

ARIC Manuscript Proposal #2813r

PC Reviewed: 04/11/2017 Status: _____ Priority: _____2
SC Reviewed: _____ Status: _____ Priority: _____

1.a. Full Title: FMO3 exome sequencing variants as quantitative trait loci for blood pressure.

b. Abbreviated Title (Length 26 characters): FMO3 and Blood Pressure

2. Writing Group:

Tyler Bryant, Priya Duggal, Tariq Shafi, Eric Boerwinkle, Georg Ehret, Bing Yu, Josef Coresh, Adrienne Tin

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

Data analysis will start immediately. A manuscript is expected to be prepared within 8 months.

4. Rationale:

With an age-adjusted prevalence of 29.1% in the United States, hypertension is a public health issue that warrants attention.¹ One study using the Atherosclerosis Risk in Communities (ARIC) cohort found a heritability estimate of 80% for systolic blood pressure.² Understanding the genetic components that explain variability in blood pressure can aid in risk prediction and future pharmacologic research for controlling hypertension. High levels of trimethylamine N-oxide (TMAO) have been associated with atherosclerotic lesions in mice and various cardiovascular disease outcomes (cerebrovascular accident, myocardial infarction, and CVD related mortality) in humans.³ The gut microbiome converts dietary L-carnitine and choline into trimethylamine (TMA). A gene named FMO3, in turn, regulates the amount of TMAO in the blood through the N-oxygenation of TMA.⁴ A few loss of function variants in FMO3 have been found to cause trimethylaminuria, a condition also called fish-odor syndrome because of the odor produced from an accumulation of trimethylamine in the blood.⁵ A study has found that those who have rare variants causing trimethylaminuria also commonly have hypertension.⁶ This is not necessarily through an accumulation of TMAO, however. It is believed that FMO3, with its ability to modify a broad range of substrates, may play a role in catecholamine metabolism.⁶ Catecholamines have the ability to regulate blood pressure and heart rate in response to stress, and FMO3 variants may contribute to this increase in blood pressure through abnormal catecholamine metabolism.⁷ A couple of studies have looked at E158K, a common variant in of FMO3, and its association with hypertension with conflicting results.^{7, 8} Looking at these common variants as well as rare variants detected in exome sequencing in a large sample will be beneficial in replicating and finding novel variants in FMO3 that may influence blood pressure values.

The primary goals of this study are to 1) characterize the number and type of variants located in the FMO3 gene, 2) detect prospective associations between rare FMO3 variants and blood pressure values, and 3) replicate the previous association of the common variant E158K with hypertension and blood pressure values found by other authors.^{7, 8}

5. Main Hypothesis/Study Questions:

There will be nonsynonymous and splice site variants in FMO3 associated with systolic and diastolic blood pressure values.

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).

Inclusion criteria: Participants with FMO3 exome sequencing data passing quality control with data on blood pressure and potential covariates.

Outcomes:

Primary outcome: Systolic and diastolic blood pressure at visit 1, taken as the average of the second and third measurements.

Secondary Outcome: Hypertension status at visit 1

Predictor: Rare and low-frequency non-synonymous and splice site variants in FMO3 exons passing quality control from the exome sequencing data with minor allele frequency of 0.1%-5%.

Other variable of interest at visit 1: age, sex, race, diabetes, BMI, smoking status, antihypertensive medication use, the first 10 principal components generated using Affymetric 6.0 autosomal genotype data to control for population substructure.

Data analysis:

The quality control for FMO3 exome sequencing data will be the same criteria as reported in Yu et al.⁹ Low frequency variants with a minor allele frequency <5% will be the focus of this analysis. The association of rare and low frequency FMO3 variants with the average systolic and diastolic blood pressure values of the second and third measurement at visit 1 will be assessed with single variant tests adjusted for potential confounders. The sequential kernel association test (SKAT) and burden test will be used to test for a significant association of variants with MAF < 5% in FMO3 with systolic and diastolic blood pressure values. In an attempt to replicate results from a previous study, the common variant E158K will be tested for an association with systolic and diastolic blood pressure, as well as hypertension status, stratified by smoking status.⁸ The analysis will be conducted in European and African Americans separately controlling for the genetic principal components within each population.

Genetic Model: Because the FMO3 activity decreases with increasing number of loss-of-function alleles, the genetic model for the variants in this study will be additive.¹⁰

Significance threshold:

Bonferroni corrected values will be used to determine significance in this study. For the single variant association test, $p < 0.025$ will be significant based on 2 variants with MAF between 0.1% to 5% in European Americans and $p < 0.0035$ based on 14 variants with the same MAF in African Americans.

For the SKAT and Burden tests, $p < 0.05$ will be significant.

For the replication study of E158K, significance will be determined by $p < 0.05$.

Power calculation:

With around 7,272 European participants with exome sequence data in our analysis, we expect to be able to detect a change in systolic and diastolic blood pressure around 5.4mmHg and 3.2mmHg, respectively, with 80% power given a minor allele frequency (MAF) of 0.5%. With the same power and minor allele frequency, we expect to be able to detect a change of 10.4mmHg for systolic and 6.1mmHg for diastolic blood pressures in the 2790 African American participants in our sample. For the replication study of the common variant E158K (MAF of 40%), we have 99% power to detect a change in systolic blood pressure 2 mmHg and 1mmHg for diastolic blood pressure.

Replication:

We plan to replicate the any positive results from the single variant analysis using data from an exome-wide analysis of blood pressure from several cohorts, including the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), and the Erasmus Rucphen Family Study (ERF).¹¹

Limitations:

Blood pressure medication use could potentially limit the findings of this analysis due a smaller number of individuals having high recorded systolic and diastolic blood pressure, even when this would truly be the case without medication. We plan to account for this by adjusting blood pressure values according to previous GWAS studies that have added 10mmHg to systolic blood pressure and 5mmHg to diastolic blood pressure.¹²

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

(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?

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

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)?

MP2259

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*)
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