ARIC Manuscript Proposal #2541

PC Reviewed: 4/14/15	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Prevalence, Penetrance and Clinical Impact of Rare Desmosomal Gene Variants Associated with Arrhythmogenic Right Ventricular Cardiomyopathy in the Community

b. Abbreviated Title (Length 26 characters):
ARVC Variants in the community

2. Writing Group:

Writing group members: **Aditya Bhonsale MD, Kunihiro Matsushita MD, PhD, Amil Shah MD, MPH, Cindy A. James PhD, Elizabeth Selvin PhD, MPH, Eliseo Guallar MD, DrPH, Eric Boerwinkle Ph.D, Dan Arking PhD, Daniel P. Judge MD, Scott D. Solomon MD, Josef Coresh MD, PhD, Hugh Calkins MD, others welcome**

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __AB__ **[please confirm with your initials electronically or in writing]**

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3. Timeline:

Analysis will begin following proposal approval. Analysis time would be 4 months after receiving necessary data for this proposal. A manuscript will be complete within 4 months of completion of analysis.

4. Rationale:

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy characterized by ventricular arrhythmias, predominantly right ventricular dysfunction, and an increased risk of sudden cardiac death (SCD) [1, 2]. It is a leading cause of sudden death among young athletes[3] with an estimated prevalence of 1 in 1000 people[4]. The past decade has witnessed the identification of causative mutations in components of the desmosome (*JUP* encoding plakoglobin[5], *PKP2* encoding plakophilin-2[6], *DSP* encoding desmoplakin[7], *DSG2* encoding desmoglein-2[8] and *DSC2* encoding desmocollin-2[9]) with mutations being identified in up to 60% of ARVC patients[10]. The phenotypic expression and clinical outcome among carriers of rare desmosomal gene variants has been evaluated in the past by tertiary referral center registries that have enrolled highly selected affected families with a potential of referral and selection bias marring our understanding of the clinical significance of harboring these variants in the general population. Data from these registries suggests that clinical course in these gene variant carriers varies widely with often incomplete age dependent penetrance[12-14].

Prior smaller studies (total n= 6,734)[15, 16] suggest that these putatively pathogenic variants that are thought to predispose to SCD and heart failure (HF) may be much more common in general populations than previously considered (1 in 200 people in a Finnish population[15] and 16% of 400 healthy controls in Kapplinger et al[16]). However, the prevalence, phenotypic penetrance (abnormalities of cardiac structure and function in allele carriers) and clinical impact of rare desmosomal gene variants in a general population sample currently remains unknown. With the ongoing effort to integrate genomic information in ARVC risk assessment[17], it is imperative to examine the clinical impact of these rare genetic variants in large general populations. These results would not only guide family screening paradigms but would provide insight on pathophysiological involvement of these genes and arrhythmic risk assessment in phenotype- genotype+ individuals.

5. Main Hypothesis/Study Questions:

Hypothesis:

1. We hypothesize that presence of these deleterious mutations would be associated with cardiac phenotypic (electrocardiographic and echocardiographic) abnormalities and cardiac markers in these community based populations.
2. We hypothesize that rare variants in desmosomal genes will be found at a substantial frequency among the population and will impact cardiovascular outcomes and overall mortality.

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).

Inclusion criteria:

This study would include all individuals from the ARIC cohort that have available genotypic data.

Exposure:

Available sequencing and Exome chip data obtained from the study participants (n=13,087 on Affy 6.0 array, and N=14,106 on Illumina HumanExome BeadChip, version 1.0) will be analyzed to identify patients with presence of rare desmosomal gene (*PKP2, DSG2, DSP, DSC2* and *JUP*) variants (minor allele frequency <1%).
The identified variants will be characterized by the gene involved and by the nature of the specific mutation (truncating, splice site, missense). The variants will be reviewed and nonsense, frame shift, and splice site variants will all be considered to be proven pathogenic unless previously identified as polymorphism or non-pathogenic[16]. Missense variants will be considered pathogenic when *in-silico* predictive programs (SIFT) and Polymorphism Phenotyping-2 (PolyPhen-2) predict the genetic variants to affect protein function by a tolerance index score of <0.02 (SIFT) and a PolyPhen-2 score of >0.900[16] with expert adjudication in discordant cases (D.P.J).

We will exclude participants with reported race other than white or black as well as those with missing genotype information.

Covariates:

Baseline socio-demographic data from Visit 1 will be obtained:

1. Age at presentation, gender, race, Body mass index (BMI), Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Hypertension, Diabetes Mellitus, prior myocardial infarction, prior heart failure, medication use (lipid lowering, antihypertensive, anticoagulant, antiarrhythmic medications), smoking status, prevalent heart failure, and prevalent coronary artery disease.
2. Laboratory analysis: Creatinine, estimated GFR,
3. Physical activity at baseline details (MET-minutes per week spent in Total sport, moderate to vigorous, and vigorous (>6.0 METs) activity, derived from modified Baecke Physical activity questionnaire on Visit1) since exercise has been shown to be related to ARVC phenotypic expression[17].
4. Measures of lung function: FEV1 and forced vital capacity (FVC).
5. FH of sibling/material/paternal stroke/CHD/premature CHD (from Visit 2).
6. Prevalent atrial fibrillation/atrial flutter (ECGs and a 2-minute rhythm strip from the first visit will be examined to identify participants with prevalent AF or atrial flutter).

Outcome variables:

Outcomes will be ascertained during follow-up visits for the ARIC study as well as through annual calls to participants, ongoing surveillance of health department certificate files, and review of local hospital-discharge lists (with outcomes determined on the basis of *International Classification of Diseases* codes) and death certificates.

