Author’s response to reviews

Title: Gene-diet interaction effects on BMI levels in the Singapore Chinese population

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The authors would like to express our sincerest appreciation to all reviewers and editors for their constructive comments, which we believe have helped us tremendously in improving the quality of this manuscript. In the following sections, we provide specific responses to the comments and indicate how the paper has been revised.
Response to Reviewers of “NUTJ-D-17-00363”

Reviewer 1

1. You are examining the relationship of gene SNP’s to BMI and the Healthy Eating Index (AHEI). Both BMI and the AHEI have limitations. BMI does not account for percent body fat. Leaner individuals with greater muscle mass often classify as obese even though they have low body fat. Additionally, there are well-known limitations in using various dietary frequency, recall, or food logs. Both of these can introduce significant variation and thereby reduce correlations. While this type of study is an important contribution to understanding these relationships, the deficiencies in using BMI and the AHEI need to be highlighted.

Response: We thank the reviewer for this comment. The following information has been added in the revised discussion section to highlight the limitations in using BMI and AHEI-2010 score (manuscript; page 10; lines 262-266):

“In addition, AHEI-2010 is constructed by a simple summation of several components scored on a scale ranging between 0 and 10 and therefore had assumed that each component affects health equally, which is not the case. Thus a score calculated in a more sophisticated manner might be needed for a comprehensive assessment of dietary effects on health outcomes. [1].”

(Manuscript; page 10; lines 270-275):

“Moreover, BMI is a surrogate measure of body composition. In certain situations, it might not be a valid reflection for body fat percentage, the excess of which is considered to be the cause of comorbid conditions, such as for people with well-developed musculature [2]. Nevertheless in the general population, there is a significant positive correlation between BMI and body fat percentage, as well as with clinical outcome such as AMI, CAD and CHD mortality, including the Chinese subjects [3-10].”

2. You describe the general methods of each study, but need more detail about BMI limitations and relationship to body fat as well as the AHEI. This should include reported validity, test-to-test reliability, and coefficient of variation type of information. You briefly mention limitations, but more detail is needed to help the reader understand this limitation.

Response: We are unable to evaluate the correlation between BMI and body fat within our datasets as body fat percentages were not measured in the subjects. However, correlations between BMI and body fat percentages are relatively high in the general population and in other Chinese populations (r2 between 0.72 and 0.98) [3-7]. We have therefore, now highlighted that BMI is a surrogate measure for body fat and this is a limitation of the study.
3. Your discussion primarily summarizes results. It should compare your results to other findings. You cite some papers that have previously identified obesity-related SNP's. How does your study compare?

Response: The following information has now been added in the revised discussion section to compare our findings with previous studies (manuscript; page 8; lines 224-229):

“The BMI wGRS showed robust association with BMI levels in our Singapore Chinese samples and individually, most of the BMI susceptibility SNPs, were directionally consistent with their previously reported effects, indicating that genetic predisposition to obesity is largely transferrable to the Singapore Chinese population [11]. A total of nine loci (TMEM18, GNPDA2, RALYL, NT5C2, OLFM4, FTO, MC4R, QPCTL and ZC3H4) were associated with the outcome and the most strongly associated locus was rs11191560 on NT5C2.”

(Manuscript; page 9; lines 239-243):

“Previous study in adults of European ancestry showed two BMI loci, LRRN6C and MTIF3, could modify the association between dietary score and BMI levels [12]. However, in our study, none of the reported risk loci significantly interacted with AHEI-2010. Differences in sample sizes, risk allele frequencies and/or dietary consumptions may explain these discrepancies.”

Reviewer 2

1. I would suggest the authors refer to the use of two sample populations rather than 6 data sets. The latter suggests independent sampling which is not the case. It is also not clear why (incident) cases and controls were examined separately for SCHS. Wasn't diet and BMI measured prior to event? Please add rational to methods section.

Response: We thank the reviewer for highlighting this. The SP2 samples were genotyped on different SNP arrays (Illumina 1M and Illumina 610 chips). Similarly the SCHS samples were genotyped on different SNP arrays (SCHS-CAD cases and controls were genotyped on the Illumina OmniZhonghua array and the SCHS-T2D cases and controls were genotyped on Affymetrix ASI (Asian) Axiom array). Also there were known associations between BMI and CAD and T2D among our datasets. As such, we had treated each dataset separately and subsequently combined the results using meta-analysis procedures to prevent possible systematic
errors due to varying genotyping methods and bias during association analyses. The following sentences have been revised to clarify this point (Manuscript; page 3; lines 77-82):

“We studied 7,817 participants from six data subsets from two adult Chinese population, Singapore Chinese Health Study (SCHS), including the SCHS coronary artery disease (SCHS-CAD) cases (N = 594) and controls (N = 1,070), SCHS-Type 2 diabetes (SCHS-T2D) cases (N = 2,004) and controls (N = 2,055), and Singapore Prospective Study Programme (SP2, N = 2,094). Since the samples were genotyped on various SNP arrays and BMI is known to associate with CAD and T2D [13, 14], we performed analysis individually in these six data subsets and combined individual results using meta-analysis procedures.”

