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Polymorphisms in the *NOS1AP* Gene Modulate QT Interval Duration and Risk of Arrhythmias in the Long QT Syndrome

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Objectives We investigated the role of nitric oxide 1 adaptor protein (*NOS1AP*) as a genetic modifier of long QT syndrome (LQTS).

Background LQTS risk stratification is complicated by the phenotype variability that limits prediction of life-threatening arrhythmic events based on available metrics. Thus, the identification of new markers is desirable. Recent studies have shown that *NOS1AP* variations in the gene modulate QT interval in healthy and 1 LQTS kindred, and occurrence of cardiac events in healthy subjects.

Methods The study included 901 patients enrolled in a prospective LQTS registry. Three *NOS1AP* marker SNPs (rs4657139, rs16847548, and rs10494366) were genotyped to assess the effect of variant alleles on QTc and on the incidence of cardiac events. We quantified the association between variant alleles, QTc, and outcomes to assess whether *NOS1AP* is a useful risk stratifier in LQTS.

Results Variant alleles tagged by SNPs rs4657139 and rs16847548 were associated with an average QTc prolongation of 7 and 8 ms, respectively ($p < 0.05$; $p < 0.01$); whereas rs4657139 and rs10494366 were associated with increased incidence of cardiac events (25.2% vs. 18.0%, $p < 0.05$ and 24.8% vs. 17.8% $p < 0.05$). Cox multivariate analysis identified rs10494366 minor allele as an independent prognostic marker among patients with QTc <500 ms (hazard ratio: 1.63; 95% confidence interval: 1.06 to 2.5; $p < 0.05$) but not in the entire cohort.

Conclusions Our results provide the first demonstration, to our knowledge, of a risk-conferring genetic modifier in a large LQTS cohort. Subject to confirmation in additional cohorts, we suggest that the *NOS1AP* tag SNP genotype may provide an additional clinical dimension, which helps assess risk and choice of therapeutic strategies in LQTS. (J Am Coll Cardiol 2010;55:2745–52) © 2010 by the American College of Cardiology Foundation

Long QT syndrome (LQTS) is a genetic disorder caused by mutations that affect genes encoding ion-channel subunits or proteins that indirectly modulate the function of ion channels. The disease derives its name from the diagnostic electrocardiographic feature of prolonged repolarization (QT interval). The heart of LQTS patients is predisposed to develop life-threatening arrhythmias when exposed to physical or psychological stress (1). Beta-blockers are the mainstay therapy in LQTS (1). The use of the implantable cardioverter-defibrillator (ICD) is recommended in patients

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Abbreviations and Acronyms

CI = confidence interval
ECG = electrocardiogram
HR = hazard ratio
ICD = implantable cardioverter-defibrillator
LQTS = long QT syndrome
MAF = minor allele frequency
NOS1AP = nitric oxide 1 adaptor protein
PCR = polymerase chain reaction
SCD = sudden cardiac death
SNP = single nucleotide polymorphism

at high risk of sudden cardiac death (SCD) and in those who continue to have cardiac events despite antiadrenergic therapy (2). The identification of markers of increased arrhythmic risk is a major goal of epidemiological research in LQTS. In addition to extent of QT prolongation, the major prognostic indicators (3), sex and genetic locus, have also proven useful in predicting adverse events (4,5). More recently, the effort to account for the large phenotypic variability of the disease (6) has focused on the search for additional genetic modifiers. Polymorphisms at additional loci in LQTS genes have been proposed as factors that may modulate QT interval (7); however, conclusive evidence that 1 or more polymorphisms influence the phenotype, including risk of cardiac events, of the disease is lacking.

Recently, a genome-wide association study identified an association between single nucleotide polymorphisms (SNPs) in the nitric oxide 1 adaptor protein (*NOS1AP*) gene and QT interval (8). The QT-modifying influence of *NOS1AP* SNPs was subsequently replicated in follow-up studies on the Framingham Heart and Rotterdam populations (9,10) as well as in other cohorts (11–16). Recently, the role of *NOS1AP* gene variants in sudden cardiac death has been reported in the general population (17), but the role of variants within this gene in modulating susceptibility to cardiac events in LQTS patients is only beginning to be assessed (18,19).