1. **Cardiac phenotype**

- ECG: Baseline Electrocardiograms (Visit 1 /first available) and follow up electrocardiograms would be reviewed for:

a) Major and minor depolarization and repolarization abnormalities as per the revised ARVC diagnostic criteria[18] (in rare variant carriers and age and gender matched (1:2) non carriers)
b) Minnesota code abnormalities: Assigned Minnesota Codes will be used to define the type and severity of ECG (‘‘major’’ and ‘‘minor’’ findings) abnormalities along with review of continuous electrocardiographic variables (rare variant carriers and all non-carriers)
c) Rhythm strip (Visit 1) will be reviewed for ectopy (SVPB, VPB)(for total atrial ectopic beats, supraventricular, ventricular complexes and ventricular runs, bigeminy, trigeminy, and multiform complex’s)
d) Heart rate (as determined from the 2 minute rhythm strip or the baseline ECG)

- ECHOCARDIOGRPAHY: Echocardiograms from Visit 3 and 5 will be reviewed for right and left ventricular quantitative and qualitative parameters.

a) RV parameters: Right ventricular dimensions on apical 4 chamber view, Right ventricular outflow tract (RVOT) dimensions on Parasternal Short Axis View, Right ventricular Fractional Area Change(FAC), Tricuspid annular plane systolic excursion (TAPSE), Right ventricular systolic pressure(estimated), and Tricuspid Regurgitation velocity

b) LV parameters: left ventricular mass, wall thickness, left ventricular diameter (End Diastolic Diameter/End Systolic Diameter),volume(End Diastolic Volume/End Systolic Volume), Stroke volume, Stroke Volume Index, cardiac output, Cardiac Index, left atrial diameter/volume, ejection fraction, Left-sided diastolic function: E wave, A wave, E/A, E wave deceleration time, tissue Doppler imaging (TDI) E’, E/E’ and strain analysis results (global, radial and longitudinal).

2. Cardiac markers: Hs-TnT and NT-proBNP measured at visits 2 and 4

3. Clinical events: We will use combination of adjudicated events and ICD-based events, as summarized below.

- SCD events (adjudicated events before 2001)
- ICD implantation (based on the presence of the following ICD 9 codes in any hospitalization: Primary ICD: 37.94, 37.95 37.96, 37.97 00.51; and ICD Removal: 37.98, 00.54)
- Pacemaker implantation based on the presence of the following ICD9 codes in any hospitalization: 37.8 (insertion, placement and revision of pacemaker), V45.01 (status post-pacemaker implantation), or V53.31 (fitting and adjustment of cardiac pacemaker)

- Ventricular Tachycardia/Fibrillation occurrence (based on the presence of the following ICD9 codes in any hospitalization: Ventricular fibrillation (427.41), ventricular flutter (427.42), Paroxysmal ventricular tachycardia (427.) or cardiac arrest (427.5)
- Incident AF (identified from study visit ECGs, hospital discharge diagnoses, and death certificates)
- HF: Incident heart failure in the ARIC study was defined as presence of an ICD code for heart failure (ICD9 428, ICD10 I50) in a hospitalization or death certificate
- All-cause mortality

Analysis: Although we will primarily analyze all participants with variables of interest, to account for potential premature death among carriers, we will also investigate age- and gender-matched group of carriers and non-carriers (1:2) when appropriate.

- Summary statistics will be expressed as mean ± standard deviation (SD), median [25th-75th Percentiles], or numbers (percentages) between carriers and non-carriers. We will use Pearson's chi-square test for categorical variables and t-test for continuous variables.
- The analysis will describe the spectrum of rare variants (gene, type of variant, frequency).
- Clinical and demographic data (covariates) will be compared between carriers and non-carriers of rare desmosomal gene variants.
- The study will describe and compare electrocardiographic parameters (categorical and continuous) in mutation carriers and non-carriers. The fulfillment of ARVC ECG diagnostic criteria will also be compared between rare variant carriers and non-carriers.
- The study will compare right and left ventricular quantitative and qualitative echocardiographic parameters between rare variant carriers and non-carriers.
- The long term clinical events (SCD, ICD/PPM implantation/VT/VF/AF, heart failure, all-cause mortality,) free survival estimates will be obtained according to the Kaplan-Meier method.
- Age- and gender-stratified hazard ratios will be used estimated to investigate the association between the rare variant carriers and the risk of death, arrhythmic outcomes and incident heart failure using Cox proportional hazards regression. Participants with prevalent outcome at the time of the first evaluation or for whom information on outcome status at baseline is missing will be excluded from the analysis.

Limitations

-Since ARVC presentation may occur with SCD in third and fourth decade, rare pathogenic variant carriers with the most severe presentation will not be evaluated in this study.
- The spectrum of rare variation identified would be limited by the genotyping platform used and may not completely represent various ethnic populations.
-The diagnostic criteria for ARVC are complex and though this study would identify key phenotypic feature that are part of diagnosis of ARVC, definite diagnosis often requires additional information that is not obtained in this study (eg. cardiac MRI or VT morphology).-
This study would advance our understanding of true impact of rare pathogenic desmosomal gene variants of ARVC in the general population and potentially be an informative tool in assessing cardiovascular risk in general population in the future.

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

b. If Yes, is the author aware that the file ICTDER03 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? __x__ Yes ____ No
(This file ICTDER 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?
__x__ Yes ____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"?
__x__ Yes ____ No

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. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

___x___ Yes _____ No

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)?

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes __x__ No

11.b. If yes, is the proposal
___ A. primarily the result of an ancillary study (list number* _____)
___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your**

responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

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