Author's response to reviews

Title: Common variants in LEPR, IL6, AMD1, and NAMPT do not associate with risk of juvenile and childhood obesity in Danes: a case-control study

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Version: 4 Date: 8 July 2015

Author's response to reviews: see over
Executive Editor of BMC Medical genetics

Dear Dr. Tim Sands

Please consider the revised manuscript MS: 3954698671669725 titled “Common variants in LEPR, IL6, AMD1, and NAMPT do not associate with risk of juvenile and childhood obesity in Danes: a case-control study” for publication in your esteemed journal (BMC Medical Genetics) as an original research article.

In this revised manuscript, we have provided all the details suggested by the reviewers. We have enclosed point-to-point replies (following this letter below) to the well-taken comments made by the reviewers and have made the necessary changes in the revised manuscript guided by the respected reviewers. The inserted text has been underlined and is in red bold font.

We would like to express our deep gratitude and acknowledge the editorial board and reviewers who have provided us with their explicit comments in order to improve the science and impact of this study.

We thank you again for your support and look forward to your reply.

Sincerely yours,

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Rebuttal Letter

Re: MS: 3954698671669725: Common variants in LEPR, IL6, AMD1, and NAMPT do not associate with risk of juvenile and childhood obesity in Danes: a case-control study

Dear editor,

We thank you for your response and the reviewer’s comments on our manuscript. We have revised the manuscript and rewritten sections in response to the comments provided by the reviewers, and we believe that the revised manuscript has improved substantially. We hope that the reviewers are satisfied with our answers. We have provided a point by point response to the comments:

Reviewer: Jose Fernandez

a. According to the letter of submission, the study seems to be a resubmission; however, it is not clear how the authors addressed the concerns of previous reviewers as the letter of response does not provide an item by item explanation.

Reply a: We thank the reviewer for this valid point; however the re-submission was performed solely due to edits in the spelling of the name of one of the co-authors.

b. The authors combined overweight and obesity into one category, taking the risk of confounding their results and missing a potential association. Physiologically, obesity as a disease may activate predisposing genes that may not be expressed during the overweight stage, which serves as a precursor stage in obesity development. Another aspect is the homogeneity, or generalizability, or the classification itself when taking into account that they used obesity prevalence dependent on a historical definition of 40 years ago (the GOYA study). It is recommended to perform some preliminary analysis with obese individuals (eliminating the overweight), and within study populations to evaluate if there are differences in the results. A reader will benefit from some description of these exploratory analyses in the discussion.

Reply b: With respect to the reviewer’s suggestion, the reason we have not sub-grouped the study individuals into overweight and obese is because we have tried to replicate similar settings as applied in the study by Tabassum et al (1). The very relevant comments to the GOYA study has been addressed in reply 1 to reviewer 2 – Professor Dwaipayan Bharadwaj

Reviewer: Dwaipayan Bharadwaj

1. The observed discrepancies between the present study and original studies might be due to differences in study designs. Age groups of the participants included in the present study differ from the original studies by Tabassum et al. Genetic variants are known to have different effects at different age groups and also at different time period (as demonstrated recently in a study by Rosenquist et al., which showed that effect of FTO variant on BMI
can vary over time). Also, the fact that only a few adult obesity genes are implicated in childhood obesity too, suggests that the genetic factors and pathophysiology are different in two age groups. This fact in case of known obesity gene FTO has been elegantly shown that effect of the same variant differs between adult and childhood in same ethnic population (PloS One (2012) 7(10): e47772). The rationale to include GOYA male that mainly comprised of young adults (>18 years of age) in the study is unclear and should be discussed in details. Moreover, GOYA samples were recruited over a period of around 35 years between 1943 and 1977. This could have also led to heterogeneity in the samples and thus could affect the association results. Also, samples in TDCOB were recruited in case-control settings that could have influenced the statistical analyses, particularly regression analysis with BMI and other quantitative traits in only normal-weight individuals. It is not clear how the samples have been selected from the whole cohort and what was the range and distribution of age in the selected subset of the samples. Authors should provide that in details. These issues should be discussed properly in the manuscript.

