Author's response to reviews

Title: No evidence for association between BMI and 10 candidate genes at ages 4, 7 and 10 in a large UK sample of twins

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Version: 3 Date: 19 December 2007

Author's response to reviews: see over
Dear Dr Kowalczuk,

Re: Revised Manuscript: MS: 9388663821535587

Please find attached our revised manuscript, with a new title: *No evidence for association between BMI and 10 candidate genes at ages 4, 7 and 10 in a large UK sample of twins.*

Thank you for the reviews of our manuscript. We have attempted to address each of the issues raised, and have summarized the alterations and comments we have made in the attached document. The reviewers’ comments are presented, along with our response.

The manuscript is intended as a research article and is not being submitted for publication elsewhere. All authors have read and approved the current version of the manuscript. We have complied with APA ethical standards in the treatment of our sample and we have no competing interests. Contact details for the corresponding author are as follows:

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I look forward to hearing from you.

Sincerely,

Claire Haworth
Reviewer 1
Reviewer’s report
Title: Associations between BMI and 10 candidate genes at ages 4, 7 and 10 in a large UK sample
Version: 2 Date: 6 September 2007
Reviewer: Hyung Doo Shin
Reviewer’s report:
General
In this manuscript, the author describes an association between BMI and 10 candidate genes at ages 4, 7, and 10 in a large UK sample. But, none of the associations were significant after correction for multiple testing. Overall, this MS is not well describing their results from large sample size. On the study, there are several points of concerns as below.
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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. There may be miss information of parents in qTDT analysis. I do not find any description about parents. Did they qTDT without information of parents?
Response: We do not have parental genotypes. We used QTDT to control for the twin-pair structure of our data. We have included this information in the methods section.

2. The discussion was very insufficient to fully address their results.
Response: We have extended the discussion to more fully address our results, including further details about the gender-specific and categorical analyses.

3. Description about statistical methods should be added.
Response: We have provided further details about the statistical methods that we used.
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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. In chi-square analysis, I wonder how to divide by three groups (obese, overweight and normal). There may be provided the range of BMI of three groups in Material and Method session.
Response: The chi-square analyses were run on the obese vs. normal weight group and then also on the obese-overweight group versus the normal weight group. We have clarified this in the text. In addition, we have provided further descriptive statistics on these groups.

2. A childhood BMI is very changeable by their age, even several months. If authors investigated the comparison between BMI z-score differences and genotype per annual or 6 months, there may be obtain more valuable results.
Response: It would be beneficial to have more frequent measurements of BMI, but because of the expense of phenotyping such a large sample we
were not able to get BMI measurements every 6 months. What we do provide is a developmental snap-shot from early to middle childhood. We have included this as a limitation of the study.

3. Clinical profiles of subjects are missing
   **Response:** Subjects are from a population representative cohort of twins. We do not have clinical information on these twins. However, we have provided further descriptive information of the sample, including zygosity and sex composition of the sample and mean BMI scores at each age.

4. The author may provide the results of analysis in form of supplementary document (not just saying "data not shown).
   **Response:** We have provided results from the chi-square analysis in the text.

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**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.
Reviewer’s report

Title: Associations between BMI and 10 candidate genes at ages 4, 7 and 10 in a large UK sample

Version: 2 Date: 8 November 2007

Reviewer: Harold Snieder

Reviewer’s report:

This study investigates 10 single-nucleotide polymorphisms (SNPs) in candidate genes for obesity (measured as BMI) in a sample of 5000 children (2500 twin pairs) with BMI data at 4, 7 and 10 years. It has a number of strengths such as the large sample size, multiple measurements at different time points and the use of FDR. I have a number of concerns.

1. The introduction is very short and very similar to the abstract. I propose the authors extend this section and include information on previous genetic association studies carried out in children. Also they could justify why they chose BMI which is surrogate marker of adiposity as the only phenotype for this study and clarify why the study was carried out in such a young population.

