Author’s response to reviews

Title: The MAP2K5-linked SNP rs2241423 is associated with BMI and obesity in two cohorts of Swedish and Greek children

Authors:

Mathias Rask-Andersen (mathiasraskandersen@gmail.com)
Josefin A Jacobsson (josefin.jacobsson@neuro.uu.se)
George Moschonis (amoschi@hua.gr)
Anna E Ek (Anna.Ek@ki.se)
George P Chrousos (chrousog@exchange.nih.gov)
Claude Marcus (Claude.Marcus@ki.se)
Yannis Mannios (manios@hua.gr)
Robert Fredriksson (Robert.Fredriksson@neuro.uu.se)
Helgi B Schiöth (Helgi.Schioth@neuro.uu.se)

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Author’s response to reviews: see over
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Dear Dr Meyre

Associate Editor, BMC Medical Genetics

Regarding our Manuscript, no: 4011183746672728, "The MAP2K5-linked SNP rs2241423 is associated with BMI and obesity in two cohorts of Swedish and Greek children":

Thank you very much for the evaluation of our manuscript. We are very happy that the paper can be considered for publication and pleased by the response from the reviewers. Below we include detailed responses to each of the reviewer’s comments. We hope that the manuscript is now acceptable for publication in BMC Medical Genetics.

Best Regards

Helgi Schiöth, Professor.
Reviewer 1.

1. The effect of this variant on obesity was recently published as the authors point out in their introduction, therefore the results of this study do not add significant value to the literature given the addition their nominal significance in both cohorts.

Author’s response: We thank the reviewer for his valuable comments which are most welcome. Replication of the meta-analysis by the GIANT consortium in independent cohorts is important as it solidifies the original findings on a population level. Our cohorts also allow the testing for relationship of genetic variants to other phenotypic variables, thus specifying the effect carried by the genetic variant. We chose to test for an association of rs2241423 with phenotypic variables describing body-fat distribution as this is very well characterized in the cohort of Greek children. Identification and replication of genetic factors underlying pediatric obesity is also of great interest as genetic variants can have differential effects on early-onset and adult obesity.

2. [...] the number of subjects differ in the different sections of the manuscripts.

Author’s response: The correct number of patients and controls (n=474 & n=519 respectively) are now stated throughout the manuscript.

3. In the abstract the authors should give the result of the analysis.

Author’s response: The main results of our analysis have been added to the abstract.
4. In the discussion section most of the text should be placed in the introduction as it is not related with the data of the study.

**Author’s response:** The parts of the discussion more related to the background of the study have been moved to and integrated with the introduction.

**Reviewer 2.**

**Major comments**

1. In the present study, authors attempted to analyze only one locus of the 32 associated with obesity in the study by Speliotes et al Nat Genet 2010. Why did authors analyze only one SNP of the GIANT consortium and not all the 32 SNPs? According to which criteria and why they selected to study MAP2K5 gene among the 32 obesity-susceptibility loci?

**Author’s response:** Replication of the GIANT consortium SNPs in independent cohorts is important as it solidifies the original associations. Smaller cohorts also allow the testing for relationship of genetic variants to other phenotypic variables, thus specifying the effect carried by the genetic variant. Due to our sample size limitation we do not have the power to analyze all the 32 obesity-susceptibility loci identified by Speliotes et al. and thus we chose only one of the 18 newly identified BMI loci. MAP2K5 was tested in our cohorts due to the high allele frequency of the variant and the effects observed in secondary meta-analysis in child cohorts performed by the GIANT consortium both in a case-control study and in a transmission disequilibrium test with extremely obese children and adolescents. We briefly
mentioned this in the introduction but have now added some more information in order to clarify the selection criteria.

2. Authors do not comment on limitations of the study. Did they apply multiple testing such as Bonferroni correction, for the Swedish cohort? Author’s should test power calculation of SNP by softwares (QUANTO etc).

**Author’s response:** We thank the reviewer for these valuable comments. Given the prior information about the role of MAP2K5 variation in obesity, we considered our evaluation of the association with BMI and obesity in our cohorts as a replication study; thus, nominal $P$ values $\leq 0.05$ were considered significant. For other phenotypes in the Greek cohort, not previously studied, we applied a Bonferroni correction and a $p$-value $<0.007$ was considered statistical significant. This information has been added to the manuscript. Furthermore, for the Swedish cohort, we performed a case/control power calculation with the CaTS power calculator ([http://www.sph.umich.edu/csg/abecasis/CaTS/index.html](http://www.sph.umich.edu/csg/abecasis/CaTS/index.html)) (Skol, Scott et al. 2006). For the Greek cohort we performed power calculations for quantitative traits assuming an additive model using the QUNATO software (Version 1.2.4) ([http://hydra.usc.edu/gxe/](http://hydra.usc.edu/gxe/)). Among the Swedish children we had 80% power to detect association with obesity with a relative risk of 1.35. Among the Greek children we had 80% power to detect an effect size of 9.6% of the standard deviation per allele for normalized quantitative phenotypes. This information has also been added to the manuscript. We have furthermore added a part in the discussion where we mention our limitations.

3. Authors provided genotype call rates and HWE p-values for both cohorts. Were HWE-testing and genotyping call rate the only criteria used for validating the genotype data?
**Author’s Response:** As the allelic distribution is performed in SDS, the fluorescence curves for the reactions are also manually inspected to check for irregularities. Allele frequencies also corresponded to allele frequencies reported in HapMap ([http://hapmap.ncbi.nlm.nih.gov/](http://hapmap.ncbi.nlm.nih.gov/)) for populations of European descent such as Utah residents with Northern and Western European ancestry from the CEPH collection (CEU C) and Tuscan in Italy (TSI (T)).

