ARIC Manuscript Proposal #2259

PC Reviewed: 11/12/13        Status: A        Priority: 2
SC Reviewed: _________        Status: _____        Priority: ____

1.a. Full Title: Association of FMO3 polymorphisms with adverse cardiac events in European and African Americans

b. Abbreviated Title (Length 26 characters): FMO3 polymorphisms and atherosclerosis

2. Writing Group:
   Writing group members:

   Ariel Brautbar MD, Marwan Shinawi, MD, Alon Keinan PhD, Salim Virani MD, Christie Ballantyne MD, and others.

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

First author: Ariel Brautbar M.D. Baylor College of Medicine, Department of Medicine the section of Atherosclerosis, Houston, TX.

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).

Christie Ballantyne, Baylor College of Medicine, Department of Medicine the section of Atherosclerosis, Houston, Tx.

3. Timeline: Analysis is to start as soon as approval is obtained. We hope that the manuscript will be prepared within 6 months from approval of the analysis.

4. Rationale:
Trimethylamine N-oxide (TMAO), a metabolite of the dietary phosphatidylcholine, was recently shown to predict risk for cardiovascular disease in animals and humans. Dietary supplementation of mice with TMAO caused upregulation of multiple macrophage scavenger receptors, augmented macrophage cholesterol accumulation and foam cell formation, and subsequently promoted atherosclerosis (Wang et al., 2011). In addition, it was shown that metabolism by
intestinal microbiota of carnitine, which is abundant in red meat, produces TMAO and accelerates atherosclerosis in mice (Koeth et al., 2013). Tang et al., quantified plasma and urinary levels of TMAO after a phosphatidylcholine challenge in healthy participants before and after the suppression of intestinal microbiota with oral broad-spectrum antibiotics and found that plasma levels of TMAO were markedly suppressed after the administration of antibiotics (Tang et al., 2013). They also found that fasting plasma levels of TMAO were associated with increased risk of major adverse cardiovascular events (death, myocardial infarction, or stroke) after adjustment for traditional risk factors (Tang et al., 2013). Interestingly, it was shown that omnivorous human subjects produced more TMAO than did vegetarians following ingestion of carnitine through a microbiota-dependent mechanism. Plasma carnitine levels in subjects undergoing cardiac evaluation were associated with increased risks for cardiovascular disease and major adverse cardiac events especially among subjects with concurrently high TMAO levels (Koeth et al., 2013).

Trimethylamine (TMA) is a volatile, fish-smelling compound that is formed in the mammalian gut via the reduction of TMAO and choline. TMA enters the enterohepatic circulation and in the liver, the hepatic enzyme FMO3 oxidizes TMA back to TMAO, an odorless, water-soluble compound excreted in the urine. Individuals with mutations in FMO3 present with excessive accumulation of TMA levels in body fluids, causing a rare genetic condition called trimethylaminuria (TMAU; aka fish malodor syndrome). FMO3 enzyme activity determines the ratio between TMA and TMAO. Upregulation of hepatic FMO3 in mice decreased TMA and increased TMAO levels in the circulation, while antisense-mediated silencing of FMO3 increased TMA and decreased TMAO levels (Bennett et al., 2013). Furthermore, TMAO levels were significantly correlated with FMO3 expression, which varied due to natural genetic variations among inbred strains of mice (Bennett et al., 2013).

Sequencing of the FMO3 gene in patients with transient TMAU revealed variant alleles, carrying two amino acid polymorphisms, c.472G > A and c.923A > G (E308G; E158K). These two variations decrease the FMO3 activity and the TMAO/TMA ratio. The frequency of these two variants is high in different populations:

1. c.472G > A (E158K): the following link shows the frequency of this SNP (rs2266782) in different populations:
http://useast.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=1:171076466-171077466;v=rs2266782;vdb=variation;vf=1846945

2. c.923A> G (p.E308G): the following link shows the frequency of this SNP (rs2266780) in different populations:
http://useast.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=1:171082742-171083742;v=rs2266780;vdb=variation;vf=1846944

Individuals who carry one or both of these SNPs are “slow metabolizers” for the TMA and therefore produce lower levels of TMAO and consequently may have lower risk for atherosclerosis and its complications as compared to controls.

5. **Main Hypothesis/Study Questions:**

Our main hypothesis is that variants in the *FMO3* affect the risk for atherosclerosis and risk for hospitalization for congestive heart failure. The study question is whether the frequency of the two SNPs mentioned above is statistically different in patients with adverse cardiovascular events as compared to controls.

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).**

1. Analysis will be performed in European Americans excluding those with cardiovascular disease at visit 1.
   Cox regression analyses adjusting for covariates to determine whether FMO3 SNPs are independently associated with future risk of CVD.

Covariates should be the traditional risk factors: age, sex, hdl cholesterol, ldl cholesterol, smoking, HTN, HTN meds, BMI.

Analysis will be done for 4 possible outcomes:

a. coronary death and MI (exclude stroke)

b. a + stroke 

c. CVD as defined in ARIC which also includes definition b plus revascularization

d. Hospitalization for heart failure
2. Each SNP will be examined separately.

3. Analysis will be done for a. ARIC whites b. ARIC blacks. c. combined white and black population.

7. a. Will the data be used for non-CVD analysis in this manuscript? ___ Yes ___ 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

8.a. Will the DNA data be used in this manuscript? ___ 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.cscc.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)?

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* 2008.10 )

___x___ 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/

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.

Agreed.

Reference