Systematic Meta-Analysis of the Association Between a Common NOS1AP Genetic Polymorphism, the QTc Interval, and Sudden Death

Xiaobiao Zang,^{1*} MD, Sisi Li,^{2*} PhD, Yonghui Zhao,¹ MD, Ke Chen,¹ MD, Xianqing Wang,¹ MD, Weifeng Song,¹ MD, Jifang Ma,¹ MD, Xin Tu,³ PhD, Yunlong Xia,⁴ MD, Shulong Zhang,⁵ MD and Chuanyu Gao,¹ MD

Summary

Contemporary studies have identified rs10494366 in the nitric oxide synthase 1 adaptor protein (NOS1AP) gene as a new genetic marker in modulating the QT interval and sudden cardiac death (SCD) in general populations. However, the conclusions were not coincident. Therefore, we conducted for the first time a system evaluation of the relativity of rs10494366, the QT interval, and sudden death by meta-analysis. In our study, the meta-analysis displayed the GG genotype of rs10494366 correlated with the QT interval in women with no heterogeneity, and in diabetes mellitus (DM) patients with minor heterogeneity. In the Caucasian population, the correlation of rs10494366 and sudden death was significant. The heterogeneity referred to the relevance between rs10494366 and sudden death in the Asian population. In conclusion, the minor allele of rs10494366 may have an impact on the QT interval in women or DM patients and may have a potential role in sudden death in the Caucasian population.

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Key words: Sudden cardiac death, Single nucleotide polymorphism

Sudden death is the leading cause of mortality in developed countries, and accordingly an important public health concern. Sudden death is mostly cardiovascular and arrhythmic in origin, with ventricular fibrillation as the ultimate precipitating mechanism of cardiac arrest. The mechanism of sudden death is complex, and currently not completely understood. Many clinical and genetic indicators have been identified and helped in distinguishing high-risk patients in previous studies. In contemporary studies, rs10494366 in the nitric oxide synthase 1 adaptor protein (NOS1AP) gene was identified as a novel genetic marker in modulating the QT interval and sudden cardiac death (SCD) in the general population.¹⁻³⁾ However, the conclusions of the studies were not coincident,^{4,5)} and meta-analysis was considered.

Methods

Databases, including PubMed, Embase, ScienceDirect, Google Scholar, WanFang, and CNKI were searched to identify relevant studies up to August 2018. The meta-

analysis included two components: the association of rs10494366 and the QT interval, as well as the association of rs10494366 and sudden death. The following terms were included in the search: “rs10494366” and “polymorphism or variant” and “nitric oxide synthase 1 adaptor protein or NOS1AP” and “QT interval or QT or QT prolongation” for the meta-analysis involving the QT interval and rs10494366. The inclusion criteria were as follows: 1) studies were in Hardy-Weinberg equilibrium (HWE) and performed in sporadic population; 2) studies that provided data involving the correlation coefficient and the 95% confidence interval (CI) or standard error for linear correlation of the QTc interval and rs10494366. Studies in which patients were enrolled with one or more mutation sites were excluded from the analysis. Studies were also excluded if the correction of the QT (QTc) interval did not use the Bazett’s formula ($QTc = QT / \sqrt{RR}$). The following search terms were used for the meta-analysis of sudden death and rs10494366: “nitric oxide synthase 1 adaptor protein or NOS1AP” and “polymorphism or variant” and “heart arrest or SCD or sudden arrhythmic death

From the ¹Zhengzhou University People’s Hospital, Fuwai Central China Cardiovascular Hospital, Zhengzhou University, Zhengzhou, China, ²Gannan Medical University, Jiangxi, China, ³Key Laboratory of Molecular Biophysics of the Ministry of Education, College of Life Science and Technology and Center for Human Genome Research, Cardio-X Institute, Huazhong University of Science and Technology, Wuhan, China, ⁴First Affiliated Hospital of Dalian Medical University, Dalian, China and ⁵Department of Cardiology, Affiliated Zhongshan Hospital of Dalian University, Dalian, China.

*These authors contributed equally to this work.

