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## Common *NOS1AP* Variants Are Associated With a Prolonged QTc Interval in the Rotterdam Study

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**Background**—QT prolongation is an important risk factor for sudden cardiac death. About 35% of QT-interval variation is heritable. In a recent genome-wide association study, a common variant (rs10494366) in the nitric oxide synthase 1 adaptor protein (*NOS1AP*) gene was found to be associated with QT-interval variation. We tested for association of 2 *NOS1AP* variants with QT duration and sudden cardiac death.

**Methods and Results**—The Rotterdam Study is a population-based, prospective cohort study of individuals  $\geq 55$  years of age. The *NOS1AP* variants rs10494366 T>G and rs10918594 C>G were genotyped in 6571 individuals. Heart rate-corrected QT interval (QTc) was determined with ECG analysis software on up to 3 digital ECGs per individual (total, 11 108 ECGs from 5374 individuals). The association with QTc duration was estimated with repeated-measures analyses, and the association with sudden cardiac death was estimated by Cox proportional-hazards analyses. The rs10494366 G allele (36% frequency) was associated with a 3.8-ms (95% confidence interval, 3.0 to 4.6;  $P=7.8 \times 10^{-20}$ ) increase in QTc interval duration for each additional allele copy, and the rs10918594 G allele (31% frequency) was associated with a 3.6-ms (95% confidence interval, 2.7 to 4.4;  $P=6.9 \times 10^{-17}$ ) increase per additional allele copy. None of the inferred *NOS1AP* haplotypes showed a stronger effect than the individual single-nucleotide polymorphisms. There were 233 sudden cardiac deaths over 11.9 median years of follow-up. No significant association was observed with sudden cardiac death risk.

**Conclusions**—Common variants in *NOS1AP* are strongly associated with QT-interval duration in an elderly population. Larger sample sizes are needed to confirm or exclude an effect on sudden cardiac death risk. (*Circulation*. 2007;116:10-16.)

**Key Words:** arrhythmia ■ death, sudden ■ electrocardiography ■ genetics ■ long-QT syndrome

Sudden cardiac death (SCD) claims 300 000 lives annually in the United States.<sup>1</sup> Although certain high-risk groups have been identified,<sup>2</sup> most SCD occurs in individuals unrecognized to be at risk.<sup>3</sup>

### Clinical Perspective p 16

Familial aggregation of SCD suggests a substantial contribution of genetic variation to SCD risk,<sup>4-7</sup> but mendelian mutations identified to date individually explain little of the population burden of SCD.<sup>8,9</sup> Until recently, the search for sequence variants contributing to SCD risk has been restricted to candidate genes known for their role in arrhythmogenesis.<sup>10</sup> The recent development of large single-nucleotide polymorphism (SNP) databases,<sup>11</sup> genotyping arrays of

great accuracy and genome-wide coverage of common variation,<sup>12</sup> together with analytical methods,<sup>13</sup> has enabled unbiased surveys of most of the common variation in the human genome. Still, the relatively small size of existing SCD collections and etiologic heterogeneity limit the statistical power to detect causal variants; therefore, initial attention has focused on quantitative SCD risk factors in large cohorts.

The electrocardiographic QT interval is a noninvasive measure of ventricular repolarization. About 35% of the variation in QT-interval duration in unselected community-based samples is heritable.<sup>14,15</sup> Mendelian congenital long- and short-QT syndromes are both characterized by SCD from ventricular arrhythmias. Moreover, nonsyndromal long QT interval<sup>16-19</sup> and short QT interval<sup>20</sup> impart increased risk of

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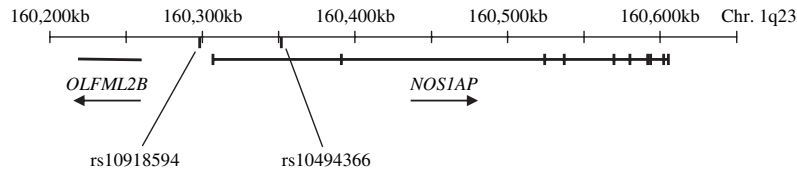
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*NOS1AP* and location of rs10494366 and rs10918594. The ruler indicates the physical position on chromosome 1. Thick horizontal lines indicate genes in the region; thick vertical lines, *NOS1AP* exons; and arrows, the direction of transcription. Thick vertical lines on the ruler indicate the positions of rs10918594 and rs10494366, which are  $\approx 55$  kb apart. The 2 SNPs were in linkage disequilibrium, with an  $r^2$  of 0.63 and  $D'$  of 0.89.

