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ARTICLE DETAILS

<table>
<thead>
<tr>
<th>TITLE (PROVISIONAL)</th>
<th>Maternal pre-pregnancy weight and externalizing behavior problems in preschool children: a UK-based twin study</th>
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<tr>
<td>AUTHORS</td>
<td>Antoniou, Evangelia; Fowler, Tom; Reed, Keith; Southwood, Taunton; McCleery, Joseph; Zeegers, Maurice</td>
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VERSION 1 - REVIEW

<table>
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<th>READER</th>
<th>Ryan Van Lieshout</th>
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<td>READER AFFILIATION</td>
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<td>READER COUNTRY</td>
<td>Canada</td>
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<tr>
<td>REVIEW RETURNED</td>
<td>09-Jul-2014</td>
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GENERAL COMMENTS

ABSTRACT:

I would soften the strength of the wording of the first sentence of the abstract since many children with behavioural problems in the preschool years do not go on to develop psychiatric disorders.

It would be helpful if they could name the scale used to assess internalizing and externalizing problems and if they could indicate what covariates they adjusted for in their analyses.

INTRODUCTION

In addition to reference 1, they could consider adding the following important reference:


References 2-4 only speak to dietary intake and fail to fully address their assertion that externalizing and internalizing problems show considerable stability across ages. This should be addressed using more relevant references.

References 5-7 don’t appear even remotely related to this manuscript and need to be checked and corrected.

I don't mean to be self-serving, but in addition to reference 19, two systematic reviews have summarized the literature on associations between pre-pregnancy adiposity and offspring neurodevelopment and I suggest that they be included:

Van Lieshout RJ. Role of Maternal Adiposity Prior to and During...


I think that reference 22 is intended to be:


On page 4 line 100, the word 'year' should be 'age'

METHODS

It would be of significant value if the authors provided more detail on the sample including the sampling frame, the number of women approached, rates of attrition, and how this sample compares to the general population in the UK (or at least in Birmingham) in terms of relevant demographic information.

It would also be of value to know how maternal pre-pregnancy BMI was determined. That is, was maternal weight self-reported? If so, at what stage of pregnancy or the puerperium was this done? Or was it derived from their medical charts? How was height determined?

Could the authors please justify why they wanted to look at BMI as both a continuous and categorical predictor?

Given the general audience served by BMJ Open, it would be helpful if the authors could more explicitly explain to the generalist reader what heritability analysis does and how its results are interpreted so that they have a better sense of the methods that are being used and how to understand their outputs. In the least, a bit more information on what the terms corresponding to A, C, and E would be useful.

It would be helpful if the authors provided rationale for and a reference to support their choice of using 60 point cutoffs for ‘clinically important behavior problems’ when the CBCL T-scores when the usual choice is 65 or higher. If this cannot be justified, they should rename what they are defining as it is not clear that 60 points defines ‘clinically important behavior problems’ as they assert.

RESULTS

In table 4, I would be inclined to list the weight categories in the following order for each CBCL subscale: Underweight, Normal Weight (reference), Overweight/Obese.

The authors could also indicate why they chose to group overweight and obese into a single category for this manuscript.

DISCUSSION

I believe that reference 31 should be:

References 41 and 42 are clearly not relevant to this paper.

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Carsten Obel</th>
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<tbody>
<tr>
<td>Institution and Country</td>
<td>Aarhus University, Denmark</td>
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<tr>
<td>REVIEW RETURNED</td>
<td>14-Jul-2014</td>
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</table>

**GENERAL COMMENTS**

This paper’s title suggest that the aim is to test the association between pre-pregnancy weight and offspring externalizing behavior problems. The paper does in fact test on internalization problems too.

There have been studies reporting a positive association with externalizing problems - and the main challenge is to rule out if this is due to genetic or social confounding.

