Genetic Variations in Nitric Oxide Synthase 1 Adaptor Protein Are Associated With Sudden Cardiac Death in US White Community-Based Populations

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Background—The ECG QT interval is associated with risk of sudden cardiac death (SCD). A previous genome-wide association study demonstrated that allelic variants (rs10494366 and rs4657139) in the nitric oxide synthase 1 adaptor protein (NOS1AP), which encodes a carboxy-terminal PDZ ligand of neuronal nitric oxide synthase, are associated with the QT interval in white adults. The present analysis was conducted to validate the association between NOS1AP variants and the QT interval and to examine the association with SCD in a combined population of 19 295 black and white adults from the Atherosclerosis Risk In Communities Study and the Cardiovascular Health Study.

Methods and Results—We examined 19 tagging single-nucleotide polymorphisms in the genomic blocks containing rs10494366 and rs4657139 in NOS1AP. SCD was defined as a sudden pulseless condition of cardiac origin in a previously stable individual. General linear models and Cox proportional hazards regression models were used. Multiple single-nucleotide polymorphisms in NOS1AP, including rs10494366, rs4657139, and rs16847548, were significantly associated with adjusted QT interval in whites (P<0.0001). In whites, after adjustment for age, sex, and study, the relative hazard of SCD associated with each C allele at rs16847548 was 1.31 (95% confidence interval 1.10 to 1.56, P=0.002), assuming an additive model. In addition, a downstream neighboring single-nucleotide polymorphism, rs12567209, which was not correlated with rs16847548 or QT interval, was also independently associated with SCD in whites (relative hazard 0.57, 95% confidence interval 0.39 to 0.83, P=0.003). Adjustment for QT interval and coronary heart disease risk factors attenuated but did not eliminate the association between rs16847548 and SCD, and such adjustment had no effect on the association between rs12567209 and SCD. No significant associations between tagging single-nucleotide polymorphisms in NOS1AP and either QT interval or SCD were observed in blacks.

Conclusions—In a combined analysis of 2 population-based prospective cohort studies, sequence variations in NOS1AP were associated with baseline QT interval and the risk of SCD in white US adults. (Circulation. 2009;119:940-951.)

Key Words: death, sudden ▪ arrhythmia ▪ genetics ▪ epidemiology

Sudden cardiac death (SCD) and cardiac arrhythmias remain a daunting public health problem. It is estimated that between 250 000 and 400 000 SCDs occur in the United States each year.1,2 Nearly half of all coronary heart disease (CHD) deaths are sudden, and approximately one third of these deaths are the first clinical manifestation of disease.3 Thus, it is important to identify risk factors, both genetic and environmental, for SCD.

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Previous studies have identified family history of SCD as a powerful risk factor for SCD that is independent of traditional risk factors.
risk factors for CHD or a family history of myocardial infarction (MI). Moreover, a number of genes have been linked to rare heritable arrhythmias that predispose to SCD. However, the genetic factors underlying SCD in the general population are largely unknown.

SCD is a complex phenotype of heterogeneous origin with multiple factors contributing to risk that can be broadly classified into 3 categories: (1) atherosclerosis and thrombosis; (2) electogenesis and propagation; and (3) initiating influences and triggers. Indeed, SCD is a multifactorial disorder that involves the interaction of multiple genes in conjunction with environmental influences. A number of pathways modulate the electrophysiology of the heart and have been associated with enhanced risk of SCD. Altered ventricular repolarization reflected in abnormalities of the ECG QT interval is an intermediate phenotype that is not only associated with an increased risk of SCD in both the presence and absence of structural heart disease but also is heritable within families and in population-based studies. Using a genome-wide association study, we have previously demonstrated that allelic variants in NOS1AP (nitric oxide synthase 1 adaptor protein), which encodes a cytosolic ligand of neuronal nitric oxide synthase, are associated with altered QT intervals in white adults. This association has subsequently been replicated in additional white populations. The objectives of the present study were to validate the association of NOS1AP variants with QT-interval prolongation in a large US population–based cohort of white and black adults and, more importantly, to establish the association between NOS1AP variants and the risk of SCD in these community-based individuals. We hypothesized that at least 1 of the NOS1AP single-nucleotide polymorphisms (SNPs) examined would be associated with the QT interval; moreover, at least 1 such SNP would also be associated with the risk of SCD.

