Author's response to reviews

Title: Fatty acid binding protein 3 (FABP3) is associated with insulin, lipids and cardiovascular phenotypes of the metabolic syndrome through epigenetic modifications in a Northern European family population

Authors:

Yi Zhang (szhang@mcw.edu)
Jack W Kent Jr (jkent@txbiomedgenetics.org)
Adam Lee (Lee.adam@mayo.edu)
Diana Cerjak (dgenz@mcw.edu)
Omar Ali (oali@mcw.edu)
Robert Diasio (diasio.robert@mayo.edu)
Michael Olivier (molivier@mcw.edu)
John Blangero (john@txbiomedgenetics.org)
Melanie A Carless (mcarless@txbiomedgenetics.org)
Ahmed H Kissebah (akisseba@mcw.edu)

Version: 6 Date: 15 February 2013

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

Thank you for your thoughtful review of our work again. I am pleased to submit to your attention our second revision of our manuscript entitled “Fatty acid binding protein 3 (FABP3) is associated with insulin, lipids and cardiovascular phenotypes of the metabolic syndrome through epigenetic modifications in a Northern European family population”.

Same as the last review, we have found the comments from you and the two reviewers’ constructive in improving our manuscripts and have tried to address the remaining questions to our best. Reviewers’ reports showed that while we had addressed all issues of one reviewer in our last revision, there are two open questions raised by the other reviewer. For the first one regarding the EMSA results, we have taken the reviewer’s suggestion and have now included it in this revision. For the second question that was raised by the reviewer on our new evidence of FABP3 expression, we have gone back to our array data and confirmed there are very few samples whose FABP3 gene expression has passed our stringent quality control analysis steps that we can perform the additional “cis-effect” analysis as the reviewer hinted. We do have expression data that were measured in some samples as part of the methylation cohort in this study. However its FABP3 transcript levels were measured by a different probe on a different version of the Illumina arrays. Furthermore, they generally exhibited low levels by this probe with detection p-values mostly >0.05 (our first QC guard). We would like to share with you and the reviewer the data that we obtained from this second set of gene expression data (please see attached Figure below). We did see a pattern in which high level of FABP3 promoter methylation correspond to lower means of FABP3 transcript levels in the same tissue. But because the reasons explained above, we would not include these data in the final manuscript.

We are acquiring new samples where we can systematically assess the effects of FABP3 methylation on its expression in different tissue types but that will require significantly greater time and support and it is beyond the scope of this paper.

In summary, our approach as described in our manuscript is that we tested the hypothesized association of CpG methylation status of FABP3 with the expression of the MetS traits in our family-based cohort. In parallel, we showed in a second group of subjects that the expression of FABP3 in the same tissue type correlate with lipid traits and the directionality from these two lines of evidence is consistent with one another, for example, adverse profiles of lipids correspond to higher methylation and lower expression of FABP3 in peripheral blood. Our EMSA experiment then suggested a mechanism by which this epigenetic tag may function to regulate its gene expression in heart cells, a tissue of physiological interest.

We hope that you now find the revised manuscript acceptable to be published in BMC Medical Genomics. Thank you again for your time in reviewing our work.

Sincerely yours,

Yi Zhang
Reviewer's report

Title: Fatty acid binding protein 3 (FABP3) is associated with insulin, lipids and cardiovascular phenotypes of the metabolic syndrome through epigenetic modifications in a Northern European family population

Version: 5 Date: 28 January 2013
Reviewer: David Serre

Reviewer's report:
The authors have discussed all issues raised previously.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests
Reviewer's report
Title: Fatty acid binding protein 3 (FABP3) is associated with insulin, lipids and cardiovascular phenotypes of the metabolic syndrome through epigenetic modifications in a Northern European family population
Version: 5 Date: 21 January 2013
Reviewer: Reid S Alisch
Reviewer's report:
This is a study testing whether DNA methylation variation at the promoter of FABP3 is associated with metabolic syndrome phenotypes. The findings from this study are suggestive and may have important implications for cardiovascular disease; however, the current version lacks a clear connection to FABP3 expression and methylation within the heart (the tissue of interest).
Major Compulsory Revisions:
1. In the "Response to the Reviewers" the authors informed us that they removed the EMSA results in lieu of the expression data. However, the authors have included the EMSA findings in both the abstract and the discussion (below), but fail to include it in the results section. I assume that this was an oversight and expect that the authors will either remove the EMSA findings from these sections or will fully describe them in the results section. I'd vote for it's inclusion in the manuscript but it needs to be explained better than it was in the original draft.