SCD in unselected populations. In addition, medication-induced prolonged QT interval and ventricular arrhythmias have led to the withdrawal of many noncardiac medications,<sup>21</sup> making the QT interval an important phenotype to study.

Previously, we identified a locus on chromosome 3 with suggestive evidence of linkage to QT-interval duration, but the genomic interval was large, and the finding has yet to be confirmed.<sup>15</sup> More recently, Arking et al<sup>22</sup> reported the finding from a genome-wide association study that a common variant (rs10494366; minor allele frequency, 38%) in the *NOS1AP* gene was reproducibly associated with QT-interval variation in several large population samples. The *NOS1AP* gene, encoding the nitric oxide synthase 1 adaptor protein, has been found to regulate neuronal nitric oxide synthase activation<sup>23</sup> and to enhance Dexras1 activation by neuronal nitric oxide synthase through a ternary complex.<sup>24</sup> Neuronal nitric oxide synthase–knockout mice have been found to have altered cardiac contractility, which suggests a role for *NOS1AP* in cardiac depolarization.<sup>25–27</sup> Furthermore, *NOS1AP* is capable of interaction with ion channels through its PDZ domain.<sup>28–30</sup> Nevertheless, the involvement of *NOS1AP* in myocardial repolarization was not known until the initial report of the association.

The impact of *NOS1AP* variants on QT-interval duration in older populations, in whom nongenetic factors might play a stronger role than heritable factors, is unknown.

The goal of the present study was to test for association of the *NOS1AP* variant with QT duration and to test for its association with SCD in the Rotterdam Study.

## Methods

### Study Population

The Rotterdam Study is a prospective population-based cohort study of chronic diseases in the elderly. All inhabitants of Ommoord, a Rotterdam suburb,  $\geq 55$  years of age ( $n = 10\,278$ ), were ascertained from the municipal register and invited to participate. Of them, 78% ( $n = 7983$ ; 58% female, 98% white) took part in the baseline examination from March 1990 through July 1993. Second and third examinations were conducted from September 1993 to August 1996 and from April 1997 to December 1999, respectively. Objectives and methods of the Rotterdam Study have been described in detail.<sup>31</sup> The medical ethics committee of Erasmus Medical Center (Rotterdam, the Netherlands) approved the study, and all participants provided signed informed consent for participation, including retrieval of medical records, use of blood and DNA for scientific purposes, and publication of data. DNA for genotyping is available for 6571 participants (82%) from the baseline visit.

Clinical characteristics, including smoking, body mass index, hypertension, diabetes mellitus, heart failure, and myocardial infarction, were ascertained as previously described.<sup>19,32–36</sup> Active surveillance for incident diabetes mellitus, heart failure, and myocardial

infarction is conducted continuously between exams. In addition, exposure of study participants to medications has been gathered continuously from January 1, 1991, to the present through computerized pharmacy records of the pharmacies in the study area.

### Genotyping

All participants were genotyped for the *NOS1AP* SNP rs10494366 T>G, previously shown to be associated with QT interval in 3 independent samples.<sup>22</sup> The correlated SNP rs10918594 C>G, which had evidence of association with QT interval in one of the original samples,<sup>22</sup> also was genotyped (see the Figure). Both were genotyped with Taqman assays C\_1777074\_10 and C\_1777009\_10 (Applied Biosystems, Foster City, Calif) in 1 ng genomic DNA extracted from leukocytes, as previously reported.<sup>37</sup>