Methods

The Atherosclerosis Risk In Communities Study and the Cardiovascular Health Study

The Atherosclerosis Risk In Communities (ARIC) study and the Cardiovascular Health Study (CHS) are both population-based prospective cohort studies of cardiovascular disease. The ARIC Study includes 15,792 persons 45 to 64 years old at baseline (1987–1989), randomly chosen from 4 US communities. ARIC cohort members completed 4 clinic examinations, conducted approximately 3 years apart between 1987 and 1998. CHS includes 5888 participants ≥65 years of age identified from 4 US communities by use of Medicare eligibility lists. The original cohort included 5201 participants recruited in 1989 to 1990, and 687 additional subjects were recruited in 1992 to 1993 to enhance the racial/ethnic diversity of the cohort.

Clinic examinations for both ARIC and CHS participants included assessment of cardiovascular risk factors, self-reported medical family history, employment and educational status, diet, physical activity, comorbidities, and clinical and laboratory measurements. ARIC and CHS participants were contacted annually by telephone for identification of all hospitalizations and deaths, and lists of discharges from local hospitals were scanned for events. Deaths were identified from death certificates, and potential out-of-hospital fatal CHD events were investigated by interviewing 1 or more next of kin and by the completion of a questionnaire by the patient’s physician. ARIC and CHS staff abstracted discharge diagnoses on all hospitalizations and conduct a standardized committee review of all CHD, stroke, and cardiovascular death events. Comprehensive data were gathered on cardiovascular events and deaths from hospital records; interviews with physicians; next of kin and/or witnesses; and autopsy reports. In addition to similar study protocols between ARIC and CHS, extensive review of the data definitions and study sources was performed with review of files by an independent set of investigators so that only comparable clinical variables were included in the present analysis of the combined cohorts.

The following exclusion criteria, based on missing exposure or outcome data, were applied to obtain the final sample for the present analysis: (1) self-reported race/ethnicity other than black or white (48 in ARIC, 39 in CHS); (2) samples not genotyped due to lack of DNA or consent for genetic research (103 in ARIC, 432 in CHS); (3) samples with <75% of genotypes called (1052 in ARIC, 305 in CHS); (4) missing ECGs (not performed or not transmitted) or poor-quality data (missing leads or artifacts) for either QT or heart rate (189 in ARIC, 124 in CHS); and (5) unconfirmed SCD (82 in ARIC, 2 in CHS). After these exclusions, 14,309 (91%) of 15,783 ARIC participants and 4986 (85%) of 5888 CHS participants were included in the present analysis, for a combined sample size of 19,295 individuals.

Assessment of SCD

Each parent study classified all cases of fatal CHD according to standard protocols. To identify cases of SCD in ARIC and CHS for the present study, all cases of fatal CHD that occurred by July 31, 2002, in CHS and by December 31, 2002, in ARIC were reviewed and adjudicated by a committee of physicians. SCD was operationally defined as a sudden pulseless condition with a cardiac origin in a previously stable individual. After review of data available from death certificates, informant interviews, physician questionnaires, coroner reports, and hospital discharge summaries, the reviewers classified each CHD death as either sudden arrhythmic death, possible sudden arrhythmic death, definite nonsudden death, or unclassifiable. We a priori sought to exclude cases with nonarrhythmic characteristics, including those with evidence of progressive hypotension or advanced congestive heart failure before death. We also excluded those cases with advanced dementia or terminal illness, such as end-stage cancer or liver disease. Each event was adjudicated independently by 2 investigators. If disagreement existed between the first 2 reviewers, a third investigator independently reviewed the event to provide final classification. As part of the event review, information was abstracted systematically with regard to duration of symptoms, whether the event was witnessed, other circumstances of the event, and any medical comorbidities of the patient to help classify whether the subject had experienced SCD. Those classified as “definite sudden arrhythmic death” were either confirmed by evidence of “instantaneous death” or, in the case of unwitnessed deaths, descriptive information was provided on the position of the body that indicated a sudden event had occurred. All suspected SCDs, defined as a sudden pulseless condition with a cardiac origin in a previously stable individual, that we could not classify as “definite” were classified as “possible SCD.” Cases were identified as either in- or out-of-hospital deaths. The primary outcome of SCD described in the present study combines both definite and possible sudden arrhythmic death. For the present analysis, 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, 2002, for CHS and December 31, 2002, for ARIC, based on the study’s adjudication schedules.

