Overrepresentation of the proarrhythmic, sudden death predisposing sodium channel polymorphism S1103Y in a population-based cohort of African-American sudden infant death syndrome

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BACKGROUND The S1103Y-SCN5A polymorphism has been implicated as a proarrhythmic, sudden death predisposing risk factor in African Americans, including one postmortem investigation of African-American infants with sudden infant death syndrome (SIDS).

OBJECTIVE The purpose of this study was to assess whether the relatively African-American-specific common polymorphism S1103Y in the *SCN5A*-encoded cardiac sodium channel is overrepresented in SIDS among African Americans.

METHODS Seventy-one cases from a population-based cohort of unexplained infant deaths among African Americans (37 females and 34 males, average age 3 ± 2 months, age range birth to 11 months) were submitted to the Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory for postmortem genetic testing. Polymerase chain reaction and a restriction digest assay were performed to genotype this cohort for S1103Y.

RESULTS Targeted mutational analysis of exon 18 in *SCN5A* of the African-American SIDS cohort (n = 71) revealed the S1103Y polymorphism in 16 (22.5%) of 71 African-American cases of SIDS compared to 135 (11.6%) of 1,161 ostensibly healthy adult African Americans ($P = .01$).

CONCLUSION This study provides an independent assessment of the prevalence of S1103Y-SCN5A among African-American infants with sudden, unexpected, unexplained death prior to their first birthday. Further scrutiny and quantification of the risk apparently associated with S1103Y appear warranted.

KEYWORDS Cardiac sodium channel gene *SCN5A*; Long QT syndrome; Arrhythmia; Sudden infant death syndrome; Sudden death

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Introduction

Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant younger than 1 year that remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history.[1] Despite the success of the national "Back-to-Sleep" campaigns, SIDS claims the lives of more than 2,500 infants in the United States each year, remaining a leading cause of death in this vulnerable population.[2–5]

There is a significant disparity in SIDS rates among racial and ethnic groups. Specifically, rates are highest among African-American infants and more than two times greater than in white infants,[6] thus suggesting a potential role for ethnic-specific genetic predisposition. Although the pathophysiologic mechanisms underlying most of these tragic deaths remain elusive, heritable cardiac arrhythmia syndromes such as long QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia appear to account for 10% to 15% of SIDS cases, with the majority of SIDS-related mutations identified in the *SCN5A*-encoded cardiac sodium channel α subunit (Nav1.5) or its interacting proteins.[7–15]

In 2002, Splawski et al[16] reported on the common African-American–specific polymorphism S1103Y-SCN5A (previously denoted as S1102Y), with a prevalence of 13% among African Americans and associated with an increased risk for arrhythmia susceptibility, particularly in the context of other acquired risk factors such as medications, hypoka-

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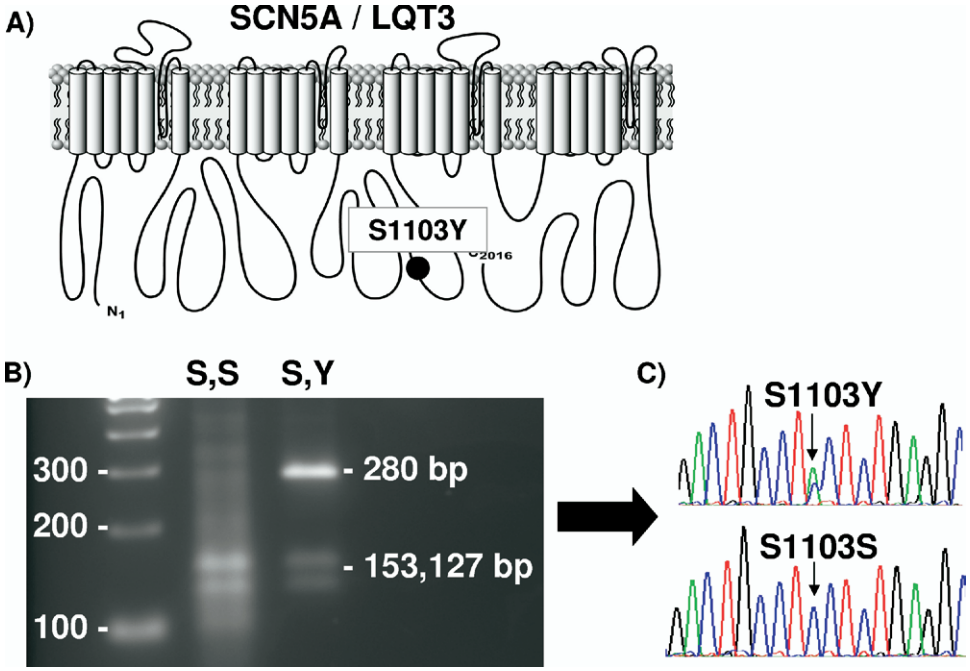

