Reviewer's report

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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Reviewer: Dwaipayan Bharadwaj

Reviewer's report:

The present study has attempted to replicate the findings of previous two studies from Indian population that reported association of four variants with childhood obesity. The authors have examined the association of these four variants with obesity and related anthropometric traits in Danish children or young adult. It is an important and interesting study as genetic variants predisposing for the risk of obesity during childhood largely remains unknown. The study did not find any association of these four variants with obesity in the Danish population. There could be many reasons for the observed discrepancies including differences in study designs, differences in phenotype characteristics and genetic architectures of two populations. These issues should be addressed and discussed adequately in the manuscript. The major concerns and suggestions are provided below:

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.

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.

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?

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.

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.

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.

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.

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?

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”.

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

**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.