Nitric Oxide Synthase 1 Adaptor Protein, an Emerging New Genetic Marker for QT Prolongation and Sudden Cardiac Death

Kuan-Cheng Chang, 1,2 Tetsuo Sasano, 3 Yu-Chen Wang 1,2 and Shoei K. Stephen Huang 4

Sudden cardiac death (SCD) is defined as sudden unexplained death due to cardiac causes with an acute change in cardiovascular status within 1 hour of onset of symptoms. Alternatively, in unwitnessed cases, SCD can also be defined as a person last seen functionally normal 24 hours before being found dead. Despite significant advances in understanding the pathophysiology of cardiovascular diseases and the resultant improvement in resuscitation science, SCD remains a major healthcare challenge worldwide. Although the most pronounced risk factor for SCD is the presence of coronary artery disease in the setting of a depressed left ventricular function, most deaths occur in the larger, lower-risk subgroups where genetic variations and other conditions may be the precipitating factors in triggering SCD. Recently, a common genetic variation in a neuronal nitric oxide synthase regulator, nitric oxide synthase 1 adaptor protein (NOS1AP), also known as carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase protein (CAPON) gene, has been identified as a new genetic marker in modulating QT interval prolongation and SCD in general populations. Animal study revealed that NOS1AP is expressed in the heart and interacts with NOS1-NO pathways to modulate cardiac repolarization via suppressing the sarcolemmal L-type calcium current and enhancing the $I_{\text{Kr}}$ current. This important genetic implication was soon replicated in other racial/ethnic populations and extended to a variety of clinical settings including diabetes mellitus, coronary artery disease, myocardial infarction, and congenital or drug-induced long QT syndrome. The purpose of this review aims to provide up-to-date information about the emerging new genetic marker, NOS1AP, in relation to QT prolongation and SCD.

Key Words: NOS1AP • QT interval • Sudden cardiac death

INTRODUCTION

Sudden cardiac death (SCD) is defined as sudden death without explanation arising from cardiac causes, involving an acute cardiovascular status change within 1 hour of onset of symptoms. In unwitnessed cases, SCD can also be defined as a person last seen functionally normal 24 hours before being found dead. The annual incidence of SCD is approximately 300,000-400,000 cases in the United States and 4~5 million worldwide.1 The underlying etiologies of SCD consist of coronary artery disease, cardiomyopathy and hereditary electrical disorders.2 Structural heart diseases secondary to coronary artery diseases or cardiomyopathy constitute the majority of SCD (90-95%), while the non-structural
heart diseases comprise the remaining 5-10% of cases in Western countries.3,4

Although the most important risk factor for SCD is the presence of coronary artery disease accompanied by a depressed left ventricular function, this highest-risk subgroup only accounts for a small fraction of SCDs.5 The largest absolute number of SCD events occurs in the relatively lower-risk subgroups where specific risk markers responsible for triggering SCD remain to be defined. On the other hand, among those patients who die suddenly without underlying structural heart disease, congenital long QT syndrome (LQTS) caused by gene mutation and resultant ion channel dysfunction leading to ventricular tachycardia and SCD has been well recognized. Apart from the rare disease-causing mutations in congenital LQTS, severe QT prolongation, torsades de pointes ventricular tachycardia and SCD can occur resulting from complex interactions among factors including common genetic polymorphisms, drugs, gender effects, metabolic/electrolytes derangement, and underlying structural heart diseases in susceptible individuals.

Recently, a common genetic variation in a neuronal nitric oxide synthase regulator, nitric oxide synthase 1 adaptor protein (NOS1AP) also known as carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON) gene, has been identified as a new genetic marker in modulating QT interval prolongation and SCD in general populations. This important genetic implication was soon confirmed in other racial/ethnic populations and extended to a variety of clinical settings including diabetes, coronary artery disease, myocardial infarction, and congenital or drug-induced LQTS. The purpose of this review is to provide up-to-date information about the emerging new genetic marker, NOS1AP, in relation to QT prolongation and SCD.

IDENTIFICATION AND PROPERTY OF NOS1AP GENE

In searching for new genetic markers responsible for QT prolongation and SCD, a whole-genome association approach has identified common genetic variants in a neuronal nitric oxide synthase regulator, NOS1AP gene, that is found to contribute to QT interval differences in a community-based population.6 This genetic finding has since provided new insights into QT prolongation and SCD.

