ARIC Manuscript Proposal #2669

PC Reviewed: 11/10/15       Status: A       Priority: 2
SC Reviewed: _______       Status: _____       Priority: ____

1.a. Full Title: The association between common genetic variants and ECG risk factors for Atrial Fibrillation

b. Abbreviated Title (Length 26 characters): SNPS & ECG markers of AF

2. Writing Group:
Writing group members: Kaylin Nguyen, Jason Roberts, Alvaro Alonso, Elsayed Soliman, Lin Y. Chen, Dan Arking, Eric Vittinghoff, Bruce Psaty, Susan R. Heckbert, Gregory M. Marcus

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

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ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

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3. Timeline:
Dr. Marcus has the majority of this data available from previously approved manuscript proposals and grant funding. Kaylin Nguyen, the first author, is a medical student at UCSF on a yearlong pre-doctoral clinical and translational research grant, simultaneously taking classes in epidemiology and biostatistics. As such, she should have sufficient time and support from Dr. Marcus and the biostatistics department at UCSF to complete this project within six months from its approval. In addition, we already have certification from the UCSF Committee on Human Research to perform this study, as they do not require specific approval to analyze de-identified data.

4. Rationale:

Atrial fibrillation (AF) is the most common arrhythmia. It affects several million Americans and contributes to heart failure, stroke, and death (1-4). There is no known prevention of AF (1,5) and available treatments have major limitations (6,7). While AF is commonly associated with underlying cardiovascular disease, a subset of patients develops AF in the absence of known risk factors (8). Recent epidemiologic studies suggest a genetic component in the development of AF (9-11) and subsequent genome wide association scans (GWAS) using multiple cohorts have linked single-nucleotide polymorphisms (SNPs) to the arrhythmia (12-17). However, the mechanisms by which the identified genetic loci are associated with AF remains unclear (18).

This project aims to study the association between ECG risk factors of atrial fibrillation and underlying genetic susceptibility. Our group has identified several predictors from the electrocardiogram (ECG) of AF that might reflect distinct mechanistic phenotypes. These ECG markers include premature atrial complexes (PACs) (19), left anterior fascicular block (LAFB) (20), and a prolonged QT interval (21). Our group has shown that PAC count alone was at least as predictive of AF risk as a validated, multivariable Framingham risk model (19). LAFB may reflect a general propensity for left heart fibrosis. The QT interval is a marker of ventricular repolarization and patients with a prolonged QT interval were at greater risk of developing AF (21), suggesting ventricular repolarization as a marker of AF risk. Additionally, changes in other ECG markers have been associated with AF. The PR interval has been associated with increased risk of AF and mortality (22) and p-wave indices such as p-wave duration have been associated with mortality (23).

GWAS have lead to the identification of several SNPs associated with increased AF risk (12-17). However, the mechanism through which these variants mediate AF risk remains unclear. In this study, we aim to study the relationship between ECG risk factors of AF and genetic risk factors, represented by the previously identified risk SNPs. This study could lead to an improved understanding of the underlying genetic mechanisms responsible for AF.
Of the fourteen susceptibility loci identified through GWAS (24), the SNP on chromosome 4q25 is most highly associated with AF (12). The gene located nearest to the SNP is *PITX2*, which encodes for a transcription factor involved in asymmetric cardiac development. *PITX2c*-deficient mice fail to develop pulmonary vein myocardium (25), where ectopic beats leading to AF are believed to originate (26), which suggests that the SNP on chromosome 4q25 may mediate their arrhythmogenic effect by influencing atrial ectopy (which may be manifested by conduction disease such as left anterior fascicular block (20), changes in PR interval (22) and p-wave indices (23).

Another susceptibility locus is located on chromosome 7q31, in the intron of the *CAVI* gene (16), which encodes for an inhibitor of the profibrotic TGF-β1 signaling pathway (27-28). TGF-β1 is a key profibrotic cytokine critical in the development of atrial fibrosis and, in animal studies, atrial fibrosis is sufficient to cause AF (29). This suggests that this SNP risk allele mediates AF risk through an effect on atrial fibrosis, which may be manifested by conduction disease such as left anterior fascicular block (20), changes in PR interval (22) and p-wave indices (23).

The susceptibility locus on chromosome 1q21 is located in the intron of *KCNN3*, which encodes the calcium-activated potassium channel SK3 (15). *KCNN3* is involved in action potential duration and cardiac repolarization in the heart (30-31). Thus the locus on chromosome 1q21 may increase susceptibility to AF through an effect on repolarization (which may be manifested by a different QT interval).

