Reviewer's report

Title: DNA methylation within the I.4 promoter region correlates with CYP19A1 gene expression in human ex vivo mature omental and subcutaneous adipocytes

Version: 1 Date: 22 January 2013

Reviewer: Mangalathu Rajeevan

Reviewer's report:

Major compulsory revisions
There are major flaws in the rationale, experimental details, analysis and interpretation of this study.

1. The purpose of the study is not adequately explained; simply stating (page 5) that “…the relationship between DNA methylation and other cell types has not been investigated” is not a sufficient reason to undertake a study. Further, the authors admit (page 14) based on reference 11 that the investigated CpG sites resist 5-aza-dC dependent demethylation to determine whether these site-specific DNA methylation alters gene expression. Except, CpG site I.4.5 for SP1 binding, none of the other CpG sites are located in the transcription factor binding sites, and thus may not be functionally important or meaningful in explaining glucocorticoid sensitivity of this promoter in relation to methylation. Thus from several angles, there is absence of biological relevance and reasoning to potentially interpret the data from this study that used valuable samples from human subjects. This is unfortunate.

2. CpG site I.4.5 showed no significant difference in methylation between omental and subcutaneous adipocytes (Figure 2). On the other hand, this CpG site showed a significant positive correlation with aromatase expression only in subcutaneous adipocytes (no correlation with omental adipocytes)? How could this be possible when the level of methylation is same in both adipocytes? The authors have not provided results on the relative aromatase gene expression between omental and subcutaneous adipocytes (like the one provided for methylation in Figure 2). This information is needed to understand the source of methylation dependent difference in gene expression, if any, between these two adipocyte tissues.

3. The authors should have provided correlation of methylation levels with estrogen levels to support the methylation dependent correlation with aromatase gene expression, and whole body composition characteristics given in Table 2. Table 2 footnote says 10 subjects used in this analysis, but how many men and women in this 10 participants?

4. The stated conclusions cannot be supported on the basis of the above three major points. Other major concerns are:

5. The method section on aromatase mRNA expression is not well described. For example, how the tissues were stored during the time between surgery and
extraction of nucleic acids? What was the quality of RNA extracted? It is assumed that the kit used is designed for simultaneous extraction of DNA and RNA, and it should be stated so in the manuscript. How much RNA was used per cDNA synthesis reaction? Why 18S was used for normalization? There is a mention of n=36 samples for promoter specific analysis. Were these samples coming from; from the same 25 subjects first stated in the methods section?

6. Method section on methylation quantification is also not well described. In Table 1, indicate which CpG sites are quantified in each of the PCR products. How much DNA was used for bisulfite treatment? It says “after conversion, 40 ng bisulfite converted DNA was amplified” How this 40ng was determined? Were the PCR conditions the same for all three PCR reactions? Were the 2% and 5% variations (page 8) experimentally determined for the assays in this study or are these figures quoted from reference 32?

7. From each subject, paired omental and subcutaneous adipocytes were used in this study. Considering this paired nature of the samples, why statistical analysis for paired samples was not used in this study. It says t-tests for independent samples were used. Is this the appropriate test?

8. Describe Figure 1 in methods, not in the introduction (see paragraph two on page 4) and results.

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests