ARIC Manuscript Proposal #2070

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

1.a. Full Title: DNA Methylation Related SNPS Interact with Fatty Acids on HDL

b. Abbreviated Title (Length 26 characters): **DNA Methylation, fatty acids, and HDL**

2. Writing Group:

Writing group members: YiYi Ma, Lyn Steffen, Weihua Guan, Mike Tsai, Brian Steffen, Rozenn Lamaitre, Dary Mozafarrian, Myriam Fornage, and others from the CHARGE fatty acid working group.

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

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3. Timeline: 2 years

February, 2013 – January 2014: data analysis at each field center; meta-analysis

February, 2013 – January 2015: manuscript preparation including literature review, describing the methods for each study, methods for meta-analysis, results, and writing the Discussion section.

4. Rationale:

RATIONALE AND OBJECTIVES:

The long-term objective of the proposed project is to investigate relationships between fatty acids, epigenetic changes and genetic variants for CVD risk factors. Epigenetic mechanisms have been shown to regulate gene function to alter phenotypes, and epigenetic events may occur as a result of environmental exposures. Evidence demonstrating relationships between epigenetic events and sequence variation is also accumulating, as allele-specific methylation has been documented at loci distributed across the genome[1, 2]. We hypothesize that sequence variants that alter the likelihood of epigenetic events provide a potential mechanistic explanation for associations between certain SNPs and phenotypes. *In vitro* evidence also shows that specific fatty acids (including palmitic, oleic, butyric and arachidonic, and n-3 fatty acids)[3-5] are correlated with epigenetic changes that may alter gene expression. We further postulate that blood fatty acids interact with SNPs to modulate phenotypes of interest via mechanisms involving epigenetic events.

Although several major epigenetic mechanisms are described (e.g., DNA methylation, histone modification, chromatin remodeling), we will focus on SNPs occurring at predicted DNA methylation sites because of established associations between methylation and multiple CVD risk factors (atherosclerosis[6, 7], dyslipidemia[7] and inflammation[8, 9]). Furthermore, methylation occurs on DNA nucleotides (rather than on chromatin or histone proteins), and is therefore more likely to be sensitive to sequence variation than other epigenetic events.

In the short-term, we seek to investigate the associations and interactions between plasma/red blood cell membrane fatty acids and candidate SNPs in modulating high density lipoprotein cholesterol (HDL), risk factor of CVD. The results from the proposed project will guide future laboratory experiments designed to evaluate SNP functionality that we hypothesize are related to fatty acid-mediated changes in methylation.

In this study, the outcome is HDL. The predictor of interest is those SNPs predicted to be related with DNA methylation or referred as "biomarkers" of DNA methylation change. The effect modifiers are plasma fatty acids. The alternative hypothesis of the study is that plasma fatty acids modify the relationship between SNPs and HDL. Confounders in this study, defined as the factor having associations with both outcome and predictor, are age, sex, BMI, smoking, alcohol, physical activity. Some potential confounders may include population structure or pedigree, education, total fat intake, dietary carbohydrate quality, total energy intake, folate, VitB12, and estrogen therapy. Precision covariates, defined as those reduce standard errors, may include center. Given the extensive gaps in understanding of the relationships between fatty acids, genetic variation and methylation-based mechanisms, we cannot categorize the covariates with complete certainty, and overlaps may exist. However, the proposed models include most standard lifestyle-related factors used for HDL.

SNP SELECTION:

A flowchart detailing selection of candidate SNPs is listed in **Figure 1**. The starting list of SNPs was obtained from GWAS[10-17] of lipids and candidate genes for lipids. Next, eight selection criteria were developed, covering five characteristics of the SNPs: 1) association with relevant phenotypes, 2) demonstrated association with fatty acids, 3) minimum minor allele frequency (MAF), 4) DNA methylation potential, and 5) potential functionality. As a result, 8 SNPs were selected and listed in **Table 1**. Of these 8, 7 SNPs will be meta-analyzed due to genotype availability of each cohort and LD between SNPs.

PRELIMINARY RESULTS IN THE GOLDN STUDY:

We obtained preliminary evidence in GOLDN that suggest that red blood cell membrane fatty acids interact with genetic variants to modulate HDL. **Table 2** and **Table 3** illustrate SNP and fatty acids associations for the three phenotypes. Results of SNPs* fatty acids interactions are listed in **Table 4**.