Response: We have included more information about the rationale of our study in the introduction. The study was carried out in a young population because we were interested to see when the associations would emerge in early development. Childhood BMI has been shown to become stable after the age of 11 years (Wardle 2006), so we felt it would be interesting to investigate whether the associations would appear as BMI became more stable.

We were only able to include a measurement of BMI as a surrogate measure of obesity, and have included this as a limitation of the study. Collecting repeated measurements of further measures of adiposity on this large sample was not economically feasible.

2. Introduction, first line obesity rather than BMI should be mentioned as a complex trait.

Response: We have made this change.

3. Methods section, the authors could give details on sampling procedure, selection process and demographics of the population. Also, total number of subjects, as well as number of boys and girls. Some of this information could be included in a Table.

Response: We have included further descriptive information on the sample, as requested by the reviewer.

4. In the same section, second paragraph, the authors mention that the BMI, weight and height were converted into age- and gender-specific standard deviation scores (SDS, Z-scores) of their distribution in the British 1990 reference data. However, children were categorized into normal, overweight or obese based on IOFT criteria. My suggestion to the authors is that it would be more appropriate to use the UK1990 BMI-for-age growth charts for classifying the
children as overweight (85th-95th percentile) and as obese (#95th percentile). IOFT definitions of obesity have been shown to have low sensitivity and they are highly sex-specific in comparison to national definitions (Chinn, Eur J Clin Nutr, 60, 1189-1194, 2006; Reilly, IJO, 30, 595-597, 2006).

Response: Although we are aware of the difficulties in using the IOTF criteria to define obesity, we felt that for this study, it was more appropriate to use the IOTF criteria for classifying the children as normal weight, overweight and obese, for the following reasons. Firstly, it was important that the cut-offs used in this study were meaningful to an international reader, and not just to a British reader; also using international criteria facilitates future comparisons between studies. Secondly, Chinn (2006) stated that in the UK, use of the 95th centile overestimates obesity compared to the IOTF criteria. Thirdly, the IOTF criteria are linked to adult cut-offs for obesity – and our study is attempting to replicate candidate genes found for adult obesity. Finally, the focus of our paper is not on the categorical analyses (but on the more powerful continuous analyses), and we are not using the IOTF criteria for clinical diagnosis of obesity. We have included a brief discussion of our rationale for using these criteria in the measures section.

5. Maybe the authors could give more details on the genotyping methods, eg what was the genotype success rate?.
Response: We have provided further details about the TaqMan genotyping methods.

6. The allele frequencies for all the SNPs could also be given.
Response: We have included the allele frequencies for the SNPs.

7. It could be interesting if more phenotypes such as body fat measurements or circumferences were included in the analysis, since BMI maybe a less direct measurement of adiposity in children. In this context, the authors could include a section with the advantages and limitations of this study and compare these findings with those in previous association studies in young children.
Response: We were only able to include a measurement of BMI as a surrogate measure of obesity, and have included this as a limitation of the study.

8. The analysis does not seem to make optimal use of the longitudinal developmental nature of the data. This needs to be discussed as a limitation. For example, in a recent similar study investigating candidate genes for obesity in youth, Podolsky et al (IJO, 2007) used a growth curve approach to capture the development in adiposity over time.
Response: We accept that there are a number of ways that we could examine these data, and growth curve analysis is an excellent way of assessing change. However, for this analysis we only have three measurements, which is less than ideal for growth curve modeling.
Nevertheless, we will certainly consider using this method in the future, when we have more repeated measurements of BMI.

9. It is not entirely clear whether FDR was applied within each age category (10 tests) or across all age categories (30 tests). Please clarify.
Response: We have clarified the procedure for the FDR correction.

