Author’s response to reviews

Title: Effects of smoking on the genetic risk of obesity: the Population Architecture using Genomics and Epidemiology Study

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Author’s response to reviews: see over
November 19th 2012

Dr. Lu Qi, Associate Editor
BMC Medical Genetics

Dear Dr. Qi,

We greatly appreciate the opportunity to resubmit our manuscript, “Effects of smoking on the genetic risk of obesity: the Population Architecture using Genomics and Epidemiology Study” (MS# 1669700221784855), to BMC Medical Genetics. I very much apologize for the delay of five days in resubmitting this manuscript, which is due to some miscommunication and one of the lead authors taking a new position.

We carefully evaluated the helpful comments of the reviewers and provide a point-by-point response to each comment below. These comments allowed us to further improve our manuscript, which we believe is of interest to a broad audience given that we are addressing an important question about the potential impact of smoking on the effect of genetic variants on obesity risk. This study was conducted within the “Population Architecture using Genomics and Epidemiology” (PAGE) study, a National Human Genome Research Institute (NHGRI)-funded study to investigate the epidemiologic architecture of well-replicated genetic variants associated with complex disease in several large, ethnically diverse population-based studies. In this manuscript we are utilizing this rich resource to investigate the potential for effect modification by smoking status on the effect of several well-established genetic risk factors on body mass index. Our study includes European and African decent populations with well characterized phenotype data from major US cohorts such as Women’s Health Initiative, Atherosclerosis Risk in Communities, Coronary Artery Risk in Young Adults, or Multiethnic Cohort.

We appreciate the opportunity to revise this paper for further consideration for publication in BMC Medical Genetics.

Best regards,

Ulrike Peters, PhD MPH
Member, Fred Hutchinson Cancer Research Center
Research Associate Professor, University of Washington
Reviewer #1:

Overall report
The authors evaluated interactions between established obesity variants and smoking status for the outcome of BMI. Data from 6 cohorts comprising the PAGE study were meta-analyzed. The investigation is of interest, given consistently demonstrated relationships between smoking and BMI, and potential overlap between CNS pathways modulating smoking and eating behaviors.

We thank the reviewer for this overall positive feedback and respond to each specific comment below.

Major Compulsory Revisions
none

Minor Essential Revisions

1. There is a typographical error in the last sentence of the Background section. The phrase “and a smoking” should be “and smoking”. (Minor issue not for publication)

Thank you, we have fixed this typo.

2. In the Statistical Analysis section and/or Results, it is not clear whether the authors tested for smoking status*SNP interaction with men and women combined (and obtained no significant interaction term) or whether it was not tested. Please clarify and if the decision was made to limit interaction testing to gender stratified files, please explain.

Rereading the statistical analysis section we realizing that we were not clear about this and appreciate this thoughtful comment. We performed all analyses stratified by sex due to potential sex-specific differences in obesity pathways. To clarify this and to provide a justification, we have added the following sentence on page 6: “Because it has been described that nicotine has antiestrogenic properties and is metabolized differently in men and women, all analyses were additionally stratified by sex [12]. ”

3. A question about data in Table 1 Current smokers, female: The number of current smokers for the white participants of the EAGLE study is listed as 688 and the number of Former/never smokers is listed as 317. If the total number of women in the EAGLE study is 1005 then the prevalence of smoking would be 68%. Is that correct? For African American women in EAGLE the value looks more plausible: the prevalence of current smokers is 89/500 or 15%. This is the only one I looked at closely. Please check other values.

Thank you for alerting us to this error. The tables for EAGLE were erroneously based on a subset of samples, instead of the entire group of subjects used in each analysis. We have
closely looked at all numbers and the tables are now revised with the correct values. We also revised the numbers in the text accordingly.

Discretionary Revisions

1. In the SNP Selection and Genotyping section, suggest that the authors refer to “African populations” as “populations with African ancestry” or “African Americans” to avoid the implication that the individuals live in Africa, and/or that they are characterized by exclusively African ancestry. Many African Americans possess African and European admixture.

