Common Variation in the NOS1AP Gene Is Associated With Drug-Induced QT Prolongation and Ventricular Arrhythmia

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Objectives
This study sought to determine whether variations in NOS1AP affect drug-induced long QT syndrome (LQTS).

Background
Use of antiarrhythmic drugs is limited by the high incidence of serious adverse events including QT prolongation and torsades de pointes. NOS1AP gene variants play a role in modulating QT intervals in healthy subjects and severity of presentation in LQTS.

Methods
This study carried out an association study using 167 single nucleotide polymorphisms (SNP) spanning the NOS1AP gene in 58 Caucasian patients experiencing drug-induced LQTS (dLQTS) and 87 Caucasian controls from the DARE (Drug-Induced Arrhythmia Risk Evaluation) study.

Results
The rs10800397 SNP was significantly associated with dLQTS (odds ratio [OR]: 3.3, 99.95% confidence interval [CI]: 1.0 to 10.8, p = 3.7 × 10^{-4}). The associations were more pronounced in the subgroup of amiodarone users, in which 3 SNPs, including rs10800397, were significantly associated (most significant SNP: rs10919035: OR: 5.5, 99.95% CI: 1.1 to 27.9, p = 3.0 × 10^{-4}). We genotyped rs10919035 in an independent replication cohort of 28 amiodarone dLQTS cases versus 173 control subjects (meta-analysis of both studies: OR: 2.81, 99.95% CI: 1.62 to 4.89, p = 2.4 × 10^{-4}). Analysis of corrected QT interval among 74 control subjects from our dataset showed a similar pattern of significance over the gene region as the case-control analysis. This pattern was confirmed in 1,480 control subjects from the BRIGHT (British Genetics of Hypertension Study) cohort (top SNP from DARE: rs12734991 in meta-analysis: increase in corrected QT interval per C allele: 9.1 ± 3.2 ms, p = 1.7 × 10^{-4}).

Conclusions
These results provide the first demonstration that common variations in the NOS1AP gene are associated with a significant increase in the risk of dLQTS. This study suggests that common variations in the NOS1AP gene may have relevance for future pharmacogenomic applications in clinical practice permitting safer prescription of drugs for vulnerable patients. (J Am Coll Cardiol 2012;60:841–50) © 2012 by the American College of Cardiology Foundation

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Currently amiodarone is the most commonly used antiarrhythmic drug, followed closely by sotalol. However, use of antiarrhythmic drugs is limited by the high incidence of bradycardia and QT prolongation, which can result in torsades de pointes (TdP) (1–3). Amiodarone is a class III antiarrhythmic agent inhibiting $I_{K_{S}}$ (4,5) and increasing action potential duration and the effective refractory period (6,7) (seen as QT prolongation on the surface electrocardiogram [ECG]). Amiodarone also decreases conduction velocity by blocking Na$^{+}$ channels (class I effect), reduces the number of beta-adrenergic receptors with a resultant antidiurenergic effect (class II effect), and suppresses Ca$^{2+}$-mediated action potentials by blocking L-type calcium channels (class IV effect). Sotalol meanwhile is an antiarrhythmic drug with class II and class III properties (5).

It has been suggested that every individual has a physiological “cardiac repolarization reserve” (8), which may be genetically determined and which compensates for any endogenous or exogenous factors (e.g., drugs) that would either decrease repolarizing or increase depolarizing currents during the action potential. It is likely, therefore, that individuals with reduced repolarization reserve are more vulnerable to developing QT-interval prolongation and TdP when exposed to potassium channel-blocking drugs such as amiodarone and sotalol.

Genome-wide analysis has consistently associated common variants of the nitric oxide synthase 1 adaptor protein ($NOS1AP$) gene with QT interval across independent replication studies (9–12). Despite attempts to identify and validate a single functional variant in $NOS1AP$ associated with QT interval, resequencing of all exons in $NOS1AP$ has not yet identified any missense mutations that explain these results, suggesting that the functional variants associated with these single nucleotide polymorphisms (SNP) are likely to be regulatory in nature (9).

