

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 Iribarren, 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]

First author: Joshua Knowles
Division of Cardiovascular Medicine
Stanford University School of Medicine
Falk CVRC
300 Pasteur Dr
Stanford, CA 94305
Phone: Tel: 650-498-4154
Fax: 650-725-1699
E-mail: knowlej@stanford.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):
Address:

Phone:
E-mail:

Fax:

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 pleiotropic effects on endothelial dysfunction and atherosclerosis¹⁻⁷ (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 pathways⁸⁻¹⁶. *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 CAD¹⁹⁻²¹. 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 SNPs 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 publications²²).
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 manuscripts²². 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
☒ 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? ☒ 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"?

☒ 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.csc.unc.edu/ARIC/search.php>

☒ 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? ☒ Yes ☐ No

11.b. If yes, is the proposal

☒ 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.csc.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.

References

1. Sawamura T, Kume N, Aoyama T, et al. An endothelial receptor for oxidized low-density lipoprotein. Nature 1997; 386:73-7.
2. Aoyama T, Sawamura T, Furutani Y, et al. Structure and chromosomal assignment of the human lectin-like oxidized low-density-lipoprotein receptor-1 (LOX-1) gene. Biochem J 1999; 339 (Pt 1):177-84.
3. Yamanaka S, Zhang XY, Miura K, Kim S, Iwao H. The human gene encoding the lectin-type oxidized LDL receptor (OLR1) is a novel member of the natural killer gene complex with a unique expression profile. Genomics 1998; 54:191-9.

4. Chen M, Inoue K, Narumiya S, Masaki T, Sawamura T. Requirements of basic amino acid residues within the lectin-like domain of LOX-1 for the binding of oxidized low-density lipoprotein. *FEBS Lett* 2001; 499:215-9.
5. Chen M, Sawamura T. Essential role of cytoplasmic sequences for cell-surface sorting of the lectin-like oxidized LDL receptor-1 (LOX-1). *J Mol Cell Cardiol* 2005.
6. Ohki I, Ishigaki T, Oyama T, et al. Crystal structure of human lectin-like, oxidized low-density lipoprotein receptor 1 ligand binding domain and its ligand recognition mode to OxLDL. *Structure (Camb)* 2005; 13:905-17.
7. Park H, Adsit FG, Boyington JC. The 1.4 angstrom crystal structure of the human oxidized low density lipoprotein receptor lox-1. *J Biol Chem* 2005; 280:13593-9.
8. Cominacini L, Pasini AF, Garbin U, et al. Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-kappaB through an increased production of intracellular reactive oxygen species. *J Biol Chem* 2000; 275:12633-8.
9. Li D, Mehta JL. Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. *Circulation* 2000; 101:2889-95.
10. Hayashida K, Kume N, Minami M, Kita T. Lectin-like oxidized LDL receptor-1 (LOX-1) supports adhesion of mononuclear leukocytes and a monocyte-like cell line THP-1 cells under static and flow conditions. *FEBS Lett* 2002; 511:133-8.
11. Li D, Liu L, Chen H, Sawamura T, Mehta JL. LOX-1, an oxidized LDL endothelial receptor, induces CD40/CD40L signaling in human coronary artery endothelial cells. *Arterioscler Thromb Vasc Biol* 2003; 23:816-21.
12. Hofnagel O, Luechtenborg B, Stolle K, et al. Proinflammatory cytokines regulate LOX-1 expression in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2004; 24:1789-95.
13. Chen J, Mehta JL, Haider N, Zhang X, Narula J, Li D. Role of caspases in Ox-LDL-induced apoptotic cascade in human coronary artery endothelial cells. *Circ Res* 2004; 94:370-6.
14. Kume N, Kita T. Apoptosis of vascular cells by oxidized LDL: involvement of caspases and LOX-1 and its implication in atherosclerotic plaque rupture. *Circ Res* 2004; 94:269-70.
15. Li D, Liu L, Chen H, Sawamura T, Ranganathan S, Mehta JL. LOX-1 mediates oxidized low-density lipoprotein-induced expression of matrix metalloproteinases in human coronary artery endothelial cells. *Circulation* 2003; 107:612-7.
16. Chen H, Li D, Saldeen T, Mehta JL. Transforming growth factor-beta(1) modulates oxidatively modified LDL-induced expression of adhesion molecules: role of LOX-1. *Circ Res* 2001; 89:1155-60.
17. Kataoka H, Kume N, Miyamoto S, et al. Expression of lectinlike oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. *Circulation* 1999; 99:3110-7.
18. Inoue K, Arai Y, Kurihara H, Kita T, Sawamura T. Overexpression of lectin-like oxidized low-density lipoprotein receptor-1 induces intramyocardial vasculopathy in apolipoprotein E-null mice. *Circ Res* 2005; 97:176-84.
19. Tatsuguchi M, Furutani M, Hinagata J, et al. Oxidized LDL receptor gene (OLR1) is associated with the risk of myocardial infarction. *Biochem Biophys Res Commun* 2003; 303:247-50.
20. Mango R, Clementi F, Borgiani P, et al. Association of single nucleotide polymorphisms in the oxidised LDL receptor 1 (OLR1) gene in patients with acute myocardial infarction. *J Med Genet* 2003; 40:933-6.
21. Ohmori R, Momiyama Y, Nagano M, et al. An oxidized low-density lipoprotein receptor gene variant is inversely associated with the severity of coronary artery disease. *Clin Cardiol* 2004; 27:641-4.
22. Volcik KA, Ballantyne CM, Coresh J, Folsom AR, Wu KK, Boerwinkle E. P-selectin Thr715Pro polymorphism predicts P-selectin levels but not risk of incident coronary heart disease or ischemic stroke in a cohort of 14595 participants: the Atherosclerosis Risk in Communities Study. *Atherosclerosis* 2006; 186:74-9.