NOS1AP, first identified in rat brain neurons, is a highly-conserved protein with an approximate 92% conceptual amino acid sequence identity between rat and human.7-9 NOS1AP protein comprises an amino-terminus phospho-tyrosine binding (PTB) domain and a carboxyl-terminus PDZ binding domain. In brain neuron, NOS1AP competes with postsynaptic density protein 95 for the binding of nitric oxide synthase 1 (NOS1) through the interaction of its carboxyl-terminus PDZ binding domain with the PDZ domain of NOS1 to uncouple the N-Methyl-D-aspartate (NMDA)-NOS1-nitric oxide (NO) mediated signaling pathways.7 NOS1AP is also an adaptor protein of NOS1, capable of directing NOS1 to other specific target proteins to exert relevant biological functions.8,9

In addition to neuronal expression in the brain, we previously have identified NOS1AP expression in the heart (Figure 1).10 The cardiac expression of NOS1AP was further confirmed by Beigi et al.11 in a study of cardiac localization of NOS1 in physiological and diseased states. They showed that the PDZ-binding domain inter-

![Immunohistochemistry staining of nitric oxide synthase 1 adaptor protein (NOS1AP) also known as carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase (CAPON, green) and actin (red) in left ventricular tissues from rats showing a cytoplasmic distribution pattern of the soluble NOS1AP protein. Myocytes nuclei (blue) are stained by 4',6-diamidino-2-phenylindole (DAPI).](image-url)
action between NOS1AP and NOS1 contributes to NOS1 localization in specific organelles within cardiomyocytes. NOS1AP co-immunoprecipitated with the mu and alpha isoforms of NOS1 in whole heart lysates, and co-localization of NOS1AP and NOS1 was present in the sarcoplasmic reticulum and mitochondria with dual immunogold electron microscopy.11

The QT interval reflects global ventricular repolarization, which is the duration of action potentials of all ventricular cardiomyocytes. Prolonged repolarization in cardiac ventricular myocytes is caused by an increase in depolarizing inward currents and/or by a decrease in repolarizing outward currents. We found that NOS1AP over-expression accelerates cardiac repolarization via suppressing the sarcolemmal L-type calcium current and enhancing the $I_{Kr}$ current, which was mediated by the interaction with NOS1-NO pathways in ventricular cardiomyocytes. These findings provide the rationale for the association of NOS1AP gene variants and the extremes of QT interval in humans.

COMMON NOS1AP VARIANTS AND RISK OF SCD IN GENERAL POPULATION

Soon after the discovery by Arking et al. in 2006 in a genome-wide association study that a common variant of NOS1AP (rs10494366) in the non-coding region is significantly associated with QTc interval variation in a community-based German population, a number of studies have confirmed similar results (Table 1). Aarnoudse et al.12 reported that in a population-based, prospective cohort study of individuals aged ≥ 55 living in the Rotterdam area in the west of Netherlands, both rs10494366 and rs10918594 variants were significantly associated with QTc interval prolongation (3.8 ms and 3.6 ms increase in QTc, respectively for each additional allele copy). Over 11.9 median years of follow-up of 233 cases involving SCDs, no significant association of both NOS1AP variants was found with the risk of SCD. However, Eijgelsheim et al.15 demonstrated that when the SCD definition was restricted to only witnessed SCD, significant associations of rs12143842 and its proxy rs16847549 with SCD were seen, despite the fact that the case number was reduced in the Rotterdam Study population. They further showed that combining the Rotterdam Study with the data from the Cardiovascular Health Study and the Atherosclerosis Risk in Communities study further strengthened the overall significance of the association between rs16847548 and SCD, irrespective of the phenotype definition. In the Atherosclerosis Risk in Communities Study and the Cardiovascular Health Study, Kao et al.16 analyzed the association of NOS1AP variants with SCD in a combined population of 19,295 black and white adults by examining 19 tagging single nucleotide polymorphisms (SNPs) in the genomic blocks containing rs10494366 and rs4657139 in NOS1AP. They found that multiple SNPs in NOS1AP, including rs10494366, rs4657139, and rs16847548, were significantly associated with adjusted QT interval in whites. In whites, both rs16847548 and rs12567209 were independently associated with SCD even after considering a variety of confounders including QT interval and coronary heart disease risk factors.

Table 1. Common NOS1AP variants in general populations

<table>
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<tr>
<th>SNPs</th>
<th>rs10494366</th>
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<tr>
<td>Author</td>
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<td>Aarnoudse et al.11</td>
<td>Post et al.12</td>
<td>Tobin et al.13</td>
<td>Kao et al.15</td>
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<tr>
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<td>2009</td>
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NA, not applicable; SNPs, single nucleotide polymorphisms.
+ denotes significant association with prolongation of adjusted QT interval or sudden cardiac death.
* Restricted to witnessed sudden cardiac death; † Only seen in women; ‡ Only re16847548; § American black and white adults.
On the other hand, there were no significant associations between tagging SNPs in NOS1AP and either QT interval or SCD in blacks.

