Author's response to reviews

Title: Adipose Transcript Networks Across Finns and Mexicans Identify Novel Triglyceride-Associated Genes

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Author's response to reviews: see over
Dear Editor,

Thank you for the opportunity to submit a revised version of our manuscript (MS: 2071192208745599) now entitled “Adipose Co-expression Networks Across Finns and Mexicans Identify Novel Triglyceride-Associated Genes” by Blake Haas et al. for publication as an original article in the *BMC Medical Genomics*. We also thank the Reviewers for their helpful and constructive comments. We have now carefully addressed all of the comments and carried out all of the revisions suggested by the Reviewers. Please find enclosed below our point-by-point responses to the Reviewers’ comments.

Taken together we feel that the Reviewers’ comments have substantially improved our manuscript, and we sincerely hope that the revised manuscript will be accepted for publication in the *BMC Medical Genomics*. All authors have read and approved the submission of the manuscript, and the manuscript is not being considered for publication elsewhere as a whole or in part.

Thank you for your consideration.

Sincerely yours,

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Responses to Reviewer 1
We thank the Reviewer for the helpful critique and comments of the manuscript and have addressed all of the issues that were raised. We hope that the revisions satisfactorily respond to all of the Reviewer's concerns.

Reviewer's report (Reviewer John Whitfield):

Point 1. The authors refer to 'triglyceride genes' in the title and to 'novel TG genes' in the first paragraph of the Discussion. This implies that the genes affect triglycerides, which may well be the case, but the method is based on association between two phenotypes and does not necessarily identify the direction of causation. For example, this method might well find associations between the level of a hormone in the serum and expression of a group of genes in its target tissue but they would not be 'insulin genes' or 'oestradiol genes' in the sense implied by 'triglyceride genes' here. Moreover it is possible that an association between gene expression and the phenotype (in this case triglyceride) could depend on some unmeasured and confounding variable.

The causation issue can also be seen in relation to BMI. Figure 1 shows that both the blue module and the yellow module are associated with BMI as well as with triglycerides. Therefore gene expression in subcutaneous adipose tissue varies with adiposity, i.e. the total mass of adipose tissue. This is interesting in itself, and also raises the question whether total fat cell mass changes gene expression or gene expression changes total fat cell mass.

The authors should give a little more space to their concept of 'triglyceride genes' and say that inferences about causation based on this method must be supported by biological arguments as well as by statistically significant associations.

Response to Point 1. We thank the Reviewer for this helpful comment and have revised the terms 'triglyceride genes' in the title and 'novel TG genes' in the Discussion (page 14, the first paragraph) to reflect an association with TGs (i.e. novel associations between gene expression and serum TG levels). As suggested by the Reviewer, we also now state in the Discussion (page 14, the second paragraph) that inferences about causation based on the WGCNA method must be supported by both biological arguments and statistically significant associations.

Point 2. The Abstract uses the term 'independent' and the Background states that 'triglyceride levels have been implicated as an independent risk factor for CHD', but whether triglycerides are independent has long been controversial. Statistical independence has mostly been judged through multiple logistic regression, and most (but not all) studies have found that adjustment for other risk factors removes the association with triglycerides. (See, for example, Major lipids, apolipoproteins, and risk of vascular disease, JAMA 2009;302:1993-2000.) The Mendelian Randomisation approach taken in reference [1] is subtly different because it addresses causation; it too has its problems because it is necessary that APOA5 variation has no effects on anything except triglycerides and it is hard to be sure of this. There is no problem in saying that triglycerides are metabolically important and their variation is of interest, but because the article starts by justifying study of triglycerides with reference to CHD the second sentence of the Background needs some qualification.

Response to Point 2. As suggested by the Reviewer, we have revised the Abstract and second sentence of the Background (page 4) to remove the claim that TGs would be an independent risk factor for CHD.
Point 3. The fourth paragraph of Background says ‘Previous studies have analysed gene expression data from subcutaneous adipose tissue …’ It would be desirable to have references to these studies.

Response to Point 3. As suggested by the Reviewer, we have added three references to support the statement in the fourth paragraph of the Background (page 5, the second paragraph).
Responses to Reviewer 2
We thank the Reviewer for the helpful critique and comments of the manuscript and have addressed all of the issues that were raised. We hope that the revisions satisfactorily respond to all of the Reviewer’s concerns.

Reviewer’s report (Reviewer Lina Chen)

Point 1. The authors used both “biological networks” and “modules” to refer to modules that are correlated with TG levels in the whole article. Are they the same meaning? If so, please use the same term. E.g. in Abstract, “hypothesized that biological networks in human adipose tissue may be correlated with serum TG levels” and “we observed a module (biological gene expression network) that was significantly associated with serum TG levels.” In addition, what is “human adipose transcript network” in the title? Does it mean “the gene co-expression network” or network modules?

Response to Point 1. We thank the Reviewer for this helpful comment. The term “module” means a gene co-expression network (i.e. a group of genes identified to be co-expressed by WGCNA). As suggested by the Reviewer, we have now clarified all of the parts the Reviewer lists above to systematically refer to a gene co-expression network. We also revised the entire manuscript to clarify this issue.