Figure 1 Flow chart of SNP selection

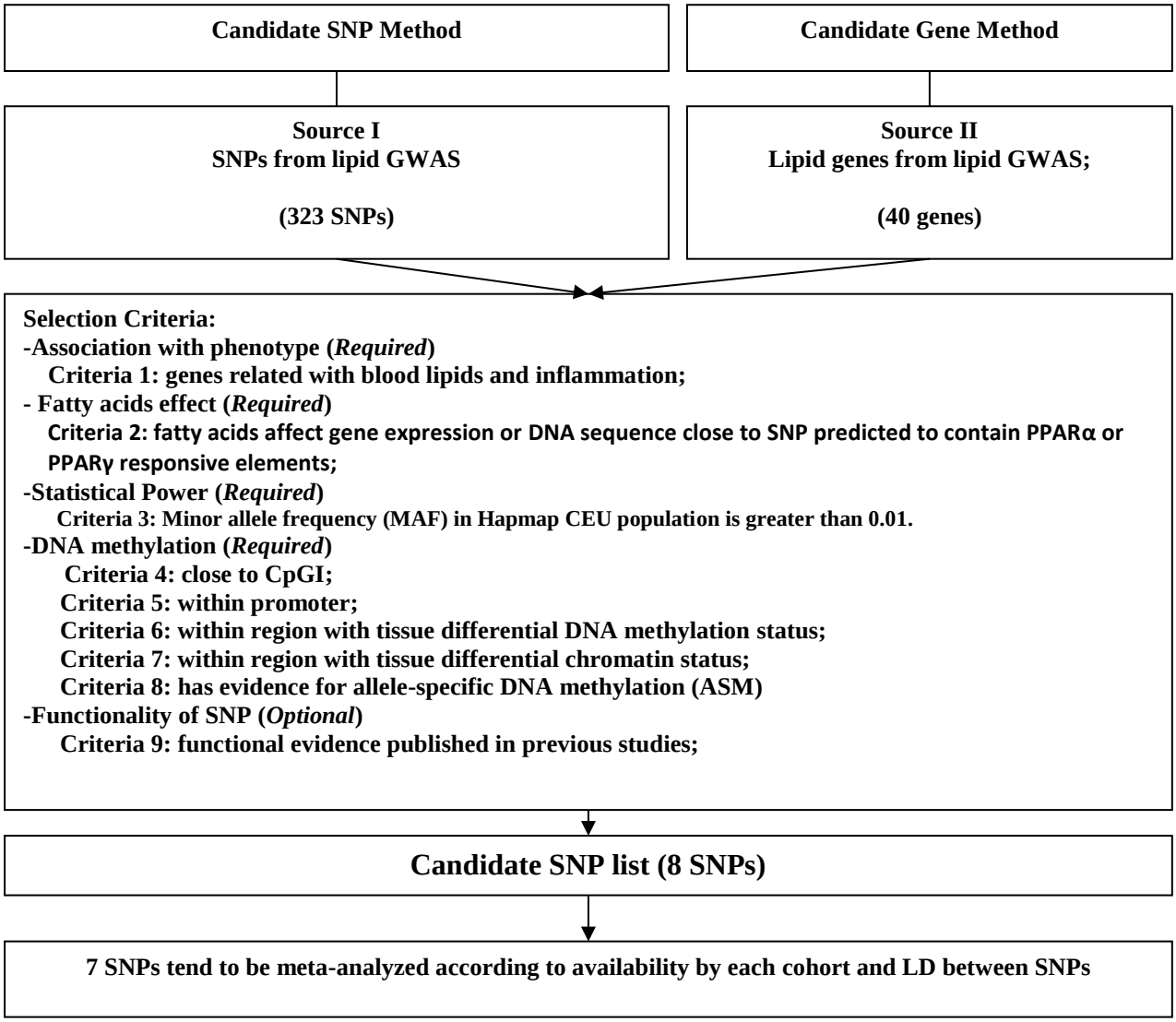

Table 1 Candidate SNPs selected

Number	SNP	Gene	MAF	Criteria	Genotype Availability*	LD with other SNP	Meta-analysis
1	rs405509	APOE	0.491	1,2,3,4,5,6,7,9	3 (2)	No	Yes
2	rs2246293	ABCA1	0.412	1,2,3,4,5,6,7	2 (2)	No	Yes
3	rs3761740	HMGCR	0.153	1,2,3,4,5,6,7,9	4 (4)	No	Yes
4	rs662799	APOA5	0.017	1,2,3,4,5,6,7,9	4 (1)	No	Yes
5	rs2479409	PCSK9	0.35	1,2,3,4,5,6,7,9	3 (3)	No	Yes
6	rs1169288	HNF1A	0.283	1,2,3,4,5,6,7,9	5 (0)	rs2244608	Yes
7	rs2244608	HNF1A	0.283	1,2,3,4,5,6,7	5 (0)	rs1169288	No
8	rs1169287	HNF1A	0.017	1,2,3,4,5,6,7	4 (0)	No	Yes

