Author's response to reviews

Title: The contribution of FTO and UCP-1 SNPs to extreme obesity, diabetes and cardiovascular risk in Brazilian individuals

Authors:

Adauto V Ramos (adautoversiani@yahoo.com.br)
Luciana Bastos-Rodrigues (lu.bastosr@gmail.com)
Bruna A Resende (brunaamr@gmail.com)
Eitan Friedman (eitan.friedman@sheba.health.gov.il)
Luciana Campanha-Versiani (campanhaversiani@gmail.com)
Debora M Miranda (debora.m.miranda@gmail.com)
Marta Sarquis (martasarquis@gmail.com)
Luiz De Marco (ldemarco@ufmg.br)

Version: 2 Date: 31 August 2012

Author's response to reviews: see over
The Editor  
BMC Medical Genetics

Sir

We thank the Associate Editor and both reviewers for their comments and suggestions. We incorporated all of them into the text which made the manuscript stronger and more readable.

We hope you will find this manuscript suitable for publication in BMC Medical Genetics.

We would be glad to provide any further information needed.

Yours sincerely

Luiz De Marco MD PhD

Luiz De Marco MD PhD  
Department of Surgery  
Universidade Federal de Minas Gerais  
Av. Alfredo Balena 190 - room 325  
Belo Horizonte – 30130-100  
Brazil  
e-mail: ldemarco@ufmg.br  
Phone/Fax: +55 31 3409-9134
Response to reviewers

We thank the Associate Editor and both reviewers for their comments and suggestions. We incorporated all of them into the text which made the manuscript stronger and more readable.

1. Associate Editor

Query: I am sharing the concern of reviewer 2 about ancestry estimation. There are chances that the selected indels are in the 1000G dataset. Please test the ability of this indels selection to separate the different 1000 G population.

My only small additional comment is that the study of Scuteri et al. appeared after the study by Dina et al. (which was sent before publication of Frayling et al.). Therefore labeling Scuteri et al as original discovery and Dina et al. as a replication sounds a bit curious.

Finally, stating that association does not mean causation is true in general but useless here and, maybe, true if understood literally. Indeed, there is few chances that phenotype influences the genotype (in the individuals, not during natural selection) so here, correlation may well mean causation (although this correlation does not show which gene exactly is involved and how it acts on obesity.

In general, the discussion is too long.

I am not sure unphased can be used as is for association studies and if yes, please explain whether the association is performed using the best-guess haplotype or with a weight given on haplotype probabilities.

Answer:

The ancestry estimation model we used was published by Bastos-Rodrigues et al. (2006) using the HGDP-CEPH Diversity Panel (1064 individuals from 52 populations with 40 biallelic markers). These same markers used in our present report discriminates well our ancestors: European, Amerindians and Africans. We included this issue into the text (lines 183 to 189).
We corrected the order of references by Scuteri, Dina and Frayling. The association/causation issue was corrected.

We tried to shorten the discussion. However, we had to incorporate text to answer queries made by the two other reviewers. Therefore, the discussion ended up roughly, with the same length.

UNPHASED implements maximum-likelihood inference on haplotype and genotype effects while allowing for missing data such as uncertain phase and missing genotypes. (Dudbridge F (2003). It has been used to analyze data similar to ours and we believe it supports our data. Other software, such as MDR, could also have been used.
Reviewer # 1: Robert Fredriksson

Major comments:

Query 1) The tables appear as supplementary information in the submission.
Corrected

Query 2) Considering the relatively small sample size in this study, the authors should present power estimations in the context of the data in Table 2.
Corrected (lines 153 to 155 and 258 to 262).

Query 3) Estimating the genetic origin of an individual based on only 40 markers seems relatively weak. The authors should discuss the power issues of this in the discussion, especially since . Also, in the context of this, the authors state that there is no difference between the obese and the controls. How was this obtained? To me there seem to be a difference in the Africans genetic contribution (case:0.087+-0.017, control: 0.037+-0.006). This should be clarified.
This issue was clarified. Please see lines 27 to 31 (above).