Response to reviewer: We have included the EMSA results in the revised manuscript and have added an explanation as suggested by the reviewer. Please see highlighted changes in Results and Discussion. And we feel that by including this EMSA, it helps to make the connection with one of the target tissues in which the product of this gene has been suggested to be functional.

Abstract:
"Further, we show that differential methylation of FABP3 affects binding activity with nuclear proteins from heart tissue."

Response to reviewer: As we have included the EMSA results, this sentence remains in the Abstract.

Discussion:
"We also found that differential methylation at these units affects the affinity of this region of DNA for binding to nuclear proteins extracted from human heart, thus indicating a possible mechanism by which methylation may regulate gene-expression in this region."

Response to reviewer: We have changed the discussion regarding the EMSA findings. Please see red ink in the Discussion.

2. Since the authors are using the expression data to demonstrate a correlation between FABP3 expression and MutS phenotype, and by association FABP3 methylation level and MutS phenotype, it would help to convince me of a correlation if the authors included any correlative observed between methylation level and expression (i.e. does promoter methylation associated with a MutS phenotype correlate with lower FABP3 expression level and that same MutS
phenotype?).

**Response to reviewer:** Thank you for the comments. We have gone back to our array data and confirmed there are very few samples whose FABP3 gene expression has passed our stringent quality control analysis steps in which we can perform the additional “cis-effect” analysis as you hinted. We do have expression data that were measured in some samples as part of the methylation cohort in this study. However its FABP3 transcript levels were measured by a different probe on a different version of the Illumina arrays. Furthermore, they generally exhibited low levels by this probe with detection p-values mostly >0.05 (our first QC guard). We would like to share with you and the editor the data that we obtained from this second set of gene expression data (please see attached Figure below). In this preliminary analysis, we examined FABP3 expression in 22 subjects with highest regional average methylation levels (which was the highest association with lipids) compared with 21 subjects with the lowest measures in methylation. And we repeated this for the other associated methylation states. Consistently, we saw a pattern in which high level of FABP3 promoter methylation correspond to lower means of FABP3 transcript levels in the same tissue. But because the reasons explained above, we would not include these data in the final manuscript. We are working to obtain new samples where we can systematically assess the effects of FABP3 methylation on its expression but it requires significant support and time before we can publish the data.

**Minor Essential Revisions:**
3. The rationale described in the first paragraph of the conclusion seems more appropriate for the introduction to help the reader better understand the approach.

**Response to reviewer:** We believe you were referring to the first paragraph of the Discussion? We have moved this paragraph to the Background to strengthen the statement of the rationale of our approach.

4. It is not clear in the "Results" section or the "Discussion" that the cohort used for the expression studies is different from the cohort used for the methylation studies. This should be made clear. We also need to see the demographics of this cohort in a table.

**Response to reviewer:** Yes they are separate cohorts. We have added a table (Table 4) to describe the related demographics of this second cohort.

5. Please make sure to include all "results" data directly into the manuscript, especially for major findings. For example; author wrote, "The strongest association was that between overall average methylation and total cholesterol level," yet they did not include the significance level, requiring the reader to refer to Table 2.

**Response to reviewer:** Thank you. We have made those changes accordingly.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I do not have competing interests.
Expression signals of FABP3 in subjects exhibiting differential regional promoter methylation levels. Raw expression signals of the 22 highest and 21 lowest methylation samples were inversed normalized and plotted. Samples with low methylation of FABP3 promoter showed higher mean expression levels than the samples with high methylation of FABP3 promoter.