Here, we assessed a large cohort of LQTS patients to address whether: 1) polymorphisms of the *NOS1AP* gene modulate the duration of QT interval in LQTS; and 2) *NOS1AP* SNPs are independent predictors of risk of cardiac events in LQTS patients and may therefore contribute to risk stratification.

Methods

Study population. From our clinical database and DNA bank of LQTS patients (4–6), we identified Caucasian individuals with a clinical and/or molecular diagnosis of Romano-Ward LQTS who had been screened for mutations in the *KCNQ1, KCNH2, KCNE1, KCNE2*, and *SCN5A* genes (4–6) and for whom we had DNA and clinical data available on natural history, cardiac events, and response to therapy. Nine-hundred and one affected patients entered the study: 520 probands and 381 family members (216 first-degree relatives from 153 families). Families with more than 1 mutation were excluded.

Local and national laws regarding biomedical research, personal data protection, and confidentiality were respected. The study protocol was approved by the Maugeri Foundation Ethical Committee, and all participants or their legal guardians signed an informed consent.

Definitions. QT interval was measured at the enrollment electrocardiogram (ECG). Heart rate QT correction was performed (QTc) in lead II (or lead I or III if it could not be measured in lead II) (4–6) from 12-lead ECGs with the use of Bazett's formula during stable heart rate (4–6). Based on previous studies (4,5), we used the QTc cutoff of ≥500 ms to define high-risk patients. Cardiac events were defined as syncope, torsade de pointes, cardiac arrest, or ICD shocks (4). As previously done in our studies (4–6), we used an observation period for cardiac events from birth to either attainment of age 40 years or the initiation of antiadrenergic therapy (beta-blockers or left cardiac sympathetic denervation). Symptomatic patients are considered those who experienced a cardiac event according to the preceding definitions.

On the basis of a recent report (17), we genotyped 3 polymorphisms (rs4657139, rs16847548, and rs10494366) located within the high linkage disequilibrium block in the 5′ region of the *NOS1AP* gene (8); 3 SNPs (rs4657139, rs16847548, and rs10494366) showed minor allele frequency (MAF) >0.10 and were included in this analysis. SNPs and haplotypes are indicated by an uppercase "A" to refer to the "major" (common) allele and a lower case "a" to refer to the "minor" (rare) allele. We have compared whether the dominant, additive, or recessive model fitted better in order to predict QT or cardiac events. To do this, we performed linear and logistic regression for QT and cardiac events, respectively, taking the SNP as the predictor variable in a dominant (2 categories: AA vs. Aa or aa), additive (3 categories: AA vs. Aa vs. aa and treated as continuous variables), or recessive (2 categories: AA or Aa vs. aa). After estimating the model, the model with highest likelihood was chosen: the largest R^2 for linear model and the Nagelkerke's pseudo-R^2 for the logistic regression model. This analysis showed that the dominant model for QTc reached the best fit for both rs4657139 ($p = 0.004$) and rs16847548 ($p = 0.008$). For rs10494366, the linear trend was not significant for QTc ($p = 0.209$), indicating no significant effect of rs10494366 allele variation on QT, and both the additive and dominant model are equally appropriate. A dominant model was the best fit for cardiac events ($p = 0.022$). The SNPs having MAF >0.10 were included in the haplotypes in the following order: rs4657139 T>A, rs16847548 T>C, and rs10494366 T>G. The D′ parameter and the frequency of haplotypes ≥0.10, defined by the unphased genotypes and a dominant model, were estimated with the haplo.stats R package (Mayo Foundation for Medical Education and Research, Scottsdale, Arizona). This program uses the expectation-maximization algorithm to estimate the probability of all and of the most likely haplotype combinations. The inclusion cutoff for haplotypes was set to an estimated probability ≥0.80.

Genetic analysis. Genomic DNA was extracted from peripheral blood lymphocytes according to standard procedures. Methods for mutation screening on LQTS genes have been reported in detail previously (4–6).