It is not made clear how a twin study can contribute here and the focus of the paper is generally a bit unclear. Most analyses are classical genetic twin analyses, and to what extend the hypothesis of an effect of maternal pre-pregnancy weight is tested is limited. Pre-pregnancy weight is included in the models but the interpretation of the effect of doing so did not seem convincing to me. It may be that I do not have the sufficient understanding. There seem to be far too little statistical power to test the previous research findings and a sibling design where there is some discordance in maternal BMI between siblings may be a more appropriate design for this purpose. The only way I see that these data could contribute to our understanding of this association would be to compare the genetic model-fitting stratified by maternal +/- overweight.

**VERSION 1 – AUTHOR RESPONSE**

Reviewer #1

Reviewer Name Ryan Van Lieshout
Institution and Country McMaster University
Canada
Please state any competing interests or state 'None declared': None declared

This is an interesting manuscript addressing an interesting topic. In the interest of full disclosure, my expertise is more in the content area that this manuscript touches on rather than the genetic methods utilized.

I will organize my review by manuscript section below:

**ABSTRACT:**

I would soften the strength of the wording of the first sentence of the abstract since many children with behavioural problems in the preschool years do not go on to develop psychiatric disorders.

-In the revised version in page 2, this sentence has been updated since the structure of the abstract has been changed according to editorial requirements.
It would be helpful if they could name the scale used to assess internalizing and externalizing problems and if they could indicate what covariates they adjusted for in their analyses.

- Agreed. The new text in the abstract contains information on the scale used to assess behavior and the covariates adjusted for. In page 2, lines 41-45 the new text reads: A total of 443 (16% of initial registered members) mothers answered questions on pre-pregnancy weight and their children’s internalizing and externalizing problems using the Child Behavior Checklist and correcting for important covariates including gestational age, twins’ birth weight, age and sex, mother’s educational level and smoking (before, during, after pregnancy).

INTRODUCTION

In addition to reference 1, they could consider adding the following important reference:


- The reference suggested by the reviewer has been added in the revised version in page 4 line 100.

References 2-4 only speak to dietary intake and fail to fully address their assertion that externalizing and internalizing problems show considerable stability across ages. This should be addressed using more relevant references.

- These references were inserted by mistake and the manuscript is now updated with new relevant references. Please see reference list numbers 3-5 and page 4 line 103.

References 5-7 don't appear even remotely related to this manuscript and need to be checked and corrected.

- The same as above applies here. Please see reference list numbers 6-8 and page 4 line 106.

I don't mean to be self-serving, but in addition to reference 19, two systematic reviews have summarized the literature on associations between pre-pregnancy adiposity and offspring neurodevelopment and I suggest that they be included:


- The references suggested by the reviewer have been added in the manuscript, page 4, line 122 and reference list numbers 22 and 23.

I think that reference 22 is intended to be:

METHODS

It would be of significant value if the authors provided more detail on the sample including the sampling frame, the number of women approached, rates of attrition, and how this sample compares to the general population in the UK (or at least in Birmingham) in terms of relevant demographic information.

The sampling frame used was the nation-wide Twins and Multiple Births Association (TAMBA). TAMBA routinely records residential information of their members, a variable that is known to correlate highly with mother's age at birth and social economic status. Both, TAMBA members and the TAMBALHS study participants show a similar and representative geographical spread of the twin families across the UK.

Some information over the sample has been included in the Methods. The text reads: An invitation letter to this study was sent to all present (n=2712), at the moment of the study, twin family registered members of the Twins and Multiple Births Association (TAMBA) beginning of July 2008. TAMBA is an association, which registers and provides support to multiple birth families across the UK. In the time period between July 2008 and May 2010, 443 (16%) mothers completed the study's online questionnaire on their twins' emotional and behavioral development. The participants show a similar and representative geographical spread of the twin families across the UK.

It would also be of value to know how maternal pre-pregnancy BMI was determined. That is, was maternal weight self-reported? If so, at what stage of pregnancy or the puerperium was this done? Or was it derived from their medical charts? How was height determined?

Some information over the BMI determination has been included in Methods in Materials. The height was also self reported. Pre-pregnancy BMI was based on maternal self-report of weight and height and was calculated by dividing their weight in kilograms by their self-report height in meters squared [(kg)/ height (m2)].