Assessment of QT Interval at the Baseline Examinations of Each Study

At the baseline visit of the ARIC study, participants were asked not to smoke or ingest caffeine for at least 1 hour before the ECG. After the participant had rested for 5 to 10 minutes while the electrodes were being placed, a standard supine 12-lead ECG and a 2-minute paper recording of a 3-lead (leads V5, II, and V4)
rhythm strip were made. The ECGs were recorded digitally, and identical methods (MAC personal computer, Marquette Electronics, Milwaukee, Wis) were used in all clinical centers. A similar protocol was used at the baseline visit of CHS. MAC PC-DT ECG acquisition units (Marquette Electronics) were used to record a 10-second, 12-lead simultaneous ECG at a sample rate of 250 per second per lead. All tracings from the baseline visits of both CHS and ARIC were transmitted over analog phone lines to a central ECG reading center in Edmonton, Alberta, Canada, for analysis. The QT interval from the digital 12-lead ECG was determined with the Novacode ECG measurement and classification program.

Assessment of Covariates
Both the ARIC study and CHS have extensive data on behavioral, clinical, and serological factors relevant to selected cardiovascular phenotypes and outcomes. At each visit, demographic, anthropometric, and cardiovascular risk factor data were collected. Data from the baseline visits of both ARIC and CHS were used for the present analyses. Participants described themselves as white or black in response to an interviewer-administered questionnaire, which also contained questions on highest education attained, smoking status, and marital status. Collection of fasting blood samples and processing for total cholesterol followed standard study protocols.20-24 Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications. Diabetes mellitus was defined as fasting glucose ≥126 mg/dL, nonfasting glucose ≥200 mg/dL, or history/treatment of diabetes mellitus. History of MI at the baseline examination was defined by either a self-reported history of physician-diagnosed MI, or a history of MI identified on the baseline examination. Individuals who did not. Because 19 SNPs were tested, we used a Bonferroni-corrected α-level of 0.003 (0.05/19) as the threshold for statistical significance in the age-, sex-, and study-adjusted model.

For SCD risk, single SNP genotype–based analyses were performed. Cumulative incidence of SCD in the presence of competing events (deaths due to other causes) was estimated overall and by SNP genotype.20 To estimate the relative hazards (RHs) and the significance of the association between each SNP genotype and SCD risk while adjusting for covariates, Cox proportional hazards models were constructed, and a Bonferroni-corrected α-level of 0.003 was used to declare statistical significance in the age-, sex-, and study-adjusted analysis. An additive model was assumed for each SNP. For significant SNPs, a model that assumed 3 genotypic risks was also constructed to confirm the use of the additive model. The proportional hazards assumption was checked with the Schoenfeld residual.31

As exploratory analyses, the role of genotypic effects across various high-risk subgroups was examined both with stratified analyses and by fitting interaction terms into the regression models. All analyses were performed with either SAS (version 9.0; SAS, Inc, Cary, NC) or STATA (version 9.2; StataCorp LP, College Station, Tex). The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Clinical Characteristics of ARIC and CHS Participants at the Baseline Examinations
Baseline demographic and cardiovascular risk factors are shown by SCD status and by self-reported race/ethnicity in Table 1. As expected, many of the well-established cardiovascular risk factors were significantly associated with SCD risk. Among both whites and blacks, individuals who died of SCD were significantly older, had higher systolic blood pressure and fibrinogen levels, and had lower HDL cholesterol. They were also more likely to be male, smokers, and less well educated and to have a history of diabetes mellitus, hypertension, and MI at the baseline examination. Individuals who experienced SCD had significantly longer mean QT intervals at baseline, before the event. In whites, the age-, sex-, heart rate-, and study-adjusted mean QT duration was 403 ms for those who did not ultimately have SCD and 411 ms for those who did (P<0.001), and the corresponding
values were 402 and 410 ms, respectively, in blacks ($P<0.001$).

Although the 2 studies are largely comparable, modest differences were found between them in the significance and magnitude of associations between cardiovascular risk factors and SCD risk. For example, smoking, body mass index, and total cholesterol were not significantly associated with SCD in CHS but were in ARIC (Table 2).

