Author's response to reviews

Title: Failure to replicate an association of SNPs in the oxidized LDL receptor gene (OLR1) with CAD

Authors:

Joshua Knowles (knowlej@stanford.edu)
Tim Assimes (tassimes@cvmed.stanford.edu)
Eric Boerwinkle (Eric.Boerwinkle@uth.tmc.edu)
Steve Fortmann (Fortmann@stanford.edu)
Alan Go (Alan.S.Go@kp.org)
Megan Grove (Megan.L.Grove@uth.tmc.edu)
Mark Hlatky (mah@stanford.edu)
Carlos Iribarren (Carlos.Iribarren@kp.org)
Jun Li (junzli@umich.edu)
Richard Myers (myers@paxil.stanford.edu)
Neil Risch (rischn@humgen.ucsf.edu)
Stephen Sidney (Steve.Sidney@kp.org)
Audrey Southwick (southwic@stanfordalumni.org)
Kelly Volcik (Kelly.A.Volcik@uth.tmc.edu)
Tom Quertermous (tomq1@stanford.edu)

Version: 3 Date: 1 April 2008

Author's response to reviews: see over
Dear Dr. Norton,

We are writing to you in regards to our manuscript “Failure to replicate an association of SNPs in the oxidized LDL receptor gene (OLR1) with CAD”.

We are delighted that it has been accepted for publication and we have revised the manuscript to address the minor editorial and stylistic comments.

Thank you,

Josh Knowles, MD, PhD
Cardiovascular Medicine Fellow, Stanford University
300 Pasteur Drive, Falk CVRC
Stanford, CA 94305
tel: 650-723-1431
fax: 650-725-2178
email: knowlej@stanford.edu

Themistocles Assimes, MD, MS
Instructor of Medicine, Division of Cardiovascular Medicine, Stanford University
300 Pasteur Drive, Falk CVRC
Stanford, CA 94305
tel: 650-498-4154
fax: 650-725-2178
tassimes@cvmed.stanford.edu

Thomas Quertermous, MD
Director, Division of Cardiovascular Medicine (Research) Stanford Cardiovascular Medicine, Falk CVRC 300 Pasteur Drive Stanford, CA 94305
tel: 650-723-5013
fax: 650-725-2178
email: tomq1@cvmed.stanford.edu

on behalf of all co-authors
Reviewer:

The first reviewer has also published in the area and supports the null hypothesis, so is also conflicted.

We respectfully disagree that someone who has published negative results on this gene as having the same conflict of interest as someone who has a strong, non-disclosed, financial conflict of interest. Furthermore, the first reviewer’s report did not focus only on LOX1 alone but rather on a large number of putative genotype-phenotype associations with CAD.

The ADVANCE study actually represents an incredible replication of the study by Mango et al. in terms of the consistency of direction on both SNPs. I find that hard to ignore. Especially when I calculate from a straight ch-square that the ADVANCE study is much more significant than they report (I calculate p=10^{-5} of the 3’ SNP).□

While indeed the Odds Ratios for both SNPs are in the same direction as the study of Mango et al., we find it hard to ignore the following additional facts:

1) both the ADVANCE results and the Mango et al. results for the non-synonymous SNP LOX1.2 (or K167N) are in the opposite direction of Tatsuguchi et al. (Pub Med ID 12646194) where the minor allele increases risk almost 3 fold.
2) Trabetti et al. could not demonstrate an association between K167N and AMI or CAD (PMID 16251892 and 16724009)
3) Sentinelli et al. could not find an association between the 3’ UTR SNPs and AMI or CAD (PMID: 16829343).

The reviewer does not specify which genotype counts he used to perform his “straight up chi square” in the ADVANCE study. However, based on the reviewer’s results (“I calculate p =10^{-5} of the 3’SNP) and our calculations, we assume this “straight up chi square” was calculated using the combined genotype counts of cases and controls across all race/ethnic groups for LOX1.3 (genotype counts of “all” at the bottom of Table 3).