$NOS1AP$ is a regulator of neuronal nitric oxide synthase (nNOS encoded by $NOS1$), an isoform of NOS, which regulates intracellular calcium levels and myocyte contraction in the heart (13–15). The $NOS1AP$ interacts with nNOS to accelerate cardiac repolarization by inhibition of L-type calcium channels (16–19), thereby providing a rationale for the association of $NOS1AP$ gene variants with QT-interval duration.

The variability of drug action in individuals can arise because of variation in genes encoding drug targets, genes modulating the overall activity of the complex biological systems within which the drugs act, and genes that are responsible for drug metabolism and elimination. In view of the role of $NOS1AP$ in cardiac repolarization, we hypothesized that genetic variation in the $NOS1AP$ gene influences the incidence of drug-induced ventricular arrhythmia and QT prolongation.

### Abbreviations and Acronyms

- **CEU** = western and northern European ancestry
- **CI** = confidence interval
- **DNA** = deoxyribonucleic acid
- **ECG** = electrocardiogram
- **IRB** = Institutional Review Board
- **LD** = linkage disequilibrium
- **LQTS** = long QT syndrome
- **nNOS** = neuronal nitric oxide synthase
- **$NOS1AP$** = nitric oxide synthase 1 adaptor protein
- **OR** = odds ratio
- **SNP** = single nucleotide polymorphism
- **TdP** = torsades de pointes

The DARE (Drug-Induced Arrhythmia Risk Evaluation) study is a national cohort of 112 patients experiencing drug-induced ventricular arrhythmias and/or severe QT-interval prolongation in the United Kingdom. A case-control study was established from the DARE study consisting of 59 Caucasian case subjects who had experienced an arrhythmic event associated with drug-induced QT prolongation, and 91 control subjects, all of whom had provided deoxyribonucleic acid (DNA) samples. Ethnicity was self-reported as Caucasian for all cases and controls.

Cases were included if they had 1 or more of the following diagnosed as secondary to a medication: documented classical TdP defined as 3 beats or more of polymorphic ventricular tachycardia associated with QT prolongation and pauses prior to onset of the arrhythmic event; ventricular fibrillation and/or cardiac arrest associated with corrected (QTc)-interval prolongation; and QTc-interval prolongation with a history consistent with cardiac syncope, excluding vasovagal syncope and seizures. After withdrawal of the culpable drug, cessation of ventricular arrhythmia and syncope and at least partial resolution of QT prolongation were required. All QTc intervals were corrected using Bazett’s formula and values $>450$ ms (men) or $>470$ ms (women) were considered prolonged. Cases were excluded if DNA was unavailable and/or arrhythmias were not documented and/or QT prolongation was absent.

Healthy control subjects were provided from primary care physicians responsible for the cases to ensure geographical matching. Inclusion criteria were no history of drug-induced arrhythmias, ventricular arrhythmias, or the congenital long QT syndrome (LQTS). Control subjects with abnormal resting 12-lead ECGs were excluded.

### Methods

The DARE (Drug-Induced Arrhythmia Risk Evaluation) study was a national cohort of 112 patients experiencing drug-induced ventricular arrhythmias and/or severe QT-interval prolongation in the United Kingdom. A case-control study was established from the DARE study consisting of 59 Caucasian case subjects who had experienced an arrhythmic event associated with drug-induced QT prolongation, and 91 control subjects, all of whom had provided deoxyribonucleic acid (DNA) samples. Ethnicity was self-reported as Caucasian for all cases and controls.

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### Clinical and ECG assessment

The case subjects’ acute presentation with arrhythmia and/or syncope and past medical history were assessed by obtaining hospital records, interview, and patient questionnaires. The QT and RR
intervals were measured manually from paper ECGs at stable heart rates by averaging them for up to 5 cardiac cycles (up to 10 cardiac cycles in atrial fibrillation) and Bazett’s formula was used to calculate the heart rate corrected QTc interval.

Case subjects, with drug exposure removed, and control subjects underwent resting 500-Hz digital 12-lead ECGs acquired using PC-based Cardiograph recorders (Del Mar Reynolds, Spacelabs Healthcare, Issaquah, Washington). Each automatically calculated QT interval was checked manually. If they were similar, the automatic measurement was not revised. If not, then the same method described for manual measurements was used.

**NOS1AP sequencing.** Gene sequencing of NOS1AP (ENSG00000198929) exons and intron/exon boundaries was carried out for all case subjects and control subjects using the ABI3130 System (Applied Biosystems, The Medical Biomics Centre, St. George’s University London [SGUL], London, United Kingdom) (primers available on request).