Multiplex-PCR amplicons were purified with exonuclease I–shrimp alkaline phosphatase (USB, Cleveland, Ohio) and used as a template for fluorescent dideoxy single-base extension of unlabeled oligonucleotide primers with the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems, Foster City, California). A post-extension treatment with calf intestine alkaline phosphatase (New England BioLabs, Frankfurt, Germany), GeneScan-120 LIZ internal size standard addition, and heat denaturation were carried out before sequencing in an ABI PRISM 310 Genetic (Applied Biosystems).

Statistical analysis. Analysis of variance, paired and unpaired *t* tests, and cross-tabulations with Fisher exact test were used as appropriate. Classification tree analysis was used as an explorative unbiased technique to understand the structure of relationships between variables and to highlight cohort stratifications (20). We specifically used this approach in order to assess whether *NOS1AP* SNPs might add information to risk stratification in our LQTS population when analyzed together with the known metrics (QTc, sex, and genotype). Classification trees were learned using the Orange open source data mining software (University of Minnesota, Duluth, Minnesota) with gain ratio as purity measure. Correction of QTc versus *NOS1AP* statistics for multiplicity of comparisons was performed by permutation test, and permutation-corrected p values were generated using an adaptive permutation approach as implemented in PLINK open source software (Massachusetts General Hospital Center for Human Genetic Research, Boston, Massachusetts). The cumulative probability of a first cardiac event before the age of 40 years and before antiadrenergic therapy was determined in the entire population and in each of the 3 most prevalent genetic loci with the use of the life-table method of Kaplan and Meier. Log-rank test with Bonferroni correction for multiplicity of comparisons was applied. Cox multivariate survivorship analyses were performed to evaluate the significance and independence of predictors of a first cardiac events or a first cardiac arrest alone. Values of $p \geq 0.05$ were considered statistically significant. Unless otherwise specified, analyses were performed using SPSS software version 16 (SPSS Inc., Chicago, Illinois). Values of $p < 0.05$ were considered statistically significant. Means are reported ± SD.

Results

The characteristics of the 901 LQTS patients (55% women, mean age at last follow-up of 31 ± 19 years) from 520 families included in the study are shown in Table 1. LQTS gene mutations were identified in 770 patients (85%): *KCNQ1* (n = 421; 55%), *KCNH2* (n = 243; 32%), *SCN5A* (n = 83; 11%), *KCNE1* (n = 21; 2.7%), and *KCNE2* (n = 2; 0.3%). In the remaining 131 patients, no mutations were found in the 5 genes. Two-hundred four patients experienced cardiac events.

Table 1 Characteristics of LQTS Patients

LQTS patients/probands	901/520
Women	498 (55%)
Age at last follow-up (yrs)	31 ± 19
Symptomatic patients	204 (23%)
Cardiac arrest	26 (13%)
Syncope	178 (87%)
QTc (ms)	476 ± 46
LQT locus (gene)	770 (85%)
LQT1 (*KCNQ1*)	421 (55%)
LQT2 (*KCNH2*)	243 (32%)
LQT3 (*SCN5A*)	83 (11%)
LQT5 (*KCNE1*)	21 (2.7%)
LQT6 (*KCNE2*)	2 (0.3%)
Not identified LQT genotype	131 (15%)
NOS1AP SNPs MAF	
rs4657139 T>A	0.42
rs16847548 T>C	0.29
rs10494366 T>G	0.45
Haplotype frequency*	
AAA	0.50
aaa	0.26
aAa	0.11

Date presented as n (%) or mean ± SD unless otherwise noted. No significant differences in allelic frequencies in the subgroups have been identified. *The order of the polymorphisms in the haplotype is rs4657139 T>A, rs16847548 T>C, and rs10494366 T>G, where uppercase letters represent the major alleles.

LQTS = long QT syndrome; MAF = major allele frequency; NOS1AP = nitric oxide 1 adaptor protein; SNP = single nucleotide polymorphism; QTc = corrected QT interval.

MAF of SNPs and haplotypes. The MAF of the SNPs rs4657139 T>A, rs16847548 T>C, and rs10494366 T>G MAF were 0.42, 0.29, and 0.45, respectively, and these were in linkage disequilibrium (D′ >0.800). Out of the 901 subjects, *NOS1AP* haplotypes could be estimated with a probability ≥0.80 for 887 patients with 197 events (22.2%). The AAA, aaa, and aAa haplotypes were the only ones presenting with >0.10 frequency (0.51, 0.26, and 0.11, respectively). SNP MAFs and common haplotype frequencies are shown in Table 1. Haplotype analysis provided no information beyond what was indicated by SNP analysis alone; thus, these data are not included here. Detailed haplotype frequencies are reported in the online supplement (Online Table 1).