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Address for correspondence: Chuanyu Gao, MD, Zhengzhou University People’s Hospital, Fuwai Central China Cardiovascular Hospital, Zhengzhou University, No. 1 Fuwai Avenue, Zhengzhou, China. E-mail: gaochuanyu01@163.com

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Figure 1. Flowchart of the study selection process of the meta-analysis of the association between rs10494366 and the QT interval.

or sudden cardiac arrest or sudden death.” The inclusion criteria were as follows: 1) case-control studies focused on the association between NOS1AP and sudden death; 2) studies that provided sufficient genotype data (TT, TG, and GG) in cases as well as control groups; and 3) all death events had definitive diagnostic criteria. Exclusion criteria were as follows: 1) no detailed genotype frequencies and 2) rs10494366 was deviated from HWE in the control group. For the two meta-analyses in this study, the abstracts, reviews, simple commentaries, editorials, case reports, and meta-analyses were excluded. When overlapping studies were obtained, the study with the largest sample size was included. If data in a selected study was lacking, the authors were contacted to see if more information could be obtained. Only studies published in the English or Chinese language were included.

Two reviewers independently extracted data, and any disagreement was resolved by joint review until a consensus was reached. For the association of the QT interval and rs10494366, author, publication year, country, ethnicity, study name, numbers of all subjects and each genotype, QT interval or QTc interval of all subjects and each genotype, minor allele frequency (MAF) in the entire group or subgroup, linear regression coefficient (β), and standard error (SE) were extracted from candidate studies. Simultaneously, in the meta-analysis of rs10494366 and sudden death, author, publication year, country, ethnicity, MAF in controls, related diseases, and the distribution of three genotypes in the case and control groups were extracted. The Newcastle-Ottawa Quality Assessment Scale (NOS) was used independently by two authors to evaluate the quality of the included studies (<http://www.ohri.ca/pro>

grams/clinical_epidemiology/oxford.asp). Studies with a score of seven stars or greater were considered of high quality and were included in the current meta-analysis.

In this study, pooled effect size β and SE were used to determine the strength of associations between rs10494366 and QT interval under an additive genetic model. β was pooled using a random-effect model if heterogeneity existed, otherwise, the fixed model was conducted. Analysis of the correlation between rs10494366 and SCD was implemented by ORs with 95% CIs in four genetic models. The heterogeneity Q-test and I-squared test were used to assess the heterogeneity between studies. $P < 0.05$ and I-squared $\geq 50\%$ were considered of significant heterogeneity. Subgroup analysis was performed to explore the sources of heterogeneity. Publication bias was calculated using Begg’s funnel plot or Egger’s plot, and the significance level for both was set at 0.05. Meta-analysis was performed using STATA 12.0 (STATA Corp, College Station, TX, USA). P -value < 0.05 was considered statistically significant.

Results

For the meta-analysis of rs10494366 and the QTc interval, a total of 266 studies were selected through a database search. A total of eight eligible studies were enrolled after removing duplicates, screening by title and abstract review, and assessment of eligibility criteria (Figure 1). The total number of subjects in the present study is 45,206, which included 27,556 Caucasian, 14,715 African, 2436 Asian, and 499 Hispanics.^{1,6-12)} Five studies consisted of more than one study cohort (Table I).

Table 1. Main Characteristics of Studies Included in the Meta-analysis Association Between G Allele of rs10494366 and the QT Interval