Reply 1: We agree with the reviewer’s comments regarding GOYA being young men, and preferably, we would have included data from the ages 7-17 years in our analyses. However, since the number of individuals (at age 7-17) with available measures of BMI was relatively fewer (n~172 in each group) compared to ages 18 and above (n_cases=739; n_controls=722), we use the data obtained at a mean age of ~20 years while arguing that the young men were already overweight/obese during childhood. We also state this in our manuscript: "the group of overweight/obese individuals already had a much increased BMI at age seven years, compared to the population sample and this deviation increased as the children grew older" (lines 323-325).

To confirm this, we have calculated the zBMI scores for the available subset retrospectively using the available data from the school health records at ages 7 through 17. The obtained values can be observed in the table and figure 1 below. The plotted values are zBMI scores for individuals from the case group (BMI>25 kg/m2; n~170) vs. the individuals from the control group (n~172) plotted for 7-17 years. (Figure and table 1).

Figure 1: zBMI: cases vs controls during childhood (ages 7-17)
To help clarify these issues for the reader, we have made some additions in the text in the revised manuscript (lines 325-329):

“...However these data were available in a much smaller subset (n~172 in each group) compared to those at mean age 19.9 years (n~722 in each group) and for that reason we define the individuals as juvenile onset obese/overweight cases and controls, in the current study.”

2. It is noteworthy that the phenotypic differences with regards to obesity measures are well known between Europeans and Indian populations and genetic variants might have different effects in different ethnic populations. Authors should discuss this as a possible reason for observed differences in the studies.

Reply 2: We thank the reviewer for this important note, and as per the reviewer’s suggestion we have elaborated the discussion in the revised manuscript (lines 334-347):

“The identified differences between the current study results and those reported among Indian children [14, 15] may be due to the following reasons: firstly, the observed phenotype at a given BMI cutoff is known to differ between various ethnic populations, and risk factors for cardiovascular disease can be identified at a lower BMI in Asian and Indian populations compared to European [48]. This suggests that applying the same BMI cut-off of 25 kg/m$^2$ in both the Indian and Danish populations may confound the association. Furthermore, genotype frequencies and LD patterns may differ between the populations, and environmental factors such as lifestyle may also modify the effects exerted by genetic variants. Finally, population stratification may also play a role. Genetic marker based clustering analyses have been applied to exclude ethnic outliers in the current study as well by Tabassum and colleagues [14, 15] ruling out the possibility that major population stratification play a role for the observed differences between the two studies.”

3. Authors mentioned that GOYA participants had a much-increased BMI at age seven groups and hence included in the present study as cases. It would make more sense to categorize participants as overweight/obese or normal-weight based on BMI measurements during childhood age groups if data available. How many of the included participants were actually overweight/obese at age 7 or during childhood? Would the results change if the analysis were done only in these participants?

Reply 3: As per our reply to comment 1, we agree with the reviewer’s comments regarding GOYA being young men, and preferably, we would have included data from the ages 7-17 years in our analyses. However, since the number of individuals (at age 7-17) with available measures of BMI was relatively fewer (n~172 in each group) compared
to ages 18 and above (n_cases=739; n_controls=722), we use the data obtained at a mean age of ~20 years while arguing that the young men were already overweight/obese during childhood. We also state this in our manuscript: "the group of overweight/obese individuals already had a much increased BMI at age seven years, compared to the population sample and this deviation increased as the children grew older" (lines 323-325).

4. There have been inconsistent reports regarding the association of rs1137100 with obesity and whether A or G is the risk allele. The present study finds that A allele of rs1137100 (LEPR) is associated with lower BMI, rather than increased BMI as reported by Tabassum et al. Consistent with Tabassum et al. report, many other studies have also suggested A alleles as the risk allele for obesity, and related measurements (Labayen et al. Obesity 19, 2038–2045, 2011; Furusawa et al. Hum Genet 127:287–294, 2010) and increased plasma leptin levels (Sun et al. Hum Mol Genet 19: 1846–1855, 2010). Authors should discuss these inconsistencies in the risk allele of this variant.

Reply 4: In regard to the reviewer’s valid comment we have elaborated the discussion (lines 278 - 284) as stated below:

“These inconsistent findings could imply that the effects of rs1137100 may get modified by additional genetic or environmental factors which may vary between populations. Recent studies in Europeans have suggested obesity related measures to be influenced by interactions between LEPR polymorphisms and a polymorphism of the tyrosine phosphatase 1B [36] and ponderal index [37], and future studies focusing on such “gene-gene” and “gene-environment” interactions are thus warranted.”

