1.a. Full Title: Interactions between dietary fat and lipoprotein lipase variants for plasma lipid and lipoprotein concentrations

b. Abbreviated Title (Length 26 characters): LPL x diet for lipoproteins

2. Writing Group:

Project Lead: Kris Richardson, Tufts
Jose Ordovas, Tufts (Kris Richardson’s mentor)
Caren Smith, Tufts (member of Ordovas lab)

ARIC analyst and author: Jennifer Nettleton, UTHSC-H (chair of CHARGE Nutrition Working Group)

Other ARIC contributors are welcome to join this effort.

And authors TBD from other contributing cohorts, ~to date~ including CHS, MESA, Framingham, Health ABC, THISEAS, Young Finns Study, and the Helsinki Birth Cohort Study. Additional author names/affiliations can be provided by Kris Richardson upon request.

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

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3. Timeline:
Data sharing deadline = July 1, 2011; Meta-Analysis complete = July 31, 2011; Additional analyses (if necessary) complete = August 31, 2011; Manuscript drafting / submission = October 1, 2011
4. **Rationale:**

Cardiovascular disease (CVD) is a complex multifactorial disease and the leading cause of mortality in the world. Many genetic factors have been identified in contributing to CVD risk; however, in light of even the most recent GWAS studies, ~ 90% of the attributable genetic risk remains unexplained [1]. A portion of the SNPs associating with CVD traits do not always replicate, an observation that may be explained by potential interactions between multiple genetic and non-genetic factors, such as diet [2,3]. It may be that the effects of these genetic variants on CVD risk are, in part, dependent on dietary factors. Therefore, there is a need to investigate these interactions to define more precisely an individual’s disease risk and the most appropriate therapeutic approach.

Lipoprotein Lipase (LPL) is a key factor in the hydrolysis of triglycerides (TAG) from nascent very low-density lipoprotein (VLDL) and chylomicron lipoproteins into free fatty acids (FFA). Importantly, LPL activity, in part, determines circulating TAG and HDL-C levels [4]. LPL activity has been shown to stimulate PPAR-alpha (PPARα) through generation of unbound fatty acids. Specifically, LPL treatment of VLDL results in PPARα activation including ligand displacement, the induction of PPARα target genes, and peroxisome proliferation *in-vivo* and *in-vitro* [5,6].

Several variants at the LPL locus have shown consistent associations with multiple lipid phenotypes [7,8]. The most studied is rs328, a variant that introduces a premature stop codon in the LPL transcript, and is thought to result in a gain-of-function phenotype (reduced triglycerides and elevated HDL-C). Furthermore, one study reported interactions between the rs328 SNP and both MUFA and SF for HDL-C levels in white adults but not in African American adults [9]. Interestingly, recent work has demonstrated that another SNP falling in the LPL 3’UTR, rs13702, which is not in LD with rs328, associates with both reduced triglycerides and elevated HDL-C levels in populations of European and African ancestry [10,11].

We undertook a preliminary investigation into a possible functional consequence of the rs13702 SNP using the microRNA prediction tool miRanda, which revealed that the C allele disrupts a predicted microRNA (miR) recognition element (MRE) for the miR-410 in the LPL 3’UTR (Figure 1, see page 5). miRs bind MRE sequences in target mRNAs and post-transcriptionally regulate protein output by dampening mRNA translation. The rs13702 SNP is predicted to fall in the 7th position of the MRE seed site, defined as positions 2-7 of the MRE. Importantly, single point mutations in MRE seed sites have shown the ability to reduce or abolish miR-mediated repression [12]. Interestingly, a role for miR-410 has been demonstrated in glucose stimulated insulin secretion (GSIS) *in-vitro*, whereby overexpression of mir-410 enhances GSIS [13]. However, information regarding miR-410 expression and function remains sparse.

The evidence presented here suggests a biological mechanism whereby the rs13072 C allele results in increased LPL levels through disruption of a regulatory MRE in the LPL 3’UTR. If miR-410 acts to down regulate LPL through a MRE, then disruption of this regulatory motif may result in increased LPL levels, and potentially a lipid profile associated with lower risk for CVD. Furthermore, published association data support this mechanism showing that those rs13702 minor alleles carriers have lower TAGs and elevated HDL-C levels compared to non-carriers [10,11].

*References on page 5*
5. Main Hypothesis/Study Questions:
We hypothesize that the rs13702 minor allele may modulate transcript levels of LPL through a novel miR interaction. As a result rs13702 may modulate lipid traits through interaction with dietary fatty acids. Specifically, if the minor allele of rs13702 increases LPL levels in some or all tissues, we would expect this to result in variability in the processing of chylomicron and VLDL lipoproteins, and subsequent unesterified FFA levels which may then contribute to variable PPARa activity between those individuals who carry the rs13702 minor allele and those who do not.

The proposed project will seek to 1) replicate main effects of rs13702 and those SNPs in linkage disequilibrium (LD) with rs13702 (rs326 and 2083637) on relevant outcomes and 2) investigate interactions between dietary fatty acids and rs13702 (and SNPs in LD) with respect to these same outcomes in cohorts participating in the CHARGE Consortium.

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).

Exclusions:
- Inadequate (failed QC) dietary data- cohort specific definition
- Non-fasting
- Missing genotype information for rs13702, rs326 and rs2083637
- Non-white race
- Taking lipid-lowering medications at the time of lipid/lipoprotein measurement

Dietary Exposures:
Saturated fatty acid intake (% of total energy), modeled as a categorical (dichotomized into high and low based on population median intake) and also as a continuous variable.