**Association Between QT Interval and SCD**

Over a median follow-up of 14.1 years in ARIC and 12.2 years in CHS, 334 whites and 164 blacks experienced SCD (cumulative incidence rate per 1000 person-years: 2.0 overall; 1.4 in ARIC and 4.5 in CHS; 1.8 in whites and 2.9 in blacks). Given the older age of the CHS cohort, 222 (45%) of the 498 SCD events occurred in CHS, whereas 276 events occurred in ARIC. Of all CHD mortality adjudicated (n=985), 40.2% deaths were classified as definite SCD and 10.4% as possible SCD. The majority of cases of SCD occurred out of the hospital (90%).

Among whites, longer QT interval was associated with the development of SCD after adjustment for age, sex, heart rate, and study. Compared with whites in the first quintile of QT interval, those in the 2nd, 3rd, 4th, and 5th quintiles were 1.43 (95% confidence interval [CI] 0.97 to 2.13), 1.77 (95% CI 1.16 to 2.69), 2.33 (95% CI 1.49 to 3.64), and 3.58 (95% CI 2.20 to 5.81) times more likely to have had SCD, respectively ($P$ for trend $<0.0001$). A similar dose-response relationship was observed when the analysis was repeated with QT-interval deciles ($P$ for trend $<0.0001$). Among blacks, a longer QT interval was also associated with SCD risk; however, the dose-response relationship was less apparent, possibly owing to the smaller number of cases. Compared with blacks in the first quintile of QT interval, those in the 2nd, 3rd, 4th, and 5th quintiles were approximately 1.75 (95% CI 1.07 to 2.88), 2.04 (95% CI 1.19 to 3.49), 1.62 (95% CI 0.86 to 3.05), and 2.54 (95% CI 1.34 to 4.82) times more likely to have had SCD ($P$ for trend=0.02).

**Association Between 19 SNPs in NOS1AP and QT Interval**

Allele frequencies of the 19 SNPs examined are shown in Table 3 by race/ethnicity. The LD pattern of these 19 SNPs
were quite different in blacks than in whites, with considerably less LD among SNPs in blacks (Figure 1). Eleven of 19 SNPs examined were significantly associated with the age-, sex-, and heart rate-adjusted QT interval in whites, with \( P \leq 0.003 \); however, no SNPs were significantly associated with the QT interval in blacks.

In whites, the most significant SNP in the present study was rs16847548 \( (P=2.2 \times 10^{-10}) \), which is in LD with rs4657139 but was not typed in previous studies.\(^{11-15,17}\) The frequency of the C allele of rs16847548 was 0.22 in whites. After adjustment for age, sex, and heart rate, the mean QT intervals of individuals with the TT, TC, and CC genotypes at rs16847548 were 399, 401, and 403 ms, respectively, in ARIC; 411, 414, and 416 ms, respectively, in CHS; and 402, 404, and 407 ms, respectively, in the combined data set. This effect size of an \( \approx 5\)-ms difference between the 2 homozygous groups is consistent with our previous observations. In whites, the percent variation \( (R^2) \) in the QT-interval distribution (uncorrected QT interval) that was explained by rs16847548 was 0.2% in both the individual studies and the combined data set. In comparison, the \( R^2 \) associated with other variables was 0.5% for age, 0.1% for sex, 0.2% for diabetes mellitus, 0.4% for history of MI at baseline, and 67% for heart rate in white ARIC participants. Among white CHS participants, the \( R^2 \) values associated with age, sex, diabetes mellitus, history of MI at baseline, and heart rate were 0.03%, 1.1%, 0.4%, 0.5%, and 63%, respectively.

### Associations Between NOS1AP Genotypes and SCD

Consistent with the observation of longer mean QT interval associated with the C allele of rs16847548, this allele was also significantly associated with increased risk of SCD in whites. Indeed, only 179 (2%) of the 9895 individuals carrying the TT genotype at rs16847548 had SCD (Table 4), whereas 25 (3.5%) of the 706 individuals with the CC genotype experienced SCD. In whites, the crude RRs that were estimated with a codominant model suggested a dose-response relationship between copies of the C allele at rs16847548 and SCD. With an additive model, the age-, sex-, and study-adjusted RR for each C allele was 1.31 (95% CI 1.10 to 1.56, \( P=0.002 \); Table 4).

