

ARIC Manuscript Proposal # 1175

PC Reviewed: 08 / 15 / 06 **Status:** A **Priority:** 2

SC Reviewed: 08 / 17 / 06 **Status:** A **Priority:** 2

1.a. Full Title: Beta2-Adrenergic Variants and Risk of Sudden Cardiac Death

b. Abbreviated Title (Length 26 characters): B2AR and SCD Risk

2. Writing Group:

Writing group members: Nona Sotoodehnia, Christina Wassel-Fyr, David Siscovick, Ron Prineas, Rozenn Lemaitre, Thomas Rea, Eric Boerwinkle, Greg Burke, Wendy Post, Aaron Folsom

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. **NS**

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3. Timeline: Once we obtain the data files from the coordinating center, we anticipate performing analyses, and writing and submitting manuscripts to the P&P Committee within 3-6 months.

4. Rationale:

Sudden cardiac death (SCD) is a major public health concern, accounting for over 400,000 deaths in the US each year.¹ While environmental factors clearly contribute to the determinants of SCD, familial aggregation studies and advances in the molecular genetics of inherited arrhythmias suggest that genetic factors confer susceptibility to SCD in the general population.²⁻⁵ Identifying these genetic factors will not only provide insight into the mechanisms of SCD, it will also have significant public health implications for risk stratification and prevention of SCD.

Experimental and epidemiologic data implicate the sympathetic nervous system as a potent stimulus for ventricular tachyarrhythmias and SCD. Direct stimulation of cardiac sympathetic nerves reduces the threshold for ventricular fibrillation in dogs and precipitates ventricular fibrillation during myocardial ischemia.^{6,7} In humans, malignant ventricular arrhythmias have been associated with cardiac sympathetic activation and with higher plasma and cardiac norepinephrine levels.⁸ Additionally, we have shown that SCD can be triggered by activities that stimulate the adrenergic system, such as vigorous physical exertion and inhaled beta-agonist medication use.^{9,10} Drugs that block sympathetic activity, such as beta-blockers, decrease the incidence of SCD particularly post myocardial infarction and in patients with congestive heart failure.^{11,12} Animal studies suggest that the beta2-adrenergic receptor (B2AR) partly mediates this response to sympathetic activation.^{13,14}

B2AR is a small intronless gene which displays polymorphic variation in multiple different structural domains of the encoded protein.¹⁵ Two common single nucleotide polymorphisms (SNPs) result in amino acid substitutions Gly16Arg and Gln27Glu and are in strong linkage disequilibrium; Glu27 is almost always paired with Gly16 in humans. Therefore, three common haplotypes exist: H1 (Gly16-Glu27), H2 (Arg16-Gln27) and H3 (Gly16-Gln27).¹⁵ The B2AR haplotypes are evolutionarily distant and functionally different. Both recombinant expression systems and human studies have indicated that these variants alter some aspect of receptor signaling.^{16,17}

We recently have shown that genetic variation in the B2AR gene is associated with SCD risk in two distinct population-based studies.¹⁸ We examined 4441 European-American and 808 African-American participants of the Cardiovascular Health Study (CHS) who were followed prospectively for SCD and replicated our findings in 155 case and 144 control European-American participants in the Cardiac Arrest Blood Study (CABS). SCD risk was higher in Gln27 (allele frequency 62%) homozygous participants than in Glu27 carriers among European-American participants in both CHS (HR=1.65, 95% CI= 1.21-2.27) and CABS (OR=1.64, 95%CI=1.02-2.63). There was a 26% non-significant increase in risk among African-American participants (HR=1.26, 95%CI=0.63-2.54), although the confidence interval was wide. Gly16Arg was not associated with SCD risk in either study. Haplotype analysis mirrored the findings with individual SNPs, where haplotypes containing the Glu27 variant were at lower risk. In conclusion, we found that Gln27 homozygous individuals have a 65% increased risk of SCD in two European-American populations.

We are now interested in further exploring the association of B2AR genetic variation with SCD risk in a bi-ethnic population-based cohort of middle-aged adults. In addition to validating our previous findings, the ARIC population allows us to more fully explore this association among African-Americans. To accomplish the study aims, the investigators helped systematically review all deaths due to heart disease from ARIC to classify SCD events.

5. Main Hypothesis/Study Questions:

Aim 1: To assess the association of SCD risk with variation in the B2AR gene.