What next?: Accept after minor essential revisions  
Level of interest: An article whose findings are important to those with closely related research interests  
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'
Reviewer 3

Reviewer's report

Title: Associations between BMI and 10 candidate genes at ages 4, 7 and 10 in a large UK sample

Version: 2 Date: 23 November 2007

Reviewer: steven wiltshire

Reviewer's report:

General

The study describes an examination of ten SNPs, previously associated with obesity, BMI or waist circumference, for association with BMI in a sample of 5000 twins at the ages of 4, 7 and 10. After correction for multiple testing, using the false discovery rate, the study finds no significant associations. The authors conclude that the stage of biological development of the subjects is important in candidate gene studies.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. More details on the models used in the QTDT analysis are needed. Was the twin-option used (to model shared twin environment)? Were familial correlations due to the major gene modelled with a specific variance component (i.e. using IBDs) or just the polygenic and environmental components? Was population stratification examined, thereby informing the decision to do a total association test rather than the orthogonal test?

Response: We have provided further information about the QTDT analyses. We tested which variance components were significant before including them in the model. In the final model we included the twin environment, and the polygenic and environmental components. We did not have parental genotypes, so were unable to include IBD status. We found no evidence for population stratification and therefore proceeded with the total association test.

2. In the three chi-square analyses of the trichotomised data, how was the relatedness between the each member of a twin pair accommodated, or was just a single twin per pair used in this part of the analysis?

Response: For the chi-square analyses we compared just two groups: obese versus normal weight, and obese+overweight versus normal weight. To overcome the non-independence of the data, these analyses were performed on one randomly selected member of each twin pair. We have clarified this in the text.

3. What proportion of the twin pairs was dizygotic and what monozygotic?

Response: We have provided further details about the composition of our sample, including zygosity information.
4. What was the gender-breakdown of the twins? A table addressing this question and question 3 would be helpful.

Response: We have provided further details about the composition of our sample, including gender information.

5. Were the parents genotyped?

Response: We have added details to the text explaining that we did not have parental genotypes.

6. Regarding the analyses including sex as a covariate, did these use the BMI z-scores that had been standardised against age and sex (in which case would an effect of sex actually be expected)?

Response: We have removed the analyses including sex as a covariate, and instead we have provided further details about the gender-specific QTDT analyses.

7. I think the authors need to include P-values at least for the chi-square analyses, and an indication of the range of significances for the gender-specific QTDT analyses.

Response: We have included further information about the results from the chi-square analyses and the gender-specific QTDT analyses in the text.

8. In the authors’ discussion, they mention the issue of power and failure to replicate (midway down page 7). Perhaps they could address the possibility that the initial finding of an association is a false positive.

Response: We have briefly addressed this issue in the discussion. However, as many of the candidate genes we have tested were found for adult BMI, our study is not a replication, but an extension of the previous finding. Therefore the results from our study are not a good test of whether the previous finding was valid.

Discretionary Revisions (which the author can choose to ignore)

9. Perhaps add in the title of the manuscript a mention that the UK sample is a sample of twins.

Response: We have added this information to the title.

10. Also, given that the authors observe no significant associations (excepting the one with $P=0.033$) maybe the authors might consider changing the title to “No evidence for association between ...”

Response: We have indicated in the title that there were no significant associations.

11. Would it help the reader for the authors to quote the BMI cutoffs according to these criteria for ages 4, 7 and 10 years?
Response: We have included more information about the IOTF cut-offs in the text. We have also provided the raw BMI scores for the three groups at each age.

12. A few more details are needed regarding the power calculations (how, which software etc.) for the benefit of other researchers?
Response: We have provided further details of our power calculation. These calculations were done by hand using Cohen (1988).

13. It would help the reader for Table 1 to include as a footnote the full name of each gene.
Response: We have included the full gene names as a footnote to Table 1.

14. What were the heritability estimates for BMI at the three ages?
Response: We have reported the heritability estimates for BMI at the three ages.

What next?: Accept after minor essential revisions
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I declare that I have no competing interests.