Author's response to reviews

Title: Association of sICAM-1 and MCP-1 with coronary artery calcification in the NHLBI Family Heart Study follow-up examination

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Author's response to reviews: see over
September 12, 2007

Melissa Norton, MD
*BMC Cardiovascular Disorders*, Editor-in-Chief

Re: Association of sICAM-1 and MCP-1 with Coronary Artery Calcification in the NHLBI Family Heart Study Follow-up Examination

Dear Dr. Norton:

On behalf of my co-authors, I thank you for your careful review of the above-referenced manuscript and for your willingness to consider a revision for publication in *BMC Cardiovascular Disorders*.

As directed, we have responded to the reviewers’ comments in detail (on separate sheets). The revisions made in response to the previous review has clarified and strengthened our paper. We have also added IRB approval information to the manuscript (page 6, 2nd paragraph).

Thank you very much in advance for your careful consideration of our revised manuscript.

Sincerely yours,

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Response to Reviewers’ Comments

We would like to thank both reviewers for their careful review of our manuscript. Their insightful comments and suggestions have helped us clarify and strengthen the manuscript. In the follow please find our response (in bold font) to their specific comments:

Reviewer #1: Iftikhar Kullo

Reviewer’s report:
General
Tang et al investigated the association of ICAM-1 and MCP-1 with CAC in 2716 subjects (2246 Whites and 470 African Americans).

Significance
The paper adds an important piece of information to the rapidly growing field of the interaction of cardiovascular biomarkers with subclinical coronary atherosclerosis.

Clarity of hypothesis and rationale
The hypothesis is based on a well established set of existing data linking some cardiovascular biomarkers with coronary atherosclerosis, and is clearly stated.

Adequacy of experimental design and methods
The design was a cross-sectional which has limitations. The manuscript is well written and methods are appropriate and well described.

Quality of data and presentation of results
Data is presented clearly and in an unbiased fashion; however the authors may want to shorten the results section.
Response: We have shortened the Results section. Specifically, we have shortened the two paragraphs presenting the results of tables 2 and 3 (page 12).

Comments to the Author
What determined the choice of biomarkers for the study? Why did the authors choose ICAM-1 among the adhesion molecules family? Have the authors measured the level of VCAM-1 or Pselectin?
Response: The level of VCAM-1 or Pselectin has not been measured in the study. The Family Heart Study Steering Committee chose the biomarkers based on prior data in the literature. Among the three adhesion molecules (ICAM-1, VCAM-1, and Pselectin), ICAM-1 had more published data regarding its association with coronary heart disease in the literature, suggesting that ICAM-1 might be a stronger biomarker of coronary atherosclerosis than the other two.
How was CRP related to CAC presence?

Response: In response to the reviewer’s question, we analyzed the association of CRP with CAC the same way as the association of CAC with MCP-1 and sICAM-1. When CAC > 0 was the outcome variable, CRP was significantly associated with CAC before and after adjustment for age and gender. But the association was no longer significant after additional adjustment for other traditional CHD risk factors. When CAC ≥ 10 was the outcome variable, CRP was only significantly associated with CAC before covariate adjustment. Our data is largely consistent with data from other studies such as the Dallas Heart Study in which CRP and CAC were significantly associated before but not after adjustment for traditional cardiovascular risk factors and estrogen and statin use (Khera A, et al, Circulation 2006, 113(1): 38-43). We did not include the data on the association between CRP and CAC in this manuscript because it is beyond the scope of the paper.

Did the authors consider measuring ICAM-1 levels by an alternative commercial human sICAM-1 ELISA (BMS201INST; Bender MedSystems)?

Response: We used the R&D assay to measure sICAM-1 because our lab at the University of Vermont had experience in using this assay in other studies and found its performance acceptable. We did not try alternative commercial assays due to limited budget.

Did the authors test the interaction of age and sex on the association between these biomarkers and CAC?

Response: We would like to thank the reviewer for raising this question. The interaction of sex on the association between these biomarkers and CAC has been tested and reported in the Results section (last line on page 10 – lines 1-3 on page 11). In response to the reviewer’s request to test age interaction, we grouped white participants by age tertile cutpoints (47 and 63 years) and African American participants by the median cutpoint (51.5 years) and tested whether the associations of CAC with sICAM-1 and MCP-1 differed across age groups. We used the median cutpoint of age in African Americans due to limited sample size. We did not observe any significant age difference in the association between CAC and MCP-1 in either race group or between CAC and sICAM-1 in whites. This result has been added to the Results section (page 11, 1st paragraph, lines 3-6).

