ARIC Manuscript Proposal # 1788

PC Reviewed: 5/10/11	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: *Genome-wide Association Study of Plasma Phospholipid N6 Fatty Acids within the CHARGE Consortium*

b. Abbreviated Title (Length 26 characters): *CHARGE n6 fatty acid GWAS*

2. Writing Group:

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

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3. Timeline: [estimated timeline]

Cohort-specific data analyses: November 1-30, 2010
Meta-analysis: January 1, 2011
Manuscript drafting complete: July 1, 2011

4. Rationale:

Background: Fatty acids in phospholipids (plasma and cell membranes) originate from the diet (e.g. essential fatty acids) or from endogenous metabolism. Phospholipid n-6 fatty acids, including linoleic aicd (LA) and arachidonic acid (AA), have been positively associated with the risk of ischemic heart disease (IHD). For example, Baylin and Campos (1) reported that AA in adipose tissue was correlated with the risk of nonfatal myocardial infarction (MI) in Costa Rica. Another study by Kark et al. (2) examined an Israeli population consuming a diet high in LA and reported a relation between risk of acute MI and adipose tissue AA but not that of LA. However, evidence from other observational studies has shown an inverse relation between LA acid and coronary heart disease (3).

The other n-6 fatty acids gamma-linolenic acid (GLA) and dihomo-gamma-linoleic acid (DGLA), intermediates in the pathway between LA and AA, have also been related to chronic disease. GLA supplementation lowered triglycerides and increased HDL-cholesterol, albeit a small sample (n=12) (4). Few studies report measurment of GLA in tissue or blood. In the Edinburgh Artery Study, DGLA in phospholipids and red cell fractions was positively associated with the odds of having an MI and stroke (5). In addition, the adjusted rate ratios (RRs) of CHD incidence for the highest versus the lowest quintile were 1.31 in CE and 1.44 in PL for dihomo-gamma-linolenic acid (p for trend: 0.05 and 0.02, respectively) in middle-aged adults enrolled in the ARIC study (6). However, other studies have reported inverse relations of DGLA and CHD (7,8) In a study of children, DGLA was associated with adverse levels of cardiovascular risk factors, including BMI, triglycerides, total cholesterol, and HDL-cholesterol (9).

Synthesis of LA to AA: LA is metabolized in a variety of tissues by Δ6 desaturase to form GLA, which is rapidly elongated to DGLA. DGLA can be further desaturated to AA by Δ5 desaturase. However, due to the limited activity of Δ5 desaturase, only a small fraction of DGLA converted to AA (10,11).

Factors influencing fatty acid metabolism: While age (12), sex (13), race (14), smoking (15), and dietary intake (ref) clearly influence levels of plasma phosholipids, evidence from the Kibbutzim Family Study suggests strong heritability of all erythrocyte fatty acids (4). Recently a case-control genome wide association study (GWAS) of fatty acids in the InCHIANTI Study reported associations between the polymorphism at the FADS gene cluster on chromosome 11 and several polyunsaturated phospholipid fatty acids (5). The SNP rs174537 was significant for AA ($p=5.95^{-46}$), but not LA. GLA and DGLA were not examined in this study. The allele (GG) associated with higher AA showed greater expression of FADS1. The minor allele (TT) associated with lower AA has also been associated with lower levels of LDL- and total cholesterol.

However, the InChianti study had a limited number of observations. Therefore, in collaboration with several other groups, we propose to participate in the CHARGE collaborative effort to

identify genetic predictors of n6 fatty acid phenotypes by analyzing the ARIC fatty acid data and integrating our results with the other participating cohorts through meta-analysis.

5. Research Hypothesis:

Using a genome wide approach, we will identify common genetic variants that are associated with plasma phospholipid fatty acid levels of n-6 fatty acids, including linolenic (LA; 18:2,n6), gamma linolenic (GLA; 18:3,n6), dihomo-gamma-linolenic (DGLA; 20:3,n6), arachidonic (AA; 20:4,n6) acids, adrenic acid (22:4,n6), and others.

1. 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

Dependent variables: plasma phospholipid fatty acid levels of n-6 fatty acids (LA, GLA, DGLA, AA, adrenic acid, and 22:4,n6, others)

Covariates of interest: age and sex, and potential cohort-specific variables if applicable to the other cohorts.

Brief analysis plan and methods:

The analysis will be a linear regression of all 2.5 M SNPs against each phenotype of interest. The primary analysis will be adjusted for age and sex. Phospholipid fatty acids are expressed as percentages of total fatty acids. Genetic variants will be modeled additively.

Association results will be meta-analyzed across the other participating cohorts in the CHARGE consortium (6). Imputation of genotypes to the HapMap will allow the comparison and integration of GWAS from multiple platforms. Fixed-effects models with inverse-variance weighted meta-analyses will be performed to summarize p-values and effect size (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 hypothesis testing ($p < 5 \times 10^{-8}$).

Summary/conclusion:

We propose to undertake a genome-wide study of plasma phospholipid n6 fatty acid levels using the ARIC cohort and integrate our results using meta-analysis with several other genomic studies (including CHS, CARDIA, MESA, and others) to identify novel genetic variants associated with these phospholipid fatty acids. These findings may identify novel candidate genes and mechanisms regulating phospholipid n6 fatty acids.

Participating cohorts (whites only) with fatty acids and genomic information

Cohort	Fatty acid compartment(s)	N=	18:2,n6	18:3,n6	20:3,n6	20:4,n6	22:4,n6
CHS	Plasma PL	2150	x	x	x	x	X
Cardia	Plasma PL	1507	x	x	X	x	
ARIC (MN sample)	Plasma PL, CE	3793	x	x	X	x	X
InCHIANTI	Plasma	1075	X	x	X	x	
MESA	Plasma PL	690*	X	x	x	x	
-Total n (fatty acids and GWAS)		9215					

PL = phospholipids; CE = cholesteryl esters

*MESA included whites, blacks, Hispanics and Chinese; however, only data from whites are included in the meta-analysis.

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7.a. Will the data be used for non-CVD analysis in this manuscript?
Plasma n6 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.cscc.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)?

#890 Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: The Atherosclerosis Risk in Communities (ARIC) Study
Lead author: Lu Wang

#890B Plasma Fatty Acid Composition and Incidence of Heart Failure in Middle Aged Adults: The Atherosclerosis Risk in Communities (ARIC) Study
Lead author: Kazumasa Yamagishi

#1600: Genome-wide Association Study of Plasma Phospholipid Fatty Acids within the CHARGE Consortium. Lead author: Rozenn Lemaitre

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 & GENVA 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.