Post et al.\textsuperscript{13} conducted another study to replicate the association between NOS1AP variants and the QT interval in a more genetically homogenous population, the Old Order Amish people, living in Lancaster County, Pennsylvania, United States. They found that two (rs1415262 and rs10494366) of the four selected SNPs were significantly associated with variation in adjusted QT interval. Tobin et al.\textsuperscript{14} assessed gender-specific NOS1AP associations with resting QTc in 919 women and 918 men from 504 representative families in the UK GRAPHIC study, and found that the minor allele (G) of the NOS1AP SNP rs10494366 significantly prolonged QTc by 4.59 ms in women, but only by 1.62 ms in men, suggesting the existence of gender-specific effects of the NOS1AP variant on QTc interval prolongation in this population. In addition to these findings, a variety of new genetic loci within NOS1AP associated with QT variation have since been identified over the past few years,\textsuperscript{17-22} and the association of NOS1AP variants and QT variation has further been shown in non-European populations.\textsuperscript{23-26} All of these studies provide strong evidence that common genetic variants of NOS1AP may influence QT interval in healthy populations across different races.

### COMMON NOS1AP VARIANTS AND DIABETES MELLITUS

Diabetes is a strong risk factor for SCD.\textsuperscript{27,28} QT interval prolongation has been associated with both type 1 and type 2 diabetes.\textsuperscript{29-33} Diabetic patients with longer QTc have a threefold increased risk of SCD after adjusting for clinical and other electrocardiographic (ECG) or autonomic characteristics,\textsuperscript{29} thus linking QTc prolongation as a particularly potent risk factor in the diabetic population. The relationship between diabetes and QT dispersion has been attributed to acquired autonomic dysfunction in humans.\textsuperscript{34} However, cardiac ion channel remodeling, predominantly involving the transient outward K\textsuperscript{+} current ($I_{to}$), slowly delayed rectifier K\textsuperscript{+} current ($I_{Ks}$) and rapidly delayed rectifier K\textsuperscript{+} current ($I_{Kr}$), and has been shown to underlie diabetic QT prolongation in animal models.\textsuperscript{30,32} Recently, Lehtinen et al.\textsuperscript{35} demonstrated that common genetic variants in NOS1AP in families with a genetic susceptibility to diabetes are independently associated with QTc prolongation, even accounted for the strong effect of diabetes on repolarization. The strength of the effect of NOS1AP SNPs on QTc prolongation in European-American diabetic subjects was 11.3 and 13.9 ms differences between minor and major homozygotes respectively for SNP rs10494366 and SNP 10918594. This difference was much greater than that observed in the previous reports by Arking\textsuperscript{6} (4-8 ms), Aarnoudse\textsuperscript{12} (6.3-7.2 ms), and Post\textsuperscript{13} (0.2-6.1 ms) (Figure 2). They thus suggest a positive synergistic interaction between diabetes and NOS1AP genetic variants that alter myocardial repolarization. Furthermore, in Lehtinen’s study, 34% of all European-American diabetic subjects in the Diabetes Heart Study were using a QT-altering medication. It is therefore possible that use of QT-prolonging medication in the diabetic population, along with the presence of QT-prolonging alleles of NOS1AP, may increase the risk of arrhythmias and SCD in this patient subset. Although the interactions between diabetes and NOS1AP genetic variants may exist and cause more QTc prolongation in this European-American diabetic population, whether these findings can be applied to nonwhite diabetic patients remains unclear. In a recent study by Lu et al.,\textsuperscript{36} three candidate SNPs rs10494366, rs12143842 and

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**Figure 2.** Differences of the adjusted QT interval between major and minor homozygotes for NOS1AP single nucleotide polymorphism, rs10494366, in four population studies. Note the greater extent of adjusted QT interval difference in a type 2 diabetes – enriched sample of European ancestry reported by Lehtinen et al.\textsuperscript{35} The QT interval was corrected by heart rate, age and sex in Arking’s study;\textsuperscript{6} by heart rate, age, sex, and family structure in Post’s study;\textsuperscript{13} by Bazett’s formula in Aarnoudse’s\textsuperscript{12} and Lehtinen’s\textsuperscript{35} studies.
rs12029454 were genotyped in 1240 Chinese Type 2 diabetic patients (631 men and 609 women) and 1196 normal controls (433 men and 763 women). Interestingly, they found that in the diabetic group, the rs12143842 T allele was associated with a 3.87 ms increase in QTc interval for each additional allele copy, while rs10494366 and rs12029454 showed no significant association with QTc. Additionally, there was no evidence of association for the three SNPs in subjects with normal glucose regulation and no significant SNP-gender and -diabetes interaction was observed. Thus, further studies with fine mapping of the association of the NOS1AP locus with QT interval are important to clarify whether NOS1AP variants potentiate QTc prolongation and risk for SCD in type 2 diabetic populations.