5. Authors discuss that this discrepancy in the risk allele of rs1137100 could arise due to population stratification in Indian samples or population specific allele frequency. Population stratification could lead to spurious association and all genetic association studies should take considerable measures to avoid it. Tabassum et al. reported that they have taken all the efforts to minimize the stratification by collecting samples from randomly selected schools from a small geographical region and as a general population samples prior to classification as normal-weight and overweight/obese. Moreover, they also provided evidence of no stratification through genetic markers analyses. Actually, there is a GWAS study from the same group on the same adult population already showed the absence of stratification in this population (Diabetes 62, 977-986, 2013). The studies of Tabassum et.al. are entirely on North Indian subjects of Indo-European Origin. There are considerable numbers of population genetics based literature available that show the myth of stratification in Indian population (J.Genet. 87(1) 3-20, 2008; Nature 461: 489-494, 2009). Both the above articles clearly showed that Indo-European people of Northern part of India are genetically clustered together as Dravidian people of Southern India. Authors should discuss the issue in the light of this. And should discuss about the possibility of population stratification in their samples which could have led to observed discrepancy and if they have taken any measures to avoid the stratification.
Reply 5: We thank the reviewer for providing references to show that the differences in results from our study compared to the studies by Tabassum et al. are not due to stratification issues in the Indian dataset and that the North Indians are genetically clustered together.

With regards to our study, we mention in the manuscript that we identified individuals with Non-European ancestry by performing PCA clustering with the HapMap CEU population and removed those individuals that were ethnic outliers in the GOYA and in the TDCOB cohorts. This filter removes the scope for population stratification among Danish dataset.

We have revised the paragraph to emphasize these important points (lines 343-347):

“Finally, population stratification may also play a role. Genetic marker based clustering analyses have therefore been applied to exclude ethnic outliers in the current study as well by Tabassum and colleagues [14, 15] ruling out the possibility that major population stratification plays a role for the observed differences between the two studies.”

6. Differences in allele frequencies or population specific effect could also lead to differences in observations. Authors have discussed this possibility briefly. However, in one paragraph authors have mentioned that the G-allele frequency reported by Tabassum et al. is quite low as compared to other reports from Asians. That does not seem to be the case. The G allele frequencies reported by Tabassum et al. (16% in overweight/obese and 21% in normal-weight children) matches with the other reports from South Asian population and large databases like HapMap and ExAC Browser. The higher G allele frequency as mentioned by the authors is observed in East Asian populations. The genetic differences in South Asians and East Asians are well established. East Asians are mainly Hun-Chinese population and genetically distant to most of the Indian populations. Authors should correct this information and be careful in reporting the populations.

Reply 6: As per the reviewer suggestions we have noted that there exist genetic differences between East and South Asian populations and therefore we cannot directly compare those with Indians. For this reason we have updated the paragraph by omitting some lines.

7. The direction of effect of rs1137100 on BMI seems to be in opposite direction in normal weight and overweight/obese individuals (though not significant in later group). Authors should discuss possible reasons? Moreover, the analyses separately in normal-weight individuals and overweight/obese group separately could reduce the variance in each group and hence could influence the results. A combined analysis could be useful.

Reply 7: The directional differences in controls and obese (although non-significant in the obese) may be merely by chance. We agree to the reviewer that this could also be due to the extreme sampling design where the variance of the outcome measure is reduced to different extents among cases and control groups.

Although a combined analysis could have been useful here, we do not apply it due to the fact of the extreme sampling design introducing two distributions when combined.
8. Please provide effect allele frequencies separately for cases and controls and for both cohorts in the table 2. Also, provide mean BMI values in each genotype group for cases and controls in the table. Do the Z-scores for quantitative traits were normalized? Was the data was adjusted for age, sex? If not then why?

**Reply 8:** As per the reviewer’s suggestions we have updated Table 2 with effect allele frequencies and mean BMI values for both cohorts. The data was adjusted for age and sex as mentioned in the methodology section (lines 199-202). No transformation of traits was performed as data followed normal or near normal distributions and this statement has been added in the revised manuscript (lines 205-207)

“No transformation of traits was performed as they followed normal distributions within case or control groups.”

9. Indian civilization is one of the most ancient civilizations on this planet and known as Indus Civilization. In light of this eternal truth the terminology “Asian Indian” is quite naive. Logically this will be always “Indian” not “Asian Indian”.

**Reply 9:** We agree with the reviewer, and we have made the desired changes in the revised manuscript.

References