Author	Year	Country	Ethnicity	Study cohorts	N-all	QT-all (ms)	N-TT	QT-TT (ms)	N-TG	QT-TG (ms)	N-GG	QT-GG (ms)	β	SE	MAF
Tobin MD	2008	UK	Caucasian	GRAPHIC-Women	918	409.93 $\pm$ 20.05	-	-	-	-	-	-	4.59	0.93	0.40 $\triangle$
Tobin MD	2008	UK	Caucasian	GRAPHIC-Men	919	398.70 $\pm$ 19.46	-	-	-	-	-	-	1.62	0.9	-
Tobin MD	2008	Finland	Caucasian	FINCAVAS-Women	699	422.51 $\pm$ 23.07	-	-	-	-	-	-	2.16	1.31	0.34 $\triangle$
Tobin MD	2008	Finland	Caucasian	FINCAVAS-Men	1225	420.91 $\pm$ 24.39	-	-	-	-	-	-	0.86	1.03	-
Lehtinen AB	2008	USA	Caucasian	Diabetes Heart	568	393.21 $\pm$ 33.22	238	386.4 $\pm$ 20.2	266	392.8 $\pm$ 19.1	64	395.7 $\pm$ 21.7	5.3	0.74	0.35
Lehtinen AB	2008	USA	African	Diabetes Heart	113	394.85 $\pm$ 31.90	22	386.1 $\pm$ 20.6	51	385.1 $\pm$ 18.2	40	391.5 $\pm$ 23.4	3.2	0.73	0.54
Raitakari OT	2009	Finland	Caucasian	Cardiovascular Risk in Young Finns	1842	369.92 $\pm$ 23.75	760	367 $\pm$ 23	845	371 $\pm$ 24	237	373 $\pm$ 22	3.3	0.82	0.36
Pfeuffer A	2009	USA, Germany, Italy	Caucasian	Five population-based cohorts ∇	15842	-	-	-	-	-	-	-	-2.2	0.2	-
Arking DE	2009	USA	African	DHS, ARIC, CHS	1497	-	-	-	-	-	-	-	1.22	0.63	0.62
Arking DE	2009	USA	Caucasian	DHS, ARIC, CHS	940	-	-	-	-	-	-	-	0.72	0.60	0.36
Arking DE	2009	USA	Hispanics	DHS, ARIC, CHS	499	-	-	-	-	-	-	-	1.52	0.84	0.41
Eijgelsheim M	2009	Netherlands	Caucasian	Rotterdam-women	2751	430.8 $\pm$ 21.6	1128	-	1262	-	361	-	3.8	0.46	0.36
Eijgelsheim M	2009	Netherlands	Caucasian	Rotterdam-men	1852	420.3 $\pm$ 23.5	755	-	848	-	249	-	3.1	0.56	0.36
Lu J	2010	China	Asian	Shanghai Diabetes-T2DM women	609	421.72 $\pm$ 38.64	-	409.68 $\pm$ 36.28 $\blacktriangle$	-	412.14 $\pm$ 40.14 $\blacktriangle$	-	413.82 $\pm$ 35.36 $\blacktriangle$	3.35	2.43	0.37 $\triangle$
Lu J	2010	China	Asian	Shanghai Diabetes-T2DM men	631	400.73 $\pm$ 33.97	-	-	-	-	-	-	0.54	2.06	-
Lu J	2010	China	Asian	Shanghai Diabetes-control women	763	400.26 $\pm$ 33.07	-	397.24 $\pm$ 33.99 $\blacktriangle$	-	397.49 $\pm$ 31.49 $\blacktriangle$	-	392.87 $\pm$ 30.35 $\blacktriangle$	2.02	1.73	-
Lu J	2010	China	Asian	Shanghai Diabetes-control men	433	390.06 $\pm$ 31.20	-	-	-	-	-	-	0.22	2.19	-
Smith JG	2012	USA	African	10 GWA studies $\blacktriangledown$	13105	-	-	-	-	-	-	-	0.83	0.26	0.59

$\triangle$ MAF in entire group; $\blacktriangle$ the length of QTc interval in entire subgroup; ∇ the cohorts include ARIC, KORA, Sardinia, GenNOVA, and HNR from Europe and the United States; $\blacktriangledown$ the cohorts include ARIC, BHS, BLSA, CFS, CHS, Health ABC, HANDLS, JHS, MESA, and WHI from the United States. MAF indicates minor allele frequency.

Figure 2. Forest plots for the association between rs10494366 and the QT interval in all groups using the additive genetic model.