4. Authors found that rs2241423 is associated with lower risk of obesity in the Swedish cohort. Did authors search for association to risk of being obese in the greek children by dividing them in subgroups (Table 1)?

**Author’s response:** An association test for rs2241423 between obese and normal weight in the Greek cohort was not performed as we assume this cohort is underpowered for this type of analysis due to the low number of obese individuals (n=263). As this cohort is designed as a cross sectional study, linear regression analysis is better suited to analyze the relationship between rs2241423 and body weight. It enables us to include all participants in the cohort.

**Minor comments**

1. Incomplete affiliation no. 3. Also, there are no email addresses for all authors.

**Author’s response:** Affiliation no.3 has been completed and email addresses for all authors have been added.

2. Abstract page: it has no structured format as requested (Background, Methods, Results & Conclusions).
a. Authors should state that the minor allele associated with protection against obesity and lower 
   BMI is allele A.

b. Authors should include statistics and genotyping methods in brief.

c. Preferably use rs2241423 instead of Rs2241423 inside the text.

d. Add ORs or b-coefficients and 95% CIs for p-values of association.

e. Authors must state the number of Swedish controls (n=519) used for comparison with obese 
   Swedish children (n=474).

**Author’s response:**

The abstract has been restructured to comply with the requested format. The minor allele has 
been specified in the abstract. Statistical methods and genotyping procedures have been added 
in brief. ‘Rs2241423’ has been adjusted to ‘rs2241423’. Odds ratio and beta value have both 
been added to the corresponding p-values. The number of Swedish controls has been added. 
95% CI has been added to the reported beta-value and OR.

3. There are no keywords after the abstract.

**Author’s response:** Keywords have been added after the abstract. **Keywords:** Obesity, 
MAP2K5, childhood obesity, genetics, rs2241423.

4. Introduction: Add abbreviation for ERK5 and CNS.

**Author’s response:** Abbreviations have been added for ERK5 and CNS.

5. **Methods and materials:**
a. While in introduction section authors say that genotyping was performed in swedish cohort in 476 obese children and 521 controls, in this section they refer to 474 obese and 519 controls. Which number is correct? Please be more precise.

**Author’s response:** The correct number of patients and controls (n=474 & n=519 respectively) are now stated in the introduction.

b. Abbreviation for BMISDS (standard deviation score).

**Author’s response:** Abbreviation for BMISDS has been added.

c. Add a sentence for written informed consent of Swedish participants.

**Author’s response:** Informed written consent was provided by all participants or their legal guardians. This information has been added to the methods section describing the cohorts.

6. In results section, 1st paragraph, p-value and 95% CI differ from those in table 2, please correct.

**Author’s response:** The p-values and 95% CI in the results section have been corrected with the correct values from Table 2.

7. Titles of tables must be above the Table and legends can then follow.

**Author’s response:** Titles to the Tables have been moved to above the tables.
8. Table 1. P-values should be included for comparisons between groups.

**Author’s response:** P-values have been added for comparisons between groups in accordance with the reviewer’s suggestions. Two-Way analysis of variance was used to test differences between groups. Group means were compared with the normal weight group in the greek cohort. Obese patients were compared with the control population in the Swedish cohort. The legend of table 1 has been updated to reflect these additions.

9. Table 3. List HWE and MAF in the same order as in Table 2.

**Author’s response:** The order of HWE and MAF in table 3 has been changed in accordance with the reviewer’s suggestions.

10. No competing interests and author’s contributions are shown.

**Author’s response:** A declaration of no competing interests has been added after the acknowledgements.

11. Change all references according to Journal’s reference style (see instructions for authors).

**Author’s response:** The reference style has been changed in accordance with the preferred style of BMC Medical Genetics. A reference for the Kyoto Encyclopedia of Genes and Genomes (KEGG) website has also been added.
Discretionary revisions:

1. As the authors say, the minor allele of rs2241423 was associated with a protection against obesity in the Swedish cohort. Was that result observed under an additive model? As in GWAs study of Speliotes et al Nat Genet 2010 evidence of non-additive (dominant or recessive effects) and heterogeneity by gender were examined, did the authors make similar tests?

Author’s response: Since no evidence of non-additive effects or heterogeneity by sex was observed by Speliotes et al. we assumed an additive model in our study and we did not conduct any test for heterogeneity by gender. Although this is a very important aspect to consider and although we previously have identified heterogeneity by gender for other obesity loci in our Swedish cohort, we have not included such analysis in our present study due to our relatively small sample size and thus limited statistical power. We have added information about our model assumption in Methods and Materials.

2. Were related subjects excluded from the study? Please add a sentence or a word in exclusion criteria.

Author’s response: In the Swedish cohort, related subjects were excluded from the study. This information was however not available in the Greek cohort and such exclusion was not possible. This information has been added to the manuscript.

Minor issues:
1. In paragraph ‘Greek child cohort – The Healthy Growth Study’, there is a mistake in the sentence: Waist and hip circumference…0.1cm…at a standing position.

**Author’s response:** This sentence has been restructured to two sentences for clarity. It now reads:

“Waist and hip circumference was measured to the nearest 0.1 cm with the use of a non-elastic tape (Hoechstmass, Sulzback, Germany). Measurements were taken with the subject at a standing position, around the trunk, at the level of umbilicus midway between the lower rib margin and the iliac crest.”

2. In paragraph ‘Genotyping and linkage disequilibrium analysis’ write Hardy instead of hardy.

**Author’s response:** The paragraph ‘Genotyping and linkage disequilibrium analysis’ has been adjusted according to the reviewer’s suggestions.

3. In results section use 95% CI instead of CI95%.

**Author’s response:** The results section has been adjusted in accordance with the reviewer’s suggestions, ‘95% CI’ is now the consistent format throughout the manuscript.