**NOS1AP Association Study**

One hundred and ninety-eight tagging SNPs, derived from the National Center for Biotechnology Information’s build 35 of the NOS1AP gene, were genotyped in all case subjects and control subjects using the Infinium Human_CVD 50K Bead Array (Illumina-IBC/CVD, Illumina Inc., San Diego, California) (20) and were analyzed using the Illumina platform 500GX (Medical Biomics Centre, SGUL). Seven SNP covered up to 4.3 kb of the upstream region of NOS1AP from the start site of the gene, and after resequencing, we found 2 more SNP, which we added to the dataset. The downstream region of the gene was not covered. Two outliers (1 case subject and 1 control subject) were excluded from the analysis as they had >10% missing data. We checked for ethnic outliers using the complete genetic dataset of the CVD chip. Multidimensional scaling analysis in PLINK (version 1.07, Shaun Purcell, Center for Human Genetic Research, Boston, Massachusetts) (21,22) revealed that 3 control subjects were not of Caucasian descent despite their self-reported Caucasian ethnicity. After these exclusions, there were 58 cases and 87 controls available for analysis. The average call rate of the remaining subjects was 99.9%. Thirty-three SNPs were nonpolymorphic, hence 167 SNPs could be analyzed for association with QT-interval prolongation and drug-induced ventricular arrhythmia.

**NOS1AP rs10919035 replication cohort.** For validation, an independent set of 28 amiodarone-treated patients collected at Vanderbilt University Medical Center, under appropriate Institutional Review Board (IRB)-approved protocols was used. Patients were of European descent from North America with drug-induced LQTS, defined as documented TdP associated with reversible QT prolongation during treatment with amiodarone. Covariates included age, sex, self-reported ethnicity, hypokalemia, and the culprit drug at the time of the index arrhythmia.

**REPLICATION DRUG-EXPOSED CONTROLS.** One hundred and five self-identified European ancestry subjects derived from a clinical study at Vanderbilt University Medical Center, under an IRB-approved protocol were included as drug-exposed control subjects. The study uses electronic medical record–based surveillance to identify patients in whom assorted QT prolonging antiarrhythmics were being initiated.

**REPLICATION NORMAL CONTROLS.** Sixty-eight self-identified European ancestry subjects derived from a clinical study at Vanderbilt University Medical Center, under an IRB-approved protocol were included as drug-exposed control subjects. Control subjects were normal, healthy volunteers recruited from the general population and challenged with an antiarrhythmic drug (ibutilide) (23).

For this study, control subjects were defined as having the absence of qualifying arrhythmias, <50 ms increase in QTc (by Bazett’s formula) interval on drug exposure, and no QTc interval exceeding 500 ms during drug treatment or ibutilide challenge.

The frequency distributions of the 2 control samples were similar, and hence the control data were pooled for analysis.

**QTc replication cohort. BRIGHT.** Over 2,000 unrelated white European hypertensive individuals from the BRIGHT (British Genetics of Hypertension Study) study (24) were genotyped using the Human_CVD BeadChip (Illumina). Of those, 1,909 individuals passed quality control checks (samples with low call rate, cryptic duplicates and relatives, outliers in ancestry principle component analysis, sex X chromosome mismatch were excluded). Of the 1,909, 1,628 individuals had 12-lead ECG recordings (Siemens-Sicard440, Siemens, Berlin, Germany) (25). For this analysis, we excluded individuals with QRS duration >120 ms (n = 83), and individuals with atrial fibrillation or persistent flutter (Minnesota codes 8-3-1 or 8-3-2; n = 25). No data were available for antiarrhythmic drug consumption. We also excluded individuals with a missing covariate (age; n = 40). Thus, data on 1,480 individuals were tested for association.

We used a normal linear model with QTc as outcome and sex, age, and 10 ancestry principal components as covariates to control for population stratification. We analyzed all SNPs within 50 kb of the NOS1AP transcript, specifically from rs4657139 (chr1:160296531) to rs457879 (chr1:160602678) inclusive. We excluded SNPs that could not be called with high confidence (4 SNPs) and 1 SNP with a call rate below 98%. The results for 195 SNPs were provided.