*Genotype Availability: Number of cohort with genotype either by chip genotyping or imputation (quality >0.8) (Number of cohort with genotype by chip genotyping)

Table 2 Association of SNPs and phenotypes in GOLDN: (yellow and bold indicate P<0.05, pink indicates 0.05<=P<0.1)*

		HDL		
Variable		Beta	Stderr	P
APOA5_m1123	Genotyped	-0.05	0.02	**0.02**
APOE_m226	Genotyped	-0.01	0.01	0.34
ABCA1_rs2246293	Genotyped	0.02	0.01	0.08
PCSK9_rs2479409	Genotyped	-0.03	0.01	**0.01**
HMGCR_rs3761740	Genotyped	0.01	0.02	0.56
HNF1A_rs1169287	Imputed	0.04	0.03	0.20
HNF1A_rs1169288	Imputed	0.01	0.01	0.68

*Model adjusts for pedigree (assuming exchangeable structure within one pedigree), sex, age, center, BMI, smoking status *(categorical: never vs. past vs. current smokers),* physical activity**(continuous, based on study-specific metric),* alcohol intake *(categorical: current vs. former/never),* current estrogen therapy *(categorical: yes/no),* current lipid-lowering medication *(categorical: yes/no),* education level *(categorical: cohort-specific metric) ,* total energy intake *(continuous, kcal/day)*

Table 3 Association of red blood cell membrane fatty acids and phenotypes in GOLDN: (yellow indicates <0.05, pink indicates 0.05<=P<0.1)*

	HDL		
Variable	Beta	Stderr	P
fa160	0.00	0.01	0.52
OleicAcidRbc	0.00	0.01	0.60
fa182cc	0.01	0.00	**0.003**
fa204n6	0.01	0.01	0.06
fa183n3	0.78	0.23	**0.0006**
fa205n3	0.09	0.04	**0.01**
fa226n3	-0.01	0.01	0.37

*Model adjusts for pedigree (assuming exchangeable structure within one pedigree), sex, age, center, BMI, smoking status *(categorical: never vs. past vs. current smokers),* physical activity**(continuous, based on study-specific metric),* alcohol intake *(categorical: current vs. former/never),* current estrogen therapy *(categorical: yes/no),* current lipid-lowering medication *(categorical: yes/no),* education level *(categorical: cohort-specific metric) ,* total energy intake *(continuous, kcal/day)*

Table 4 Interactions of red blood cell membrane fatty acids and SNPs: (Yellow background with bold red fonts are with P<0.01; Yellow background are with 0.01<=P<0.05. Pink background are with 0.05<=P<=0.10)*

SNP/FA		Fa160 Palmitic acid	OleicAcidRbc Sum of fa18111c, fa18112c, fa1819c	fa182cc c/c linoleic acid	fa204n6 Arachidonic acid	fa183n3 Alpha-linolenic acid	fa205n3 EPA	fa226n3 DHA
APOA5_rs662799	Genotyped		HDL					
APOE_rs405509	Genotyped		HDL					
ABCA1_rs2246293	Genotyped						HDL	
PCSK9_rs2479409	Genotyped				**HDL**			
HMGCR_rs3761740	Genotyped		HDL					
HNF1A_rs1169287	Imputed				HDL			
HNF1A_rs1169288	Imputed							

*Model adjusts for pedigree (assuming exchangeable structure within one pedigree), sex, age, center, BMI, smoking status *(categorical: never vs. past vs. current smokers),* physical activity**(continuous, based on study-specific metric),* alcohol intake *(categorical: current vs. former/never),* current estrogen therapy *(categorical: yes/no),* current lipid-lowering medication *(categorical: yes/no),* education level *(categorical: cohort-specific metric) ,* total energy intake *(continuous, kcal/day)*

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5. Main Hypothesis/Study Questions:

To investigate the associations and interactions between plasma/red blood cell membrane fatty acids and candidate SNPs in modulating high density lipoprotein cholesterol (HDL).

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).

Study Design: Meta-analysis
Study population: ARIC participants (Minnesota) who have baseline phospholipid fatty acid values.

Exclusions: those with missing fatty acids and HDL-cholesterol values; non-white race

ANALYSIS PLAN:

OUTCOMES:

Baseline level of HDL (mg/dL), preferred fasting level but also accept non-fasting level.