***NOS1AP* and cardiac repolarization.** SNP analysis showed that carriers (Aa or aa) of minor alleles tagged by SNPs rs4657139 and rs16847548 had a longer QTc of 7 ms ($p = 0.047$) and 8 ms ($p = 0.009$), respectively, in this cohort, whereas there was no difference in QT associated with the minor allele of rs10494366 (Table 2).

***NOS1AP* and cardiac events.** We assessed whether tag SNP variation in the 5′′*NOS1AP* gene were associated with an increased risk of cardiac events in the absence of antiadrenergic therapy. The percentage of symptomatic patients was higher among rs4657139 rare allele carriers as compared with AA homozygotes (25.2% vs. 18.0%; sex-

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Table 2 QTc Duration in the Overall LQTS Patient Cohort Shown by *NOS1AP* Single SNPs

	AA		a-Carriers		
SNP	QTc (ms)	n	QTc (ms)	n	p Value
rs4657139	472 ± 44	322	479 ± 46	579	0.047
rs16847548	472 ± 45	455	480 ± 46	446	0.009
rs10494366	475 ± 47	275	477 ± 45	626	0.486

Abbreviations as in Table 1.

adjusted hazard ratio [HR]: 1.38, 95% confidence interval [CI]: 1.01 to 1.86; p = 0.040). Similarly, rs10494366 rare allele carriers were more symptomatic than AA homozygotes (24.8% vs. 17.8%; sex-adjusted HR: 1.42, 95% CI: 1.03 to 1.96; p = 0.031). Patients with the rs16847548 minor allele did not show an increase in risk of cardiac events. Similar univariate associations between SNPs and events were also observed with Kaplan-Meier survival analysis (Online Figs. 1 to 3).

QTc, sex, and genetic locus in LQTS. Cohort records were analyzed over an observation period of 23 ± 14 years, during which 204 patients (23%) experienced a cardiac event before 40 years of age or initiation of treatment with beta-blockers (Table 1). The mean age at first cardiac event was 14 ± 10 years. Syncope occurred in 178 of 204 (87%) patients, whereas cardiac arrest occurred in the remaining 26 individuals (13%).

Among LQT1, LQT2, and LQT3 patients (n = 747), Cox regression analysis showed that QTc, sex, and genetic locus were significantly associated with clinical outcome as previously reported (4,21). Symptomatic patients had a longer QTc (500 ± 48 ms, n = 156 vs. 466 ± 40 ms, n = 591; p < 0.0001; HR for QTc ≥500 ms: 2.81, 95% CI: 2.02 to 3.90; p < 0.0001) and were more likely to be female (68.5% females in the symptomatic group vs. 51.7% among asymptomatic cases; p < 0.0001; HR for females: 1.54, 95% CI: 1.02 to 2.16; p = 0.014). Genetic locus was also associated with the outcome since LQT2 and LQT3 patients had more cardiac events as compared with LQT1 cases (HR: 1.67, 95% CI: 1.20 to 2.32; p < 0.002).

***NOS1AP* and outcome prediction in LQTS.** We assessed the value of *NOS1AP* genotyping as a predictor of adverse outcomes and as a novel additional metric for risk stratification in LQTS. To this end, we first performed classification tree analysis for unbiased identification of risk clusters. The results showed that *NOS1AP* genotype becomes informative regarding event risk only in patients with QTc <500 ms, whereas QTc and sex dominated the model for patients with QTc ≥500 ms. Accordingly, Cox regression in the cohort of 901 patients showed that none of the *NOS1AP* SNPs is a significant independent predictor of risk of events.