COMMON NOS1AP VARIANTS AND CORONARY ARTERY DISEASE

Approximately 80% of SCDs occur in the setting of coronary artery disease with ventricular tachycardia or ventricular fibrillation as the major arrhythmias. Recent candidate gene association studies for SCD in patients with coronary artery disease have identified several susceptible common variants including β2 adrenergic receptor gene, angiotensin-converting enzyme pathway genes and the transforming growth factor β-receptor 2 gene. However, these studies are either lacking in unified SCD phenotype definitions or have yet to be validated. Aouizerat et al. conducted a genome-wide association case-control study comparing 89 patients with coronary artery disease and SCD due to ventricular tachycardia or ventricular fibrillation to 520 healthy controls, and found that rs4292933 in NOS1AP was among the 14 SNPs spanning 11 genes that were associated with an elevated likelihood of SCD. Westaway et al. conducted a candidate-gene approach using haplotype tagging SNPs to identify genes associated with SCD risk in the context of coronary artery disease in 291 subjects from the Oregon Sudden Unexpected Death Study. They reported that common NOS1AP variants (rs12084280 and rs10918859) are associated with increased risk of SCD in patients with coronary artery disease. Further studies are needed to verify whether common genetic variants in NOS1AP play a role in SCD risk stratification of non-Caucasian patients with coronary artery disease.

COMMON NOS1AP VARIANTS AND CONGENITAL LONG QT SYNDROME

Congenital LQTS is the most common genetically heterogeneous disorder predisposing affected individuals to SCD. Common genetic variants other than the primary mutation have been shown to modify the probability of life-threatening events. Crotti et al. investigated whether common variants in NOS1AP modify the risk of clinical manifestations and the magnitude of QT prolongation in a South African LQTS population segregating a founder mutation in KCNQ1 /A341V using a family-based association analysis. They found that NOS1AP variants (rs4657139 and rs16847548) were significantly associated with the occurrence of symptoms and disease severity, as manifested by a greater probability for cardiac arrest and sudden death. The QT interval also tends to be longer (in the top 40% of values) among all mutation carriers (rs4657139 and rs16847548). Tomás et al. investigated the role of NOS1AP as a genetic modifier of congenital LQTS in 901 patients enrolled in a prospective LQTS registry by genotyping three NOS1AP marker SNPs (rs4657139, rs16847548, and rs10494366). They found that SNPs rs4657139 and rs16847548 were associated with an average QTc prolongation of 7 and 8 ms, respectively, whereas rs4657139 and rs10494366 were associated with increased incidence of cardiac events. Interestingly, the rs10494366 minor allele was identified as an independent prognostic marker among patients with QTc < 500 ms, but not in the entire cohort according to Cox multivariate analysis. However, in a recent clinical and genetic analysis of a single Saudi family, Shinwari et al. reported that both rs4657139 and rs16847548 NOS1AP variants did not correlate with the cardiac symptoms or with the QTc intervals. Results from the single family study appear inconsistent with the large LQTS cohort analysis, suggesting that the LQTS phenotypes may be modified by varying NOS1AP SNPs across races. Nevertheless, emerging evidence supports the notion that NOS1AP not only affects the QTc interval in a general population, but also

221 Acta Cardiol Sin 2013;29:217–225
influences sudden death risk in subjects with LQTS. Therefore, NOS1AP can be regarded as a genetic modifier and is clinically useful for risk stratification in patients with congenital LQTS.