### Assessment of QTc Interval and Other Electrocardiographic Measurements

The electrocardiography (ECG) phenotype studied was the heart rate–corrected QT interval (QTc) in milliseconds using Bazett's formula ( $QTc = QT/\sqrt{RR}$ ).<sup>38</sup> As in previous studies of QTc in the Rotterdam Study<sup>19</sup> we used a 10-second resting 12-lead ECG (average of 8 to 10 beats), which was recorded on an ACTA ECG (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. All ECGs were processed by the Modular ECG Analysis System (MEANS) to obtain ECG measurements.<sup>39–41</sup> MEANS determines the QT interval from the start of the QRS complex until the end of the T wave. MEANS also determines the presence of right or left bundle-branch block and left ventricular hypertrophy. To study the association between *NOS1AP* variants and QTc duration, all eligible ECGs from subjects with DNA available were used. ECGs with right or left bundle-branch block were excluded from analyses. In addition, to minimize confounding by nongenetic influences on QT duration, all ECGs taken while the subject was on any QT-altering drugs were excluded from analyses. Drugs were considered possibly QT prolonging if they appeared on any of lists 1 through 4 at [www.qt drugs.org](http://www.qt drugs.org).<sup>42</sup> We also excluded ECGs if subjects were on flupentixol, levomepromazine, mefloquine, olanzapine, or sertindole, which may prolong QT interval, or digoxin, which shortens the QT interval. Up to 3 QTc measurements were recorded across the 3 examination cycles.

Finally, in additional analyses, the mean QTc interval per individual was divided into 3 gender-specific categories as previously described. For women, the cut points were  $\leq 450$  ms (normal), 451 to 470 ms (borderline), and  $> 470$  ms (prolonged); for men, the cut points were  $\leq 430$  ms (normal), 431 to 450 ms (borderline), and  $> 450$  ms (prolonged).<sup>19,43</sup>

### Adjudication of SCD

For the SCD analyses, all genotyped subjects were included. The ascertainment of SCD cases in the Rotterdam Study has been described previously.<sup>19</sup> SCDs were defined operationally as a witnessed natural death attributable to cardiac causes, heralded by abrupt loss of consciousness, within 1 hour of onset of acute symptoms, or as an unwitnessed, unexpected death of someone seen in a stable medical condition  $< 24$  hours previously with no evidence of a noncardiac cause.<sup>44,45</sup>

TABLE 1. Baseline Characteristics

Characteristic	Genotyped Sample		QTc Sample		SCD Cases	
	Men (n=2666, 40.6%)	Women (n=3905, 59.4%)	Men (n=2191, 40.8%)	Women (n=3183, 59.2%)	Men (n=116, 49.8%)	Women (n=117, 50.2%)
Age at baseline, y, mean±SD	68.2±8.2	70.4±9.6	67.0±7.7	69.0±9.1	71.8±7.8	74.4±7.7
Follow-up time, y, mean±SD	10.0±3.8	10.5±3.7	10.6±3.4	11.1±3.2	6.4±3.8	7.3±3.8
Current smoking, n (%)	774 (29.0)	680 (17.4)	634 (28.9)	582 (18.3%)	32 (27.6)	15 (12.8%)
Past smoking, n (%)	1635 (61.3)	1040 (26.6)	1343 (61.3)	872 (27.4)	75 (64.7)	38 (32.5%)
Body mass index, kg/m <sup>2</sup> , mean±SD	25.7±3.0	26.7±4.1	25.7±3.0	26.7±4.0	25.3±3.0	27.3±3.9
Systolic blood pressure, mm Hg, mean±SD	138.7±21.7	139.8±22.6	138.3±21.5	139.2±22.2	144.6±24.2	152.8±27.7
Diastolic blood pressure, mm Hg, mean±SD	74.6±11.5	73.2±11.4	74.9±11.3	73.2±11.1	74.0±12.5	77.0±14.1
Hypertension, n (%)	780 (29.3)	1415 (36.2)	621 (28.3)	1102 (34.6)	53 (45.7)	65 (55.6)
Diabetes mellitus, n (%)	281 (10.5)	422 (10.8)	213 (9.7)	309 (9.7)	14 (12.1)	27 (23.1)
Myocardial infarction, n (%)	447 (16.8)	320 (8.2)	345 (15.7)	243 (7.6)	44 (37.9)	19 (16.2)
Heart failure, n (%)	81 (3.0)	131 (3.4)	34 (1.6)	75 (2.4)	17 (14.7)	7 (6.0)

Shown are characteristics of all individuals with DNA available for genotyping (genotyped sample), of the subset of genotyped subjects with ECGs without bundle-branch block or use of a QT-prolonging drug or digoxin (QTc sample), and of the SCD cases. The SCD source sample includes all genotyped subjects.

### Statistical Analysis

Genotype frequencies were tested for Hardy-Weinberg equilibrium with a  $\chi^2$  test.