Overall, cardiac event distribution was remarkably different (p < 0.002) in the 4 subgroups of patients when further stratified by QTc and sex: females with QTc ≥500 ms were at highest risk of events (incidence 52%, n = 128), followed by a risk of 25% in males with QTc ≥500 ms (n = 77), a risk of 21% in females with QTc <500 ms (n = 370), and a risk of 13% among males with QTc <500 ms (n = 326). Kaplan-Meier analyses showed significant differences of event rates in the 4 groups (Fig. 1, Online Figs. 1 to 6).

When we focused on patients with QTc <500 ms, Cox regression analysis identified 2 independent markers of cardiac events: rs10494366 genotype (HR: 1.63; 95% CI: 1.06 to 2.50; p = 0.03) (Fig. 2) and female sex (HR: 1.61; 95% CI: 1.10 to 2.35; p = 0.01). Note that these data indicate female carriers of the minor allele of rs10494366 have a 2.6-fold increase in risk of arrhythmic events. Multiple regression analysis confirmed the lack of significant interaction between QTc and rs10994366. Interestingly, when we restricted the analysis to cardiac arrest only, there were 10 events in carriers of the rs10494366 minor allele and none in the common allele (log-rank p = 0.031) (Online Fig. 7).

***NOS1AP* and LQTS mutation.** Based on the published risk stratification schemes for LQT1, LQT2, and LQT3 patients (2,4), we investigated whether *NOS1AP* SNPs can add new insights for risk stratification in this group of patients. Univariate analysis showed that in the 747 patients genotyped as LQT1, LQT2, or LQT3, variant alleles in rs10494366 and rs4657139 are significantly associated with risk of cardiac events (log-rank p = 0.016 and p = 0.037, respectively). Cox multivariate analysis demonstrated that:

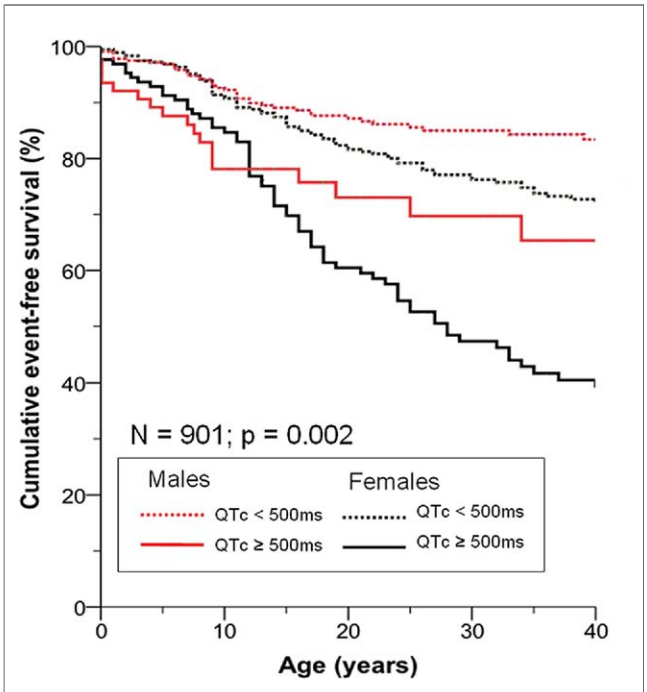

Figure 1 Survival According to QTc and Sex

Kaplan-Meier survival analysis showing the cumulative incidence of cardiac events according to sex and corrected QT interval (QTc) interval duration in long QT syndrome (LQTS) population (n = 901). Females with QTc ≥500 ms **(black continuous line)** demonstrate the worst outcome.

