ARIC Manuscript Proposal #1945

PC Reviewed: 5/8/12  Status: A  Priority: 2
SC Reviewed: _________  Status: _____  Priority: ____

1.a. Full Title: *Sex Interaction Genome-wide Association Study of Plasma Phospholipid Polyunsaturated Fatty Acids*

b. Abbreviated Title (Length 26 characters): *Sex x GWAS for plasma fatty acids*

2. Writing Group:

Writing group members:

- **Millennia Foy** (CARDIA)—MANUSCRIPT LEAD (postdoctoral fellow at the University of Texas Health Science Center, Graduate School of Biomedical Sciences)
- Myriam Fornage (CARDIA)—mentor to Dr. Foy
- **Lyn Steffen** (ARIC/CARDIA/MESA)
- Weihua Guan (ARIC/MESA)
- Weihong Tang (ARIC)
- Mike Tsai (MESA) (ARIC/MESA)
- Ani Manichaikul (MESA)
- Stephen Rich (MESA)
- Donna Arnett (GOLDN/MESA)
- Rozenn Lemaître (CHS/MESA)
- Jason Wu (CHS)
- Dariush Mozaffarian (CHS)
- Caren Smith (GOLDN)
- José Ordovás (GOLDN)
- Edmond Kabagambe (GOLDN)
- Toshiko Tanaka (InCHIANTI)

**Please note: This is a multi-cohort collaboration including cohorts from the CHARGE Plasma Fatty Acids Working Group—ARIC is one of several participating groups (others are listed above with potential for additional collaborators to join. Authorship from each group is expected; thus, the present list is somewhat abbreviated.**

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

**First authors:** **Millennia Foy, PhD**  
Address: University of Texas Health Science Center, Houston  
Graduate School of Biomedical Sciences  
Phone: (713) 500-2463  
E-mail: millennia.foy@uth.tmc.edu

**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).  
Name: **Lyn Steffen, PhD, MPH**  
Address: University of Minnesota, School of Public Health, Division of Epidemiology and Community Health; Minneapolis, MN  
Phone: 612-625-9307  Fax: 612-624-0315  
E-mail: steffen@epi.umn.edu

3. **Timeline:** [estimated timeline]  
Manuscript pen draft anticipated by end of summer 2012
4. **Rationale:**

Fatty acids in phospholipids (plasma and cell membranes) originate from the diet (e.g. essential fatty acids) or from endogenous metabolism. The pathway of n-3 and n-6 fatty acid synthesis is depicted in Figure 1. In animals linoleic acid (LA; 18:2n-6) and alpha-linolenic acid (ALA; 18:3n-3) are obtained from the diet and then can be synthesized to longer chain n-3 and n-6 fatty acids polyunsaturated fatty acids (PUFAs) in parallel through a series of competitive elongations (red arrows) and desaturations (blue arrows). LA synthesizes to gamma-linolenic acid (GLA; 18:3n-6), dihomo-gamma-linolenic acid (DGLA; 20:3n-6), and arachidonic acid (AA; 20:4n-6) in the omega 6 family. ALA heads the omega 3 which includes eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). Studies have shown that intakes of n-3 PUFAs, especially EPA and DHA are related to lower risks of a number of health outcomes including cardiovascular disease, inflammatory diseases, and mental and neurodegenerative disease [1-5]. Some Medical research suggests that excessive levels of n-6 PUFAs compared to n-3 PUFAs can increase the risk of a number of diseases and depression [6-8]. However, recent literature questions the usefulness of the ratio between n-6 and n-3 fatty acids and suggests that clinical endpoints are directly related to specific fatty acids instead [9]. The authors note that although it has been suggested that n-6 PUFAs increase the risk of certain diseases through the ratio, it has been shown that the n-6 PUFAs decrease the coronary heart disease, and provide benefits in brain development, and the lowering of blood pressure [10].

A recent study has shown strong heritability of erythrocyte fatty acids [11]. Further, a case-control genome-wide association study linked PUFAs to a polymorphism in the FADS gene cluster [12].