Because the QTc in subsequent ECGs of the same subject are correlated, we used repeated-measures analyses implemented in PROC MIXED (SAS 8.2, SAS Institute, Cary, NC). Both allelic and general genotype models were tested for the 2 polymorphisms, although the allelic model was considered primary because of the previously reported rs10494366–QT relationship.<sup>22</sup> Haplotypes were estimated with the expectation-maximization algorithm implemented in PHASE 2.0 (University of Washington, Seattle),<sup>46,47</sup> and only individuals with successful genotyping for both SNPs and a posterior probability of  $P>0.95$  for assigned haplotypes were included in haplotype analyses. In total, we identified 2245 double heterozygotes, all of whom were phased as heterozygous haplotype TC-GG because these are the major haplotypes, with posterior probabilities in excess of 0.95. In haplotype analyses, the haplotype with major alleles for both SNPs was considered the reference to which the other 3 haplotypes were compared individually. QTc was tested for association with genotype as the sole predictor (crude) and with adjustment for age and gender (multivariable). To compare the outcomes of haplotype analysis with individual SNP analysis, the latter analyses also were performed restricting the analysis to subjects in whom genotyping was successful for both SNPs. Finally, a sensitivity analysis was carried out, excluding ECGs with an abnormally prolonged QTc and using gender-specific cutoff points of  $>450$  ms for men and  $>470$  ms for women. Jonckheere-Terpstra tests were used to test whether individuals carrying *NOS1AP* minor alleles had an increased frequency of borderline and abnormal mean QTc.

Hazard ratios for time to SCD from baseline were estimated with Cox proportional-hazards models. Again, both allelic and general genotype models were tested for the 2 polymorphisms. In addition to *NOS1AP* genotype, known SCD risk factors—including age, gender, body mass index, smoking, hypertension, diabetes mellitus, heart failure, and myocardial infarction at baseline and time-dependent incident diabetes mellitus, heart failure, and myocardial infarction—were included as predictors. To minimize misclassification of SCD, we additionally performed a subanalysis restricting the case definition to witnessed deaths only. As we have previously shown, the risk of SCD for increasing QTc is stronger in the younger than in the older age group,<sup>19</sup> so we determined the hazard ratios for time to SCD separately in groups stratified by age above and below the median age at baseline. Finally, we performed a sensitivity analysis, excluding subjects with a history of myocardial infarction at baseline from the analysis. All Cox proportional hazards analyses were

performed with SPSS for Windows, version 11.0 (SPSS Inc, Chicago, Ill).

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

### Study Subjects

Baseline characteristics for the total study population, consisting of all genotyped subjects from the Rotterdam Study (n=6571), are summarized in Table 1. Within the study population, 12 967 ECGs were available from 6052 subjects across up to 3 examination cycles. After exclusion of ECGs with right or left bundle-branch block (n=640) and those performed in individuals taking QT-prolonging or -shortening drugs (n=1334) or both, a total of 11 108 ECGs from 5374 subjects remained (on average, 2.1 ECGs per individual). The 5374 subjects included in the QTc analyses were 1.3 years younger at baseline, reflecting exclusions among older participants (Table 1). Women had an 8.9-ms-longer age-adjusted QTc interval (431.4 versus 422.5 ms;  $P<0.0001$ ), as previously shown,<sup>38,48</sup> and were 2.2 years older than men (70.4 versus 68.2 years at baseline;  $P<0.0001$ ). The numbers of abnormally prolonged QTc in men and women of our study population were slightly higher than expected on the basis of numbers from reference populations.<sup>48,49</sup> However, our study population was on average already considerably older at baseline (69.5 versus 53 and 61 years, respectively), and this mean further increased when follow-up ECGs were taken.

### Genotyping

The G-allele (minor) frequency of rs10494366 T>G was 36.4% and of rs10918594 C>G was 31.4%. Successful genotype calls were made in 95.8% and 95.9% of subjects, respectively. Both SNPs were in Hardy-Weinberg equilibrium ( $P=0.32$  for rs10494366 and  $P=0.89$  for rs10918594). The 2 SNPs were in linkage disequilibrium, with an  $r^2$  of 0.63 and  $D'$  of 0.89. On phasing, we observed 2 common 2-SNP haplotypes, TC (61.4%) and GG (29.1%), consisting of the 2