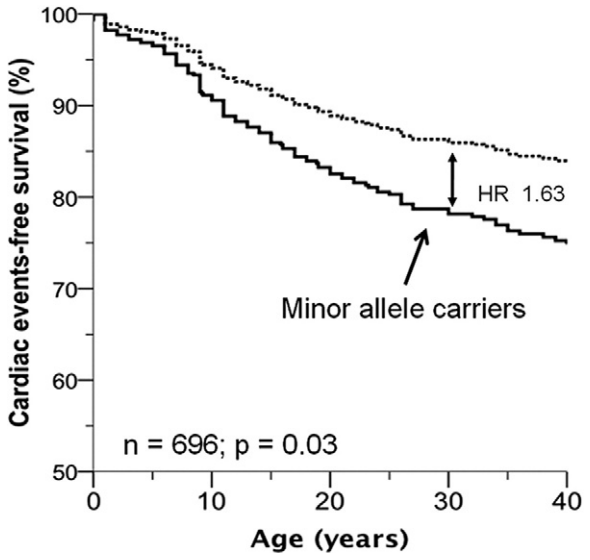

Figure 2 **Effect of rs10494366 Minor Allele in LQTS With QTc <500 ms**

Cox regression analysis showing the independent effect of *NOS1AP* rs10494366 on cumulative events-free survival in LQTS population with QTc <500 ms. The presence of at least 1 rs10494366 minor allele confers a 1.63-fold increased risk of cardiac events as compared with major allele homozygotes. **Dashed line** = n = 216 of 27 events; **solid line** = n = 480 of 92 events (19%). Abbreviations as in Figure 1.

1) the rs10494366 rare allele (HR: 1.47; 95% CI: 1.02 to 2.13; p = 0.04); 2) LQT2 or LQT3 genotype (HR: 1.76; 95% CI: 1.25 to 2.48; p = 0.001); 3) QTc ≥500 ms (HR: 2.78; 95% CI: 1.99 to 3.85; p < 0.0001); and 4) female sex (HR: 1.53; 95% CI: 1.09 to 2.16; p = 0.01) were each significant predictors of increased risk of cardiac events (Fig. 3). No significant multivariate associations were observed with rs4657139 rs16847548 (rs4657139 rare allele: HR: 1.27; 95% CI: 0.89 to 1.80; p = 0.18; rs16847548 rare allele: HR: 1.10; 95% CI: 0.89 to 1.51; p = 0.58).

Discussion

Heritability of QT interval. Dissecting how genetic variation alters the electrical substrate has been the target of many investigations and an area of intense current research. Identifying the genes that modulate QT interval and their effect on the occurrence of cardiac events has, accordingly, become one of the primary goals of molecular studies of cardiac excitability. Several groups have performed candidate gene or genome-wide association studies (7,8,11,22–24) to identify genetic modifiers of repolarization. The candidate gene approach was initially used with the idea that variants of ion channels causing also the full-blown LQTS were potential modifiers. However, observations based on this hypothesis have been either inconsistent or remained unconfirmed (7,23–25). More recently, data from genome-wide association studies identified several variants of the *NOS1AP* gene that play a major role in the modulation of cardiac repolarization (8,26,27). The link between *NOS1AP* and QTc was provided first by Arking et al. (8), who identified SNPs associated with QT duration in the upstream 5′ regulatory region of *NOS1AP*. Such association came as a surprise since this gene had not been associated with cardiac electrogenesis, although it had been implicated in schizophrenia and dystrophic muscular atrophy (28,29). *NOS1AP* encodes the CAPON protein, an adaptor molecule, which binds the neural nitric oxide (NO) synthase (Nos1) via a C-terminal PDZ domain, with scaffold and signaling ligands (e.g., dexras, dystrophins). Functional studies show that CAPON is a spatial controller of NO production, and it modulates action potential duration via an effect on Ca^{2+} and K^{+} currents (30,31).

Reproducible evidence obtained in a total of more than 15,000 subjects shows that not only is QT interval duration modulated by *NOS1AP*, but that naturally occurring polymorphisms in this gene exert the strongest influence of any of the 10 loci now known to modulate QT in humans (27). Very recently, Kao et al. (17) showed a positive association between some *NOS1AP* SNPs with incidence of SCD in a large cohort of community SCD cases.

***NOS1AP* DNA variants: from population studies to LQTS.** Given this robust evidence for the role of *NOS1AP*, it becomes important to assess whether the same common variants account for the phenotypic variability among patients with LQTS (6,32), a disease in which cardiac repolarization is profoundly affected by deleterious mutations. A recent study has suggested a role for *NOS1AP* in modulating LQTS phenotype in an ethnically isolated population carrier of *A341V* mutation in the *KCNQ1* gene (19); however, it is unknown whether the result also applies to carriers of different mutations and, most importantly, whether *NOS1AP* SNPs may be used for risk stratification. One major challenge for such studies in LQTS is the difficulty of collecting a cohort of adequate size for which clinical data and DNA samples are available. With this objective in mind, we queried the clinical database and DNA Biobank at the Maugeri Foundation and identified 901 patients in whom *NOS1AP* polymorphisms and changes in QTc and cardiac events could be studied. We chose to screen *NOS1AP* polymorphisms with a MAF higher than 10% among those that had been previously associated with QTc prolongation and/or increased risk of sudden death in different populations worldwide (8,17,26,27). We studied a population in the drug-free state in order to avoid the confusing effect of therapy on outcome; as a consequence, our results are pertinent to the role of *NOS1AP* SNPs on the natural history of LQTS.