Table II. Subgroup Meta-analysis of the Association Between rs10494366 and the QT Interval

	Study number	Heterogeneity statistic	I-squared	Z	P
Gender					
Men	5	6.16	35.0%	3.13	0.002
Women	5	3.31	0.0%	9.76	0.000
Mixed	8	222.07	96.8%	1.85	0.064
Subjects number					
≤ 1000	11	33.65	70.3%	4.19	0.000
> 1000	7	250.02	97.6%	1.52	0.130
Age (years)					
< 45	9	162.98	95.1%	1.66	0.097
≥ 45	9	38.56	79.3%	4.72	0.000
Diseases					
DM	4	7.18	58.2%	3.93	0.000
Others	14	286.23	95.5%	2.42	0.015
Study design					
PB	12	283.23	96.1%	2.26	0.024
HB	6	15.48	67.7%	3.35	0.001
Ethnicity					
Caucasian	10	319.80	97.2%	2.10	0.035
Asian	4	1.23	0.0%	1.45	0.146
African	3	9.39	78.7%	2.45	0.014
Hispanics	1	0.00	-	1.81	0.070

DM indicates diabetes mellitus; HB, hospital-based; and PB, population-based.

Figure 2 shows the results of the random-effect model combining the β for QTc interval. Among the 18

studies, seven showed a significant positive relation between SNP rs10494366 and QTc interval. For the correlation, the pooled effect size β was 3.07, but with a huge heterogeneity in statistics ($Q = 350.87$, $I^2 = 95.2\%$). Subsequently, meta-analysis of effects in the subgroup was performed, and the results are shown in Table II. The subgroup analyses included gender, subject number, age, diseases, study design, and ethnicity. There were five cohorts that focused on gender differences. The pooled effect size was 9.76 for the women cohort in which no heterogeneity emerged ($Q = 3.31$, $I^2 = 0.0\%$). The I^2 was 35.5% and 96.8%, respectively, for men and mixed individuals. All studies were divided into two subgroups based on whether the number was greater than 1000, and all showed significant heterogeneity. Similarly, when studies were divided into two subgroups based on whether the age was > 45 years, whether subjects were combined with DM, and the type of study design, all pooled effect sizes showed heterogeneity. The association of rs10494366 and the QT interval in DM patients was significant with heterogeneity statistic equaling 7.18 ($P = 0.066$) and $I^2 = 58.2\%$. In the Asian population, the pooled effect size was not significant, with a z-value being 1.45. However, in Caucasian and African populations, the associations were significant but with a huge heterogeneity. Only one case focused on Hispanics and the results indicated a negative association. The Begg's and Egger's tests were conducted for women and DM subgroups, respectively (Supplemental Figures 1, 2), and our data showed that there was no publication bias in these two subgroups.

Figure 3. Flowchart of the study selection process in the meta-analysis of the association between rs10494366 and sudden death.

Table III. Main Characteristics of Studies Included in the Meta-analysis of rs10494366 and Sudden Death

Author	Year	Country	Ethnicity	Case			Control			MAF	Related diseases
				GG	GT	TT	GG	GT	TT		
Osawa M	2009	Japan	Asian	16	18	8	99	97	14	0.298	Sudden infant death
Becker ML	2009	Netherlands	Caucasian	12	27	15	134	473	452	0.350	Cardiovascular mortality
Huang L	2014	China	Asian	65	46	12	74	67	25	0.352	SUNDS
Huang JL	2014	China	Asian	30	18	12	33	27	20	0.419	Sudden unexpected death
Earle N	2014	New Zealand	Caucasian	8	9	4	30	106	63	0.417	Resuscitated SCD/SCD
Marcsa B	2015	Hungary	Caucasian	50	160	132	40	129	110	0.375	Cardiac death

MAF indicates minor allele frequency; SCD, sudden cardiac death; and SUNDS, sudden unexplained nocturnal death syndrome.

In the meta-analysis of association between rs10494366 and sudden death, a total of 285 articles were initially selected based on the search strategy. After screening and assessment (49 duplicates, 197 studies excluded based on title and abstract review, 33 articles excluded due to failure to meet the inclusion criteria), a total of six studies were included^{3,13-17} (Figure 3). Characteristics of the included studies are presented in Table III. Three studies were based on the Caucasian population. The related diseases included sudden infant death, sudden cardiovascular mortality, sudden unexplained nocturnal death syndrome, sudden unexpected death, resuscitated SCD/SCD, and cardiac death.