ASSOCIATION TEST

(1) ASSOCIATION TEST FOR SNP:

A regression coefficient (β± robust SE) for the main effect of SNP and outcome will be calculated in each cohort and values meta-analyzed.

Note: an additive genetic model will be used

(2) ASSOCIATION TEST FOR BLOOD FATTY ACIDS:

A regression coefficient (β± robust SE) for the main effect of plasma or red blood cell membrane fatty acids and outcome will be calculated in each cohort and values meta-analyzed.

(3) ASSOCIATION MODEL COVARIATES:

Model 1:

sex, age *(continuous: years),* center *(if applicable),* population structure or pedigree *(if applicable).*

Model 2:

Covariates from Model 1 plus BMI, smoking status *(categorical: never vs. past vs. current smokers),* physical activity**(continuous, based on study-specific metric),* alcohol intake *(categorical: current vs. former/never),* current estrogen therapy *(categorical: yes/no),* current lipid-lowering medication *(categorical: yes/no),* education level *(categorical: cohort-specific metric)* , total energy intake *(continuous, kcal/day),*

dietary total fat intake (*continuous, %total energy intake/day*), glycemic load (*if applicable*) (*continuous,g/day*), dietary total folate intake (*if applicable*) *(continuous, mcg/day)*, dietary VitB12 intake *(if applicable) (continuous, mcg/day)*.

INTERACTION TEST:

(1) INTERACTION TEST :

A regression coefficient (β± robust SE) for the interaction term for plasma or red blood cell membrane fatty acids*SNP will be calculated in each cohort and values meta-analyzed.

(2) INTERACTION MODEL COVARIATES:

Model 1:

sex, age *(continuous: years),* center *(if applicable),* population structure or pedigree *(if applicable).*

Model 2:

Covariates from Model 1 plus BMI, smoking status *(categorical: never vs. past vs. current smokers),* physical activity**(continuous, based on study-specific metric),* alcohol intake *(categorical: current vs. former/never),* current estrogen therapy *(categorical: yes/no),* current lipid-lowering medication *(categorical: yes/no),* education level *(categorical: cohort-specific metric)* , total energy intake *(continuous, kcal/day),* dietary total fat intake (*continuous, %total energy intake/day*), glycemic load (*if applicable*) (*continuous,g/day*), dietary total folate intake (*if applicable*) *(continuous, mcg/day)*, dietary VitB12 intake *(if applicable) (continuous, mcg/day)*.

(3) Exposures : Plasma MEMBRANE FATTY ACIDS

Palmitic acid (16:0) (continuous, % of total fatty acids),
Oleic acid (18:1) (continuous, % of total fatty acids),
Linoleic acid (18:2n6) (continuous, % of total fatty acids),
Arachidonic acid (20:4n6) (continuous, % of total fatty acids),
Alpha-linolenic acid (18:3n3) (continuous, % of total fatty acids),
EPA (20:5n3) (continuous, % of total fatty acids),
DHA (22:6n3) (continuous, % of total fatty acids).

FA will be modeled continuously for association and interaction analysis.

SNPS TO BE EVALUATED:

No.	SNP	NAME	PROTEIN	Function of Protein
1	rs405509	*APOE -219G/T*	APOE	Catabolism of TG-rich lipoprotein constituents
2	rs2246293	*ABCA1*	ABCA1	Cholesterol efflux from peripheral cells to nascent HDL particles
3	rs3761740	*HMGCR -911C/A*	HMGCR	Rate limiting enzyme for cholesterol synthesis
4	rs662799	*APOA5 -1131T/C*	APOA5	Component of HDL and regulation on TG
5	rs2479409	*PCSK9*	PCSK9	Proprotein convertase for cholesterol homeostasis
6	rs1169287	*HNF1A*	HNF1 homeobox A	Transcription factor required for the expression of several liver-specific genes
7	rs1169288	*HNF1A*	HNF1 homeobox A	Transcription factor required for the expression of several liver-specific genes

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

__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/search.php

   ___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)?**

    a) MS 1928; J Bressler; Genome-wide methylation analyses of cardiovascular disease (CVD) and its risk factors

    b) MS 1929 ; J Pankow; Genome-wide DNA methylation profiling in peripheral blood: quality control and association with demographic characteristics

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No

GWAS via STAMPEDE & GENEVA, #2006.03

11.b. If yes, is the proposal

_X__ A. primarily the result of an ancillary study (list number* _____)
___ 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/

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. OK

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.