NOS1AP rs4657139 and rs16847548 rare alleles were associated with a QTc prolongation of 7 and 8 ms, respectively, in this cohort. At first glance, such an effect seems small; however, it is within the range of the prolongation induced by drugs known to induce "acquired long QT

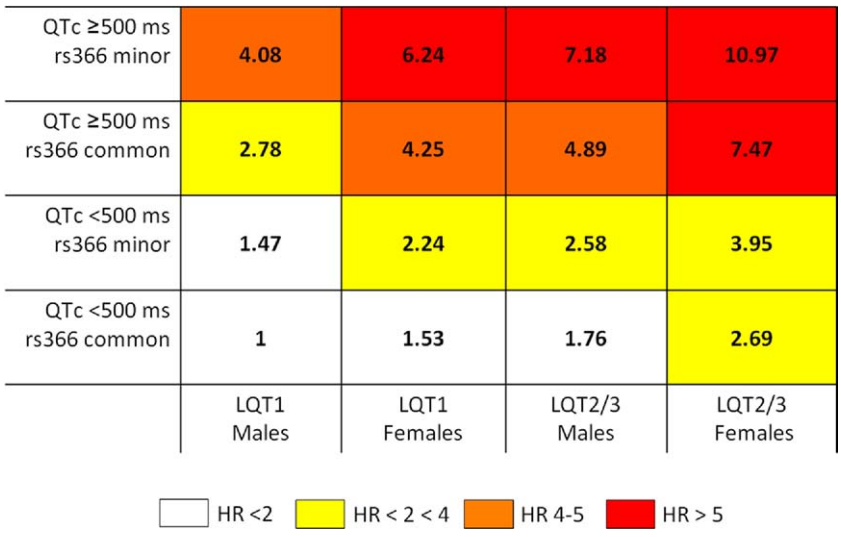

Figure 3 **LQTS Risk Stratification Scheme**

Combined hazard ratios (HRs) from Cox regression model identifies risk categories in the LQTS population. Each **box** shows the combined HR for patients with different clinical and genetic profile. To calculate the combined HR, we multiplied the HR of each independent predictor of events derived from Cox multivariate analysis with the following reference category (HR = 1) and the following features: LQT1, males, QT <500 ms homozygous for the common allele of *NOS1AP* rs10494366. Accordingly the combined HR for the category "LQT1, Males, QTc ≥500 ms, carriers of minor allele of *NOS1AP* rs10494366" is calculated as follows: LQT1 corresponds to HR = 1, Male corresponds to HR = 1, QTc ≥500 ms corresponds to HR 2.78, and carrier of minor allele of *NOS1AP* rs10494366 correspond to HR = 1.47. The combined HR is, therefore, 1 × 1 × 2.78 × 1.47 = 4.08 as shown in the corresponding **upper left corner orange box** in the figure. Reference category is represented by Males LQT1 with QTc <500 ms homozygous for the rs10494366 common allele ("TT"). Abbreviations as in Figure 1.

syndrome," such as terfenadine or cisapride, drugs that have been removed from the market by regulatory authorities based on an excess mortality related to an average QT prolongation of 6 ms (33). As shown by studies performed in different databases in the last decades, QT interval duration is the most powerful predictor of risk of arrhythmic events in LQTS (3,4). Thus, *NOS1AP* can represent a meaningful modifier in this disease.

***NOS1AP* and risk of events in LQTS.** To assess whether, besides prolonging the QT interval, *NOS1AP* SNPs contribute to modulation of susceptibility to cardiac events, we first used classification tree analysis that showed a significant stratification role for *NOS1AP* in patients with QTc <500 ms. Accordingly, Cox multivariate analysis showed that a QTc ≥500 ms is the strongest predictor of events followed by sex whereas *NOS1AP* SNPs had a nonsignificant association in the entire 901 patient cohort.

