Reviewer’s report

Title: Association of adipocyte genes with ASP expression: a microarray analysis of subcutaneous and omental adipose tissue in morbidly obese subjects

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Reviewer: Corneliu Henegar

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- Major Compulsory Revisions

MacLaren et al propose a descriptive study of the adipose tissue (AT) expression profiles in obese subjects distinguished by their circulating levels of triglycerides (TG) and of acylation stimulating protein (ASP) known to be involved in TG storage in adipocytes. Although the editorial quality of the manuscript is acceptable, it is unclear what kind of really new findings this study provides. Moreover the functional analysis and the assessment of the transcriptional co-expression interactions in the microarray data are inappropriate and/or affected by analytical biases that preclude any conclusion on the validity of their statements. No data other than those resulting from microarrays expression profiling is available to comfort these results.

1. As the authors acknowledge in the introduction the role of the ASP in the storage of TG and its local synthesis by the AT are already well known, as are the relations of this protein with various metabolic or “inflammatory” pathways that compose the functional profile of the AT. The data presented in this paper appears to provide nothing more than a simple confirmation of the previous findings by microarrays expression profiling. The authors must state clearly the rationale of the study, their objectives, the means employed in following them and what exactly is original in their findings.

2. The microarray data has not been made available on a public repository previous to the submission of the manuscript. To declare only the intention of doing so is not enough, as their findings cannot be verified in any way during the reviewing process in the absence of the experimental data. Also detailed results of the differential expression analysis should be provided as supplementary data as a complete list of differentially expressed genes together with the associated fold-changes and their statistical significance.

3. The functional analysis of the genes showing significant differential expression in between the LAT and HAT patients appears to be inappropriate and biased by strong a priori assumptions. In fact the authors limited their assessment to a manual annotation and selection of differentially expressed genes through a so termed “direct approach of analysis” based on the annotations provided by the microarray producer and on their previous knowledge of the pathways potentially related to the ASP function. Besides this no statistical analysis of any kind seems
to have been performed to test the overrepresentation of the manually selected pathways. Given the plethora of resources, tools and approaches available these days for the functional profiling of the gene expression data I feel that there is really no excuse to perform such manual ad-hoc analyses. It is mandatory that the authors carry on an adequate analysis of the functional profiles by using available international resources and recommended standards for this type of analyses.

4. The analysis of transcriptional co-expression interactions (i.e. expression profiles' similarities in between selected genes of interest) is equally inappropriate for at least two reasons:

a. Pearson correlation coefficients are not robust to non normality of the distributions and sampling anomalies related to the small number of samples (10 or 11 in this case), Spearman or Kendall coefficients being more appropriate in these cases; also there is no indication on the measures taken to address type I risk of false positives related to multiple testing errors during co-expression analyses.

b. Besides this the proportion of spurious correlations affecting microarray data is known to be very high (i.e. correlations that are not due to authentic biological relations between the studied transcripts but mostly to errors in expression profiling, sampling or even random variation). Therefore adequate techniques should be used in these cases to determine thresholds of significance from experimental data previous to analyzing such correlations (for details see Zhang, B. and Horvath, S. (2005) A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol, 4, Article17).

5. The results presented in this study suggest a different ASP profile in subcutaneous and omental AT. It is well known that the morphology and the function of these tissues are profoundly altered in morbidly obese subjects, although with a number of particularities depending on the type of AT depot (i.e. overexpression of inflammatory factors associated to a down-regulation of genes involved metabolic pathways, the local accumulation of immune cells including macrophages, an increased secretion of extracellular components resulting eventually in the constitution of an excessive interstitial fibrosis of the AT). This study would have greatly benefited from a morphological analysis conducted in parallel with the microarray expression profiling. The absence of such an analysis and/or a more detailed functional study of the AT, as well as some discordances with previous results reported in similar studies (acknowledged in the manuscript) and the analytical shortcomings mentioned earlier are disputing the credit that can be given to the results presented here.

- Minor Essential Revisions
  None

- Discretionary Revisions
  None
Level of interest: An article of limited interest

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.