ARIC Manuscript Proposal #1212

PC Reviewed: _2_/_13/06	**Status: __A__**	**Priority: __2_**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Polymorphisms in the LOX-1 gene *OLR1* and the risk of coronary disease

b. Abbreviated Title (Length 26 characters): LOX-1 and CAD

2. Writing Group:

Writing group members: Joshua W. Knowles, Themistocles L. Assimes, Kelly Volcik, Thomas Quertermous

Other authors will include: Eric Boerwinkle, Megan Grove, Audrey Southwick, Carlos Irbibarren, Alan S. Go, Steve Sidney, Mark A. Hlatky, Stephen P. Fortmann, Richard M. Myers, Neil Risch

Although there are a considerable number of authors, all of them have contributed to this work. The studies described in the paper (ADVANCE, ARIC and CARDIA) are all large, multi-center studies with their own publication requirements. All of the proposed authors have read this manuscript proposal and have given their permission for it to be submitted to the ARIC publications committee.

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

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3. Timeline:
ARIC statistical analysis: Dec-Jan 2006
Manuscript preparation: Jan 2006-Feb 2007
Manuscript revision: March 2007
Manuscript submission: April 2007

4. Rationale:
To uncover novel genetic modifiers of CAD, the ADVANCE study (Atherosclerotic Disease, VAscular functioN, and genetiC Epidemiology) a collaborative effort between Stanford and Kaiser Permanente of Northern California (KPNC), was initiated. ADVANCE is a large population based candidate gene association study of subjects receiving care within KPNC. Single nucleotide polymorphism (SNP) discovery and sequencing was performed on ~100 candidate genes.

One of our candidate genes was the lectin-like oxidized LDL receptor (LOX-1) encoded by *OLR1*. LOX-1 specifically binds and internalizes oxLDL and has pleotropic effects on endothelial dysfunction and atherosclerosis [1-7] (Figure 1). *In vitro*, oxLDL binding to LOX-1 results in: increased expression of cellular adhesion molecules, monocyte chemoattractant protein 1 (MCP-1), CD40/CD40L and matrix metalloproteinases (MMPs); and activation of pro-apoptotic pathways [8-16]. *In vivo*, LOX-1 is found at high concentrations in human atherosclerotic lesions and overexpression of LOX-1 in apolipoprotein E -/- mice results in increased cholesterol deposition in coronary arteries [17, 18]. Small human association studies have been conflicting regarding whether polymorphisms in this gene are associated with CAD [19-21]. As part of the overall goals of ADVANCE we sought to test whether SNPs in the LOX-1 gene alter susceptibility to CAD.

We first resequenced the promoter, exonic, and splice site regions of *OLR1* and then genotyped four single nucleotide polymorphisms (SNPs) in 1,547 cases with clinical CAD and 1,583 controls. We did not find any association between one of these SNPs and CAD. Three other SNPs had nominal associations with CAD. One of these SNPs is a previously known non-synonymous coding SNP (rs11053646, Lys167Asn), referred to as LOX1.2. The other two SNP were in complete LD (r^2 =0.99) and included an intronic SNP (rs3736232, referred to as LOX1.3) as well as a SNP in the 3'UTR referred to as LOX1.16 (rs1050286**)**. In the ADVANCE cohort after adjustment for traditional risk factors, LOX1.2 was associated with a lower odds ratio (OR) of CAD across all major ethnic groups studied (white/European, African American, East Asian and Hispanic)(combined OR 0.76, CI 0.63-0.91, P = 0.003). LOX1.3 and LOX1.16 were nominally associated with an increased risk of CAD (OR 1.14, CI 0.97-1.33, P = 0.04).

To avoid reporting spurious associations, we sought to replicate our findings in an independent cohort from the Atherosclerosis Risk in Communities study (ARIC), a prospective investigation of atherosclerosis in 15,792 white and African American individuals begun in 1987. We genotype LOX1.2 and LOX1.3 in the ARIC cohort and

will perform an analysis in the ARIC cohort to look for an association of these SNPs with CAD. Our power to detect an association in the ARIC cohort is > 80%.

5. Main Hypothesis/Study Questions:

1. To estimate the frequency distribution of two LOX-1 SNPs in the ARIC cohort
2. In a race specific manner to evaluate the association of these SNP with CHD events as well as CVD events (as defined by previous ARIC publications[22]).
3. To compare the HRR for this SNP in incident fatal CHD vs. all incident CHD.

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).

Please note that this manuscript is part of an ongoing collaboration with the ARIC group. We will follow the same strategy as was used in our recently approved manuscript: ARIC MS# 1168, "A near null variant of 12/15-LOX encoded by a novel SNP in ALOX15 and the risk of coronary artery disease". The main people responsible for the ARIC data analysis are Kelly Volcik and Eric Boerwinkle and they were also involved in the preparation of MS# 1168.

The usual DNA restriction, ethnic group and missing data exclusion criteria will be used. Analysis will generally be performed as has been done in previous ARIC manuscripts [22]. Exclusions will include the following: 1) positive or unknown history of prevalent CHD or stroke or history of TIA/stroke, 2) prohibited use of DNA, 3) ethnic background other than white or African American, as well as African Americans not from Jackson or Forsyth. For incident CHD analyses, we will use the variable in_02sp; analyses for CVD will combine incident CHD and incident stroke cases (in02dp). Covariates to be included in the analyses include age, gender, race, field center, HDL and total cholesterol, BMI, smoking, diabetes and hypertension status.

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 ICTDER02 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 ICTDER02 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 ICTDER02 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)?

Our groups will be collaborating on several manuscripts as governed by the ancillary study proposal listed below.

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 (list number* 2006.01__)

___ 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/

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.

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