**Statistical analysis.** Case subjects and control subjects from the DARE study were compared for population characteristics using a chi-square test (sex) and Mann-Whitney U test (age and QTc interval). Genotype frequencies were tested for Hardy-Weinberg equilibrium using the chi-square test with 1 degree of freedom. The genotype frequencies, assuming an
additive model, were compared between the whole group of case subjects and control subjects, between subjects on amiodarone and control subjects, and between subjects on sotalol and control subjects (case-control analysis) using logistic regression analysis in PLINK (version 1.07) (21,22). Age and sex were not used as covariates because the sex distribution appeared not to be significantly different between case subjects and control subjects, and control subjects appeared to be older than case subjects were; hence, they were more likely to develop QT prolongation or ventricular arrhythmia but, nevertheless, did not demonstrate either. As QTc interval appeared to be significantly longer among the case subjects after removal of the drug than among the control subjects, we also tested the model where QTc interval was included to correct for possible mediating effects. Case subjects who had a ventricular- or atrioventricular-paced ECG or a left branch bundle block were excluded from this analysis (n = 12). The same analysis was performed in the replication study for our top SNP rs10919035.

In addition, we studied QTc interval as a quantitative trait in the population-based DARE control sample (74 of 87 controls had data on QTc interval available) in order to replicate published associations. Case subjects were not included in this analysis as they had a longer QTc interval even after removal of the drug and hence were not representative of the population. A linear regression was performed for each SNP following an additive model on QTc interval with SNP, age, and sex as covariates. A similar analysis was done in the BRIGHT cohort (n = 1,480) and the results of the 2 cohorts were meta-analyzed using the fixed-effect inverse variance method in PLINK (version 1.07) (21,22).

Because multiple SNP were tested, a multiple testing correction was applied using SNP Spectral Decomposition (26,27). This method calculates the effective number of independent marker loci accounting for linkage disequilibrium between the SNP. With this number, a Bonferroni correction is applied to assess the significance threshold. For our dataset of 167 SNP in the NOS1AP gene, the effective number was 98.8, and hence a p value less than 0.00052 was considered statistically significant. Odds ratios (OR) and 99.95% confidence intervals (CI) were calculated to assess the strength of the association. Because only 1 SNP was genotyped in the replication study for amiodarone-induced

| Table 1 Characteristics of Case Subjects and Control Subjects Included in the Analyses |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | DARE Case Subjects | DARE Control Subjects | Vanderbilt Case Subjects | Vanderbilt Healthy Control Subjects | Vanderbilt Control Subjects | BRIGHT          |
| n                              | 58               | 87               | 28               | 68               | 105             | 1,480           |
| Age, yrs                       | 62.5 ± 15.5      | 71.1 ± 10.5      | 64 ± 15.16       | 26.8 ± 5.61      | 62.2 ± 14.26    | 57.7 ± 10.18    |
| Female                         | 39 (67)          | 46 (56)          | 21 (75)          | 37 (53)          | 38 (36)         | 916 (61.9)      |
| Culpable drug exposure†        |                  |                  |                  |                  |                 |                 |
| Amiodarone                     | 27 (47)          | —                | 28 (100)         | —                | —               | —               |
| Sotalol                        | 15 (26)          | —                | —                | —                | —               | —               |
| Diuretics                      | 6 (11)           | —                | 9 (32)           | 0                | 43 (41)         |                 |
| >1 drug                        | 16 (28)          | —                | 0                | 0                | 0               | 0               |
| Presentation                   |                  |                  |                  |                  |                 |                 |
| Documented torsades de pointes | 50 (89)          | —                | 28 (100)         | NA               | 0               |                 |
| Ventricular fibrillation/cardiac arrest | 12 (21)      | —                | 0                | NA               | 0               | 0               |
| Syncope only                   | 2 (4)            | —                | 0                | NA               | 0               | 0               |
| Hypokalemia                    | 11 (20)          | —                | 7 (25)           | NA               | 0               | 0               |
| Other medical history          |                  |                  |                  |                  |                 |                 |
| Prior myocardial infarction    | 14 (25)          | —                | 3 (10)           | NA               | 14 (14)         | 0 (0)           |
| Heart failure‡                 | 12 (21)          | 0 (0)            | 9 (32)           | NA               | 1,009           | 0 (0)           |
| Atrial fibrillation and/or flutter | 29 (52)       | —                | 18 (64)          | NA               | 97 (92)         | 0 (0)           |
| Congenital long QT syndrome*   | 2 (4)            | 0 (0)            | 0                | NA               | 0               | 0 (0)           |
| Hypertension                   | 32 (57)          | —                | 13 (46)          | NA               | 65 (62)         | 1.480 (100)     |
| Hypothyroidism                 | 11 (20)          | —                | 0                | NA               | 18 (17)         | 0 (0)           |
| Diabetes mellitus              | 14 (25)          | —                | 0                | NA               | 20 (19)         | 0 (0)           |
| Maximal QTc during drug exposure, ms | 592 ± 73.3       | 584              | 375–492          | 403–556          | 464             | 467             |
| Mean ± SD                      | 359 ± 73.3       | 584              | 375–492          | 403–556          | 464             | 467             |
| Range                          | 466–850          | 523–840          | 428             | 464             | 467             | 467             |
| Median                         | 590             | 613             |                 |                 |                 |                 |
| QTc without drug exposures, ms‡ |                  |                  |                  |                  |                 |                 |
| Mean ± SD                      | 441 ± 25.9       | 426 ± 18.2       | 430             | 388             | 443             | 417 ± 12.0      |
| Median                         | 435             | 423             | 428             | 406             | 442             | 420             |