The susceptibility locus on chromosome 10q22 is 5 kb upstream of the *SYNPO2L* gene (16), which is part of a gene family that has been implicated in synaptic plasticity (31) and, thus may influence autonomic innervation of the atria. Cardiac autonomic effects can be examined using heart rate variability (HRV) on the 10-second ECG (32-37) and altered HRV has been associated with risk of AF (33-34). Therefore, the locus on chromosome 10q22 may increase susceptibility to AF through an effect on autonomic activity (which may be manifested through an effect on heart rate variability on the standard 12-lead ECG).

5. **Main Hypothesis/Study Questions:**
   - Aim 1: To determine if the relationship between rs2200733 on chromosome 4q25 and AF is mediated by atrial ectopy (represented by PACs).
   - Aim 2: To determine if the relationship between rs3807989 on chromosome 7q31 and AF is mediated by fibrosis (represented by conduction disease such as LAFB, PR interval, and p-wave indices).
   - Aim 3: To determine if the relationship between rs6666258 on chromosome 1q21 and AF is mediated through an effect on repolarization (represented by the QT interval).
   - Aim 4: To determine if the relationship between rs10824026 on chromosome 10q22 and AF is mediated through an effect on autonomic tone (represented by HRV measurements).
• **Aim 5:** To take a “hypothesis-free” approach to determine whether SNPs previously associated with AF are associated with ECG risk factors for AF.

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).**

   This will be a secondary data analysis of existing ARIC data. The AF-risk SNPs of interest are rs2200733, rs10821415, rs3903239, rs3807989, rs10824026, rs11525911, rs7164883, rs210626, rs6666258 (from the Affymetrix 6.0 array). The ECG risk factors of interest will be determined from the baseline ECG.

**Relationship between SNPs (primary predictors) and AF (primary outcome):**

   The relationship between each SNP and incident AF has already been demonstrated in meta-analyses of multiple cohorts (16). Of note, the current analysis will utilize data from both the Cardiovascular Health Study and ARIC—it is however understood that despite the past literature and the use of these two cohorts, that a statistically significant relationship between each of these SNPs and AF may not be observed in every case using the data available.

**Relationship between ECG predictors (proposed mediators) and AF (primary outcome):**

   Importantly, PACs, LAFB, PR interval, and QT interval have already been shown to be associated with incident AF (19-22). Additionally, we will examine P-wave indices (including p-wave duration, time-to-peak P, and abnormal P-terminal force in V1) as predictors of the risk of AF to determine the aspects of the PR interval that may explain the relationship with AF. We will also examine the standard deviation of normal to normal R-R intervals (SDNN), a HRV measurement, and the risk of AF. Additional HRV measurements, heart rate and the root mean square differences of successive R-R (rMSSD), will be examined as supporting evidence. For these analyses, we will use multivariable Cox Proportional Hazards models, adjusting for the suspected SNP and other potential confounders, and taking into account any interaction between the ECG risk factor and SNP (38-39). Potential confounders to include in these models will include age, race, education, field center, body mass index, systolic and diastolic blood pressure, use of hypertension medications, smoking, heart rate, physical activity, diabetes, coronary disease, MI, and heart failure. Only ECG markers found to be statistically significant in these analyses will be considered for mediation analysis.

**Relationship between SNP (primary predictors) and ECG marker (proposed mediators):**
Therefore, given the established relationships described above, we will examine the relationship between particular SNPs and ECG risk factors, guided by our study hypotheses. We will then perform an exploratory analysis to investigate if associations are found between the other AF risk SNPs and ECG risk factors. We will exclude patients on anti-arrhythmic drugs that affect the heart rate, PR interval, or QT interval. SNPs will be assessed using additive models, then tested in dominant and recessive models as secondary analyses.

**Mediation analysis:**

We will formally test for mediation by assessing the change in the hazard ratio of a given SNP as a predictor of AF in multivariate models with and without adjustment for the ECG marker of interest. We will determine the percent change in the hazard ratio with 95% confidence intervals using the "proportion treatment explained" method (40). While this may not be considered valid for SNP-AF associations that are not significantly associated in this relatively limited dataset, we will acknowledge this limitation and explain the reliance on past medical literature as needed.

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?
   ___ 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.cscce.unc.edu/ARIC/search.php](http://www.cscce.unc.edu/ARIC/search.php)
Yes ___ No (See comment)

Comment: There is partial overlap with proposal #1459 given that heart rate variability is being examined in both studies. However, the other proposal is to establish a relationship between HRV and AF and in this proposal we seek to determine how genetic variants relate to that association. This was discussed with Dr. Alvaro Alonso. We agreed that we would not submit a manuscript that includes these overlapping data until the manuscript for the other proposal has been accepted for publication.

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)?


Magnani J, et al. P-wave indices and atrial fibrillation: cross-cohort assessments from the Framingham Heart Study (FHS) and Atherosclerosis Risk in Communities (ARIC) study. Am Heart J. 2015

MS#1459. Autonomic imbalance and AF

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