In addition, a downstream neighboring SNP, rs12567209, which was not correlated with rs16847548 \( (r^2=0.02) \), was also associated with SCD in whites (age-, sex-, and study-adjusted RR for each A allele=0.57 assuming an additive model; 95% CI 0.39 to 0.83, \( P=0.003 \)). Because of the low frequency of the A allele (minor allele frequency=0.07), a dominant model was also used for analysis of rs12567209. The age-, sex-, and study-adjusted RR of SCD comparing those with at least 1 copy of the A allele to those with the GG genotype was 0.53 (95% CI 0.36 to 0.79, \( P=0.002 \)), and thus,
both the additive and the dominant models were consistent with the data. The present study is not able to distinguish whether 1 model was a better fit than the other (additive model shown in Table 4). Surprisingly, rs12567209 was only modestly associated with QT interval (Table 3), which suggests that the effect on risk for SCD was not necessarily conveyed through modulation of QT interval. The mean age-, sex-, heart rate–, and study-corrected QT intervals for GG, AG, and AA genotypes were 403, 403, and 401 ms, respectively.

To explore whether the effect of NOS1AP SNPs on the risk of SCD is mediated entirely through modulation of the QT interval, we added both QT interval and heart rate as variables into the Cox proportional hazards model in whites, after adjusting for age, sex, and study. The RH of SCD associated with each additional copy of the C allele at rs16847548 decreased from 1.27 (model 2 in Table 4) to 1.22 (model 3 in Table 4), and the RH for each additional A allele at rs12567209 changed from 1.27 (model 2 in Table 4) to 1.22 (model 3 in Table 4). Further adjustment for existing cardiovascular risk factors that were associated with SCD modestly attenuated the significance of the associations for rs16847548 (RH=1.17, 95% CI 0.97 to 1.42).

### Table 3. Genotypic Association Between 19 SNPs in NOS1AP and QT Interval and SCD by Self-Reported Race/Ethnicity

<table>
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<th>Distance From Neighboring SNP, kb</th>
<th>SNP</th>
<th>Alleles*</th>
<th>Whites MAF</th>
<th>QT-Interval P</th>
<th>SCD P</th>
<th>Blacks MAF</th>
<th>QT-Interval P</th>
<th>SCD P</th>
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<td>0.03</td>
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<td>0.64</td>
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<td>0.85</td>
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MAF indicates minor allele frequency.

QT interval refers to an age-, sex-, heart rate–, and study-corrected QT interval. SCD analysis was adjusted for age, sex, and study.

*Allele listed first represents the minor allele in whites.

†Genotypes out of Hardy-Weinberg equilibrium, P<0.01, in blacks.

‡Genotypes out of Hardy-Weinberg equilibrium, P<0.01, in whites.

§Most significant SNPs from the present study.
Additional analyses were performed to examine the impact of either comorbidities or other SNPs on the robustness of the association between rs16847548 and rs12567209 and SCD risk in whites. First, exclusion of whites with a previous history of MI strengthened both associations (for each C allele of rs16847548, RH = 1.39 for model 1, 95% CI 1.13 to 1.69, \( P = 0.002 \); for each A allele of rs12567209, RH = 0.49 for model 1, 95% CI 0.30 to 0.78, \( P = 0.003 \)). Second, exclusion of 623 individuals with an ECG QRS complex > 120 ms, which is indicative of a bundle-branch block or other conduction defect, also resulted in a stronger association between rs16847548 and SCD risk (RH = 1.40 for model 1, 95% CI 1.16 to 1.68, \( P < 0.001 \)). However, when QRS duration was included in the fully adjusted model (model 3), the RH changed minimally from 1.23 to 1.25 (95% CI 1.04 to 1.51). The age-, sex-, and study-adjusted RH for rs12567209 changed from 0.57 to 0.62 (95% CI 0.42 to 0.92, \( P = 0.02 \)) on exclusion of QRS complex > 120 ms. Finally, among whites, none of the tests of interaction between either SNP, SCD, and known cardiovascular risk factors (study, history of MI, sex, diabetes mellitus, age at last follow-up, diabetes mellitus, hypertension, family history of cardiovascular disease, obesity, dyslipidemia, and smoking) was statistically significant (online-only Data Supplement Tables II and III).