Values are mean ± SD or n (%) unless otherwise indicated. Dashes indicate that data are not available. *p < 0.05. †Available for 57 case subjects. ‡From questionnaire data only. §Available for 44 case subjects and 74 control subjects.

BRIGHT = British Genetics of Hypertension Study; DARE = Drug-Induced Arrhythmia Risk Evaluation; NA = not applicable; QTc = corrected QT interval.
QTc interval, a p value <0.05 was considered significant in this cohort.

Results

Study characteristics. Fifty-eight subjects experiencing drug-induced QT prolongation and ventricular arrhythmias (50 [89%] with documented TdP), and 87 healthy control subjects from the DARE study (Table 1) were available for the primary analysis. Control subjects were on average almost 9 years older than case subjects were (p < 0.001). No sex difference was observed. Twenty-seven (46%) cases were treated with amiodarone and 15 (27%) with sotalol, whereas 15 (27%) had received more than 1 culpable drug. Eleven cases (20%) were associated with hypokalemia. The cases also had a higher frequency of accompanying structural heart disease and, when the drug exposure was removed, demonstrated greater QTc prolongation than the control subjects did (p = 1.0 x 10^-3).

DNA sequencing and association analysis. Sequencing of the NOS1AP exonic regions and intron/exon boundaries on chromosome 1 did not identify any novel coding mutations or polymorphisms. However, resequencing 2 kb upstream of the ATG site identified 2 additional SNP that were not included on the Human_CVD 50K Bead Array and these were included in the association analysis.

SNP rs10800397 was significantly associated with drug-induced ventricular arrhythmia and QT prolongation (OR: 3.3, 99.95% CI: 1.0 to 10.8, p = 3.7 x 10^-4) (Fig. 1, Table 2). This association was driven by the group of amiodarone users (rs10800397: OR: 4.5, 99.95% CI: 1.0 to 19.8, p = 4.3 x 10^-4; case subjects: 37.0%, control subjects: 14.4%) (Fig. 1, Table 2). For this subgroup of cases, 3 noncoding SNPs were significantly associated with drug-induced ventricular arrhythmia and QT-interval prolongation (most significant SNP rs10919035: OR: 5.5, 99.95% CI: 1.1 to 27.9, p = 3.0 x 10^-3; allele frequencies: case subjects: 27.8%, control subjects: 7.1%). These SNPs did not include either of the SNP rs10494366 (OR: 1.1, p = 0.79; allele frequencies amiodarone-induced: case subjects: 37.0%, control subjects: 35.1%) and rs16857031 (OR: 1.8, p = 0.13; allele frequencies amiodarone-induced: case subjects: 27.8%, control subjects: 19.0%) known to be significant.
Table 2: Top Drug-Induced Arrhythmia Associated SNPs (p < 0.01) in the Group of 27 Amiodarone Users, the Group of 15 Sotalol Users, and the Whole Group of 58 Case Subjects Compared With 87 Control Subjects

