A Common *SCN5A* Variant Is Associated with PR Interval and Atrial Fibrillation Among African Americans

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rs7629265 and AF Risk. ***Objective:*** **We examined the association of rs7626962 (S1103Y) or rs7629265, a variant in high linkage disequilibrium with S1103Y ($r^2 = 0.87 – 1$), with sudden cardiac death (SCD) and atrial fibrillation (AF) among African Americans.**

Background: **The *SCN5A* missense variant S1103Y has been associated with SCD among African Americans in small case-control studies, but larger population-based studies are needed to validate these findings. The association of this variant with AF has not been fully explored.**

Methods: **Using genotyping data on over 7,000 African Americans from 5 cohorts (Atherosclerosis Risk in Communities [ARIC], Cleveland Family Study [CFS], Jackson Heart Study [JHS], Multi-Ethnic Study of**

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Atherosclerosis [MESA], Cardiovascular Health Study [CHS]), we examined the association of rs7629265 with electrocardiographic PR, QRS, and QT intervals, and with incident AF and SCD. We examined association of S1103Y (rs7626962) with SCD using a population-based case-control study of SCD Cardiac Arrest Blood Study (CABS).

Results: **Meta-analyses across 5 cohorts demonstrated that rs7629265 was significantly associated with PR duration ($\beta = -4.1$ milliseconds; $P = 2.2\times10^{-6}$), but not significantly associated with QRS or QT intervals. In meta-analyses of prospectively followed ARIC and CHS participants (n = 3,656), rs7629265 was associated with increased AF risk (n = 299 AF cases; HR = 1.74, $P = 1.9 \times 10^{-4}$). By contrast, rs7629265 was not significantly associated with SCD risk in ARIC (n = 83 SCD cases; P = 0.30) or CHS (n = 54 SCD cases; P = 0.47). Similarly, S1103Y was not significantly associated with SCD risk in CABS (n = 225 SCD cases; P = 0.29).**

Conclusion: **The common *SCN5A* variant, rs7629265, is associated with increased AF risk and shorter PR interval among African Americans. In contrast to prior reports, we found no evidence of association of rs7629265 or rs7626962 (S1103Y) with SCD risk in the general population.** *(J Cardiovasc Electrophysiol, Vol. 25, pp. 1150-1157, November 2014)*

atrial fibrillation, electrocardiogram, genetics, PR interval, sudden death

Introduction

Sudden cardiac death (SCD) is a major public health concern, particularly among African Americans where risk of cardiac arrest is higher than in the general population, and survival is poor. Three small case-control studies have identified a missense variant of the *SCN5A* cardiac sodium channel gene (rs7626962, C->A, S1103Y), common among African Americans (allele frequency 6.8%) but absent among those of European descent, to be associated with arrhythmia susceptibility and SCD risk.[1-3] Splawski *et al.* found an 8-fold increase in risk of arrhythmias, syncope, and sudden death among 23 patients who were heterozygous or homozygous for the variant allele (referred to as S1102Y in their study).[1] Computational modeling suggested that Y1103 increases the likelihood of QT prolongation in the setting of drugs or hypokalemia that inhibit cardiac repolarization.[1] Whether the S1103Y variant is associated with SCD in the general population, or in the setting of specific risk factors, such as hypokalemia or diuretic medications, has not been validated in larger population-based studies. Furthermore, whether S1103Y is associated with risk of atrial fibrillation (AF), the most common sustained cardiac arrhythmia, has not been fully explored.

We, therefore, sought to investigate whether rs7629265 (C->T), an intronic variant in high linkage disequilibrium (LD), with S1103Y (rs7626962), is associated with risk of SCD and AF in the general population by examining 2 large African American cohorts followed prospectively. We further examined association of SCD directly with S1103Y (rs7626962) in a large population-based case-control study of the determinants of SCD. Using the National Heart Lung and Blood Institute's (NHLBI) Candidate-gene Association Resource (CARe) Consortium, we also examined the association of the intronic variant rs7629265 with electrocardiographic (ECG) parameters of PR, QRS and QT intervals–intermediary electrophysiologic measures that influence AF or SCD risk.