### Lack of Association Between rs16847548 and rs12567209 and Non-SCD in Whites

It is possible that the association between rs16847548, rs12567209, or both with SCD is due to an association with overall CHD mortality. Therefore, survival analyses were also conducted for CHD deaths that were not coded as SCD (non-SCD CHD mortality) and all other deaths that were neither SCD nor CHD (non-SCD and non-CHD mortality). Figure 2 shows the cumulative incidences of SCD, non-SCD CHD, and non-SCD and non-CHD mortality, with accounting for each other as competing causes.

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**Figure 1.** Plots showing the LD pattern and association results for both QT interval and SCD in whites (A) and blacks (B) for 19 SNPs genotyped to tag the NOS1AP locus and surrounding region that exhibited the strongest association with QT interval in previous studies. The bottom panels in A and B are plots showing the pairwise LD between SNPs. The value within each diamond represents the pairwise correlation between SNPs (measured as \( r^2 \)) defined by the top left and top right sides of the diamond. Shading represents the magnitude and significance of the pairwise LD, with a black-to-white gradient reflecting higher to lower LD values; see http://www.broad.mit.edu/mpg/haplovew/ for further details. NOS1AP exons 1 and 2 are shown in orange. The top panels in A and B are plots showing the significance for each SNP, with genomic position on the x-axis and the negative base-10 logarithm of the \( P \) value on the y-axis. Information on genomic position was taken from Human Genome Build 35.
of death, by rs16847548 or rs12567209 in whites. The cumulative incidence of SCD per 1000 person-years in whites with the TT, TC, and CC genotypes at rs16847548 was 1.5, 2.0, and 2.8, respectively (Figure 2A). On the other hand, rs16847548 was not associated with non-sudden CHD mortality (age-, sex-, and study-adjusted RH=0.98, 95% CI 0.83 to 1.17, \( P=0.86 \); Figure 2C) or with non-SCD and non-CHD mortality (age-, sex-, and study-adjusted RH=1.00, 95% CI 0.94 to 1.07, \( P=0.94 \); Figure 2E). For rs12567209, the cumulative incidence of SCD per 1000 person-years in whites with the GG, AG, and AA genotypes was 1.9, 0.9, and 2.2, respectively (Figure 2B). As for rs16847548, no association was observed with non-sudden CHD mortality (age-, sex-, and study-adjusted RH=0.83, 95% CI 0.62 to 1.11, \( P=0.21 \); Figure 2D) or with non-SCD and non-CHD mortality (age-, sex-, and study-adjusted RH=1.00, 95% CI 0.94 to 1.07, \( P=0.94 \); Figure 2E).

Table 4. Unadjusted and Adjusted RH of SCD by rs16847548 and rs12567209 Genotypes in Whites From ARIC and CHS

<table>
<thead>
<tr>
<th></th>
<th>TT</th>
<th>TC</th>
<th>CC</th>
<th>P</th>
<th>GG</th>
<th>AG</th>
<th>AA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No SCD, n (%)</td>
<td>8726 (61.1)</td>
<td>4885 (34.2)</td>
<td>681 (4.8)</td>
<td>12 197 (85.9)</td>
<td>1932 (13.6)</td>
<td>69 (0.5)</td>
<td>8197 (86.0)</td>
<td>1773 (18.0)</td>
</tr>
<tr>
<td>SCD, n (%)</td>
<td>179 (54.1)</td>
<td>127 (38.4)</td>
<td>25 (7.6)</td>
<td>303 (91.8)</td>
<td>25 (7.6)</td>
<td>2 (0.6)</td>
<td>303 (91.8)</td>
<td>25 (7.6)</td>
</tr>
<tr>
<td>RH (95% CI)</td>
<td>1.00 (Reference)</td>
<td>1.31 (1.10–1.56)</td>
<td>1.00 (Reference)</td>
<td>0.002</td>
<td>1.00 (Reference)</td>
<td>0.57 (0.39–0.83)</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00 (Reference)</td>
<td>1.27 (1.06–1.51)</td>
<td>1.00 (Reference)</td>
<td>0.008</td>
<td>1.00</td>
<td>0.62 (0.42–0.90)</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.22 (1.03–1.46)</td>
<td>1.00</td>
<td>0.02</td>
<td>1.00</td>
<td>0.63 (0.43–0.92)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00</td>
<td>1.17 (0.97–1.42)</td>
<td>0.09</td>
<td>0.09</td>
<td>0.60 (0.40–0.91)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 4</td>
<td>1.00</td>
<td>1.17 (0.97–1.42)</td>
<td>0.09</td>
<td>0.09</td>
<td>0.60 (0.40–0.91)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( P \) obtained from regression model assuming additive genetic model.