Polyunsaturated fatty acid intake (% of total energy), modeled as a categorical (dichotomized into low and high based on population median intake) and also as a continuous variable.

Monounsaturated fatty acid intake (% of total energy), modeled as a categorical (dichotomized into low and high based on population median intake) and also as a continuous variable. We would also like correlation statistics for MUFA and SFA, as depending on the population, the sources may be similar or different resulting in higher or lower correlation, respectively.

**Categorical definitions for all fatty acids: Low is defined as < population median intake and high is defined as ≥ population median intake. Please code low as 0 and high as 1 for evaluation of fatty acids as categorical variables.
SNPs to be investigated:

<table>
<thead>
<tr>
<th>No.</th>
<th>SNP</th>
<th>Alleles</th>
<th>R2 with rs13702</th>
<th>Chr:Position</th>
<th>MAF in CEU (Europeans) from HapMap</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs13702</td>
<td>A/G</td>
<td>NA</td>
<td>8: 19824492</td>
<td>0.288</td>
</tr>
<tr>
<td>2</td>
<td>rs326</td>
<td>A/G</td>
<td>0.958</td>
<td>8: 19819439</td>
<td>0.250</td>
</tr>
<tr>
<td>3</td>
<td>rs2083637</td>
<td>C/T</td>
<td>0.917</td>
<td>8: 19865175</td>
<td>0.274</td>
</tr>
</tbody>
</table>

Outcomes: *modeled in SI units*

**Fasting HDL-C, Triglycerides, LDL**

*And if available:* VLDL (total and subfractions) and Chylomicrons (total)

**Main effect Test:**

Cohorts will provide regression coefficients ($\beta \pm SE$) for the main effect of each SNP on each outcome. These values will be meta-analyzed.

*We propose a correlated Bonferroni test, to account for multiple testing:*

3 SNPs
3 types of fat (SFA, PUFA, MUFA)
2 types of lipid outcomes (LDL type and HDL/TG/VLDL/Chylomicrons type)

$\Rightarrow$ $P$ for significance: 0.05/18 = .00278.

**Interaction Test:**

A regression coefficient ($\beta \pm SE$) for the interaction term for fatty acids*SNP will be calculated in each cohort and values meta-analyzed.

*Note: Fatty acid intake will be modeled categorically (high and low based on median intake [%total energy]) and also continuously and an additive genetic model will be used. For categoricals: Low is defined as < population median intake and high is defined as ≥ population median intake. Please code low as 0 and high as 1 for evaluation of fatty acids as categorical variables.*

*We propose a correlated Bonferroni test, to account for multiple testing:*

3 SNPs
3 types of fat (SFA, PUFA, MUFA)
2 types of lipid outcomes (LDL type and HDL/TG/VLDL/Chylomicrons type)

$\Rightarrow$ $P$ for significance: 0.05/18 = .00278.

**Model Covariates for all analyses (Confounders)**

sex, age (continuous years), BMI, and cohort-specific population substructure variables as needed (e.g., field center)

**Other descriptive information requested:**

SATURATED fat intake (% total energy)
POLYUNSATURATED fat intake (% total energy)
TOTAL FAT intake (% total energy)
HDL-C (mmol/L)
Triglycerides (TAG) (mmol/L)
LDL (mmol/L)
VLDL (mmol/L)
Chylomicrons (μmol/dl)
Figure 1:
Note the sequences depicted here are the mRNA 3’UTR complement of the genomic sequence. The rs13702 SNP in the LPL 3’UTR is bolded. Capital letters indicate complementary nucleotides. When the minor G allele is present binding between the LPL 3’UTR and miR-410 is disrupted at the 7th position.

Major A allele
3’- ugccggUAGACACAAUAUaa -5’ miR-410

5’- uccgaAAAACUUUGUUUAUA 3’ LPL 3’UTR

Minor G allele
3’- ugccggguagacAAUAUaa -5’ miR-410

X

5’- uccgaaaaacuUGCUUAUua 3’ LPL 3’UTR

REFERENCES
7.a. Will the data be used for non-CVD analysis in this manuscript?  **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**

(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](http://www.cscc.unc.edu/ARIC/search.php)

**YES—no overlap**

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 related projects are also from the CHARGE Nutrition working group:

1675 “Low density lipoprotein receptor related protein 1, fatty acids and anthropometric traits”

Other ARIC proposals**: **NO OVERLAP**

#1305, NETTLETON et al. “Interaction between ANGPTL4 and Dietary Fat and Carbohydrate in relation to Triglycerides and HDL-cholesterol in the Atherosclerosis Risk in Communities (ARIC) Study” Published *Atherosclerosis* 203: 214-220, 2009. PMCID: PMC2649986

#1101, NETTLETON et al. “LIPC polymorphisms, dietary fat, and plasma HDL cholesterol in adults with and without type II diabetes” Published *Atherosclerosis* 194: e131-e140, 2007. PMCID: PMC2248232

#1130, VOLCIK et al. “Association of peroxisome proliferator-activated receptor a (PPARa) polymorphisms with lipid levels and possible effect modification of polyunsaturated fatty acid intake” Published *American Journal of Clinical Nutrition* 87:1926-1931, 2008. PMCID: PMC2661261

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*  **AS 2006.03**

*ancillary studies are listed by number at [http://www.cscc.unc.edu/aric/forms/](http://www.cscc.unc.edu/aric/forms/)
ARIC is one of several cohort studies contributing data to the CHARGE/MAGIC-based meta-analysis.

Since this work is a product of CHARGE which utilizes GWA data, ancillaries related to STAMPEDE & GENVA are also acknowledged.

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.

Understood, and we will meet this deadline