Methods

CARe Study Samples

Individuals of self-reported African American ancestry from 5 cohort studies (the Atherosclerosis Risk in Communities [ARIC] study, the Cleveland Family Study [CFS], the Cardiovascular Health Study [CHS], the Jackson Heart Study [JHS], and the Multi-Ethnic Study of Atherosclerosis [MESA] study) were genotyped as part of the CARe Consortium. Descriptions of these cohorts are detailed in the supplemental section and have been published previously.[4] The institutional review board at all participating institutions approved the study. Only individuals who provided informed consent to genetic testing were included.

CARe Genotyping and Quality Control

DNA samples and phenotype information from the CARe cohorts were shipped to the Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, and harmonized across studies (http://www.cdisc.org/models/suds/v3.1/index.html). Genotype quality control procedures were performed separately for each cohort. We used a cardiovascular 50K single nucleotide polymorphism (SNP) IBC array (ITMAT, Broad, and CARe) to type samples at the Broad Institute (MIT, Cambridge, MA, USA). While the rs7626962 (S1103Y) variant was not directly measured in CARe, a surrogate, rs7620265, which is in strong LD with rs7626962, was typed.

TaqMan Genotyping of rs7626962 (S1103Y) in CHS

In CHS, in addition to genotyping with the IBC array described above, direct genotyping of rs7626962 (S1103Y) was performed after PCR amplification using a high-throughput TaqMan assay (Applied Biosystems, Foster City, CA, USA). The PCR product was sequenced using big dye terminator chemistry and an ABI310 DNA sequencer.

CARe ECG Recordings and Phenotype Definition

Twelve-lead ECGs with standard lead placements were recorded during 10 seconds in all cohorts at baseline using Marquette MAC PC, MAC6 or MAC1200 machines (GE Healthcare, Chalfont St. Giles, UK). For our ECG parameters analysis, PR, QRS, and QT intervals were measured electronically using either the Marquette 12SL algorithm or the MC MEANS algorithm. We excluded from all ECG analyses individuals with missing covariates, or who were <18 years of age, or pregnant.

For the PR interval analysis, participants were excluded for prevalent AF on ECG; pacemaker in place; ventricular pre-excitation; second or third degree heart block; history of myocardial infarction or heart failure; and for extreme PR trait values of ≤80 milliseconds or ≥320 milliseconds. Consistent with prior studies assessing common genetic variation (9; 20), PR duration cutoffs were chosen to exclude extreme values. For the QRS analysis, participants were excluded for prevalent AF on ECG; pacemaker in place; ventricular preexcitation; second or third degree heart block; history of myocardial infarction or heart failure; and QRS ≥120 milliseconds. For the QT analysis, participants were excluded for prevalent AF on ECG; pacemaker in place; QRS ≥120 milliseconds. A clinical definition of hypokalemia was used (K^+ level < 3.7 milliequivalents per liter [mEq/L]), obtained on baseline blood draw. Diuretic use was defined by use of loop (furosemide) or thiazide (hydrochlorothiazide) diuretic at baseline evaluation.

ARIC and CHS Event Ascertainment: AF

Two of the 5 cohorts (ARIC and CHS) have systematically ascertained AF and SCD events, and were included in the analyses of these phenotypes. AF in both cohorts was limited to incident cases. In ARIC, AF was ascertained from 3 different sources: ECGs, hospitalizations, and death certificates. Study participants underwent 12-lead ECG at baseline and at each follow-up exam (3 exams; 1 exam every 3 years). All ECG recordings coded as AF by machine recording were visually rechecked by a cardiologist to confirm the diagnosis. Trained abstractors obtained and recorded all ICD-9 hospital discharge diagnoses from a participant's hospitalizations reported in annual follow-up interviews and from surveillance of local hospitals. AF was defined as the presence of ICD-9 code 427.31 or 427.32 in the discharge codes or at clinic follow-up visits. In addition, ARIC participants were classified as AF cases if AF was listed as one of the diagnoses in the death certificate.[5] AF has been reported to be accurately ascertained from ICD-9 codes and death certificates with high validity.[6] In CHS, the diagnosis of AF was made if AF or atrial flutter was present on an annual clinic visit ECG between 1989 and 1999, or when a hospital discharge ICD-9 code for AF or atrial flutter was present. The date of incident AF was taken as the earlier of the date of the clinic visit for AF identified on clinic ECG, or the date of hospital admission for AF identified from hospital discharge diagnosis. In both CHS and ARIC, AF that occurred during the same hospital stay as coronary artery bypass surgery or valve surgery was not included as an AF event.

