ARIC Manuscript Proposal #2781

PC Reviewed: 7/12/16	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Thyroid function and atrial fibrillation: a Mendelian randomization study

b. Abbreviated Title (Length 26 characters): Thyroid function and AF

2. Writing Group: ARIC Investigators: Alvaro Alonso, Dan Arking, Liz Selvin, Anna Köttgen, others welcome

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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3. Timeline:
Analysis to be conducted soon after manuscript approval. Meta-analysis of ARIC results with other results from other cohorts and drafting of a manuscript will occur over the next 6 months.

4. Rationale:
Thyroid disease is associated with organ dysfunction, whether the thyroid is hypo- or hyper-functioning. Measurement of thyroid function is primarily by the hypothalamic thyroid stimulating hormone TSH (thyrotropin) and the thyroid hormones thyroxine (T4) and triiodothyronine (T3). T4 is a pro-hormone, which is converted peripherally or intracellularly by deiodinases to T3, which is the active hormone. Measurement of the non-protein bound hormone concentrations (free T4 [fT4]) and free T3[fT3]) are a better reflection of thyroid status than the

total amount of (free plus bound), because the total concentrations are influenced by changes in thyroid binding proteins as well as hypothalamic-pituitary-thyroid axis regulation. The three major hormone binding proteins are thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin (1).

Overt primary hyperthyroidism (low TSH, high fT4) is associated with atrial fibrillation (AF) and heart failure (2). Subclinical hyperthyroidism (low TSH, fT4 within reference range) has also been shown to be associated with increased risk of AF (3), and there is even an increased risk of AF with higher fT4 levels within the normal range (4), even after controlling for other AF risk factors. The overall prevalence of hyperthyroidism in the USA is 1.3% (5), and is higher in women than in men. Smoking increases the risk of developing hyperthyroidism (2, 6), and smoking is also associated with increased risk of AF (7).

Many rare genetic variations in the TSH-receptor, thyroid hormone receptors, and other important targets have been associated with rare hereditary genetic syndromes or congenital hypothyroidism. However, only recently have common SNPs been identified for variation in concentrations of TSH, fT4, and TPO-Ab, respectively, by GWAS studies or whole genome sequencing (8-11). Also, a moderately rare variant (rs28933981, Thre139Met, freq: 0.4%) in the transthyretin gene *TTR* results in a tighter binding of thyroxine to the binding protein transthyretin (TTR) (12) and has a large effect on fT4 levels (Beta 1.7 ng/dL) (9). However, no previous studies have the genetic score for fT4 to assess the association of fT4 with the incidence of AF.

In order to obtain further evidence for a causal association between fT4 and AF, we propose a Mendelian randomization analysis (13). If fT4 is directly involved in the development of AF, then inherited genetic variation influencing fT4 should affect AF risk in the direction and magnitude predicted by the observational associations, given the assumption that any additional pleiotropic effects of the genetic variation do not also influence AF risk.

5. Main Hypothesis/Study Questions:

1. Using a genetic risk score calculate from common variants (from GWAS data):
    a. To determine whether a fT4 genotype score based upon published alleles is associated with AF in the CHARGE Consortium.
    b. To use a genotype score in an instrumental variable analysis to assess the effect of fT4 on AF risk.
2. Using information on rare variant *TTR* SNP rs28933981 (Thr139Met) (exome chip data only):
    a. To determine whether the moderately rare *TTR* SNP rs28933981 (Thr139Met) is associated with AF in the CHARGE Consortium.
    b. To use the rs28933981 allele as an instrumental variable to estimate the effect of circulating fT4 on AF risk.

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 analysis is conducted in the context of the AFGen consortium. Cohort-specific analysis will be conducted and summary results will be meta-analyzed.

Study population

We will include ARIC participants of European ancestry, with genotyping data, and free thyroxine (fT4) measured at visit 2. We will exclude individuals with prevalent AF at visit 2 (baseline for this analysis).

Primary exposures

- fT4, in ng/dL, measured as part of AS #2009.14 (ARIC Thyroid Ancillary Study, PI: Selvin) in serum on a Roche Elecsys 2010 Analyzer using a competition immunoassay method.
- fT4 common genetic risk score (wGRS): calculated from adding the number of risk alleles in 4 SNPs (see table below) previously associated with higher concentrations of fT4:

  wGRS1 = [0.154***DIO1(rs2235544_A)* + 0.137***AADAT* (rs7694879_A) + 0.087***LHX3* (rs11103377_G) + 0.098***FOXE1*(rs7045138_T)]

Locus	SNP	Effect/Other allele	Code			Beta	Freq (effect)
DIO1	rs2235544	A/C	AA=2	AC=1	CC=0	0.154	0.499
AADAT	rs7694879	T/C	TT=2	TC=1	CC=0	0.137	0.095
LHX3	rs11103377	G/A	GG=2	GA=1	AA=0	0.087	0.496
FOXE1	rs7045138	T/C	TT=2	TC=1	CC=0	0.098	0.553

Betas DIO1, AADAT, and LHX3 from Taylor 2015; beta FOXE1 from Porcu 2013. **fT4 betas are in ng/dL**

- rs28933981 (rare variant in the *TTR* gene), used as a separate instrumental variable

Locus	SNP	Coded/ Other allele	Code			Beta (ng/dL)	Freq (coded)
TTR	rs28933981	T/C	TT=2	TC=1	CC=0	1.7	0.004

Main outcome variable

Incident AF as usually defined in ARIC (from ECGs at study visits, hospital discharge diagnostic codes, or death certificates) (14, 15).

Other covariates

Age, sex, center, height, smoking status, BMI, systolic and diastolic BP, antihypertensive medication use, diabetes mellitus, alcohol intake, prevalent heart failure, prevalent coronary heart disease.

Statistical analysis

An instrumental variable (IV) approach will be used. Three sets of analyses will be performed:

1. Association of the IV (wGRS and rs28933981) with fT4 concentrations using linear regression
2. Association of the IV (wGRS and rs28933981) with incident AF using Cox regression

3. Association of fT4 with incident AF using Cox regression

Models will be adjusted for the following variables:

1. Model 1: age, sex, center
2. Model 2: Model 1 + smoking status (current vs. past/never), alcohol intake (>=2 drinks/day vs. <2), BMI (continuous), height (continuous), systolic blood pressure (continuous), diastolic blood pressure (continuous), antihypertensive medication (yes/no), prevalent diabetes mellitus (yes/no) at baseline, prevalent heart failure (yes/no) at baseline, and prevalent CHD (yes/no) at baseline.
3. Model 3 (pleiotropy test): Model 2 + TSH (continuous, µIU/ml or mIU/L (conversion factor 1.0))
4. Model 4 (mediation analysis): Model 2 + fT4 (continuous, ng/dL)

Separate analyses will be conducted for men and women, and for current smokers / current non-smokers.

IV estimates of causal hazard ratios (HR) will be derived using the Wald-type estimator, which involves taking the ratio of the AF-allele score log HR to the standardized fT4-allele score coefficient and then exponentiating to express as a causal HR. We will do this for each model above to determine contribution of confounding. With reasonable assumptions, the significance of any difference between the observed and estimated relationships can be derived by a z-statistic test.

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.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)?

MS #2492: Thyroid Dysfunction, Cardiovascular Risk Factors, and Incident Events: The Atherosclerosis Risk in Communities (ARIC) Study

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* 2009.24)

___ 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.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes _X_ No.

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