Figure 1 Molecular characterization of S1103Y polymorphism. **A:** S1103Y polymorphism within the SCN5A sodium channel. **B:** Restriction digest yielding the associated fragments and subsequent DNA sequencing **(C).**

lemia, and structural heart disease. Subsequently, Burke et al[17] observed an overrepresentation of S1103Y among African-American adolescents and adults whose deaths were classified as autopsy-negative sudden unexplained death.

Plant et al[18] reported an overrepresentation of S1103Y homozygotes (YY) in a large cohort of African-American SIDS cases (n = 133), suggesting a 24-fold risk for SIDS in infants who are homozygous for the Y1103-encoding allele (YY genotype). In addition, elegant heterologous expression of Y1103-containing sodium channels yielded significant late sodium current when environmentally stressed with acidosis, fulfilling the triple-risk hypothesis of SIDS consisting of a vulnerable host (S1103Y positive), an exogenous stressor (acidosis), and a critical development period.[18]

As is the case for any association study, population stratification can create false-positive associations, and Plant et al[18] stated unequivocally that their findings "need to be replicated in a separate SIDS cohort of African Americans." Here we assess the prevalence of S1103Y-SCN5A in a smaller African-American SIDS cohort.

Population-based cohort of SIDS

Between January 1994 and December 2000, 71 cases of unexplained sudden deaths among African-American infants (37 females and 34 males, average age 3 ± 2 months, age range birth to 11 months) derived from three population-based cohorts of SIDS were submitted to the Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory for postmortem genetic testing. To be considered SIDS, the death of the child younger than 1 year had to be sudden, unexpected, and unexplained following a comprehensive medicolegal autopsy.[1] Infants whose death was due to asphyxia or specific disease were excluded.

The study was approved by Mayo Clinic's Institutional Review Board as an anonymous study. As such, only limited medical information was available, including sex, ethnicity, and age at death. Time of day, medication use, and position at death were not available. By definition, the infant's medical history and family history were negative.

S1103Y-SCN5A genotyping

DNA was extracted from autopsy blood using the Puregene DNA Isolation kit (Gentra, Minneapolis, MN, USA) or from frozen necropsy tissue using the Qiagen DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA, USA). Genomic DNA derived from 100 healthy African-American subjects was obtained from the Human Genetic Cell Repository sponsored by the National Institute of General Medical Sciences and the Coriell Institute for Medical Research (Camden, NJ, USA) and served as ethnic-matched controls.

The S1103Y genotype was determined by polymerase chain reaction (PCR) amplification of SCN5A exon 18, restriction enzyme analysis, and gel electrophoresis. PCR reactions were performed in 20-μL volumes using 50 ng DNA, 16 pmol of each primer[19] (sense primer 5'AGGGTCTGAAACCCCCAGGGTCA3' and antisense primer 5'CCAGCTGGCTTCAGGGACAAA 3'), 200 μM of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.0 mM $MgCl_2$, and 1.0 U Amplitaq Gold (Applied Biosystems, Foster City, CA, USA). Restriction enzyme analysis was performed using 1 μL PCR product, 1 μL enzyme digestion buffer (10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM dithiothreitol [pH 7.9 at 25°C]), 2 units BseRI (New England Biolabs, Ipswich, MA, USA), and 7.5 μL deionized water. The reaction mixture was incubated at 37°C for 2 hours, followed by 65°C for 20 minutes. Digested samples were separated on a 3% agarose gel and visualized with an ultraviolet transilluminator (Figure 1). Direct sequencing of samples showing representative gel profiles confirmed the presence of S1103Y as described previously.[20]