ARIC and CHS Event Ascertainment: SCD

Both ARIC and CHS classified all cases of fatal cardiovascular death according to standard protocols, and causes of death were adjudicated by respective ARIC and CHS events committees. To identify cases of SCD in ARIC and CHS for this study, all cases of fatal cardiovascular death that occurred by July 31, 2006, in CHS and by December 31, 2001, in ARIC were reviewed and adjudicated by physicians. SCD was similarly defined in both ARIC and CHS as a sudden pulseless condition presumed due to a ventricular tachyarrhythmia in a previously stable individual without evidence of a noncardiac cause of cardiac arrest. We *a priori* sought to exclude cases with nonarrhythmic characteristics, including those with evidence of progressive hypotension or advanced decompensated congestive heart failure before death. All SCD events in this analysis occurred out of the hospital or in the emergency room. Available data from death certificates, informant interviews, physician questionnaires, coroner reports, and hospital discharge summaries were reviewed, in addition to circumstances surrounding the event, to help classify whether the subject had experienced SCD. For unwitnessed deaths, the participant must have been seen within 24 hours of the arrest in a stable condition and without evidence of a noncardiac cause of cardiac arrest.

In ARIC, each event was adjudicated independently by 2 investigators, and classified as "definite sudden arrhythmic death," "possible sudden arrhythmic death," "definite nonsudden death," or "unclassifiable." If disagreement existed between the first 2 reviewers, a third investigator independently reviewed the event to provide final classification. In CHS, each event was adjudicated by a cardiologist's record review, and classified as "definite SCD," "probable SCD," and "not SCD or unclassifiable." A blinded second physician review of a random sample of 70 of these death records showed an 88% interreviewer agreement and $\kappa = 0.74$ for SCD. Both of these physicians also participated on the ARIC SCD review panel, to ensure consistency of phenotype across studies.

For this analysis, SCD was defined as deaths that were definite or probable/possible sudden deaths. Participants were censored at time of loss to follow-up or death if the cause of death was other than SCD. The administrative censoring date was July 31, 2006, for CHS and December 31, 2001, for ARIC, based on the study's adjudication schedules.

Cardiac Arrest Blood Study (CABS)—Population

We examined a third population-based study of SCD risk determinants in order to directly examine the association of rs7626962 (S1103Y) with SCD incidence. Cases were selected from the CABS Repository, a large population-based repository of data and specimens from adult out-of-hospital cardiac arrest patients who were attended by paramedics in Seattle and King County, Washington. SCD was defined as a sudden pulseless condition in an otherwise stable person in the absence of a noncardiac cause of arrest. The records of 6,003 persons identified by paramedics to suffer cardiac arrest were reviewed and classified as definite, probable, possible, or non-SCD based on initial rhythm (e.g., VF vs. asystole vs. pulseless electrical activity), circumstances (e.g., witnessed vs. unwitnessed), and possible contribution of comorbidities to the event. We excluded nursing home residents to avoid misclassification as to the cause of death. For the current analysis, we restricted our case population to those of African descent with a cardiac arrest classified as definite or probable SCD. We excluded individuals with a presenting rhythm of pulseless electrical activity as these cases often have a different underlying pathophysiology than those with VF. We restricted the analysis to African Americans using the following 2-step algorithm. First, only cases and controls were genotyped that were identified by paramedics (cases) as "African American/Black" or self-identified (controls) as "African American/Black." Second, we excluded 1 ethnic outlier using principal components analysis.

We identified 225 SCD cases between the years of 1988 and 2007 that met these criteria. Controls (n = 198)

were identified from 2 sources: (1) random digit dialing in the community and (2) random selection from enrollees of Group Health, a large health maintenance organization in Western Washington State. The combined controls were frequency-matched to cases on age and gender. The Human Subject Review Committees of the University of Washington and Group Health Cooperative approved the study.