Model 1 included age, sex, and study. Model 2 included model 1 plus both rs16847548 and rs12567209. Model 3=model 2 plus heart rate (continuous) and QT interval (quintiles). Model 4=model 3 plus current marital and smoking status, education, body mass index, total cholesterol and fibrinogen levels, hypertension, diabetes mellitus, and history of MI.
Discussion

In the present study, common sequence variations in NOS1AP were associated with both interindividual variation in the QT interval and risk of SCD in whites in 2 large cohorts of adults in the United States. The at-risk allele (C at rs16847548) is common, with 39% of the general white population carrying 1 copy of the C allele, and 5% carrying 2 copies. More specifically, in the combined population, white US adults carrying the CC genotype at rs16847548 of NOS1AP were \( \approx 72\% \) more likely to die of SCD and had a mean QT interval that was \( \approx 5 \) ms longer than their counterparts with the TT genotype, even after accounting for age, sex, and heart rate. On the other hand, the less common A allele of rs12567209 (of which \( \approx 13\% \) of the general white population are carriers) was independently associated with a decreased risk of SCD (RH=0.57) in whites. Notably, both of the genetic effects were specific for SCD rather than other forms of death due to CHD. Finally, in spite of demographic differences between ARIC and CHS whites, the genetic effect estimates were comparable in the 2 populations separately, and no evidence was
found of significant heterogeneity (online-only Data Supplement Tables II and III).

The present study identifies a novel gene, NOS1AP, along with a new set of cellular interactions, that can potentially affect SCD risk in the general population. The multivariate analyses show that even after adjustment for QT interval and heart rate, a significant association remains between both rs16847548 and rs12567209 and SCD risk. This result was somewhat unanticipated given that our initial hypothesis evolved from a model in which SNPs influence QT interval and that increasing QT interval would increase risk for SCD. However, given that adjustment for QT interval largely does not attenuate the risk for SCD associated with these SNPs, this suggests an alternate model in which these SNPs modulate an unmeasured, or hidden, factor, which itself modulates both QT interval and risk for SCD, and that they need not do so equivalently. However, the possibility exists that the remaining significant association between these SNPs and SCD risk is due to the QT interval as assessed by ECG representing an imperfect measure of cardiac repolarization, the potential misclassification of the actual QT-interval measurements and SCD definition, or the presence of additional genetic variation (ie, not having identified the causal SNPs). Although a recent article reported no association between NOS1AP and SCD in the Rotterdam Study, rs16847548 and rs12567209 were not studied directly or efficiently tagged, and the number of SCD events was small. In addition, the positive association between rs16847548 of NOS1AP and SCD risk supports the approach of using either precursors or intermediate phenotypes in genetic studies of complex diseases, because NOS1AP was first identified to be a candidate gene for SCD through a previous genome-wide association study of QT interval. The results of the present study, together with previous reports of associations between NOS1AP and the QT interval in multiple populations of European descent, suggest novel and potentially causal mechanisms linking NOS1AP and SCD risk. As an adapter protein, the gene product of NOS1AP (CAPON) serves to physically bridge neuronal nitric oxide synthase and its targets and modulator proteins. In guinea pig ventricular myocytes, CAPON is localized near ryanodine receptors, and the overexpression of CAPON results in shortening of the cardiac action potential, a decrease in L-type Ca current, and a smaller increase in the delayed rectifier potassium current, \( I_{Kr} \), which results in prolongation of the QT interval.

