1.a. Full Title: Renin-Angiotensin System Gene Polymorphisms and Risk of Atrial Fibrillation in the Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): RAS Gene Polymorphisms and AF

2. Writing Group:
   Writing group members: Alvaro Alonso, Dan Arking, Eric Boerwinkle, others welcome.

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

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3. Timeline: Statistical Analysis: 3 months Manuscript preparation: 3 months

4. Rationale:
Atrial fibrillation (AF) is associated with activation of the renin-angiotensin system (RAS) in human atria (1). Mechanistically, angiotensin II triggers the MAP kinase pathway, causing fibroblast proliferation and cardiomyocyte hypertrophy, and thus promotes AF (2). Additionally, clinical trials have demonstrated the effectiveness of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) in preventing recurrences of AF, both in patients with common acquired AF (3, 4) and lone AF (5). Efficacy of ACE inhibitors and ARBs in the latter group supports the hypothesis that these agents prevent AF beyond the lowering of blood pressure, lending more credence to the role of RAS in regulating atria arrhythmogenicity.

Collectively, these observations suggest that genetic variation in RAS may confer susceptibility to AF. Indeed, at least two observational studies (6, 7) have reported positive associations of variants in \textit{AGT} and \textit{ACE} with AF. The first study was conducted in an entirely Caucasian population: Copenhagen City Heart Study (6). Multifactorially adjusted hazard ratios for AF for A-20C, AC, and CC versus AA genotype in \textit{AGT} were 1.1 (95% confidence interval (CI): 1.0–1.3; \textit{P}=0.05) and 1.5 (95% CI: 1.1–2.1; \textit{P}=0.01), respectively. The second study was a hospital-based case-control investigation comprising Chinese patients (7). The \textit{ACE} gene I/D polymorphism, the T174M, M235T, G-6A, A-20C, G-152A, and G-217A polymorphisms of the \textit{AGT} gene, and the A1166C polymorphism of the \textit{AGTR1} gene were interrogated. In single-locus analysis, M235T, G-6A, and G-217A were significantly associated with AF. The odds ratios for AF were 2.5 (95% CI: 1.7 to 3.3) with M235/M235 plus M235/T235 genotype, 3.3 (95% CI: 1.3 to 10.0) with G-6/G-6 genotype, and 2.0 (95% CI: 1.3 to 2.5) with G-217/G-217 genotype.

Previous studies, however, have not addressed the extent that single nucleotide polymorphisms (SNPs) in RAS, individually or collectively, improve risk discrimination beyond traditional risk factors in prediction of AF. Moreover, interactions between RAS gene variants and modifiable environmental factors in conferring AF susceptibility have
not been explored in a community-based cohort. ARIC, with 731 incident AF cases among 8,086 White subjects, is well suited to address these issues.

5. Main Hypothesis/Study Questions:

We hypothesize that genetic variation in RAS modifies susceptibility to AF. To test this hypothesis, we aim to:

1) Determine association between candidate SNPs in RAS genes: *ACE, AGT, and AGTR1* with incident AF
2) Construct a genotype score on the basis of the number of risk alleles and test its ability to provide incremental risk discrimination in AF prediction
3) Demonstrate interaction between the individual SNPs and the genotype score with hypertension and pulse pressure in modifying susceptibility to 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).

AF ascertainment

Incident paroxysmal, persistent, and permanent AF cases will be identified from:

1) Hospital discharge records (ICD-9 code 427.31 – Atrial fibrillation)
2) ECGs performed during study visits 1 – 4
3) Death certificates

Candidate SNPs in *ACE, AGT, and AGTR1*

Twenty-two SNPs that were genotyped by the Affymetrix 6.0 platform and 32 haplotype-tagging SNPs imputed using the HapMap CEU reference panel and MACH v1.0.16
software as part of Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) AF Consortium will be analyzed. Haplotype tagging SNPs were selected based on HapMap phase 1 and 2 using the software Tagger and the following tagging criteria: multimarker tagging of the HapMap CEU population, $r^2$ cut-off: 0.8, and minor allele frequency: 0.05. The SNPs are listed in the Appendix.

Covariates

1) Demographic variables: age, gender, study center
2) Clinical variables: body mass index, hypertension, pulse pressure, heart failure, diabetes mellitus, coronary artery disease, use of ACE inhibitors or ARBs

Statistical analyses

Cumulative incidence of AF will be estimated using the Kaplan-Meier method. After confirming the proportional-hazards assumption, proportional-hazards models will be constructed to examine the association of genotype with incident AF; hazard ratios will be adjusted for covariates of interest. A first model will include the individual SNPs (in an additive scale) as main predictor, and age, sex, and study center as covariates. A second model will additionally adjust for other risk factors for AF. SNPs that are associated with AF but are not correlated with each other will be selected for construction of a genotype score; scores will be assigned to individual SNPs based on their hazard ratios. Cumulative incidence curves will be constructed according to genotype score using Cox regression analysis, adjusting for covariates of interest. Receiver-operating-characteristic curves for the baseline covariates will be plotted, and the C statistic will be calculated, with or without the genotype score to evaluate the ability of the genotype score in discriminating risk. Finally, interaction of the individual SNPs and genotype score with hypertension and pulse pressure will be evaluated by a likelihood ratio test.
Validation

We will replicate positive associations in other cohorts in the CHARGE consortium.

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 ICTDER03 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

8.c. If yes, is the author aware that the participants with RES_DNA = ‘not for profit’ restriction must be excluded if the data are used by a for profit group? _____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.csec.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)?

Manuscript Proposal # 1396: CHARGE GWAS for atrial fibrillation

Manuscript Proposal # 1397: CHARGE GWAS for lone atrial fibrillation

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? _____X__ Yes _____ No
11.b. If yes, is the proposal
  __X__ A. primarily the result of an ancillary study (list number* 2008.09)
  __X__ B. primarily based on ARIC data with ancillary data playing a minor
role (usually control variables; list number(s)* 2006.03, 2007.02

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

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.

References

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patients with long-lasting persistent atrial fibrillation: a prospective and randomized

4. Pedersen OD, Bagger H, Kober L, Torp-Pedersen C. Trandolapril reduces the
incidence of atrial fibrillation after acute myocardial infarction in patients with left