CABS—Genotyping

Genotyping was performed using Affymetrix Axiom panel. Exclusion criteria at the sample level were call rates <90%, gender mismatches or non-African descent by ancestry informative markers. Exclusion criteria at the SNP level were call rate <95%, out of Hardy-Weinberg equilibrium ($P < 0.01$). The rs7626962 (S1103Y) was directly genotyped on the Axiom panel.

Statistical Analysis—ECG Phenotypes

Hardy-Weinberg equilibrium was assessed by chi-squared test. For continuous ECG phenotypes, linear regression using an additive model was performed in each cohort, adjusted for age, gender, ancestry using 10 principal components, and study site. Additional adjustments for the PR and QRS analyses included body mass index, height, and systolic blood pressure. Consistent with prior studies evaluating these ECG variables, QT and PR analyses were also adjusted for RR interval.[7-10] The JHS contains a family-based subcohort. We accounted for family structure using mixed linear models with a random effect term for pedigree. Cohort-specific results were combined using fixed effects meta-analyses with inverse variance weights.

Statistical Analysis—AF and SCD

For the analyses of SCD and AF, participants with missing covariate or genotype data at baseline were excluded. For analyses of incident AF, participants with prevalent AF at baseline (7 from ARIC and 12 from CHS) were excluded. Loss to follow-up was a censoring event. Because few study participants were homozygous for the minor allele, we used a dominant model for the outcomes of AF and SCD. Cox proportional hazards regression using a dominant model was employed, adjusting for age, gender, ancestry using 10 principal components, and study site in each cohort. Sensitivity analyses were performed using an additive model. For the incident AF analysis, the outcome was time to AF (or censor) from baseline. For the SCD analysis, the outcome was time to SCD (or censor) from baseline. We used a fixed effects meta-analytic approach with inverse variance weights to combine the regression parameter estimates from CHS and ARIC.

For the case-control analyses of SCD in the CABS study, associations of genotype with SCD risk were assessed using logistic regression with robust or "sandwich" standard errors to obtain odds ratios and their 95% confidence intervals (CI). These regressions were adjusted for age, gender, and 10 principal components derived from ancestry informative markers to control for potential residual population stratification.

Significance and Secondary Analyses

A P value of less than $0.01 = 0.05/5$ (for the number of outcomes examined in this manuscript) was deemed statistically significant for all primary analyses (ECG phenotypes, AF, and SCD). In exploratory secondary analyses, we examined whether these associations were modified by diuretic use (loop or thiazide diuretics), hypokalemia, or gender in ARIC and CHS. We tested for interaction of the genotype–phenotype association by diuretic use (diuretic users vs. nonusers), hypokalemia ($K^+ < 3.7$ mEq/L) versus normokalemia, or gender, using multiplicative models.

Results

Baseline characteristics by cohort are shown in Table 1. The mean age of the study participants across the 5 cohorts examined ranged from 37 to 73 years. Overall, approximately 40% of the participants were male. Minor allele frequencies (MAF) for rs7629265 were similar across cohorts, and there was no decline in allele frequency in older cohorts. The variant examined in the CARe study participants, rs7629265, was in high LD ($r^2 = 0.88$) with S1103Y, rs7626962, in CHS where both variants were directly genotyped. The average age of CABS study participants was 62 years and 38% were male. In CABS, MAF of S1103Y was 0.072.

Association of rs7629265 with ECG Parameters

Approximately 7,000 study participants were examined for each ECG phenotype at baseline across the 5 cohorts, after exclusion criteria were applied. rs7629265 was significantly associated with shortening of the PR interval by 4.1 milliseconds for each copy of the minor (T) allele (Table 2; 95% CI = −5.9 to −2.3 milliseconds; meta-analysis $P = 2.2 \times 10^{-6}$). Note that rs7629265 was nominally associated with QRS shortening ($\beta = -0.7$ milliseconds; 95% CI = −1.3 to −0.1 milliseconds; meta-analysis $P = 0.021$) and QT lengthening ($\beta = 1.6$ milliseconds; 95% CI = 0.2–3.0 milliseconds; meta-analysis $P = 0.019$), Table 2, but these associations were not significant after adjustment for multiple testing.