   Thank you for this suggestion, we have made the change on page 6. We also checked the entire manuscript and did not find another place where we should change the description based on this careful comment.

2. In the Results section, “borderline significant” overstates the relationship, particularly since adjustment for multiple testing was not taken into account.

   We have edited the text on page 7 to remove the phrase “borderline significant” and simply report the results and p-values for each analysis. The text no longer uses a p-value cutoff of ≤0.10 to indicate evidence for interaction.

3. The cohorts contributing to the PAGE study represent a set of populations that are heterogeneous with respect to age (age ranges from 25-100 years) and these differences are likely to be related to differences in the phenotypes and exposures. For example, mean BMI ranges from 23-27 in white women and smoking prevalence ranges from 8% to 26% (if the reported EAGLE current smoking prevalence is in error). In addition, the wide age ranges are not always distributed across the cohorts, but instead some cohorts are limited to elderly and some to young adults. Although evaluation of the I2 did not reveal heterogeneity, the authors may consider whether interaction is detectable in a subset of cohorts that are more similar in traits that affect BMI and smoking (such as elderly vs. non-elderly). The authors may want to consider meta-analyses of a subset of cohorts with more similar ages (for example, excluding CARDIA – which is limited to young adults - and CHS – which is limited to elderly adults).

   Thank you for this suggestion. We repeated the meta-analyses excluding CARDIA, a study with substantially younger participants than the other studies (mean age ~25). We found no notable differences in the results. To report on this interesting sensitivity analysis, we updated the statistical analysis section (page 7) as following: “Finally, as a sensitivity analysis to explore the effect of age, we repeated all analyses excluding subjects enrolled in CARDIA, who tended to be younger than subjects enrolled at the other PAGE sites (Table 1).” Furthermore, we added the following to the result section (page 7): “…, and the sensitivity analysis revealed that excluding subjects enrolled in CARDIA (i.e., younger subjects) did not substantially alter results (data not shown).”
4. In the Discussion section, it is not clear that the association of the SNPs with BMI eliminates the necessity for considering multiple testing adjustments as the authors state, however, the authors are appropriately guarded about the level of evidence they obtained.

We appreciate this thoughtful comment and we have reviewed the text to ensure that we do not overstate the significance of our results and address that none of the findings remain significant when accounting for the number of tests. Please note reviewer #2 made a similar comment. Accordingly, we have changed the language throughout the manuscript to reflect that our findings are null findings.

5. In Supplementary Table 1, the allele frequency (AF) is included in three columns. Given that all three values are essentially the same suggest that the authors list it once in the table and state in the text that the AF does not vary by smoking status. Suggest replacing the label “Combined” with “Combined by sex and smoking status” to be most clear. The title of the table refers to “change”. Use of “difference” instead of “change” is more appropriate to cross-sectional data.

Thank you for the suggestion, we have made these changes in the tables. We added to the result section on page 8: “Allele frequencies did not differ substantially by smoking status or sex, and thus combined frequencies are presented in all tables.”

Reviewer #2:

Overall report:
In this study, the authors examined the interactions between 10 BMI associated SNPs and smoking on BMI in a total of 13,994 African Americans and 34,233 European Americans. The overall results for SNP-smoking interaction on BMI were negative: none SNP showed significant interaction, although two SNPs showed some potential interactions with smoking status in some sub-groups stratified by race or sex. These non-significant results could be expected because of the limited statistical power for interaction analysis with individual SNPs. For FTO, the strongest BMI loci identified so far, a recent meta-analysis of ~220,000 individual only found a modest interaction between FTO and physical activity on BMI and obesity, suggesting that we may need very large sample size to detect such an interaction. The current study provides some data for gene-environment interaction on BMI, though they were negative, which might be useful for future meta-analysis. However, I have major concerns about the data presentation and interpretation. Thus, I suggested the authors to re-present the results and re-organize the paper, as their finding should be described and interpreted as negative results.