<table>
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<th>SNP</th>
<th>Minor Allele</th>
<th>MAF (%)</th>
<th>p Value</th>
<th>OR</th>
<th>99.95% CI</th>
<th>AF (%)</th>
<th>p Value</th>
<th>OR</th>
<th>99.95% CI</th>
<th>AF (%)</th>
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<td>44.4</td>
<td>0.0098</td>
<td>2.6</td>
<td>0.7</td>
<td>8.1</td>
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<td>8.1</td>
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<tr>
<td>rs10399680</td>
<td>C</td>
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<td>39.9</td>
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<td>0.7</td>
<td>8.1</td>
<td>2.6</td>
<td>0.7</td>
<td>8.1</td>
</tr>
</tbody>
</table>

SNPs are marked according to the significant association with drug-induced arrhythmia. Significant SNPs (p < 0.00052) are marked in bold.
meta-analysis), and neither were SNPs that were in strong LD with other previously QTc-associated nontyped SNPs rs12143842 and rs12029454.

Discussion

This study is the first to demonstrate that common variations in the NOS1AP gene are associated with a significant increase in the risk of drug-induced, and in particular amiodarone-induced, ventricular arrhythmia and QT prolongation. Prolongation of the QT interval associated with TdP is currently the most common cause of withdrawal or restriction of the use of antiarrhythmic drugs (29).

We performed a comprehensive screen of 167 SNPs in and close to the NOS1AP gene in order to investigate association of NOS1AP variations with drug-induced ventricular arrhythmia and prolongation of the QT interval. SNP rs10800397 reached a significance level of \( p = 3.7 \times 10^{-4} \) in the overall DARE case-control study and this was predominantly explained by the subgroup of amiodarone users (\( p = 4.3 \times 10^{-4} \)). For this subgroup of cases, 3 SNPs were significantly associated with drug-induced ventricular arrhythmia and QT-interval prolongation. The most significant one, rs10919035, was in moderate LD with rs10800397 (\( r^2 = 0.49 \)). We carried out a replication study using amiodarone-treated cases from a second study (Vanderbilt) and these were compared with a drug-challenged control group. Although we could not fully validate our results from the DARE cases and nondrug-challenged control subjects, meta-analysis of the results of rs10919035 from both studies revealed an OR of 2.81 for each T allele (99.95% CI: 1.62 to 4.89, \( p = 2.4 \times 10^{-4} \)). Interestingly, the allele frequency among the replication and DARE cases was identical. The nonsignificance in the replication study appears to be caused by the unexpectedly higher frequency among the control subjects (17% versus 7% in DARE control subjects, 8% in 1000Genomes CEU, and 11% in HapMap Phase 2 CEU (28)) and might therefore be a population-specific effect. It is also important to note that some of the drug-challenged control subjects were challenged with ibutilide. Unlike amiodarone, ibutilide does not produce its prolongation of action potential via inhibition of potassium channels including \( I_{Kr} \), nor does it have a sodium-blocking, antiadrenergic, and calcium-blocking activity.

In contrast, ventricular arrhythmia and QT prolongation induced by sotalol were not significantly affected by NOS1AP, although the same SNPs demonstrated the largest genotype differences with smaller ORs. This subgroup was too small (\( n = 15 \)) to draw any certain conclusions about drug-specific interactions.

A common pathway between amiodarone-induced LQTS and NOS1AP common variation could be the role of the NOS regulator pathway in cardiac L-type Ca\( _V \) currents. In the prospective population-based Rotterdam Study, van Noord et al. (28) associated minor alleles of the NOS1AP SNP rs10494366 and rs10918594 with the modification of QTc prolonging effect of verapamil. Furthermore, Chang et al. (16) found that overexpression of the NOS1AP gene product in isolated guinea pig myocytes causes attenuation of the L-type Ca\( _V \) current, a slight increase in rapid delayed rectifier current (\( I_{K1} \)), and shortening of action potentials.

