ARIC Manuscript Proposal # 1168

PC Reviewed: _06_/_20_/06	**Status: _A_**	**Priority: _2_**
SC Reviewed: _06/23/06__	**Status: _A_**	**Priority: _2_**

1.a. Full Title: **A Single Nucleotide Polymorphism in Exon 13 of the 12/15-Lipoxygenase Gene (ALOX15) is associated with an increased risk of clinical coronary artery disease and a marked reduction in enzymatic function**

b. Abbreviated Title (Length 26 characters): **ALOX15 and CAD**

2. Writing Group:
Principle writing group: Themistocles L. Assimes, James R. Priest, Joshua W. Knowles, Hartmut Kuhn, Thomas Quertermous
Other authors include: Megan Grove, Kelly Volcik, Eric Boerwinkle, Astrid Borchert, Raymond Tabibiazar, Holly K. Tabor, Audrey Southwick, Carlos Iribarren, Alan S. Go, Steve Sidney, Mark A. Hlatky, Stephen P. Fortmann, Richard M. Myers, Neil Risch

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

First author:
Themistocles L. Assimes
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: tassimes@cvmed.stanford.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author): Include info for corresponding author: Same as above (T.L. Assimes)

3. Timeline:
ARIC statistical analysis: July-September 2006
Manuscript preparation: July-September 2006
Manuscript revision: September 2006
Manuscript submission: September-October 2006

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 >3,600 subjects receiving care within

KPNC. Using a candidate gene approach, single nucleotide polymorphism (SNP) discovery and sequencing was performed on over 80 genes in 1,892 cases presenting with incident symptomatic CAD and 1,766 control subjects. We have found single nucleotide polymorphisms (SNPs) in several genes that are strongly associated with CAD.

One of our most promising findings was in the 15-lipoxygenase gene (*ALOX15*). 15-lipoxygenase is a key enzyme in eicosanoid biosynthesis has been implicated in vascular inflammation and atherosclerosis [1-9]. In ADVANCE, we genotyped three SNPs in *ALOX15* in 1547 cases with clinical CAD and 1583 controls. In 1309 cases and 1117 controls of white/European or Hispanic ancestry, a SNP in Exon 13 of *ALOX15* (termed ALOX15_R_18) with a MAF of 1.2% in white/Europeans and 8% in Hispanics was associated with an increased risk of symptomatic CAD independent of all traditional risk factors (OR, 1.68; 95% confidence interval, 1.11-2.59; P = 0.015). The minor allele was very uncommon (< 1%) in our subjects reporting Black/African American ancestry and non-existent in East Asians. This coding SNP leads to a change in an evolutionary conserved threonine in position 560 of the protein to methionine (T560M). Through collaboration with Dr. Hartmut Kuhn we conducted functional assays of this SNP. *In vitro* studies on the T560M mutant expressed in pro- and eukaryotic systems displayed a 20-fold decrease in catalytic activity, suggesting that the methionine allele creates a functionless enzyme.

However, due to issues related to multiple hypothesis testing, replication of our findings is essential to minimize the chances of reporting spurious associations. Therefore, we were very interested in replicating these results in ARIC (along with findings in *ALOX5*, *OLR1* and *ELN)* and earlier this year we submitted an ancillary study proposal to attempt these replication studies (ancillary study #2006.01).

5. Main Hypothesis/Study Questions:

1. To estimate the minor allele frequency of the ALOX15_R_18 SNP in ARIC
2. To evaluate the association of this SNP with CHD events as well as CVD events.
3. To calculate Cox Proportional HRRs for incident CHD adjusted for race and all traditional risk factors.
4. To perform subgroup analysis using the Framingham Risk score.
5. To compare the HRR for this SNP in incident fatal CHD vs. incident non fatal CHD.

6. Data (variables, time window, source, inclusions/exclusions):

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

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. primiarly based on ARIC data with ancillary data playing a minor role

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

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