Reviewer #2: Robert MacFadyen

Reviewer's report:

General
This is a well written manuscript. If the authors are interested in testing ethnicity and its impact on CAD then this is good and the authors should say so but the experiment should focus on KNOWN coronary disease in African Americans not surrogate endpoints of vague vascular risk.

Response: We would like to thank the reviewer for raising this point. We agree with the reviewer that it would be more straightforward to investigate the association of inflammation markers with known coronary heart disease. But this is a cross-sectional study in which only prevalent CHD cases were ascertained. It is possible that the association between risk factors and disease may have been changed in prevalent cases due to survival factors, treatment and/or behavioral interventions that were adapted after the
onset of disease. Therefore, we decided to investigate subclinical atherosclerosis in those without known CHD which is less likely subject to the influence of these factors than prevalent cases which have been diagnosed clinically.

The experimental design is flawed as it simply tests known mediator associations with major problems in the assay technology employed. A clear statement of the sample population is required in the title (e.g. it does not mention hypertension anywhere!).

Response: In the study, only African American participants were ascertained based on hypertension status of siblings. White participants were from the largest FHS families which were mainly ascertained randomly in the parent cohort studies. We have added to the Results section the data on the prevalence of hypertension in African Americans and whites (page 12, 1st paragraph, lines 5-6) and also provided more detailed information on blood pressure measurement (page 6, last paragraph, lines 5-7). Since the title is already long and study design is clearly described in the Population section, we decided to keep the title as is.

Is it really the case that the general cardiological community assumes the biology of coronary disease in African, African-American or Afro-Carribeans is equivalent between each other far less to that seen in Caucasians? I don't think we do but equally I don't think many feel it is likely to represent a simplistic one mediator difference.

Response: We would like to thank the reviewer for raising this question. We agree with the reviewer that one mediator difference between ethnicity does not necessarily represent the whole picture of coronary disease biology in different race groups. In our case, even if we did not detect significant differences in the association of CAC with MCP-1 between African Americans and whites, it is still possible that the biology of coronary heart disease differs to some extent between the two groups. In addition, the lack of a significant race interaction may be due to limited statistic power associated with the small sample size in African Americans. We have added to the Limitation paragraph the interpretation of the lack of race difference in the association between CAC and MCP-1 (page 17, 1st paragraph, last 3 lines). Furthermore, we have deleted the sentence “the mechanisms involved in mediating the association between MCP-1 and CAC might be similar in African Americans and whites.” in the following places of the original submission: ABSTRACT, page 3, last two lines; page 15, last two lines; page 18, last two lines under Conclusions.

These basic faults are however clearly admitted in the text. It might be helpful to gather them together in a formal headed Limitations paragraph.

Response: As mentioned in the above and following response, we have extended the discussion of the limitations and added the subtitle “Limitations” to the limitations paragraph (1st paragraph on page 17).

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The design cannot be altered retrospectively. I would question the principle of testing multiple mediator associations in unconfirmed CAD. It is well established that electron beam calcification is a very poor marker (not very sensitive and not very specific) for stenotic or
rather prognostically significant coronary disease. The error in assay technology is presumably irreversible?

**Response:** We agree with the reviewer that CAC measured by computed tomography is a poor marker for stenotic or prognostically significant coronary disease. In the study, we intended to use CAC as a marker for the burden of subclinical atherosclerosis, not clinically obvious outcomes. It would be ideal to include better markers for coronary stenosis or prognostically significant coronary disease in the study. We have added the acknowledgement of this limitation in the limitation paragraph (page 17, 1st paragraph, lines 8-9). Regarding the reviewer’s comment on the error in assay technology, we assume it was referred to sICAM-1 assay. The error in the sICAM-1 R&D assay was due to a K29M mutation at the ICAM-1 gene, which occurs at a much higher frequency in African Americans than Whites. Since this genotype was not measured in the African American participants of the study, we were not able to correct the bias in sICAM-1 measures based on genotype.

Discretionary Revisions (which the author can choose to ignore)
Personally I fee tables of data are immensely poor at illustrating relationships. Show us the data as a graph even a scatter plot! The first rule of statistics is to look at the data (and let everyone else see it too).

**Response:** We would like to thank the reviewer for the suggestion. In conducting the statistical analysis, we have examined the data graphically as well as in tabular format. Due to large amount of data presented in the tables, it would take more space if we convert tables to graphs. Therefore, we decided to keep the format of data presentation as is in the manuscript.