In the forest plot stratified by ethnicity (Figure 4), the association of rs10494366 G allele and sudden death was significant in the Caucasian population, however, slight heterogeneity existed ($Q = 4.86$, $I^2 = 58.9\%$), which assumed an allele model. The dominant model did not show significance without obvious heterogeneity in Cau-

casians ($Q = 2.24$, $I^2 = 10.6\%$). In the Caucasian population, the OR was 1.19 (0.70-1.67) with $z = 4.80$, $P < 0.001$, thereby assuming a dominant model. Analogous results appeared in a recessive model with $Q = 2.94$, $I^2 = 31.9\%$ in Caucasians and $Q = 2.86$, $I^2 = 30.1\%$ in the Asian population. The associations were significant in both the Asian and Caucasian populations with OR = 1.40 ($z = 3.09$, $P = 0.002$) and 1.04 ($z = 3.40$, $P = 0.001$), respectively. No heterogeneity was observed in Caucasians for the homozygote model and OR = 1.46 ($z = 2.43$, $P = 0.015$). However, in the Asian population, the heterogeneity was significant in the dominant, recessive, and homozygote model. The pooled OR was 1.10 ($z = 5.47$, $P < 0.001$) in the overall population without homozygosity in the recessive model (Supplemental Table). No statistically significant data was observed by Begg's and Egger's tests in all four genotype models (Supplemental Table).

Figure 4. Forest plots for four models of the association between rs10494366 and sudden death stratified by ethnicity.

Discussion

Recently, many studies revealed that common variants in the NOS1AP gene were associated with QTc interval variation in several large population samples. The NOS1AP gene which encodes the nitric oxide synthase 1 adaptor protein has been demonstrated to regulate neuronal nitric oxide synthase activation, and enhanced Dexas1 activation.¹⁸⁾ Neuronal nitric oxide synthase protein expression has been found in cardiac myocytes, and may play a role in controlling basal and adrenergically stimulated cardiac contractility, as well as in autonomic control of the heart rate.^{19,20)} Overexpression of neuronal nitric oxide synthase protein results in shortening of the cardiac action potential, a decrease in L-type Ca^{2+} current, and a smaller increase in the delayed rectifier potassium current, resulting in prolongation of the QTc interval.²¹⁾ In previous studies, it has been demonstrated that rs10494366, which is located in the intron region of NOS1AP, was associated with the QT interval and sudden death in independent populations.^{1,6-12)} However, the conclusions were inconsistent.^{4,5)} SNP rs10494366 may influence the QT interval in women and DM patients as revealed by the present meta-analysis. Furthermore, our meta-analysis also indicated an association between rs10494366 and sudden death in the Caucasian population.

The QT interval, which represents the electrocar-

diographic correlation of ventricular depolarization and repolarization, is susceptible to many factors. In the QT interval, approximately 35% of the variation duration is heritable, and a majority of identified SNPs implicate gene regulatory dysfunction in QT prolongation.^{22,23)} As revealed by our meta-analysis, the GG genotype of rs10494366 correlated with the QT interval in women in whom no heterogeneity is found, and in DM patients with slight heterogeneity. It has long been recognized that the QTc, which is corrected by the heart rate, is of a longer duration in women when compared to men, whereas such gender differences are not observed neonates and older children.²⁴⁾ In men, testosterone may contribute to QT interval shortening by modifying ion channels, specifically potassium and calcium channels,²⁵⁾ whereas estradiol may have an acute dose-dependent blocking effect on the potassium channel, which leads to QT prolongation.²⁶⁾ Therefore, it is reasonable to speculate that rs10494366 may share a common pathway with estradiol, which possibly influences ion channels.