Association of rs7629265 with Incident AF

Among 2,866 ARIC and 790 CHS participants, 169 (5.9%) and 130 (16.5%) incident AF cases were identified during follow-up, respectively. The higher incidence of AF in CHS compared with ARIC is consistent with the older age of the CHS cohort participants. In meta-analyses, ARIC and CHS participants heterozygous or homozygous for the rs7629265 variant (T) allele had a significantly higher risk of AF (meta-analysis HR = 1.74; 95% CI = 1.30–2.33; $P = 1.9 \times 10^{-4}$; Table 3) than those homozygous for the C allele. In sensitivity analyses, results for the additive model minimally differed from those for the dominant model, due to the very few individuals homozygous for the variant allele.

Association with SCD

During follow-up, 83 (2.9%) and 54 (6.8%) SCD cases were identified in ARIC and CHS, respectively. There was no evidence of association of the rs7629265 variant allele with SCD risk in these 2 large African American cohorts followed prospectively ($P > 0.30$; Table 4). To further examine association with SCD risk among African Americans, we examined a large population-based case-control study (n = 225 cases; n = 198 controls) where rs7626962 (S1103Y)

TABLE 1
Baseline Characteristics of African Americans by Cohort

	ARIC	CFS	CHS	JHS	MESA
Total number	2,874	632	822	2,232	1,754
Age in years (SD)	53 (6)	37 (19)	73 (6)	50 (12)	62 (10)
Male (%)	37	43	37	39	45
MAF of rs7629265	0.074	0.064	0.076	0.076	0.070
PR in milliseconds (SD)	171.8 (28)	169.0 (26)	170.5 (30)	170.6 (26)	170.8 (26)
QRS in milliseconds (SD)	90.0 (10)	89.4 (10)	87.5 (11)	91.9 (10)	91 (10)
QT in milliseconds (SD)	400.2 (31)	397.5 (30)	408.4 (35)	413.0 (31)	410.3 (32)

SD = standard deviation; MAF = minor allele frequency; SCD = sudden cardiac death.

TABLE 2
Association of rs7629265 and ECG Parameters by Cohort

	ARIC	CFS	CHS	JHS	MESA	Meta	Meta-Analysis P Value
Number of participants in PR analysis	2,146	264	612	1,985	1,549	6,556	2.2×10^{-6}
β PR in milliseconds (SE)	−1.6 (1.5)	−9.9 (5.0)	−1.8 (3.2)	−5.9 (1.5)	−4.7 (1.8)	−4.1 (0.9)	
Number of participants in QRS analysis	2,487	360	613	1,912	1,655	7,027	0.021
β QRS in milliseconds (SE)	−0.4 (0.5)	−0.5 (1.4)	−1.1 (1.1)	−0.6 (0.6)	−1.4 (0.7)	−0.7 (0.3)	
Number of participants in QT analysis	1947	340	601	2021	1527	6436	0.019
β QT in milliseconds (SE)	0.4 (1.3)	3.3 (2.7)	2.6 (2.5)	−0.05 (1.2)	4.0 (1.3)	1.6 (0.7)	

SE = standard error. Coded allele (T); referent allele (C).

TABLE 3
Association of rs7629265 with Incident Atrial Fibrillation

Cohort	rs7629265 CC AF/ Total	rs7629265 CT or TT AF/Total	HR (95% CI)	P Value
CHS	108/689	22/101	1.65 (1.03 – 2.64)	0.038
ARIC	133/2455	36/411	1.80 (1.24 – 2.61)	0.002
Meta-analysis	241/3144	58/512	1.74 (1.30 – 2.33)	1.9×10^{-4}

AF = atrial fibrillation.

was directly genotyped, and similarly found no evidence of an association with SCD risk (P = 0.29; Table 4).