Query 4) Regarding the genetic background, wouldn’t it be more relevant to consider only the markers close to relevant SNPs in UCP-1 and FTO? This way the ancestry of the genetic blocks under study could be determined and analyzed. This should be discussed.

There were no differences in the genetic background between cases and controls with rs6536991. Regarding rs9939609, please see below . We have not included this data into the text.

Marker rs6536991: (Mann-Whitney test)

<table>
<thead>
<tr>
<th></th>
<th>Africans</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>0.11</td>
<td>0.02</td>
<td>0.49</td>
</tr>
<tr>
<td>TC</td>
<td>0.10</td>
<td>0.03</td>
<td>0.54</td>
</tr>
<tr>
<td>CC</td>
<td>0.02</td>
<td>0.03</td>
<td>0.83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Europeans</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>0.80</td>
<td>0.97</td>
<td>0.37</td>
</tr>
<tr>
<td>TC</td>
<td>0.86</td>
<td>0.95</td>
<td>0.48</td>
</tr>
<tr>
<td>CC</td>
<td>0.95</td>
<td>0.94</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Marker rs9939609: (Mann-Whitney test)

<table>
<thead>
<tr>
<th></th>
<th>Africans</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.11</td>
<td>0.02</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>0.09</td>
<td>0.03</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>0.05</td>
<td>0.01</td>
<td>0.0087</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Europeans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0.84</td>
<td>0.95</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>0.85</td>
<td>0.93</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>0.92</td>
<td>0.98</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

**Query 5)** early in the discussion the authors gives the impression that FTO is only associated with BMI in Europeans. This is not true, there a numerous showing association of FTO with obesity in other populations. These are just examples I know, there are many others, also from other populations: Africans (PMID: 21317302, PMID: 20299471)

We corrected this issue and added references.

**Minor comments:**

**Query 1)** line 221: Here FTO should be replaced with the FTO SNP number investigated.

Corrected (line 251)

**Query 2)** line 237: This sentence should be rephrased to match 3) above.

The text was rephrased (lines 267 to 275)

**Query 3)** Figure 1 should have error bars and would probably be more readable in 2D rather than 3D.

Figure corrected
Reviewer # 2: Cecile Lecoeure
Major Compulsory Revisions

Query # 1: According to the previous studies mentioned by the authors, two variants (A-3826G and A-1766G), located in the 5’ flanking region of UCP1, are shown associated to obesity related phenotypes. If the polymorphisms genotyped here are different from the two mentioned above could the authors explain their choice?
   rs6536991 is a Tag SNP; rs2270565 is a functional missense mutation (Leu229Met). rs12502572 is a commercially available SNP, validated; it was found as a Tag SNP in an older HapMap version.

Query # 2: Could the authors add the location of the SNPs with regard to their related genes?
   Incorporated in the text (lines 153 to 155) and in table 2.

Query # 3. The lack of association with UCP1 polymorphisms rs12502572 and rs2270565 could be due to low power. Could the authors estimate the power of their study design?
   We included this issue into the text (lines 153 to 155 and 258 to 262).

Query # 4: 4. Could the authors give the name of the programs used to test the Hardy-Weinberg equilibrium? To estimate the linkage disequilibrium?
   Added(see lines 123 to 126)

Query # 5: 5. In “statistical analyses”, first paragraph, the authors mention that the linkage disequilibrium between the three SNPs related to UCP1 is estimated. Could the authors show the result?
   We included the data (lines 165 to 169).

Query # 6. In “statistical analyses”, last sentence, the authors declare a test as significant for any p-value < 0.05 but in “results”, fifth paragraph, they conclude to a significant association between diabetes and the rs9939609 with a p-value of 0.05. One may consider this as a borderline association.
   These sentences were corrected (lines 172-176).