DM patients are usually complicated with QT prolongation, which is considered a specific sign of autonomic cardiac dysfunction, and a high mortality risk. However, regardless of the fact whether DM is complicated with autonomic neuropathy, several general abnormalities, including QT prolongation in the ECG have been repeatedly reported in DM patients. Although DM has proven to pro-

mote a significant prolongation in the QTc interval in streptozotocin-induced diabetes in rats, data that focus on DM patients were a little bit controversial.²⁷⁾ The fact that genetic variants in NOS1AP reproducibly alter the QT interval duration in families, aggregating DM demonstrated that genetic effects still play an important role regardless of the strong effect of diabetes on repolarization.⁷⁾ The present meta-analysis showed a significant association between rs10494366 and the QTc interval in DM patients with a z-value of 3.93, which assuredly added a new genetic biological predictor for QTc interval in DM although the mechanism between rs10494366 and the QTc interval in DM patients is still not clear.

As described in previous studies, abnormal cardiac repolarization plays an important role in rapid ventricular arrhythmia, which results in SCD.²⁸⁾ Therefore, in this study, we conducted meta-analysis on the relation between rs10494366 and sudden death. Our data showed an association between rs10494366 and sudden death in the Asian population as well as in the overall population that was ambiguous due to the heterogeneity or negative conclusion that existed in the pooled studies. However, the correlation was significant in the Caucasian population without significant heterogeneity. Although associations have been shown between NOS1AP SNPs and the QTc interval, this is not yet known for the effect of the same SNPs on the risk of cardiac events.^{2,29)} On the other hand, several studies showed that NOS1AP had a distinct association with cardiac events and SCD, but not with the QTc.³⁰⁾

Three studies focused on the Asian population in the present meta-analysis on SCD. Osawa, *et al.*¹³⁾ demonstrated that the T allele of rs10494366 may be associated with the occurrence of sudden infant death syndrome (SIDS) in a manner of recessive inheritance. One of the speculated causes for SIDS is hereditary disease, in which a long QT in the electrocardiogram has been investigated in view of the mutations in various genes that represent ion channels. In two studies^{15,16)} that were performed in China, a negative conclusion was observed in patients with sudden unexplained nocturnal death syndrome or sudden unexpected death, however, the number of subjects was limited. Becker, *et al.*¹⁴⁾ studied 1113 participants in the Rotterdam study, and investigated whether this polymorphism was associated with cardiovascular mortality in users of calcium channel blockers. The data revealed that the G allele of rs10494366 in the NOS1AP gene was associated with increased all-cause and cardiovascular mortality in the Caucasian population. In another study,³⁾ a total of 273 patients with LQTS were included from the New Zealand Cardiac Inherited Disease Registry, among which 31 patients had experienced death or resuscitated SCD (RSCD). The study showed that modifier SNPs including rs10494366 in NOS1AP were associated with an increased risk of SCD and cardiac events in patients with LQTS. However, in a study that aimed to identify a genetic polymorphism that may potentially be used as a universal predictor of cardiac death risk in Caucasian, rs10494366 did not show significance.¹⁷⁾

Our meta-analyses have the following limitations: firstly, the QT interval duration is a complex trait with

many environmental and genetic determinants. Multiple factors can influence the QT interval, which displayed distinct results in enrolled studies. Although strict filter criteria were set, detailed subgroup analysis was performed, heterogeneity of enrolled studies cannot be avoided. Secondly, the number of enrolled studies was too small to provide sufficient verification for the association of this SNP and sudden death. Moreover, studies that focused on the general population are scarce. Thirdly, data directly obtained from SCD subjects are of utmost importance although SCD is a main form of different types of sudden death. At last, the mechanism underlying the current association is unknown. Rs10494366, located in intron 1 of NOS1AP, does not have a known biological function, thereby suggesting that a causal untyped variant that correlated with rs10494366 may exist.

Nevertheless, the present first coherent meta-analysis, which involves an association between rs10494366, the QT interval, and sudden death, suggested that rs10494366 may have impact on the QT interval in women or DM patients, and have a potential in clinical risk stratification for sudden death. Obviously, additional studies on the association of rs10494366 and sudden death are warranted, and the underlying biological and electrophysiological mechanism need to be revealed.

Disclosures

Conflicts of interest: The authors declare no conflicts of interest.

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Supplemental Files

Supplemental Table

Supplemental Figures 1, 2

Please see supplemental files; <https://doi.org/10.1536/ihj.19-024>