Secondary Analyses

In secondary analyses in ARIC and CHS, where information on drug use and potassium levels was available, there was a suggestion that the rs7629265 variant allele was associated with increased risk of SCD among diuretic users (n = 1,035 total, 42 SCD cases; HR = 2.05; 95% CI = 0.95–4.47; P = 0.07), and a decrease in risk of SCD among diuretic nonusers (n = 2,604 total, 95 SCD cases; HR = 0.33; 95% CI = 0.13–0.81; P = 0.02), meta-analysis interaction P = 0.006. A similar difference in risk was not seen among those with and without hypokalemia (K+ < 3.7 mEq/L) at baseline. Furthermore, there was no interaction of diuretic use or hypokalemia on the outcomes of AF, or ECG parameters of PR interval duration, QRS duration, or QT interval. There was also no evidence of an interaction with gender.

In CHS, where both variants (rs7626962, S1103Y, and rs7629265) were directly genotyped, findings for all ECG parameters (PR, QRS, QT intervals) and outcomes (AF and SCD) examined were similar for both variants, as would be expected by their high LD.

Discussion

Examining several large population-based cohorts, we found that a common cardiac sodium channel gene (*SCN5A*) variant, rs7629265, is associated with a 74% increase in risk of AF and shortening of the PR interval among African Americans. In contrast to prior reports examining S1103Y,[1-3] the 2 variants examined in this study, rs7629265 and rs7626962 (S1103Y), were not associated with risk of SCD among African Americans in 3 large population-based studies of SCD. Our findings lend support to the contribution of genetic factors to AF among African Americans, and, importantly, temper prior reports suggesting a strong association of S1103Y with SCD.

AF, the most common sustained cardiac arrhythmia, increases risk of stroke and overall mortality. A number of tools for prediction of AF have been developed, primarily among those of European descent,[11-13] which to date do not include genetic markers. It is noteworthy that a 74% increase in risk is one of the largest common single risk markers, with its effect estimate being greater than or comparable to that of tobacco use, prior myocardial infarction, hypertension, and diabetes.[14] It remains to be investigated whether including this genetic marker in risk models may help better identify African Americans at high risk of AF and could lead to preventive measures that decrease the health-related and financial burden of AF.[15]

While the burden of risk factors associated with AF, such as diabetes and hypertension, is higher among African Americans than among those of European descent, the prevalence of AF is paradoxically lower among African Americans.[16-18] Our finding of a variant found commonly among African Americans and absent among European Americans that increases risk of AF, adds to this paradox, and supports the hypothesis that African Americans may have different underlying mechanisms for arrhythmogenesis than those of European ancestry.[19]

TABLE 4
Association of rs7629265 and rs7626962 (S1103Y) with Sudden Cardiac Death

Cohort Study	rs7629265 CC SCD/Total	rs7629265 CT or TT SCD/Total	HR (95% CI)	P Value
CHS	49/698	5/104	0.71 (0.28 – 1.79)	0.47
ARIC	74/2,433	9/407	0.69 (0.34 – 1.40)	0.30
Case-control Study	**rs7626962 S1103/S1103 cases/controls**	**rs7626962 Y1103/S1103 or Y1103/Y1103 cases/controls**	**OR (95% CI)**	**P Value**
CABS	190/175	35/23	1.37 (0.77 – 2.43)	0.29

SCD = sudden cardiac death.

Our findings of an association of this variant with shortened PR interval and increased risk of AF is consistent with prior reports examining the effect of variation in the *SCN5A* gene on atrial conduction and arrhythmias.[8-10,20] Investigating the physiologic foundations for this discordant PR-AF relationship could be particularly informative in elucidating the mechanisms by which *SCN5A* influences atrioventricular conduction and atrial arrhythmia risk.

Importantly, despite initial small case-control studies that implicated S1103Y with ventricular arrhythmias and SCD,[1-3] our findings from 3 large population-based studies (n = 362 SCD total cases) did not reveal an association of SCD with either rs7629265 or rs7626962 (S1103Y), highlighting the importance of validating findings in larger and prospective studies. Our discrepant results with prior reports may be due to the differing populations and outcomes examined. While Splawski examined 23 cases with arrhythmia-related phenotypes and found an 8-fold increase risk with the S1103Y variant, only 10 of those cases were adults.[1] Whereas we examined SCD among adults in the general population, most of the 10 adults examined in the Splawski study had significant cardiomyopathy and were taking a repolarization influencing medication such as amiodarone. Furthermore, the case phenotype definition in the Splawski report included syncope and QT prolongation, and not just SCD, as outcomes.[1] A report by Sun *et al.* examined African American patients with heart failure with defibrillator implantation, and found that the S1103Y variant was more common among the 23 cases who received an appropriate defibrillatory shock therapy compared with the 89 controls who did not (35% vs. 13%, respectively; P = 0.03), but there was no association with mortality.[3] In contrast to these 2 reports examining a small number of cases with structural heart disease, a larger study by Burke *et al.* found no association between the S1103Y variant and SCD risk among 117 cases with structural heart disease compared with 107 non-SCD controls, but did find increased risk in subgroups without any heart disease on autopsy examination (n = 25), or with only mild cardiomegaly (n = 40).[2]

