Author's response to reviews

Title: No evidence for copy number and methylation variation in H19 and KCNQ10T1 imprinting control regions in children born small for gestational age

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Author's response to reviews: see over
Response to reviewer comments on MS: 3315821141207641 Copy number and methylation variation in H19 and KCNQ10T1 imprinting control regions in children born small for gestational age.

We would like to thank the associate editor for seeking clarification as to whether ethical approval for the ABC study also extends to this work, which it does. We have revised the ethics statement in the methods section to reflect this. “Written informed consent was obtained for genetic analysis relating to risk factors for SGA and associated health, from the parent or guardian on behalf of the children enrolled in the study. The Northern X ethics committee approved the study.”

We would like to thank the reviewer for their helpful comments relating to minor revisions which have now been included in this revised manuscript. These changes are detailed below:

1. Preference for title that are a statement of the overall result rather than the topic.
   [Changed as suggested to “No evidence for copy number and methylation variation in H19 and KCNQ10T1 imprinting control regions in children born small for gestational age”]

2. Abstract line 4: comma added after: “chromosome 11p15.5,”

3. Abstract line 8: full stop added after “associated with SGA.”

4. Abstract: “IGF2 differentially methylated region (DMR)” Each time the specific DMR is now specified as DMR0 etc.

5. Intro Line 5: space is added after “successive generations [3,4]”

6. Intro para 2, line 8: “severe hypomethylation of the H19 or IGF2 DMR is rewritten to clarify this means the H19 DMR “severe hypomethylation of the H19 DMR or IGF2 DMR”

7. Intro Para 2, “The IGF2/H19 ICR contains several differentially methylated regions (DMRs), which are all paternally methylated” With respect to the parent of origin of DMR0 methylation it is now considered that this DMR is paternally methylated although there are conflicting data.
   [This sentence has been altered to “The IGF2/H19 ICR contains several differentially methylated regions (DMRs), which are all predominantly methylated on the paternally inherited allele”. We have also added a sentence to clarify the important role of IGF2 DMR0 here: “The methylation status of the IGF2 DMR0 is more likely to be indicative of changes in IGF2 transcription from the active allele given it has been suggested to possess promoter activity”, reference Monk et al HMG 2006.]

8. Methods: regarding HhaI, this enzyme name is no longer italicized
9. IGF2 DMR in the last line of results: “within the IGF2 DMR” and each time the IGF2 DMR is mentioned, it is now specified that it is IGF2 DMR0.

10. List of abbreviations “CpG” is removed as suggested

11. List of abbreviations gene names for “H19” and “KCNQ10T1” have also been removed as suggested.

12. Table 1: Methylation by SGA status – the numbers are specified as % mean methylation and standard deviation in brackets.

13. There should be some discussion of the multiple testing. Would the result for IGF2 DMR0 still be significant if corrected for multiple testing.

   [We agree this is a concern and have now acknowledged this in the discussion: “This association would not have reached the level of statistical significance after correction for multiple testing, and we cannot exclude that this result occurred by chance. However, our result is consistent with a previous study...”]

We have also made a number of very minor grammatical corrections formatting changes in keeping with the journal style.

Thank you

Rinki Murphy
(on behalf of all coauthors)