**Gender differences in fatty acids**

A recent study of plasma phospholipid fatty acids in EPIC showed significant gender differences in levels of n-3, and n-6 PUFAs after adjustment for BMI, age, alcohol intake, smoking, region, season, and storage time [13] among a study of Europeans. In particular, highly significant gender differences were observed in levels of LA, AA, and DHA. Another study of plasma fatty acid levels in Tunisians found significant gender differences in levels of total PUFA, total n-3 PUFA, ALA, EPA and DHA [14].

A study of young women [15] showed that conversion of ALA to EPA and DHA was substantially higher (2.5 fold and 200 fold, respectively) when compared to a study of men of similar age [16]. Burdge and Calder [17] hypothesize that there are gender differences in the activation of the desaturation and elongation pathway of PUFAs synthesis and suggest that sex hormones influence the activity. These studies imply that there could be a biological basis to the gender differences observed in fatty acid levels.
Summary/Conclusions
We propose to undertake a genome-wide study of the interaction of gender and SNPs in association with PUFA levels using the CARDIA cohort and integrate our results with several other cohorts (including ARIC, CHS, MESA, and others) using meta-analysis to identify novel variants that interact with gender to modify levels of these fatty acid phospholipids. To our knowledge, there has not been a previous study of gender-gene interaction on fatty acids.

REFERENCES PG 6

Brief description of methods and analysis (full plan below)
The analysis will be a linear regression of 2.5 million imputed HapMap SNPs against each phenotype of interest. Analyses will be conducted on subjects of European descent. Genetic variants will be modeled additively. The primary analyses will be adjusted for age, gender, center (if applicable) and principal components (if applicable) and will include a gene-gender interaction term. Each fatty acid will be expressed as a percentage of total fatty acids. Two statistical tests of association will be carried out, analyzing (1) gender-differentiated associations between SNPs and fatty acid levels (a chi-square 2 degree of freedom test), and (2) gender-gene interaction (1 degree of freedom test). Phenotypes of interest include:
- n-3 PUFAs: ALA (18:3n-3), EPA (20:5n-3), DPA (22:5n-3), and DHA (22:6n-3)
- n-6 PUFAs: LA (18:2n-6), GLA (18:3n-6), DGLA (20:3n-6), and AA (20:4n-6)

Association results will then be meta-analyzed across the other participating cohorts in the CHARGE consortium [18]. Fixed-effects models with inverse-variance weighted meta-analyses will be performed to summarize p-values and effect sizes (β-coefficients) from individual cohorts if the fatty acid measurements are consistent across studies; when there are heterogeneities in fatty acid measurements or assays, sample-size weighted meta-analysis approach will be used. Significance thresholds for genotype-phenotype association p-values will be adjusted to account for multiple testing.

5. Research Hypothesis:
Using a genome-wide approach, we can identify common genetic variants that interact with gender to modify plasma phospholipid polyunsaturated fatty acids including the n-6 fatty acids LA(18:2), GLA(18:3), DGLA(20:3), and AA(20:4), and the n-3 fatty acids ALA(18:3), EPA(20:5), DPA(22:5), and DHA(22:6).

6. Design & Analysis
MN Sample: Participants (n=3,793) with fatty acid data and genomic data
Exclusions: missing fatty acid components, non-White race, no genetic consent, extreme outliers for the fatty acids of interest

Independent variables: genome-wide genetic information by imputation (build 36, MACH for example), ie about 2.5 million SNPs imputed to the HapMap European American panel; sex (interaction term)

Dependent variables:
a. 18:3n-3 (ALA)
b. 20:5n-3 (EPA)
c. 22:5n-3 (DPA)
d. 22:6n-3 (DHA)
e. 18:2n-6 (LA)
f. 18:3n-6 (GLA)
g. 20:3n-6 (DGLA)
h. 20:4n-6 (AA)

Covariates of interest: age and sex + field center and PCs (where applicable).
**Brief analysis plan and methods:**