Ours is the largest study to examine SCD outcomes among African Americans in the general population (n = 392 SCD cases across 3 studies). The 3 studies examined in this report were population based: 2 (CHS and ARIC) were cohorts followed prospectively and one (CABS) was a population-based case-control study of SCD. Furthermore, using ARIC and CHS African American participants, we examined the conclusions by Splawski *et al.* that the Y1103 allele may be associated with ventricular arrhythmias in the setting of additional acquired risk factors, such as medications or hypokalemia.[1] Whereas there was no overall association with SCD in our study, in secondary analyses, we did find an interaction between the rs7629265 allele and use of diuretic medications on the outcome of sudden death. This finding, although intriguing, needs to be replicated. Taken together, these findings do not provide compelling evidence for a large effect of S1103Y (rs7626962) or rs7629265 on SCD risk in the general population. Whether the S1103Y variant is associated with more modest risk of SCD, and in what clinical populations, deserves further investigation.

The mechanism by which either of the 2 *SCN5A* variants examined in our study, rs7629265 and rs7626962 (S1103Y), influence PR interval or AF incidence is unknown. *SCN5A* encodes the α-subunit of the voltage-gated cardiac sodium channel responsible for initiating the cardiac action potential.[21] Common and rare variation in this gene influences cardiac conduction, repolarization, and arrhythmia risk.[22-25] The 2 variants examined in our study, rs7629265 and rs7626962 (S1103Y), 28 kb apart, are in complete LD ($r^2 = 1.0$) among African Americans in HapMap3 (American South Western population) and in strong LD in CHS African Americans ($r^2 = 0.88$), where both variants were directly genotyped. No other SNP in HapMap3 or in 1,000 genomes is in moderate or high LD ($r^2 > 0.35$) with S1103Y (rs7626962). Which of these 2 variants is likely to be the functional allele in relation to PR interval and AF is unknown, although there is functional support for the missense SNP S1103Y (rs7626962). Electrophysiologic studies have shown that the S1103Y variant undergoes minimal kinetic shifts at baseline, but when exposed to other factors, such as cellular acidosis, late I_{Na} current is increased.[26] By contrast, rs7629265 is an SNP in intron 8, and examination of the RegulomeDB database, which catalogues annotations from the Encyclopedia of DNA Elements (ENCODE) project, provides no compelling evidence for a functional regulatory role for rs7629265.[27] Additional functional studies are needed to further elucidate the mechanism of action of the functional variant.

Several limitations should be considered. First, AF cases were captured through annual ECGs and medical records. Asymptomatic paroxysmal AF would have been missed by these surveillance methods. Moreover, although ours is the largest study of SCD among African Americans, we were underpowered to identify modest associations. Larger studies are needed, with a particular focus on interactions with medications.

In conclusion, we found a 74% increase in risk of AF with an intronic variant in *SCN5A*, rs7629265, which is in strong LD with a missense variant (S1103Y, rs7626962) found commonly among African Americans. Including this genetic factor in risk models of AF among African Americans may help better identify those at elevated risk for developing AF and could lead to preventive measures that decrease the health-related and financial burden of this disease. Larger scale, prospective studies are needed to address these questions. Importantly, in contrast to previous small case-control studies examining S1103Y, we also found no strong association between rs7629265 or rs7626962 (S1103Y) with SCD risk.[1] Taken together, our findings underscore the importance of prospective replication, and evaluation of potential genetic markers of arrhythmia susceptibility in larger studies to better understand and refine their role in arrhythmia risk prediction.

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Appendix

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