On the other hand, in patients with QTc <500 ms (n = 696), we found that the minor *NOS1AP* rs10494366 allele contributes quite significantly to risk stratification (HR: 2.6) (Fig. 2). Of note, not only the rate of cardiac events (syncope and cardiac arrest) but also the rate of cardiac arrests (hard end point) was significantly higher in the carriers of the minor allele (Online Fig. 7). Unfortunately, the total number of events was insufficient to reach multivariate statistical significance.

The new finding that variants in the *NOS1AP* region signal potential for increased risk of cardiac events and cardiac arrest in patients with QTc <500 ms has significant epidemiological and clinical implications as this group of patients represent between 65% and 75% of the total population (3,34). The more pronounced effect in patients with QTc <500 ms is conceivable since the risk of events is already so high in patients with longer QTc that it is unlikely that the presence of a single SNP can substantially modulate the risk.

The observation here that *NOS1AP* rs10494366 minor allele has not been associated with risk of arrhythmias or sudden death in other investigations (17) is reminiscent of findings regarding positional variation in different individual *NOS1AP* SNP associations reported in prior studies on other populations (8,10,11,26,27,35). As suggested by Kao et al. (17), genotyped SNPs may be considered proxy measures that are exposed to variable strength of the association with QT interval and SCD independently due to unequal linkage to the actual causal allelic variants. Therefore, we consider our finding on rs10494366 likely reflects population-specific (36) and/or disease-specific factors, as well as the possibility that there may be multiple functional elements within the *NOS1AP* region. This latter concept, if validated experimentally, would also be consistent with the presence of markers for increased risk of arrhythmic events independent from *NOS1AP* effects on QTc (17).

Based on the risk stratification model that we proposed in 2003 (4), which is now part of recommended practice guidelines for treatment of these patients (2), we analyzed our data to assess whether consideration of *NOS1AP* variation would provide additional strength to that model. As

shown in Figure 3, genotyping variation in the NOS1AP gene has much potential to expand event risk stratification for many previously identified LQT1, LQT2, and LQT3 patients.

Practical implications. Although our findings will have to be replicated in an independent LQTS population before their clinical use is proposed, it is possible to comment on the potential role of *NOS1AP* SNPs in treating LQTS patients.

When clinicians have to decide on therapy for LQTS patients, the dilemma of deciding whether the patient may need an implantable defibrillator, besides receiving beta-blockers, should be based on risk stratification schemes derived from validated epidemiological studies. We previously showed (4) that QT interval, sex, and genetic locus are the most important determinants of risk in LQT1, LQT2, and LQT3. Now, we provide evidence suggesting that *NOS1AP* SNP genotype may be a novel metric, which could help to refine risk stratification. This approach has particular potential to identify individuals at higher risk among those with QTc <500 ms; until now, these patients, who represent the majority of LQTS clinical diagnoses, have been grouped in a lower-risk category. It is thus possible that the introduction of the screening for *NOS1AP* SNPs may help the clinicians to identify subset of "QTc <500 ms patients" at higher risk, a supposition which will have to be validated by additional studies.

The potential of the discovery that *NOS1AP* variant genotypes may have validity towards this goal is illustrated in Figure 3. Based on the results of others, and our own (4) prior findings as extended here, we would consider that patients with a HR ≥6 be further evaluated as potential high-risk individuals who may benefit from ICD implantation. Patients with a HR between 4 and 6, depending on other clinical variables, may also be appropriate ICD candidates, whereas patients with a HR <4 in the absence of other risk factors (e.g., coronary or structural compromise) may be best treated with beta-blockers, again considering other cardiac risks and best clinical practices. Such considerations should obviously be in full compliance with clinical recommendations within the current American College of Cardiology, American Heart Association, and the European Society of Cardiology guidelines (2).

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Key Words: cardiac arrhythmias ■ long QT syndrome ■ single nucleotide polymorphisms ■ genetics ■ risk stratification.

APPENDIX

For a supplemental table and figures, please see the online version of this article.