**TABLE 2. Difference in QTc by NOSIAP Genotype**

	Genotypic Model*				Allelic Model†	
	Genotype	Genotype	Genotype	P	Per G allele	P
rs10494366 (36.4% MAF)	TT	TG	GG			
Subjects, n‡	2100	2334	704		5138	
Crude, ms	Ref	4.2 (3.0–5.5)	7.1 (5.3–8.9)	2.2×10 <sup>-17</sup>	3.7 (2.9–4.6)	3.3×10 <sup>-18</sup>
Age and gender adjusted, ms	Ref	4.2 (3.0–5.4)	7.2 (5.5–9.0)	5.9×10 <sup>-19</sup>	3.8 (3.0–4.6)	7.8×10 <sup>-20</sup>
rs10918594 (31.4% MAF)	CC	CG	GG			
Subjects, n‡	2456	2217	530		5203	
Crude, ms	Ref	4.3 (3.1–5.5)	6.4 (4.4–8.3)	1.7×10 <sup>-15</sup>	3.6 (2.7–4.5)	6.9×10 <sup>-16</sup>
Age and gender adjusted, ms	Ref	4.3 (3.2–5.5)	6.3 (4.4–8.2)	1.5×10 <sup>-16</sup>	3.6 (2.7–4.4)	6.9×10 <sup>-17</sup>

MAF indicates minor allele frequency; Ref, reference. Values are difference from reference group (95% CI) in milliseconds.

\*Linear regression model using dummy variables per genotype.

†Linear regression model entering genotype as an ordinal variable.

‡Because of failures in genotyping for the individual SNPs, genotype counts do not add up to the total of 5374 individuals.

major and 2 minor alleles, respectively, and 2 remaining haplotypes containing 1 major and 1 minor allele each, GC (7.2%) and TG (2.3%). Genotype distributions did not differ between men and women and between quartiles of age at baseline.

**NOSIAP Polymorphisms and QTc**

Minor alleles of both NOSIAP SNPs were significantly associated with an increase in QTc duration. SNP rs10494366 T>G was associated with a 3.8-ms increase in multivariable-adjusted QTc interval for each additional G allele, and SNP rs10918594 C>G was associated with a 3.6-ms increase per additional G allele (Table 2). Additional adjustment for ECG left ventricular hypertrophy did not alter the results (data not shown). We observed no difference in effect of the SNPs between men and women. A sensitivity analysis excluding ECGs with an abnormally prolonged QTc (using gender-specific cut points) resulted in slightly lower estimates (2.9 and 2.7 ms for the allelic models); however, the association of NOSIAP genotypes with QTc duration remained highly significant (all P<10<sup>-11</sup>).

All 3 haplotypes containing 1 (GC and TG) or 2 (GG) minor alleles for the 2 SNPs were associated with increased QTc compared with the homozygous TC reference haplotype. The GG haplotype was associated with a 4.1-ms-longer

multivariable-adjusted QTc per additional GG haplotype copy (P=2.0×10<sup>-18</sup>) using the TC haplotype as reference. The GC and TG haplotypes were associated with a 3.2-ms-longer (P=7.0×10<sup>-4</sup>) and 4.1-ms-longer (P=0.01) multivariable-adjusted QT interval per additional copy, respectively. None of the haplotypes had a more significant effect than the individual SNPs.

Furthermore, rs10494366 and rs10918594 were associated with a larger proportion of borderline and prolonged QTc intervals using gender-specific cut points<sup>19</sup> (test for trend, both P<0.0001; Table 3).

**NOSIAP Polymorphisms and SCD**

Within the study population (n=6571), we identified 233 sudden cardiac deaths, 121 of which were witnessed. Baseline characteristics of all adjudicated SCD cases are shown in Table 1. After adjustment for known risk factors, the NOSIAP polymorphisms rs10494366 T>G and rs10918594 C>G showed nonsignificant trends in the direction of increased hazard of SCD, with hazard ratios per additional minor allele for time to SCD of 1.09 (95% confidence interval, 0.90 to 1.33) and 1.10 (95% confidence interval, 0.90 to 1.34), respectively. In the subset of 121 adjudicated SCD cases that were witnessed, a similar nonsignificant trend toward increased SCD risk was found (Table 4). Stratification

**TABLE 3. Number of Individuals With Normal, Borderline, and Abnormal Mean QTc per Genotype Group Using Gender-Specific Cut Points**