We appreciate this detailed feedback. We agree that our findings should be interpreted with greater caution and as negative results. We have made the changes throughout the manuscript as detailed below.
1. The authors examined 10 previously identified BMI SNPs, while more than 30 SNPs have been established (in 2010). Why the author only selected 10 SNPs? For most of these cohorts participated in the current study, GWAS data should be available. Thus, it might be easy to get genotype data on other SNPs.

   While a subset of the studies included in this analysis have GWAS data, most of the included studies, do not have scan or only in small subsets of the cohorts, such as WHI, MEC or NHANES. SNPs were selected for genotyping in early 2009, and accordingly we do not have data on recently-reported SNPs. We have updated the text on page 5 to explain our SNP selection process, as follows: “SNPs were selected from GWAS studies published online as of December 31, 2008, based on prior GWAS findings of positive association with BMI or obesity. We analyzed a total of 10 SNPs, after excluding correlated SNPs. Details of the SNP selection process, DNA extraction and genotyping procedures, as well as the association between each of these SNPs and BMI in PAGE have been reported elsewhere [Fesinmeyer et al., 2012].”

2. The authors stated that a p-value <0.10 were considered suggestive of interaction, which might be more clear explanation, why? Since the authors test multiple SNPs the p-values even need adjustment for multiple tests.

   We appreciate this thoughtful comment and based on this we have eliminated the p ≤0.10 cutoff for “evidence of interaction” and have simply reported effects and unadjusted p-values. We have also edited the text on pages 7-9 to ensure that the significance of our results is not overstated.

3. None of the SNPs showed significant interaction with smoking on BMI. I was surprised that the conclusion is that smoking status may modify genetic effects on BMI. The authors should re-conclude their findings based on the observed negative result.

   We agree with the reviewer. To avoid overstating the significance of our results, we have revised the text throughout the manuscript.

   We added the following to the abstract (page 3): “We did not observe strong evidence for interactions and only observed two interactions with p-values <0.1:....” “Conclusions: These analyses provide limited evidence that smoking status may modify genetic effects of previously identified genetic risk factors for BMI. Larger studies are needed to follow up our results.”

   We revised the text in the result section as follows: “Out of all analyses performed in EA and AAs, none of the SNP*smoking interaction was statistically significant at p-value ≤ 0.05 (Tables 2 and 3). We observed only two interactions that had p-values <0.1:....”
In the discussion section on page 8 it now read as follows: “In our results, we found little evidence for effect modification by smoking status, although 2 SNPs (rs6548238/TMEM18 and rs9939609/FTO) showed weak evidence for interaction that should be followed up in a larger study.”

And on page 9: “In our analyses of EA females, the A allele of rs9939609/FTO was more strongly associated with BMI among current smokers compared with former/never smokers, although the analysis was underpowered to detect a statistically significant difference between the two groups.”

On page 10 and 11 we revised the conclusion as following: “We provide an investigation into the hypothesis if genetic predisposition to obesity may be modified by tobacco use among EA and AA men and women. We observed no strong evidence for SNP*smoking interaction. Despite the relative large samples size of over 50,000 participants power was limited and future larger studies should investigate the potential sex-specific effects of smoking on each variant’s association with energy balance.”

4. The authors presented some “suggestive” interaction results in some sub-group analysis as their main results. However, I think these results are not convinced. It would be better to describe all the data for each SNP rather than these 2 SNPs in sub-group analysis. Thus, table 2 could be removed. Please provide the results for 10 SNPs in European Americans for all and stratified by sex as Table 2. Similarly, please make another table for data for African Americans. Although these data were negative, they were very informative and useful. In table 2, the columns “effect size” and “%difference in mean BMI with 1 copy risk allele” are the same meaning, right? If so, please keep one in the tables.

We appreciate this suggestion, and have reorganized the tables accordingly. We have eliminated the supplementary table, and now present one table for each race / ethnicity, including the full analysis results. We have also reformatted the tables to list simply the percent difference in mean BMI with 1 copy of the risk allele.