COMMON NOS1AP VARIANTS AND DRUG-INDUCED LONG QT SYNDROME

Verapamil use has been shown to be associated with a significant increase in the QTc interval,\(^{49-51}\) whereas the QTc prolonging effect was not consistently confirmed for other types of calcium channel blockers, including amlodipine, isradipine, nifedipine, and diltiazem. A high-affinity blockage of the HERG current by verapamil (IC\(^50\) range 0.14-0.83 mmol/l), compared to weak or no blockage of this current by other calcium channel blockers and might be responsible for these differential effects.\(^{49-51}\) We have previously shown that NOS1AP overexpression activates the NOS1-NO pathway, resulting in suppression of the L-type calcium current and enhancement of the rapidly delayed rectifier potassium current in ventricular cardiomyocytes.\(^{10}\) Barouch et al.\(^{52}\) observed that NOS1 stimulates sarcoplasmatic reticulum Ca\(^{2+}\) release, which leads to increased intracellular calcium. The elevation of intracellular Ca\(^{2+}\) suppresses the Ca\(^{2+}\) entry pathway, the L-type calcium channels,\(^{53}\) and selectively enhances the delayed rectifier current, which leads to increased outflow of potassium.\(^{53}\) Consequently, it is reasonable to assume that calcium channel blockers may potentiate the QTc prolonging effect in subjects with common NOS1AP variants. This concept has been proven by van Noord et al.\(^{54}\) in the prospective population-based Rotterdam Study. van Noord et al.\(^{54}\) found that use of verapamil was associated with a significant QTc interval prolongation of 6.0 ms compared to non-users. Furthermore, verapamil users with the rs10494366 GG genotype had significantly more QTc prolongation [25.4 ms (95% confidence interval: 5.9-44.9)] than those with the TT genotype. Compared to verapamil, dihydropyridine calcium channel blockers (amlodipine, nifedipine and others), have a higher affinity for vascular calcium channels with a greater effect of vasodilatation. Becker et al.\(^{55}\) showed that the minor G allele of rs10494366 in the NOS1AP gene is associated with increased all-cause and cardiovascular mortality in Caucasian users of dihydropyridine calcium channel blockers. The putative mechanism is possibly related to changes of NOS-NO mediated vasodilatation rather than via the QT prolonging effects.

Antipsychotic drugs are among the drugs causing QT prolongation, which may trigger lethal ventricular tachyarrhythmias in susceptible patients. Identifying the genetic variants that mediate or potentiate antipsychotic-induced QT prolongation may help to minimize this risk. Aberg et al.\(^{56}\) performed candidate gene analysis and five drug-specific genome-wide association studies to search for genetic variation mediating antipsychotic-induced QT prolongation in 738 schizophrenia patients from the Clinical Antipsychotic Trial of Intervention Effectiveness study. They found that, though it may not be directly linked to the metabolic pathways of antipsychotic medications, NOS1AP was involved in antipsychotics-induced QT prolongation. Furthermore, they identified SLC22A23 with high genome-wide significance, which mediates the effects of quetiapine on QT prolongation.

Use of antiarrhythmic medications is often limited by the potential risk of serious adverse events including drug-induced QT prolongation and torsades de pointes. Jamshidi et al.\(^{57}\) performed an association study using 167 SNPs spanning the NOS1AP gene in 58 Caucasian patients experiencing drug-induced LQTS and 87 Caucasian controls from the DARE (Drug-Induced Arrhythmia Risk Evaluation) study. They found that, the minor G allele of rs10919035 in the NOS1AP gene is associated with increased all-cause and cardiovascular mortality in Caucasian users of dihydropyridine calcium channel blockers. The putative mechanism is possibly related to changes of NOS-NO mediated vasodilatation rather than via the QT prolonging effects.

These results provide the evidence that common NOS1AP variants are associated with a significant increase in the risk of drug-induced LQTS including use of calcium channel blockers (notably verapamil), antipsychotics and antiarrhythmics (Table 2). These studies also suggest that common variations in the NOS1AP gene are important for future pharmacogenomic applications in clinical settings to allow safer prescription of these drugs in susceptible patients.

In addition to the previous reports investigating the association of NOS1AP variations and drug-induced
LQTS, we recently conducted a prospective study to evaluate the interactions between selected NOS1AP SNPs and methadone in a heroin-dependent population. Because both methadone and NOS1AP affect $I_{Kr}$ current, we therefore hypothesized that NOS1AP might play a role in regulating methadone-associated QTc prolongation. Our preliminary results (unpublished data) showed that methadone maintenance therapy is associated with significant QTc lengthening, and NOS1AP variant at rs10918594 may further modify the QTc prolonging effects by methadone in 122 patients with complete genotyping data who received an ECG examination at baseline and 51 days after starting maintenance methadone treatment.

**CONCLUSIONS**

NOS1AP, has emerged as a new genetic marker in modulating QT interval prolongation and SCD in multi-racial/ethnic general populations and in a variety of clinical settings including diabetes mellitus, coronary artery disease, myocardial infarction, and congenital or drug-induced LQTS. Although the detailed mechanistic pathways and mediators remain to be defined, the new information derived from genomic approaches has provided new insights into the global public health problem of SCD. Such advances offer not only new directions for risk stratification but also additional opportunities for discovery of novel antiarrhythmic targets and therapies.

**REFERENCES**