Genotype	Normal	Borderline	Prolonged	P, Test for Trend
rs10494366, n (% within genotype)	...	...	...	<0.0001
TT	1679 (80.0)	329 (15.7)	92 (4.4)	
TG	1715 (73.5)	447 (19.2)	172 (7.4)	
GG	498 (70.7)	144 (20.5)	62 (8.8)	
rs10918594, n (% within genotype)	...	...	...	<0.0001
CC	1945 (79.2)	390 (15.9)	121 (4.9)	
CG	1609 (72.6)	448 (20.2)	160 (7.2)	
GG	385 (72.6)	96 (18.1)	49 (9.2)	

QTc interval divided into 3 gender-specific categories. For women, the cut points were ≤450 ms (normal), 451 to 470 ms (borderline) and >470 ms (prolonged); for men, they were ≤430 ms (normal), 431 to 450 ms (borderline), and >450 ms (prolonged).<sup>19,43</sup>

**TABLE 4. Hazard Ratio of All Adjudicated SCD and Witnessed SCD per *NOS1AP* Genotype or Allele**

	Genotypic Model*				Allelic Model†	
	Genotype	Genotype	Genotype	P	Per G Allele	P
All SCD, HR (95% CI)						
rs10494366	TT (n=90)	TG (n=95)	GG (n=36)			
Crude	Ref	0.97 (0.72–1.30)	1.26 (0.85–1.87)	0.41	1.08 (0.89–1.32)	0.42
Full model	Ref	0.99 (0.74–1.33)	1.27 (0.85–1.89)	0.44	1.09 (0.90–1.33)	0.37
rs10918594	CC (n=101)	CG (n=103)	GG (n=24)			
Crude	Ref	1.13 (0.85–1.50)	1.11 (0.70–1.76)	0.69	1.08 (0.88–1.32)	0.46
Full model	Ref	1.16 (0.88–1.54)	1.13 (0.71–1.80)	0.58	1.10 (0.90–1.34)	0.37
Witnessed SCD, HR (95% CI)						
rs10494366	TT (n=47)	TG (n=43)	GG (n=26)			
Crude	Ref	0.82 (0.54–1.24)	1.66 (1.02–2.70)	0.02	1.20 (0.93–1.56)	0.17
Full model	Ref	0.84 (0.55–1.28)	1.68 (1.04–2.74)	0.02	1.22 (0.94–1.58)	0.14
rs10918594	CC (n=52)	CG (n=51)	GG (n=16)			
Crude	Ref	1.11 (0.75–1.64)	1.43 (0.80–2.54)	0.47	1.17 (0.89–1.53)	0.25
Full model	Ref	1.14 (0.77–1.68)	1.45 (0.81–2.59)	0.44	1.18 (0.91–1.55)	0.22

Cox proportional hazards model. HR indicates hazard ratio; Ref, reference; and n, number of cases. Crude model was age and gender adjusted. Full model included age, gender, body mass index, smoking, hypertension, diabetes mellitus, heart failure, and myocardial infarction.

\*Genotype-specific HR.

†HR entering genotype as an ordinal variable under an allelic model.

for baseline age above and below the median showed no difference between age groups (data not shown). Finally, a sensitivity analysis excluding 767 subjects with a history of myocardial infarction at baseline did not result in a substantial change of the effect estimates or confidence intervals (data not shown).

## Discussion

We observed strong replication in the Rotterdam Study, a large, well-phenotyped cohort of European ancestry, of the finding from a prior genome-wide association study<sup>22</sup> that common *NOS1AP* variants are associated with increased age-, gender-, and heart rate-adjusted QT-interval duration. None of the haplotypes showed a more significant effect than the individual SNPs, which were not specifically selected to characterize haplotype variation at the locus. The 2 SNPs, which are 55 kb apart, are not known to be functional, nor are they highly correlated with any known functional SNP. These results support the existence of a causal untyped SNP that is correlated with both rs10494366 and rs10918594.

The association with SCD was not statistically significant. Although we cannot fully exclude survival bias because of the older age of our study population, we did not find that the genotype distribution differed between different age groups at baseline, making this less likely. The modest QTc prolongation associated with *NOS1AP* variation, despite the strong effect of prolonged QTc on SCD risk, suggests that a much larger study is needed to definitively confirm or rule out an increased risk of SCD by *NOS1AP* variants. At least 510 cases would be needed to detect an odds ratio of 1.2 per minor allele with 80% power. Even if no association with SCD is ultimately identified, the 7.2-ms increase in QTc interval in minor homozygotes compared with major homozygotes ap-

proximates the effect of medications that delay myocardial repolarization and increase liability to ventricular arrhythmias.