**A. Genotype-phenotype association within cohorts:**

a. Regression models:
   i. Linear regression with robust variance estimators
   ii. SNP-gender interaction term (Gender coded as male=0 and female=1)
   iii. Separate models for each fatty acid

   Example of a regression model including gene-gender interaction effect:

   \[ ALA = \alpha + \beta_1 \text{age} + \beta_2 \text{sex} + \beta_3 \text{study site (where applicable)} + \beta_4 \text{PC (where applicable)} + \beta_5 \text{SNP} + \beta_6 \text{sex x SNP} \]

b. Covariates for adjustment in the carbohydrate interaction regression model:
   i. Age in years
   ii. Sex (males=0, females=1)
   iii. Study site (where applicable)
   iv. PC (where applicable)

c. Genetic model: One degree of freedom/ additive model

   The choice of specified allele does not matter, but combining the results will require that the specified allele is stated.

d. Imputation
   i. Imputation to 2.5M SNPs
   ii. The 1df model is implemented with imputed estimates of the minor allele count (the ‘estimated dosage’)

e. QC methods at cohort levels
   i. Please see [http://depts.washington.edu/chargeco/wiki/QCprocedures](http://depts.washington.edu/chargeco/wiki/QCprocedures)

f. Sharing:
   i. Unstandardized regression coefficients for each main effect SNP term and for the interaction term between each SNP and gender
   ii. Standard errors, and p-values for the main and interaction terms
   iii. Covariance between the main and interaction effect terms
   iv. Coded allele and MAF
   v. See [http://depts.washington.edu/chargeco/wiki/ResultsSharing](http://depts.washington.edu/chargeco/wiki/ResultsSharing) for instructions and formatting of results. Include additional columns for the regression coefficient, standard error and p-value of the interaction term, and the covariance between the SNP main and interaction terms.

**B. Meta-analysis:**

a. Inverse- variance Fixed Effects model

b. Significance threshold: \(5 \times 10^{-8}\)

c. Final QC step (e.g. filtering MAF at 1%) at meta-analysis stage
7.a. Will the data be used for non-CVD analysis in this manuscript?  
*Plasma phospholipid fatty acid levels are the phenotype of interest*

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*  
(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?  
*Yes*

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*

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.cscucc.unc.edu/ARIC/search.php  
*Yes*

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)?

Other projects from the CHARGE fatty acids working group involving ARIC plasma FA data:

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Number</th>
<th>Title</th>
</tr>
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<tbody>
<tr>
<td>Wu</td>
<td>1710</td>
<td>Genome-wide association study of plasma lipids in DNL pathway</td>
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<tr>
<td>Steffen &amp; Tsai</td>
<td>1788</td>
<td>Genome Wide Association Study of Plasma Phospholipid n-6 Fatty Acids within the CHARGE Consortium</td>
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<tr>
<td>Smith</td>
<td>1785</td>
<td>GWAS for plasma N-3 Fatty Acids - Interactions with dietary fatty acids</td>
</tr>
<tr>
<td>Lemaitre &amp; Friedlander</td>
<td>1926</td>
<td>Genome-wide Association Study of Plasma Phospholipid Long-chain Saturated Fatty Acids within the CHARGE Consortium</td>
</tr>
<tr>
<td>Sun</td>
<td>1854</td>
<td>Genome-wide association study of odd-numbered chain saturated fatty acids in plasma phospholipids: CHARGE Fatty Acid Consortium</td>
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11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  
*Yes*  
GWAS via STAMPEDE & GENEVA, #2006.03

11.b. If yes—is the proposal a primarily the result of an ancillary study  
*ARIC is one of several cohort studies contributing data to the CHARGE-initiated meta-analysis. Since this work is a product of CHARGE which utilizes GWA data, ancillaries related to STAMPEDE & GENEVA are also acknowledged (AS2006.03).*

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.  
*The lead author is aware of, and will comply with, this stipulation.*
References