Could the authors please justify why they wanted to look at BMI as both a continuous and categorical predictor?

The analysis of BMI as a continuous variable tests our assumption of a linear effect on the outcome. The categorization of BMI, on the other hand, was conducted in order to further explore the observed effect on the outcome, regardless of any linear assumption and facilitate the interpretation of the results on a more detailed level of the effect of each BMI category on behavior problems.

Given the general audience served by BMJ Open, it would be helpful if the authors could more explicitly explain to the generalist reader what heritability analysis does and how its results are interpreted so that they have a better sense of the methods that are being used and how to understand their outputs. In the least, a bit more information on what the terms corresponding to A, C, and E would be useful.
In order to respond to this comment we have added in the Methods, page 7, lines 205-216 some information about the twin design and heritability. The text reads: The classical twin study design relies on studying twins raised in the same family environments. Monozygotic twins share all of their genes, while dizygotic twins share only about half of them. So, if a researcher compares the similarity between sets of identical twins to the similarity between sets of fraternal twins for a particular trait, then any excess likeness between the identical twins should be due to genes (that is the A component in a genetic model fitting) rather than environment. Researchers use this method, and variations on it, to estimate the heritability of traits: The percentage of variance in a population due to genes. Modern twin studies also try to quantify the effect of a person's common environment (family, that is the C component in the genetic model fitting) and non-shared environment (the individual events that shape a life, that is the E component in a genetic model fitting) on a trait.

It would be helpful if the authors provided rationale for and a reference to support their choice of using 60 point cutoffs for 'clinically important behavior problems' when the CBCL T-scores when the usual choice is 65 or higher. If this cannot be justified, they should rename what they are defining as it is not clear that 60 points defines 'clinically important behavior problems' as they assert.

In order to respond to this comment some text has been added in the Methods, pages 6-7, lines 192-196: The selection of lower cut-off scores for the broadband scales was based on the notion that these scales encompass more numerous and diverse problems than any of the syndrome scales, with the latter comprising smaller, more homogeneous sets of problems. Therefore, higher scores are needed for the syndrome scales in order to conclude that a behavior is clinically deviant (Achenbach & Rescorla, 2000).

RESULTS

In table 4, I would be inclined to list the weight categories in the following order for each CBCL subscale: Underweight, Normal Weight (reference), Overweight/Obese.

-We agree with the reviewer’s proposed layout of the table. Please see Table 4 for changes.

The authors could also indicate why they chose to group overweight and obese into a single category for this manuscript.

-In the methods section a sentence has been added in page 6, lines 169-171: Overweight and obese mothers were combined in one category due to the limited number of obese mothers.

DISCUSSION

I believe that reference 31 should be:


-The reference has been updated in the revised version. Please see page 17, line 39 and in the reference list number 34.

References 41 and 42 are clearly not relevant to this paper.
Review of Manuscript

Reviewer Name: Carsten Obel
Institution and Country: Aarhus University, Denmark
Please state any competing interests or state 'None declared': None declared

This paper's title suggests that the aim is to test the association between pre-pregnancy weight and offspring externalizing behavior problems. The paper does in fact test on internalization problems too.

We chose to refer in the title to maternal pre-pregnancy weight and externalizing behavior problems only, since our findings support an association between the two and not with internalizing problems. We certainly explored the associations with maternal pre-pregnancy weight in both behavior problem scales. If the editor, however, prefers to include the term internalizing behavior in the title, we are willing to change that.

There have been studies reporting a positive association with externalizing problems - and the main challenge is to rule out if this is due to genetic or social confounding. It is not made clear how a twin study can contribute here and the focus of the paper is generally a bit unclear.