We remind the reviewer that there are significant differences in the proportions of the 6 race/ethnic groups between cases and controls (please review Table 1, Ancestry) despite an attempt to keep these proportions roughly equal by frequency matching controls to cases on race/ethnic group (this imbalance is mentioned in the first paragraph of the results section). Since there are also very significant differences in the frequency of the minor allele of LOX1.3 (and LOX1.2) across race/ethnic groups (please review Table 2), the criteria for confounding are met (i.e. the outcome and the exposure are both associated with a third variable which in this case is race/ethnic group). Therefore, calculating a straight up chi square is not valid for our data because the result is seriously confounded by race/ethnic group. Chi squares across all race/ethnic groups should only be calculated after stratification by race/ethnic group (for example using a Mantel-Haenszel approach to calculating a chi square). When this is done, the p-values are not much different than the p-values obtained from our logistic regression analyses (see details in table below). In general they are slightly higher but this loss of precision is to be expected when some variables that are not confounders are forced into multivariate analyses. We note the logistic regression p-values differ the most from the chi square p values in the two “mixed” race ethnic groups because even within these race/ethnic groups there are significant differences in admixture between cases and controls (which we accounted for using STRUCTURE, see Statistical Analyses section).
### Comparison of Chi square derived P values to Logistic Regression derived p values

<table>
<thead>
<tr>
<th></th>
<th>LOX1.2 P values</th>
<th></th>
<th>LOX1.3 P values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi Square</td>
<td>Logistic Regression</td>
<td>Chi Square</td>
<td>Logistic Regression</td>
</tr>
<tr>
<td>Whites</td>
<td>0.00089295</td>
<td>0.00750688</td>
<td>0.03942583</td>
<td>0.05535660</td>
</tr>
<tr>
<td>Blacks</td>
<td>0.26849548</td>
<td>0.32966687</td>
<td>0.10925578</td>
<td>0.05416108</td>
</tr>
<tr>
<td>Hispanics</td>
<td>0.976462339</td>
<td>0.92022260</td>
<td>0.69715393</td>
<td>0.6979905</td>
</tr>
<tr>
<td>East Asians</td>
<td>0.09997030</td>
<td>0.06884809</td>
<td>0.58176476</td>
<td>0.35065268</td>
</tr>
<tr>
<td>Mixed Hispanics</td>
<td>0.02265435</td>
<td>0.36830961</td>
<td>0.51323483</td>
<td>0.95572910</td>
</tr>
<tr>
<td>Mixed Other</td>
<td>0.67503064</td>
<td>0.32460031</td>
<td>0.37657637</td>
<td>0.68209565</td>
</tr>
<tr>
<td>All Races Combined not adjusting for race*</td>
<td>0.00000132</td>
<td>never done</td>
<td>0.00000177</td>
<td>never done</td>
</tr>
<tr>
<td>All Races Combined adjusted for race**</td>
<td>0.00307521</td>
<td>0.00678478</td>
<td>0.00703596</td>
<td>0.03349490</td>
</tr>
</tbody>
</table>

**AMI cases only**

<table>
<thead>
<tr>
<th></th>
<th>LOX1.2 P values</th>
<th></th>
<th>LOX1.3 P values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi Square</td>
<td>Logistic Regression</td>
<td>Chi Square</td>
<td>Logistic Regression</td>
</tr>
<tr>
<td>Whites</td>
<td>0.04007555</td>
<td>0.09455477</td>
<td>0.17781053</td>
<td>0.39476610</td>
</tr>
<tr>
<td>Blacks</td>
<td>0.25641330</td>
<td>0.31537443</td>
<td>0.03801985</td>
<td>0.04182657</td>
</tr>
<tr>
<td>Hispanics</td>
<td>0.892925595</td>
<td>0.78117706</td>
<td>0.80030931</td>
<td>0.50534691</td>
</tr>
<tr>
<td>East Asians</td>
<td>0.42392268</td>
<td>0.23159592</td>
<td>0.39507936</td>
<td>0.23197710</td>
</tr>
<tr>
<td>Mixed Hispanics</td>
<td>0.08133134</td>
<td>0.99172909</td>
<td>0.61786031</td>
<td>0.98971375</td>
</tr>
<tr>
<td>Mixed Other</td>
<td>0.50713719</td>
<td>0.39188447</td>
<td>0.29139705</td>
<td>0.67557940</td>
</tr>
<tr>
<td>All Races Combined not adjusting for race*</td>
<td>0.00017359</td>
<td>never done</td>
<td>0.00038331</td>
<td>never done</td>
</tr>
<tr>
<td>All Races Combined adjusted for race**</td>
<td>0.02737023</td>
<td>0.04197147</td>
<td>0.04612313</td>
<td>0.19941740</td>
</tr>
</tbody>
</table>