Aim 2: To examine the association of B2AR variants with inter-individual variations in intermediate electrocardiographic phenotypes, such as QT interval duration, heart rate, and heart rate variability.

Secondary Aim: To investigate the impact of demographic (e.g. age, gender), environmental (e.g. beta-blocker usage), and clinical (e.g. presence or absence of subclinical cardiovascular disease) factors in modifying the association between B2AR genetic variants and SCD risk in exploratory analyses.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

All subjects who have given informed consent to be involved in genetic analyses will be included in this study. Variables of interest will include demographic, clinical, exam, medication, diagnostic, and genetic characteristics. Demographic characteristics include age, gender, ethnicity, and geographical region. Clinical characteristics include common cardiac risk factors such as diabetes, smoking, diet, lipids, and hypertension, and baseline and incident clinical heart disease (congestive heart failure, myocardial infarction) status. Exam information includes resting heart rate and blood pressure. Diagnostic information include echo and electrocardiogram variables, such as heart rate and QT interval, as well as subclinical markers of atherosclerosis such as results of carotid ultrasound, C-reactive protein, and baseline measures of renal function. Medication exposures include antihypertensive (in particular, beta-blocker usage), digoxin, and antiarrhythmic medications. Genotype variables include the variants at codons 16 (Arg16Gly) and 27 (Gln27Glu) of the B2AR gene. The outcomes of interest include the sudden cardiac death variable, resting heart rate, and QT interval. Sudden cardiac death is defined as a sudden pulseless condition in an otherwise stable individual presumed due to an arrhythmic cardiac etiology occurring out of the hospital or in an emergency room. SCD cases have been adjudicated by a physician panel as part of the Reynolds SCD working group.

Hardy-Weinberg equilibrium will be assessed in each ethnic group using the chi-squared test. Haplotypes and their frequencies will be estimated from genotypes by the expectation maximization method using the Arlequin program. Differences in participant characteristics by genotype will be assessed using the chi-squared and ANOVA tests. The exposures of interest are genotype and haplotype. Exposure will be coded as 0 (zero copies of the candidate allele), 1 (one copy of the candidate allele), or 2 (two copies of the candidate allele). All analyses will be stratified by ethnicity to test for interaction. If no interaction is detected, pooled analyses will be performed adjusted for ethnicity. All analyses will be adjusted for age, a potential confounder. Analyses will be stratified by gender to test for interaction. If no interaction is detected, pooled analyses will be performed adjusted for gender. Confounding will be assessed empirically.

The overall association of genotype with SCD risk will be assessed using Cox regression with indicator variables representing the heterozygous and each of the respective homozygous genotypes. Secondary analyses will be performed stratified by gender, median age, , hypertension, diabetes, presence of subclinical disease at baseline (as defined by the Kuller criteria) , and baseline and incident myocardial infarction and congestive heart failure and

interactions will be assessed using the likelihood ratio test. Multivariable analyses will be performed adjusting for these covariates. The association of diplotype with SCD risk will be assessed using Cox regression with indicator variables comparing each diplotype with the most common diplotype. For the continuous intermediate phenotypes of heart rate and QT interval, mean values will be estimated. Heart rate will be used to illustrate the analysis. Mean heart rate values will be compared for the three genotypes (0, 1, 2) using multivariable linear regression with indicator variables representing the three genotypes.

7.a. Will the data be used for non-CVD analysis in this manuscript? No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ___ Yes ___ No

(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? Yes

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"? Yes

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.

No overlap exists.

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

There are no related manuscript proposals. There is an ancillary study proposal (Wendy Post, PI) to examine genomic risk factors for sudden cardiac death. There is no overlap in the genes being examined by the current manuscript proposal with this prior ancillary study. Several investigators on the prior ancillary study will also participate in the current activity, including Nona Sotoodehnia, Thomas Rea, David Siscovick, Ronald Prineas, Greg Burke, and Wendy Post.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes

11.b. If yes, is the proposal

A. primarily the result of an ancillary study (Boerwinkle, AS# 1995.07)

B. primarily based on ARIC data with ancillary data playing a minor role _

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

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human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry* 1994; 33:9414-9.

- 18. Sotoodehnia N, Siscovick DS, Vatta M, et al. Beta2-adrenergic receptor genetic variants and risk of sudden cardiac death. *Circulation* 2006; 113:1842-8.**