJ) [1].

Genomic DNA preparation and exon amplification. Genomic DNA was extracted from frozen heart, brain or kidney (~1 cm^3). Solid organ tissue was macerated on ice, resuspended (3 ml of 75mM NaCl and 24mM EDTA), subjected to alkaline lysis (300 μL of 5% SDS) and proteinase K digestion (100 μL of 10 mg/mL) at 37° C overnight. Genomic DNA was isolated by equal volume Tris saturated phenol-chloroform extraction, two chloroform-isoamyl alcohol (49:1) washes, precipitated with 1.5 mL 7.5M Na acetate and two volumes of 100% ethanol. Spooled DNA was washed twice in 70% ethanol and resuspended in 500 μL dHPLC grade water. The known coding regions of *SCN5A* (33 exons) were parsed into fragments ranging in size from 200-500 base pairs and PCR primers were designed to amplify these intervals. Primers and PCR conditions are shown in Supplemental Table 5. For the exon with codon 1103, PCR buffer solution I (Epicentre Technologies, Madison, WI) and an annealing temperature of 55˚C were used. Thermocycler conditions were an initial 5 minute denaturation at 94˚C, followed by 40 cycles of: 30 seconds denaturation at 96˚C, 30 seconds annealing at either 55˚C, 58.7˚C, 65˚C, 60 seconds extension at 72˚C and a single 10 minute terminal extension at 72˚C. PCR was completed with 50 ng DNA in a PCR volume of 30-50 μL.

Mutational analysis. Denaturing high-performance liquid chromatography (dHPLC) heteroduplex analysis (WAVE, Transgenomic Corp., Omaha, NE) was performed both in the presence and absence of confirmed wild-type control DNA. To identify homozygous variants, 30 uL of amplified proband DNA was mixed with 10 μL of confirmed wild-type control and redenatured prior to dHPLC; 8 μL was loaded onto a preheated cartridge (DNASep HT, Transgenomic Inc) and was eluted under partially denaturing conditions by an increasing acetonitrile gradient. For the amplicon containing codon 1103, mutation detection was conducted using a gradient of acetonitrile and a flow rate of 0.9 mL/min in rapid DNA mode

(WAVE 3500HT) at 63.8 ^{o}C and 64.8 ^{o}C for partial denaturation, as determined with WAVEmaker 4.0 software (Transgenomic Corp., Omaha, NE); this amplicon was reamplified from all cases for direct sequencing in both forward and reverse direction at Yale's W. M. Keck Laboratory using dye-terminator reactions on an Applied Biosystems 3700.

Electrophysiology. The Y1103 change was introduced to *SCN5A*-pcDNA1 plasmid using the *Pfu* quick change kit (Stratagene, La Jolla, CA). Wild type or mutant constructs were transiently co-transfected with GFP-tagged *SCN1B* (the β-subunit of cardiac SCN5A channel) in HEK-293 cells using lipofectamine (Invitrogen, Carlsbad, CA). Twenty-four to 60 hr after transfection, patch-clamp whole-cell recording was performed using an Axon 200-B amplifier (Axon Instruments, Claremont, CA) at filter and sampling frequencies of 5 kHz and 20 kHz, respectively. Voltage errors were minimized using 80% series resistance compensation. Internal (pipette) solution contained (in mM): CsCl 60, CsF 80, EGTA 10, $CaCl_2$ 1, $MgCl_2$ 1, Na_2ATP 5, HEPES 10 (pH as indicated with CsOH). The bath solution contained (in mM) NaCl 130, CsCl 5, $CaCl_2$ 2, $MgCl_2$ 1.2 HEPES 10, glucose 5 (pH 7.4 with NaOH), as used by others [1]. Mexiletine, propranolol and amiodarone were obtained from Sigma Chemical Company (St. Louis, MO). Stock solutions were made by dissolving the compounds in bath solution (mexiletine) or dimethyl sulfoxide (propranolol and amiodarone).

Single channels were recorded from inside-out membrane patches excised from HEK293 cells. Currents were stimulated every 2.5s by a 50 ms depolarizing pulse to –30mV from a holding potential of –120mV. Data were recorded with an Axopatch 200B amplifier and pCLAMP 8.0 software (Axon Instruments, Claremont, CA) at filter and sampling frequencies of 5 kHz and 50 kHz, respectively. Borosilicate pipettes (Clark, Kent UK) were coated with Sylgard (Dow Corning, MI) and filled with bath solution as described above. Cells were perfused with the pipette solution described above. For each cell null sweeps, with no channel activity, were identified offline averaged and subtracted away from data sweeps before analysis. For display purposes data were re-filtered offline using a 2 kHz Bessel filter (pCLAMP). All experiments were performed at between 20-22°C.

1. Splawski I, Timothy KW, Tateyama M, et al. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. Science 2002; 297:1333-6.
2. Chen S, Chung MK, Martin D, Rozich R, Tchou PJ, Wang Q. SNP S1103Y in the cardiac sodium channel gene SCN5A is associated with cardiac arrhythmias and sudden death in a white family. J Med Genet 2002; 39:913-5.