Several limitations are warranted in the interpretation of these findings. First, although we have identified the association of sequence variation at the NOS1AP locus with QT interval and SCD risk, it is not known whether we have identified the functional variants. For example, it is likely that rs12567209 is only in LD with the causal SNP, because its association with SCD in whites was in the opposite direction of its association in blacks. Even though rs16847548 had the strongest association (judging by \( P \) values of all 19 SNPs) with both QT interval and SCD in both the ARIC and CHS cohorts, simulation studies have shown that the causative SNP may not necessarily have the smallest \( P \) value, because \( P \) values fluctuate by chance owing to the nature of random sampling, dependent on the sample size and allele frequency. Thus, it is possible that rs16847548 is also in LD with another causal SNP. Second, no significant association between the 19 NOS1AP SNPs and QT interval or SCD was observed in the black participants at a conservative \( \alpha = 0.003 \). The discordance in the associations between blacks and whites may represent the result of lower statistical power (due to inappropriate tagging SNPs and smaller numbers of events) in the blacks, or it may represent a real genetic difference. Given the observed carrier frequency for rs16847548 of 34% (based on allele frequency of 0.19 in blacks) and assuming an overall genotypic RH of 1.37, as observed in whites, at least 649 SCD cases in the black participants in the present study would be necessary for the study to have 80% power with an \( \alpha \)-level of 0.003. If the correlation between genotyped SNPs in the present study and the putative ungenotyped functional variant is lower in blacks, then we would have less power to detect an effect in blacks. On the other hand, it is possible that the causal allele in blacks is not rs16847548, or there exists only 1 causal allele in NOS1AP, but the pattern of LD between the causal variant and rs16847548 differs between blacks and whites. Third, despite corroborating functional data from guinea pig cardiomyocytes, it is still possible that the associated variants in the NOS1AP locus actually influence (or are in LD with) a distant gene rather than NOS1AP, because this “action at a distance” has on rare occasions been observed in other diseases. Fourth, as with all genetic association studies of complex traits, an independent replication study of comparable size and phenotype is not only the best defense against possible false-positive reporting in the present study but is necessary before certainty of the observed associations can be established. Lastly, the present study was not ideal for assessing the utility of genetic risk prediction among those already at high risk, because the number of high-risk individuals is relatively modest in these studies.

In summary, in the present study, we report that sequence variations in NOS1AP, a novel candidate gene that was previously identified through a genome-wide association study of the QT interval, are associated with both QT interval and the subsequent risk of SCD in a large cohort of 14 737 white US adults. As expected, individuals carrying the at-risk allele at rs16847548 had a modestly increased risk of SCD, with each allele increasing SCD risk by \( \approx 30\% \) compared with those who did not carry the risk allele. On the other hand, the A minor allele of rs12567209 was associated with a reduced risk of SCD, and the associations of these 2 SNPs were independent of one another. Although the genetic effects described here are modest, if replicated in other populations, this effort may be an important step toward the identification of a panel of susceptibility alleles that potentially may be used for risk assessment in the general population. Future studies that explore the pathways mediating the association between variations of NOS1AP and SCD risk will also be crucial and may shed light on both targeted prevention
strategies and novel therapeutic targets for abnormal cardiac repolarization and SCD risk.

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Disclosures

None.

References

Nearly half of all coronary heart disease deaths are sudden. Family history of sudden cardiac death (SCD) is a powerful risk factor for SCD; however, the genetic factors underlying SCD in the general population are largely unknown. The ECG QT interval is associated with risk of SCD. A previous genome-wide association study reported that allelic variants in Nitric Oxide Synthase 1 Adaptor Protein (\textit{NOS1AP}), which encodes a ligand of neuronal nitric oxide synthase, are associated with the QT interval in white adults. The present analysis was conducted to validate the association between \textit{NOS1AP} variants and the QT interval and to further examine the association with SCD in a combined population of 19,295 black and white adults from 2 population-based cohort studies. Among whites, we found that multiple single-nucleotide polymorphisms in \textit{NOS1AP} were associated with adjusted QT interval in whites ($P<0.0001$), and 2 single-nucleotide polymorphisms were independently associated with SCD. One single-nucleotide polymorphism, with a minor allele frequency of 22\%, was associated with a 31\% greater risk of SCD for each copy of the variant allele, whereas a neighboring single-nucleotide polymorphism (minor allele frequency 7\%) was associated with a 43\% lower risk for SCD. No associations between single-nucleotide polymorphisms in \textit{NOS1AP} and either QT interval or SCD were observed in blacks.

Although the genetic effects described here are modest, if replicated in other populations this effort may represent 1 step toward the use of genetic risk markers, along with other risk factors, to help identify patients who warrant our most aggressive SCD preventive strategies.
Genetic Variations in Nitric Oxide Synthase 1 Adaptor Protein Are Associated With Sudden Cardiac Death in US White Community-Based Populations

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