Author’s response to reviews

Title: Physical activity, smoking, and genetic predisposition to obesity in people from Pakistan: The PROMIS Study

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Author’s response to reviews:

Dear Dr. Tregouet,

Thank you for the opportunity to revise and resubmit our manuscript to BMC Medical Genetics. We have responded to each point raised by the reviewers with a point-by-point rebuttal (below) and in making narrative changes to the manuscript (highlighted in red text).

We hope that you and the reviewers will agree that the comments raised are addressed adequately and that you feel our paper is likely to be of interest to the readership of the Journal.

Reviewers’ remarks:

Reviewer #1: No comments provided.

Reviewer #2:

The manuscript presents an interesting study about obesity and BMI-associated loci with Pakistani population. The study dates were from 16,157 Pakistani adults (8,232 controls; 7,925 diagnosed with myocardial infarction [MI]) enrolled in the PROMIS Study. The statistics analyses used were appropriate to study's objectives.

Response R 2: We thank the Reviewer for her careful evaluation of our paper.

Reviewer #3:

Reviewer # R3.1 comment: The title does not describe what was done in reality (which lifestyle factors were assessed).

Response: R 3.1: We have modified the title to address the reviewer’s concern:

“Physical activity, smoking, and genetic predisposition to obesity in people from Pakistan: The PROMIS Study”

Reviewer # R3.2 comment: The related data used for SNPs selection were not defined in materials and methods section.

Response: R 3.2: We have rewritten the closing paragraph of the Introduction to clarify that the SNP selection was based on a previous GWAS meta-analysis in European adults:
“This study was undertaken in 16,157 ethnic Pakistani adults from the Pakistan Risk of Myocardial Infarction Study (PROMIS). The aim of the study was to examine genetic associations and gene-lifestyle interactions for BMI-associated variants previously identified and replicated in European-ancestry populations [8]. We focused on comparing the direction and magnitude of the genetic association signals between European and Pakistani adults; we also sought to determine if smoking or physical activity modified these effects.”

Reviewer # R3.3 comment: The reference or validity of physical activity questionnaire was not mentioned.

Response: R 3.3: To quantify physical activity and tobacco exposure, we first developed a pilot questionnaire. For exposure to tobacco consumption, with the help of local dietician and physicians, we came up with list of all tobacco items that are typically consumed in the Pakistani population. Similarly, for physical activity, with the help of an exercise physiologist, questions pertaining to level of activity at work, at home, mode of transportation used for commuting to work (e.g., bicycle, walking), nature of activities engaged at leisure time and nature of job were assessed in the pilot questionnaire. The pilot questionnaire was further used to assess the mode and frequency of tobacco consumption and nature of physical activity in 300 participants who were randomly chosen from an urban resident population in Karachi. The pilot questionnaire also sought information on any other forms of tobacco consumption or physical activity through open ended questions to capture information not covered by the pilot questionnaire. Based on the responses received from the participants, the questionnaire was finalized. Exposure to tobacco consumption was divided into: “ever” or “never” or “current” and information on “smoked”, “chewable”, or “snuffed” forms of tobacco was separately sought. For physical activity, participants were categorized to have “low”, “moderate”, or “intense” physical activity based on their responses. We recognize that we have not used any objective measures to quantify “physical activity” or “exposure to tobacco”; however the magnitude of the inverse association between our estimates of physical activity and smoking with BMI in PROMIS is comparable to what has been reported elsewhere for validated instruments, strongly supporting the validity of our measures.

Table 1 Physical activity in association with BMI in PROMIS (N= 16,157)

<table>
<thead>
<tr>
<th>Beta</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity</td>
<td>-0.321</td>
<td>0.054</td>
</tr>
</tbody>
</table>

Table 2 BMI across three strata of physical activity in PROMIS (N= 16,157)

<table>
<thead>
<tr>
<th>BMI (kg/m2)</th>
<th>Strata</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>25.94</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Moderately Active</td>
<td>25.78</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>25.26</td>
<td>3.9</td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Smoking in association with BMI (N= 16,157)

<table>
<thead>
<tr>
<th>Beta</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking*</td>
<td>-0.318</td>
<td>0.035</td>
</tr>
</tbody>
</table>

*smoking variable was categorized as (0= never + ex-smokers, 1= current smokers)

Table 4 BMI across three strata of smoking in PROMIS (N= 16,157)

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never + Ex-smokers</td>
<td>26.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Current smokers</td>
<td>25.4</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Reviewer # R3.4 comment: In the figures 4 and 5 Y titles and error bars should be corrected.

Response: R 3.4: We thank the reviewer for carefully looking through the figures; we have corrected the figures accordingly in updated version of manuscript.

Reviewer # R 3.5 comment: Why interaction analyses were not performed in additive model like other analyses for association of genotypes (individuals SNPs or GRS) with BMI.

Response: R 3.5: Interaction tests for individual SNPs and the GRS with physical activity and smoking (for outcomes BMI) were performed by including a SNP (or GRS) × physical activity/smoking interaction term in the multiple regression models, with the marginal effect terms also included. The regression model can be described as follows:

Outcome trait = α+βSNP + βphysical activity + βage+ βage2+ βsex + βPC1-4+βSNP *physical activity + ε

Where βPC1-4 is the term for the genetic principal components derived to control for population stratification and all other betas refer to the variables in lower case. A pairwise interaction term was fitted to model the multiplicative interaction of the genetic and lifestyle variables. Comparable models were fitted to test for interactions between smoking and the GRS. In each case, an additive mode of inheritance was assumed and the models fitted accordingly, as in the genetic association analyses. However, in Fig 5A we present the results for the CLIP1 variant (rs11057405) with carriers of the A allele pooled. We did this because the minor allele count was very low in A allele homozygotes (MAF 2%), as indicated in the supplementary tables.

Additional comments from the Editorial staff
The authors mentioned that the study sample has been imputed for 1000G. If so, why 2 SNPs were not included in their analysis? They must have been imputed. What was their imputation quality criteria (as well as that of all other SNPs studied in the manuscript)? Unless the analysis was performed on raw genotypes. If so, the message is not clear.

Response: Additional comments from the Editorial staff: The SNPs in question were directly genotyped, but deviated substantially from Hardy-Weinberg expectations and were thus excluded from the analyses.