

ARIC Manuscript Proposal # 1568

PC Reviewed: 10/13/09
SC Reviewed: _____

Status: A
Status: _____

Priority: 2
Priority: _____

1.a. Full Title:

Common variants in cardiac connexin-40 (Cx40) gene GJA5 are associated with early-onset atrial fibrillation

b. Abbreviated Title (Length 26 characters):

GJA5 and lone AF

2. Writing Group:

Writing group members:

Alvaro Alonso, Dan E. Arking, Jonathan Smith, Mina Chung, Patrick Ellinor, other investigators from Cleveland Clinic, Massachusetts General Hospital and Framingham Heart Study.

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

This manuscript proposal only requires replication in the ARIC cohort of two associations found in an independent sample. Therefore, we expect that it will be only a few weeks after manuscript approval before we have a final manuscript for review.

4. Rationale:

Cardiac connexin-40 (Cx40) is found exclusively in the atria and conduction system, where it comprises the gap junction hemi-channels that electrically couple atrial myocytes. Previous studies investigating the effect of genetic alteration in the Cx40 gene *GJA5* gene have suggested that deleterious alterations in this gene can result in conduction abnormalities in mice and atrial fibrillation in humans.¹

The Cx40 gene *GJA5* contains two alternative first exons (exons 1A and 1B), utilizing separate promoters (promoters A and B), resulting in two alternative transcripts (*Cx40* transcript A and *Cx40* transcript B) that share a common second coding exon. Groenewegen and colleagues identified a common haplotype in the *Cx40* promoter A which exhibited reduced promoter activity in vitro and was associated with AF in small case-control association studies.² A research group at the Clinical Clinic Foundation (CCF) sought to discover additional common *Cx40* polymorphisms with an effect on *Cx40* expression in human atrial tissue, and to determine if these polymorphisms predispose to AF.

This group identified a SNP in the promoter of *Cx40* transcript B that alters a putative TATA box element and strongly influences expression of *Cx40* transcript B and total *Cx40* expression in human atria. Further, they measured allelic expression imbalance in human atria and used reporter gene assays in cell culture to show that the promoter B SNP is indeed a cis-acting regulatory variant. Importantly, the promoter B SNP exhibited a trend towards association with the lone AF phenotype in a study of 500 lone AF cases and 3200 population controls ($p=0.032$, $OR=1.16$), using the additive genotype model. They found that the previously described promoter A SNP was not significantly associated with *Cx40* expression in vivo and, in contrast to previous reports, the promoter A SNP was not significantly associated with the AF phenotype.

The objective of this proposal is to replicate these results in the ARIC cohort. Specifically, we will include results from the ARIC cohort together with the analyses mentioned above.

5. Main Hypothesis/Study Questions:

We would like to use the ARIC cohorts to:

- 1) replicate our positive findings for promoter SNP B and our negative findings for promoter SNP A
- 2) increase the power of our analyses by combining the CCF and ARIC cohorts (along with data from MGH/FHS)

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).

Exclusion criteria

We will exclude ARIC participants who had ECG-defined AF at baseline, did not have a baseline ECG or the ECG was of insufficient quality to determine prevalence of AF.

For the analysis of early onset AF, we will include incident cases of AF occurring in individuals younger than 65 without history of cardiovascular disease.

Exposures of interest

Both SNPs A and B are available in the Affymetrix 6.0 platform.

AF ascertainment

As in previous analysis, we will identify cases of AF from ECGs done at study visits, from hospitalization discharge codes and from death certificates.³

Statistical analysis

We plan to carry out association analyses of both the promoter A and B SNPs separately, using the ARIC cohort in three ways:

- 1) Comparing all AF cases versus individuals not developing AF
- 2) Comparing early onset AF cases versus individuals not developing AF. Early onset defined as cases in individuals younger than 65 without history of cardiovascular disease.
- 3) Combined CCF Lone AF + ARIC AF cases versus combined CCF and ARIC noncases.

Specifically, for each SNP and each combination of cohorts, we plan to carry out the following analyses:

- 1) Allele frequency model (chi-squared 1DF analysis)
- 2) Three-genotype model (chi-squared 2DF analysis)
- 3) Additive genotype model (logistic regression 1DF analysis)
- 4) Dominant model of each allele (chi-squared 1DF analysis)

Additionally, in separate analyses, we will use an interaction term for SNPs A and B in each of the above analyses.

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”?

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?

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.csc.unc.edu/ARIC/search.php>

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)?

MS #1396 CHARGE GWAS for atrial fibrillation

MS #1397 CHARGE GWAS for lone atrial fibrillation.

In this proposal, we will use results from the GWAS of atrial fibrillation and lone atrial fibrillation.

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* 2008.09)

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.csc.unc.edu/anic/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.

1. Tsai C-T, Lai L-P, Hwang J-J, Lin J-L, Chiang F-T. Molecular genetics of atrial fibrillation. J Am Coll Cardiol 2008;52:241-250.

2. Firouzi M, Ramanna H, Kok B, et al. Association of Human Connexin40 Gene Polymorphisms With Atrial Vulnerability as a Risk Factor for Idiopathic Atrial Fibrillation. Circ Res 2004;95:e29-33.

3. Alonso A, Agarwal SK, Soliman EZ, et al. Incidence of atrial fibrillation in whites and African-Americans: the Atherosclerosis Risk in Communities (ARIC) study. Am Heart J 2009;158:111-117.