ARIC Manuscript Proposal #3481

PC Reviewed: 10/8/19	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Genetically determined fibrinogen levels and risk of venous thromboembolism and ischemic stroke: evidence from Mendelian randomization

b. Abbreviated Title (Length 26 characters): Fibrinogen MR

2. Writing Group:
Jillian Maners, Nathan Pankratz, Weihong Tang, Eric Boerwinkle, Alanna Morrison, Paul de Vries

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

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3. Timeline: We plan add results from the ARIC study relating to an existing manuscript that otherwise close to being ready for submission. We therefore expect to submit a manuscript to a peer-reviewed journal within 1-3 months of this proposal being approved.

4. Rationale: Fibrinogen is a key protein in coagulation, serving as the precursor of fibrin and promoting platelet aggregation. Fibrinogen is also part of the acute phase response, serving as an inflammatory mediator. The alpha, beta, and gamma chains that comprise fibrinogen are encoded by the FGA, FGB, and FGG genes, which are located together in a single genetic region. Alternative splicing of FGG produces an isoform of the gamma chain known as gamma prime, resulting in the production of gamma prime fibrinogen, which makes up about 8-15% of total

plasma fibrinogen. It is unclear whether gamma prime fibrinogen levels or total fibrinogen levels have a causal effect on venous thromboembolism (VTE) or ischemic stroke subtypes, including cardioembolic stroke, large artery stroke, and small vessel stroke. Mendelian randomization is an approach that, when assumptions are met, estimates the causal effect of an exposure on an outcome, by using genetic variants as instrumental variables for the exposure.

5. Main Hypothesis/Study Questions:

The aims of our study are:

1) To identify genetic variants association with gamma prime fibrinogen levels
2) To estimate the causal effect of gamma prime fibrinogen levels and total fibrinogen levels on VTE and ischemic stroke subtypes using Mendelian randomization.

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).

The genome-wide association study of gamma prime fibrinogen levels will use gamma prime fibrinogen level measurements from visit 3 that were performed using ELISA. The phenotype will be log-transformed before analyses, and participants with gamma prime fibrinogen levels > 3 SD from the mean will be excluded. We will use genotypes assessed using the Affymetrix (Santa Clara, CA, USA) Genome-wide Human SNP Array 6.0 assay, and additional genotypes imputed using IMPUTE based on the 1000 Genomes Project phase 1 version 3 reference panel. Genetic variants with P-value < 5E-8 will be considered genome-wide significant.

Our Mendelian randomization analyses will be based solely on summary statistics: we will use the beta and standard error of the association of each genetic variant with our exposures (gamma prime and total fibrinogen levels) and our outcomes (VTE and ischemic stroke subtypes). All of these summary statistics are publicly available and have already been obtained, except for those pertaining to gamma prime fibrinogen. In order to obtain summary statistics for gamma prime fibrinogen, we propose to perform a genome-wide association study of gamma prime fibrinogen levels in the ARIC study.

All analyses involving participant-level ARIC phenotype and genotype data will be carried out by Nathan Pankratz and Weihong Tang at the University of Minnesota. Only summary-level statistics for the association of each genetic variant with gamma prime fibrinogen will then be shared with Jillian Maners and Paul de Vries at the University of Texas Health Science Center at Houston, and will be used to perform Mendelian randomization.

In order to select sets of independent genetic instruments for gamma prime fibrinogen and total fibrinogen levels we will: 1) restrict the summary statistics to genetic variants also present in the summary statistics for the outcomes (VTE and ischemic stroke subtypes), 2) restrict to only genome-wide significant variants (P-value < 5×10-8), and 3) use linkage disequilibrium clumping in the “TwoSampleMR” R package to prune these variants according to their pairwise

linkage disequilibrium by removing the variant with the least significant P-value from each pair of variants with a LD r2 > 0.1.

The primary Mendelian randomization approach for producing a single estimate of the causal effect for each exposure-outcome association will be inverse variance weighted meta-analysis. However, we will also use a range of alternative approaches as sensitivity analyses, including MR-Egger, Weighted Median, and Weighted Mode. The "TwoSampleMR" R package will be used to carry out these analyses.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __x__ No

VTE and ischemic stroke are cardiovascular diseases.

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/mantrack/maintain/search/dtSearch.html

___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)?

No related manuscript proposals were identified.

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 (2006.03 and 1998.03)
___ 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 https://www2.cscc.unc.edu/aric/approved-ancillary-studies

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.