Reviewer’s report

Title: Association between the DNA methylations of POMC, MC4R, and HNF4A and metabolic profiles in the blood of children aged 7-9 years

Version: 0 Date: 09 Jan 2018

Reviewer: Eunice Adei-Atiemo

Reviewer’s report:

BACKGROUND

Line 48-49: Suggest rewording sentence as follows: "It has been noted that around 40% of overweight children continue to be overweight during adolescence and that up to 80% of obese adolescents become obese adults"

Line 49-50: Suggest rewording: "Obesity in childhood can therefore severely impact future health outcomes."

Line 52-4: Suggest combining the sentences "...remain largely unknown, one study concluded that heritability could account for 85% of obesity."

Line 54: Did you mean HEREDITY accounted for <2% instead of "obesity only accounted for <2%"?

Line 60: "rats" instead of "rat"

Line 63: Should read as "exposure to famine led" or "leads"

Line 77: Providing some references of the studies you are referring to that have focused on the associations between genetic variants of POMC, MC4R, HNF4A and metabolic phenotypes

STUDY DESIGN.

Are there any specific reasons why the age group of 7-9 years was selected?

According to your study, children with a BMI above the 85th percentile were classified as obese. Internationally children with BMI's ≥ 85th centile are defined as overweight, and those ≥ the 95th centile classified as obese. If the 85th centile is to be used then the terms "overweight and obese" throughout the document would be more accurate than referring to all children with a BMI above the 85th centile as "obese". How many of the "obese" children in the study had BMI's above the 95th centile?

Line 121: should read "…P2 promoter of HNF4A was almost fully methylated.."
Line 130: Use past tense for sentence

Line 134: "adjusting" instead of "adjusted"

Line 139: Suggest "p values of <0.05 were considered as significant"

RESULTS

Line 143: Adjust sentence structure

Lines 149-154 and Table 2.

The text is a little difficult to follow. Would suggest rewording to make it easier for the reader. For example, "The following methylation levels were significantly less methylated in obese children (p<0.001): POMC methylation at all 4 CpG sites, MC4R methylation at CpG3....." I would also suggest highlighting or indicating on the table the lower (or higher) methylation figure for greater ease of reference.

In the text (line 151) it is indicated that the CpG1 methylation level of HNF4A-P2 was significantly less methylated in obese children. According to the table, it is in fact more methylated in overweight and obese children (97.55 ± 0.91) than in normal weight children (96.93 ± 1.07)

Therefore, both CpG1 and CpG3 sites in P2 were noted to be significantly more methylated in overweight and obese children as reported in table 2, and not just at CpG3. (Line 153)

Some of the methylation figures between normal and overweight/obese children seem very close together with very marginal differences. Were all of them really that significantly different (with p-values <0.001)? May want to run the ANCOVA values again to confirm. Additionally, were there any other methylation sites that could have been tested?

Table 3 is a very busy table. It would help to highlight or star the statistically significant correlations to make identification easier. I presume that the data presented here assesses for correlations between the methylation of POMC, MC4R and HNF4A and the metabolic profiles of all the children included in the study regardless of their BMI.

It would help to clarify which of the significant correlations are related to hypomethylation and which are related to hypermethylation.

Line 160: The references to "marginal significant" correlations is not accurate. In your methodology it was stated that p values of <0.05 were to be considered as statistically significant. As such, p values of 0.06 and 0.07 cannot be marginally significant. In the discussion, one could note that a larger sample size or greater statistical power may result in more clarity regarding the significance of identified associations.
Line 163: Suggest stating which 3 metabolic variables you are specifically referring to. It is unclear which p value of 0.07 is being referred to at the end of the sentence.

Lines 165: "To control for the effect of BMI"

Table 4: It would be helpful to indicate which of the methylations were hypermethylation and which were hypomethylation

Line 170: Were the HNF4A methylations hypomethylations or hypermethylation?

Line 172-175: The use of the term marginally significant is not accurate. Can state that associations were found but were not found to be statistically significant. You may suggest ways in which the study may be amended (e.g. increased sample size) in the discussion which may clarify or improve the statistical significance.

DISCUSSION

Kindly include some discussion regarding the differences between hypomethylation and hypermethylation. Have there been any consistent correlations with either hypo or hypermethylation and metabolic profiles in the literature?

Line 178: Suggest to read as follows: "Epigenic variations in metabolism-related genes partly contribute to the development of obesity and MetS.

Lines 179-180: Incomplete sentence structure

Line 185: Suggest "may" instead of "can" influence metabolic profiles

Lines 189-194: In these studies obese children were those with a BMI greater than 95%. Note that your study participants were overweight and obese.

Line 194: Suggest starting a new paragraph beginning with "Our results indicate average..." which continues with the first sentence of the paragraph below.

Lines 196 and 197: Were the same CpG sites studied in the earlier study on term and preterm infants as in this current study?

198: Most (or the majority) of the CpG sites actually differed by <1%

199: remove the "and", suggest sentence to read .. "although the differences were quite marginal, they were found to be statistically significant."

Lines 199-202: The sentence not very clear. Use past tense. Is the implication that a lower rate of overweight/obese children may have reduced the bias and possibly increased the difference in methylation levels between the two groups?
The study being referred to (Reference number 3) does not seem to correlate with the information provided. Reference 3 refers to a study of secular trends of body sizes in Korean children and adolescents.

There was a positive correlation with insulin levels but to note that it wasn't found to be statistically significant.

Suggest sentence to read ".. a study on MC4R-null mice reported elevated insulin levels".

Were HNF4A methylation levels in P2 negatively or positively correlated with TC levels (Refer to Table 3)

"independent" instead of "independently"

Note that it was however not statistically significant.

Use past tense

The sentence is difficult to follow. Additionally only HNF4A-CpG4 (P1) and HNF4A-CpG3 (P2) were found to be significantly associated with TC levels ( and not CpG3 in P1). And only HNF4A-CpG3 was found to be marginally associated with Insulin levels.

Longitudinal studies with larger sample sizes would be needed to investigate further.

"status" instead of statuses

Suggest "… larger cohort studies are required for further evaluation and verification"

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

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