In order to support the research question of the manuscript that a genetic analysis can provide some evidence for the environmental influence of maternal pre-pregnancy weight on behavior problems and how the twin genetic design facilitates that, some text has been added in the Methods, page 7, lines 205-216 some information about the twin design and heritability. The text reads: The classical twin study design relies on studying twins raised in the same family environments. Monozygotic twins share all of their genes, while dizygotic twins share only about half of them. So, if a researcher compares the similarity between sets of identical twins to the similarity between sets of fraternal twins for a particular trait, then any excess likeness between the identical twins should be due to genes (that is the A component in a genetic model fitting) rather than environment. Researchers use this method, and variations on it, to estimate the heritability of traits: The percentage of variance in a population due to genes. Modern twin studies also try to quantify the effect of a person's common environment (family, that is the C component in a genetic model fitting) and non-shared environment (the individual events that shape a life, that is the E component in a genetic model fitting) on a trait.

Most analyses are classical genetic twin analyses, and to what extend the hypothesis of an effect of maternal pre-pregnancy weight is tested is limited. Pre-pregnancy weight is included in the models but the interpretation of the effect of doing so did not seem convincing to me. It may be that I do not have the sufficient understanding.

We understand that some clarity on the twin genetic design may be needed. In the twin genetic design the influence of the latent factors A (genes), C (common environment) and E (non-shared environment) on the outcome (i.e, externalizing behavior) is examined. The model that can best describe the data is a full ACE. When adjusting for maternal pre-pregnancy weight there was a non-significant decrease in the C factor. If we assume that pre-pregnancy weight is a proxy for the intrauterine environment, that is, the common environment between the twins, we can conclude that some of this variation can be explained by maternal pre-pregnancy weight.

There seem to be far too little statistical power to test the previous research findings and a sibling design where there is some discordance in maternal BMI between siblings may be a more
appropriate design for this purpose.

-We agree with the reviewer that a sibling design is a very good alternative genetically sensitive research design to test these associations because it takes into account more detailed environmental factors. However, that was not possible with this population-based dataset, since we did not collect information on siblings. It could certainly be a question to explore in the future. The twin design can enable a heritability analysis, so we can not only quantify the percentage of the outcomes accounted for by genes and environment but also test for the significance of the intrauterine environment using maternal pre-pregnancy weight as a proxy. Most studies on this research topic have been conducted in singletons; a twin genetic design by comparing the similarities between MZ and DZ twin sets can give us some information about the influence of the environment and by including maternal pre-pregnancy weight in the models we can test the importance of the in utero environment.

The only way I see that these data could contribute to our understanding of this association would be to compare the genetic model-fitting stratified by maternal +/- overweight

-In order to respond to this comment we repeated the genetic analyses stratifying the twins by maternal overweight. We compared the model fitting for the genetic and environmental influence comparing two groups of twins, those of overweight mothers and those of normal weight mothers. We repeated the analyses for the externalizing problems only, since there was a decrease of the variation in externalizing problems that could be explained by common environment. The model fitting for overweight mothers suggested that genes accounted for 39% (CI's: 22%-60%) of the variation, common environment for 50% (CI's: 29%-67%) and non-shared environment for 11% (CI's: 7%-16%) of the variation of externalizing disorders. The model fitting for normal weight mothers suggested that that genes accounted for 50% (CI's: 30%-75%) of the variation, common environment for 35% (CI's: 10%-53%) and non-shared environment for 15% (CI's: 11%-22%) of the variation of externalizing disorders. In the discussion, page 16, lines 11-16, the following text has been added: In order to further explore the role of overweight in externalizing problems we repeated the analysis by comparing the twins of overweight to the twins of normal weight mothers. The results suggested that 50% (CI's: 29%-67%) of the variation in externalizing problem behavior in twins of overweight mothers could be explained by common environment compared to 35% (CI's: 10%-53%) in twins of normal weight mothers.
Maternal pre-pregnancy weight and externalising behaviour problems in preschool children: a UK-based twin study

Evangelia E Antoniou, Tom Fowler, Keith Reed, Taunton R Southwood, Joseph P McCleery and Maurice P Zeegers

BMJ Open 2014 4:
doi: 10.1136/bmjopen-2014-005974

These include:

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