*Non logistic regression Chi Square calculations performed but not valid estimates of association because of confounding by race/ethnic group
**chi square based on Mantel-Haenszel method

The discussion centres around lack of replication and chance findings and does not discuss the differences in the study design appropriately. The fact that the 3' SNP is highly significantly associated with risk in retrospective studies of MI survivors but is significantly protective in a cohort of incident cases immediately suggests that the case controls suffer from "reverse causality" issues. In this case it is important that the role of the SNP in MI survival should be assessed in the ARIC study. This is a very difficult issue. My personal opinion, and that of many in the literature, is that differential inclusion rates of angina into the CAD case definition will dilute any gene effects especially in older cohorts, and renders replication very difficult. I would prefer to see the MI data.

It is not clear to us what the reviewer means by “reverse causality issues”. We assume that the reviewer is making reference to reverse causality bias which is also known as protopathic bias. This type of bias is a consequence of not recognizing that the outcome has already occurred at the time of measuring the exposure and is actually responsible for the exposure. In the case of genetic association studies (regardless of study design), reverse causality bias is impossible as the genotype (and therefore the exposure of interest) is determined at conception and the outcome always occurs after this time.

In case the reviewer is referring to a selection bias induced by studying only the subset of subjects surviving their first ever episode of CAD (MI or angina) for a period long enough to be seen enrolled in the ADVANCE study and seen in clinic, we remind the reviewer that the cases in the study of Mango et al. were also survivors of MI. Furthermore, the mean period of time between symptom onset and enrollment into the study was significantly longer in Mango et al. than the ADVANCE study (page 933 of PMID 14684693, first column, last sentence “The mean age of theAMI patients was 64.4 (5.8 years) (mean (SD)) with a mean age at onset of symptoms 60.4 (4.5) years”).
We respectfully disagree with the reviewers comments “differential inclusion rates of angina into the CAD case definition will dilute any gene effects especially in older cohorts, and renders replication very difficult”. The reviewer states that this is the opinion of many in the literature but provides no references. The reasons we disagree are outlined in the detailed responses to reviewer #2 from our original submission. Our study defined the outcome as clinically significant complications of CAD which are not limited to MI but include angina. This is a very valid composite endpoint used in many clinical trials (too many to list) and observational studies (including ARIC, CHS, MESA, Framingham and CARDIA studies) of coronary atherosclerosis as long as non-MI presentations are carefully adjudicated (as was the case in the ADVANCE and ARIC studies).

Nevertheless, as suggested by the reviewer, we have performed additional analyses using only cases that have presented with an MI in both the ADVANCE and ARIC studies. These data are included in the supplementary materials. As seen from the supplementary tables, in ADVANCE the results for both SNPs (LOX1.2 and LOX1.3) are not substantially different in any of the three models (recessive, additive or dominant either minimally or fully adjusted) whether cases include all subjects with clinically significant CAD versus cases only presenting with MI. While the point estimates for the odds ratios are quite similar there is a loss of significance in the MI comparison presumably because of a decrease in power caused by excluding subjects with incident angina. For instance, LOX 1.2 in our overall analysis has a fully adjusted, additive model OR 0.8 (0.68-0.95, P < 0.05) for the “clinically significant CAD vs control” as compared to an OR 0.84 (0.69-1.02, P = 0.07) for the “MI vs control” comparison. Similar results were found for LOX 1.3 (OR 1.13, 1.0-1.25, P < 0.05 vs 1.08, 0.96-1.22, P = 0.19).

In ARIC the results are also not substantially different comparing “incident CHD” vs “incident MI + fatal CHD” vs “incident non-fatal MI”. For these three comparisons the overall HRR, CI and P values in fully adjusted additive model for LOX 1.2 are: 0.99, 0.9-1.1, NS; 0.98, 0.9-1.1, NS; 0.98, 0.8-1.2, NS and for LOX 1.3 are: 0.92, 0.9-1.0, P = 0.04; 0.88, 0.8-1.0, P = 0.01; 0.91, 0.8-1, NS.

Therefore, restricting analyses to subjects presenting with MI does not strengthen the argument that these SNPs play a significant role in disease. We have added text describing the results of the analyses restricted to MI in the text and have included them as supplementary tables. However, we do not feel that these tables need to be added to the manuscript proper.