To address the Reviewer’s questions about the title, we revised the title as follows: “Adipose Co-expression Networks Across Finns and Mexicans Identify Novel Triglyceride-Associated Genes” to clarify that we mean gene co-expression networks in adipose tissue identified by WGCNA.

Point 2. In the Background, the authors said “WGCNA is a well-established tool that identifies novel biological networks and searches for associations between a biological network and a phenotypic trait”. Does WGCNA identify novel networks?

Response to Point 2: In accordance with our response to point 1 above, we have now clarified that WGCNA identifies novel gene co-expression modules and revised this sentence in the Background on page 5 as follows: “Weighted Gene Co-expression Network Analysis (WGCNA) is a well-established tool that identifies novel gene co-expression networks and searches for associations between a network and a phenotypic trait [10-14]”.

Point 3. The authors used a two-tailed Fisher’s exact test to determine the significance of the TG module overlap. Please explain the procedure in details.

Response to Point 3: As suggested by the Reviewer, we have revised the methods (the last paragraph, page 22) to describe the procedure in more depth.

Point 4. In Results, the authors said “It is worth noting that in WGCNA the grey module always represents background genes outside of modules, i.e. genes that cannot be clustered into one of the modules are assigned to the grey module.” I noted that the grey module was correlated with TG levels in Figure 1. There is no further explanation about the grey module. Please make it specific.
Response to Point 4. As suggested by the Reviewer, we revised the Results (page 7, the last paragraph) to better explain that the grey module may contain genes that are associated with TGs but are not part of a WGCNA module.

Point 5. In Results, the authors used “pathways” to explain functions of TG module genes in “The observation that the WGCNA TG module genes significantly overlap with conventional differential gene expression analysis provides additional evidence that the TG regulatory pathways in human adipose tissue are highly shared across Finns and Mexicans.” They used only GO function enrichment analysis, not pathway analysis.

Response to Point 5. To address this point, we revised the Results (page 9, the second paragraph and page 11, the second paragraph) by changing ‘pathways’ to ‘GO functional enrichment’.

Point 6. The authors said “expression of all 4 MGAT genes tested (MGAT1, MGAT2, MGAT4A, MGAT4B) were significantly correlated (P<0.05) with TG module kME (i.e. the connectivity based on the module eigenvector, see methods) in two or more of the study samples, although they were not placed into the module using WGCNA.” in Results. What is “kME”? Besides, how to explain the results that genes that were not in the module were correlated with TG level, since the module is significant correlated with TG levels? Does the method miss some genes that are TG level-related in the adipose tissue?

Response to Point 6: To address this point, we revised our definition of ‘kME’ in the Results (page 11, the last paragraph) as follows: module membership (aka kME) is defined as the correlation between a gene expression value and the module eigengene (the average module expression value for an individual). We also revised the manuscript (page 12, the first paragraph) to better explain that not all TG-associated genes are found in the same module. Modules are formed based on similar gene expression patterns across multiple samples, so TG-associated genes that do not exhibit highly correlated gene expression will be found in different modules. Furthermore, the grey module may contain genes that are associated with TGs but are not part of a WGCNA module (please see our response to point 4 above).

Point 7. The authors did not discuss any limitations of their work.

Response to Point 7. We have now discussed the limitations of our work in the ‘Discussion’ section (page 14, the second paragraph - page 15, the first paragraph). As suggested by the other Reviewer, we also now state in the Discussion (page 14, the second paragraph) that inferences about causation based on the WGCNA method must be supported by both biological arguments and statistically significant associations.

Minor point 1. The authors used two Finnish and one Mexican study population, which were referred to as “sample” in the article. The concept is different from what were used in “Study Samples”. I recommend use “set of samples” to replace the word.

Response to minor Point 1: As suggested by the Reviewer, we have changed the phrase ‘study samples’ to ‘sets of samples’ throughout the manuscript.

Minor point 2. ME was short for “module eigengene”. What does ME mean in “Relating modules (MEs) instead of genes” in Results?
Response to minor Point 2. We thank the Reviewer for this helpful comment. We have now revised and clarified this part of the Results (the first paragraph of page 7) as follows: “The first principal component represents the summary of the module and is referred to as the module eigengene (ME)[8,9,11,12]. ME is the average module expression value for an individual. Relating MEs instead of genes to a clinical trait is a major advantage of WGCNA as it diminishes the multiple testing from thousands of transcripts to the number of modules. “

Minor point 3. Explain “high module expression” in “TGs are higher in individuals with high module expression” in Results.

Response to minor Point: As suggested by the Reviewer, we have now explained the term “high module expression” in this sentence of the Results (the first paragraph of page 7) as follows: “TGs are higher in individuals with high ME values. “ We have also now clarified earlier in the same paragraph that the term module expression (ME) means the average module expression value for an individual and explained how the ME value is used in WCGNA (the first paragraph of page 7). Please see our response to Point 2 above.

Minor point 4. There are several grammar errors in the article that make it hard to understand.

Response to minor point 4: As suggested by the Reviewer, we have corrected the grammatical errors in the manuscript.

Discretionary Revisions:
What is “During a fast”? 

Response to the suggested Discretionary Revisions: We have now revised “During a fast” to “During fasting” in this last sentence of page 4 in the Background.