Query # 7: In “results”, fifth paragraph, the authors conclude that there is a significant association between the FTO variant and BMI but give a p-value of 0.420. Could they check this? They also find a significant association with UCP1. What is the p-value?
   Corrected (lines 177-179)

Query # 8: In table 1, did the authors also use the Mann-Whitney test for comparing the distributions of gender and disease status? In case of dichotomous variables the Chi² test is more appropriate. Could they precise this table includes mean and standard deviation?
   Information added to Table 1
Query # 9: The authors tested the effect of the variants on BMI. Did they perform the tests in each group or in the overall sample? As mentioned in “statistical analyses”, the gender is used as covariate but that would also be interesting to include the age.

Age was included. We observed that for each increase of one year of age, the chance of the patient being thin increases by 30% (95% CI: 1.12 to 1.49) (data not shown).

Query # 10: In “results”, last paragraph, could the authors give the p-value when testing the proportions of genomic ancestries?

Included (lines 183 to 189)

Query # 11: When testing rs6536911 with the obesity status, the genotype TC is more frequently observed in cases than in controls. When testing that variant with BMI, the highest mean BMI is observed with the genotype TT. Could the authors discuss that point?

Genotyping was performed three times. Checked and re-checked. We do not have a plausible explanation for this finding. We believe further studies will help clarify this issue.

Query # 12: In different published studies, the minor allele A of rs9939609 is shown associated with higher BMI mean. In table 3, in the obese group, the lower BMI mean is associated with the minor genotype AA. Could the authors discuss that point?

Genotyping was performed three times. Checked and re-checked. We do not have a plausible explanation for this finding. We believe further studies will help clarify this issue.

Query # 13: In tables 3 and 4, could the authors indicate that mean is given and add the 95% confidence interval of the mean?

Added to tables. Now tables 4 and 5.

Query # 14: Among the samples, some were probably under treatment. How were considered the values observed under treatment when testing? Could the authors say a word about that in the “methods and procedure” part?

This information was added (lines 93 to 96).

Minor Essential Revisions

Query # 1: For one SNP of UCP1, one can find three spellings: rs22705565 in the text, rs22700565 in the title of table 2, rs2270565 in table 2. The last one seems the correct one.

We apologize for the misspelling. Corrected throughout

Query # 2: As the authors tested the effect of the SNPs on diabetes, I suggest not restricting the title to obesity and cardiovascular risk.

Changed as suggested

Query # 3: In the methods part of the abstract, when the authors give the BMI mean of control and case groups please precise this is the mean. Symbol # is not
appropriate. At the beginning of the “results” part, please also precise it is the mean of BMI given into brackets
Corrected as suggested (line 87 onwards)

Query # 4: In “statistical analyses”, first paragraph, “permutation tests”.
Corrected (line 126)

Query # 5: In “statistical analyses”, “Unphased to analyze the polymorphisms and their association…”
Corrected (line 135)

Query # 6: There’s a discrepancy between the age characteristics of the control group in “results”, first paragraph, and in the table 1 (17.3 vs. 17.1).
Corrected (line 148)

Query # 7: In “statistical analyses”, no s required to covariate in “with gender as covariate for BMI”.
Corrected (line 140)

Query # 8: In “discussion”, fifth paragraph, a t is missing in “weighing”, and the term BMI would be more appropriate in reference to the written 3kg/m². Further in the paragraph I think the word year is missing “…weight loss one after…”.
Corrected (lines 252 and 264)

Query # 9: Could the authors check spelling of the reference 20 (polymorphism in the plural, the word obesity is missing)
Corrected (now reference 26, by Shin et al.)

Query # 10: Several errors occurred in table 2. There are 262 copies of both alleles for rs12502572 in the case group. Only 252 are expected, and according to the genotypes there are only 111 copies of allele A. The percentage of allele A of rs2270565 in the case group is 7% not 4%. In the legend, the spelling of rs12502572 is not correct.
Mistyping corrected (now, Table 3)

Query # 11: Could the authors check the MAF of rs9939609 given in “results”, third paragraph?
This was corrected, using the latest dbSNP data.