2. -The FFQ for SP2 captured diet during the month prior to the interview. What time period did the FFQ for SCHS capture? The SCHS FFQ serving options ranged from never/hardly to 2 or more times/d. What serving options were allowed in the SP2 FFQ?

Response: The FFQ for SCHS captured diet during the year prior to the interview. Related information has been added to the manuscript (Page 5, line 128). In SCHS, three portion sizes were available for each food. For items that could be counted conveniently, the three portion sizes were number-based: 1) one-half egg or less, 2) one egg, and 3) two eggs or more. For most items, colored photographs of three identical dishes of various amounts are shown to the subjects during the in-person interviews. Subjects were asked to express quantities in units of household measurements, e.g., Chinese spoon and rice bowl [15]. In SP2 FFQ, the participants were asked to estimate the frequency of consumption of each food group based on a reference portion size and to indicate consumption on a per-day, per-week or per month basis or as never/rarely. Pre-defined standard portion sizes were included and expressed in terms of cups, spoons, and slices and corresponding measuring utensils were supplied to aid estimation [16].

3. -Please provide justification for the choice of 10 dietary factors. Previous high impact papers have reported interactions with sugar sweetened beverages. Might the latter be tested for replication in the current study? This would be an important analysis and aligns with the authors' broader motivation for conducting the study. This would further qualify the authors' conclusions: stated in the abstract and discussion: "In conclusion, similar to studies performed in large-scale European ancestry samples, our data indicates that, in aggregate, most known BMI risk loci do not interact with dietary intake to modify BMI levels in East-Asian subjects."

Response: The reviewer has raised an excellent point. Although, as the reviewer correctly highlights, the servings of specific food groups such as sweetened beverage have been previously shown to interact with specific BMI loci (such as the FTO locus), we are unable to perform such
analyses as the information is sparse in the samples utilized in the present study. Also the serving portions for type of food between SCHS and SP2 were not directly comparable. As such we had focused our analyses on the dietary score and the 10 nutrient components (macronutrients and micronutrients), as these were available for the majority of the samples tested in the study.

4. -The significance threshold chosen is unclear. The authors state that 89 tests were conducted when in fact at least 78 SNPs x 11 dietary variables = 858 tests were conducted.

Response: We thank the reviewer for highlighting this. We have changed the stringent threshold for 858 tests (78 SNPs x 11 dietary variables). This still yields a nominally significant association between cholesterol intake and rs4740619 in our study (adj Pvalue = 0.043). We have now made the required changes in the manuscript:

(Manuscript, Page 7, line 182)

“Bonferroni adjusted P value of < 0.05 (2 tailed) was considered statistically significant after adjusting for multiple comparison for 858 tests (78 BMI SNPs × 11 dietary variables).”

(Manuscript, Page 8, line 210)

“adjusted Pinteraction = 0.043”

5. -Discussion page 9 lines 227-233. These are 'results' and should be moved to the results section.

Response: We thank the reviewer for highlighting this and this section has been moved into the result section (Manuscript; page 8; lines 210-215).

6. - Only 9 of the 78 BMI SNPs were 'significantly' associated with BMI in the current study. Were these tests corrected for multiple testing? The supplementary table suggests they were not and thus even a few the 9 'significant' snp-bmi associations might be chance findings. Regardless, can the authors speculate on the low reproducibility of these SNPs in the current sample? The very weak association between FTO and BMI is especially surprising. Might this have impacted power to detect or replicate gene-diet interactions?
Response: These associations were not corrected for multiple testing. However, the small numbers of significant replications for the individual BMI-associated SNPs in our study is not highly surprising given the relatively modest sample set and statistical power to replicate all known BMI SNPs in the present study, compared to the sample sizes in the large-scale BMI GWAS studies that these SNPs were identified from. Nevertheless most of these BMI loci showed consistent effect directions in our samples (with original GWAS studies, 62 out of 78 SNPs, Binomial p value ≤ 0.000001) and the overall BMI gene-risk score was strongly and robustly associated with BMI levels in our study (p=1.55×10-15). As such, in aggregate, we believe that the known genetic predisposition to obesity is largely transferrable to the Singapore Chinese population.

We have previously evaluated the effects of known BMI loci in the Singaporean populations [17, 18]. As previously indicated, the lower risk allele frequency of the FTO SNP is likely to have resulted in weaker associations with BMI levels in the East-Asian and Singapore Chinese samples (A allele of rs1558902, MAF=15% in Singapore Chinese vs 43% in Europeans). As indicated by the reviewer, differences in risk allele frequencies at specific risk loci in different ethnic groups may indeed affect power to identify interactions and we have now highlighted this in the discussion section of the manuscript (Manuscript; page 9; lines 241-242).

“Differences sample sizes, risk allele frequencies and/or dietary consumptions may explain these discrepancies.”

References