The mechanism by which a common variation in *NOS1AP* affects QTc interval duration is unknown at present. However, the statistical evidence supporting the association with QTc interval of rs10494366 ( $P < 10^{-19}$ ) and rs10918594 ( $P < 10^{-16}$ ) in 5374 individuals confirms that this is a genuine association, consistent with evidence from 4 independent cohorts totaling >13 000 individuals of European ancestry. Our study examined the relationship of genetic variation, present at birth, in an elderly cohort in whom one might assume that genetic factors play a smaller role than in younger cohorts. However, these results demonstrate that genetic factors continue to play a role even at older age.

One major advantage of our study was the availability of up to 3 ECGs per subject at regular intervals during follow-up, resulting in more precise long-term ECG measures. Furthermore, the use of digital ECG recordings all measured with the MEANS system likely reduced systematic differences in assessment of the QTc interval. In addition, the intersection of the Rotterdam Study with detailed pharmacy exposure data allowed us to exclude ECGs recorded in individuals on QT-prolonging or -shortening drugs, which could have attenuated the power to detect the association. Although no information on long-QT syndrome cases was available, the number of relatives in the Rotterdam Study is low, and the sensitivity analysis excluding abnormally prolonged QTc further minimized influence of potential familial long-QT syndrome cases. Another advantage of the Rotterdam Study is the prospective ascertainment of risk factors and the active surveillance for SCD events over a relatively long period of follow-up. Thus, extensive information surrounding

SCD events was available, including the time between start of symptoms and death, which enabled rigorous adjudication of SCD events.

One limitation of the study resides in the variety of competing causes of abrupt death at increasing age, which may have led to misclassification, especially in cases in which death was unwitnessed. Because SCD coding was blinded to *NOS1AP* genotype, this would likely have biased our study to detect no effect. This might explain our finding of a slightly increased, but still nonsignificant, hazard ratio when the analyses were restricted to witnessed sudden cardiac deaths. Our results and those of the prior study by Arking et al<sup>22</sup> were restricted to population samples of European ancestry. Further testing in samples of African and Asian ancestry is needed to establish the role of genetic variation at the *NOS1AP* locus in myocardial repolarization in these population groups. Moreover, substantial frequency differences are observed among European, African, and Asian HapMap samples, which raises the possibility of natural selection in the region.<sup>50</sup> Attempts to validate the *NOS1AP* association in recently admixed populations, such as African Americans, will need to account for global and local chromosomal differences in ancestry because of the strong association with continental ancestry and the risk of population stratification.

### Conclusions

We have strongly confirmed the association of *NOS1AP* variants with QT-interval duration. With the limited number of SCD cases in our cohort, it was not possible to demonstrate that this association translates into an influence on risk of SCD, although the point estimates suggest that such a risk increase may truly exist. Additional larger studies are required to determine whether *NOS1AP* genotype is associated with SCD.

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### Disclosures

None.

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### CLINICAL PERSPECTIVE

Sudden cardiac death (SCD) claims 300 000 lives annually in the United States. The ECG QT interval is a noninvasive measure of ventricular repolarization, and prolongation of the QT interval is an important risk factor for SCD and drug-induced arrhythmias. Approximately 35% of the variation in QT-interval duration is attributable to heritable factors. Until recently, the search for sequence variants contributing to QT-interval duration and SCD risk has been restricted to candidate genes known for their role in arrhythmogenesis. However, in a recent genome-wide association study, a common variant in the nitric oxide synthase 1 adaptor protein (*NOS1AP*) gene was found to be associated with QT-interval variation. *NOS1AP* was not previously known to play a role in repolarization. In the present study, we have strongly confirmed the association of *NOS1AP* variants and QT-interval duration with a difference between minor homozygotes and major homozygotes of 7.2 ms ( $P < 10^{-19}$ ). We did not find an association of *NOS1AP* variants with SCD cases in our cohort, but with 228 SCD events, our study was underpowered to demonstrate such an effect. Even if no association with SCD is ultimately identified, the 7.2-ms increase in QTc interval in minor allele homozygotes compared with major homozygotes approximates the effect of medications that delay myocardial repolarization and increase liability to ventricular arrhythmias. The study underscores the power of association methods to identify novel genes and pathways involved in myocardial repolarization and to identify genetic variants that could contribute to the risk of cardiac arrhythmias.