Data from the congenital LQTS may also support such a mechanism. Rare variants in the LQT8 gene CACNA1C, encoding a subunit of the L-type Ca\( _V \) channel, cause an unusual form of LQTS by increasing calcium influx into the myocyte (17,18). The recently identified LQT12 gene,
alpha-1-syntrophin (SNTA1), has also been shown to be involved in the nNOS pathway (19). A mutation in the gene causes inhibition of nNOS and is associated with increased peak and late sodium currents. Therefore, genes encoding proteins interacting with nNOS have the potential to alter cardiac repolarization, perhaps by influencing calcium cycling in cardiac myocytes. NOS1AP minor allele variants have more recently been associated with modification of the severity of presentation of LQTS (31,32) and the risk of sudden death in coronary artery disease (33).

In addition, we studied QT interval as a quantitative trait in population-based control subjects. In the meta-analysis of the DARE control subjects (n = 74) and the BRIGHT cohort (n = 1,480), 22 SNPs reached significance (p < 5.2 × 10^{-4}). Although the results in the BRIGHT cohort were more significant as a result of the larger sample size, the effects of the SNPs were 2× to 4× smaller than in the DARE control subjects. Furthermore, many of the top hits of this analysis overlap with the top hits of the drug-induced case-control analysis. This implies that the effect of NOS1AP on drug-induced LQTS is not independent of the effect of NOS1AP on QT interval in general. Because case subjects and control subjects already demonstrated significantly different mean QTc intervals after drug removal, we also corrected the case-control drug-induced LQTS interval analysis for baseline QTc interval. Although the results became less significant, the ORs only diminished slightly.

This suggests that the investigated QT-interval prolonging drugs and in particular amiodarone interact with NOS1AP variants. Unfortunately, our study design does not allow testing for interactions directly as all case subjects used drugs, whereas none of the DARE control subjects did and the drug-challenged control subjects from the Vanderbilt study were not on amiodarone specifically. The findings do lend support, however, to repolarization reserve being influenced by common genetic variation.

Another explanation for the larger QTc interval among case subjects after removal of the drug may be that the case subjects demonstrated a higher frequency of hypertension and underlying cardiac disease than the healthy control subjects.
subjects did. These are known acquired risk factors for QT-interval prolongation and TdP (34).

There are currently no published functional studies investigating variable expression of \textit{NOS1AP} polymorphisms. Given the shared effects of \textit{NOS1AP} and amiodarone on L-type calcium and potassium currents, however, one might hypothesize that individuals carrying genetic variants in \textit{NOS1AP}, which impair its expression, and in turn, result in increased L-type calcium currents and/or QT prolongation, may have additional arrhythmogenic risk with amiodarone therapy. These common variants may have relevance for future pharmacogenomic applications in clinical practice permitting safer prescription of amiodarone for vulnerable patients. Development of safer novel drugs may also benefit from this improved understanding of the biological pathways underlying the variation in drug response.

\textbf{Study strengths.} First, the DARE case subjects were collected prospectively and nationally in a systematic manner with identical comprehensive phenotyping, a strength compared with other series. Second, the coverage of the \textit{NOS1AP} gene was comprehensive and included 167 polymorphic SNPs in and close to the \textit{NOS1AP} gene. Third, the high ORs and level of significance despite small numbers provides compelling support for the association as does the similar frequency of SNPs in cases in the replication case-control study and the trend toward a significant association.

\textbf{Study limitations.} First, we were limited by the rarity of subjects treated with amiodarone that presented with ventricular arrhythmia and QT prolongation. Second, whereas the DARE control subjects were originally matched for age, sex, and ethnicity, it was difficult to also match for drug exposure and other comorbidity, which results in difficulties in determining whether the associations identified are caused by drug exposure or by the underlying arrhythmic event. The replication case-control study was however able to use controls exposed to QT-prolonging drugs although not amiodarone specifically. Third, as with previous resequencing efforts of \textit{NOS1AP} (9), we did not identify any missense mutations that explain the association results. The associated \textit{NOS1AP} SNPs are therefore not functional variants and are only in linkage disequilibrium with the causal SNP or regulatory DNA element.

\textbf{Conclusions}

Our study shows that common variants in the \textit{NOS1AP} gene play a role in the pathogenesis of drug-induced, and particularly amiodarone-induced, LQTS. These variants may be used in the future as markers to predict and avoid risk for drug-induced TdP in Caucasian patients who may require amiodarone therapy.

\textbf{Acknowledgments}

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