PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	RESPIRATORY CHAIN POLYMORPHISMS AND OBESITY IN THE SPANISH POPULATION, A CROSS-SECTIONAL STUDY
AUTHORS	de marco, griselda; Garcia-Garcia, Ana Barbara; Real, Jose; Gonzalez-Albert, Veronica; Briongos-Figuero, Laisa; Cobos-Siles, Marta; Lago-Sampedro, Ana; Corbaton, Arturo; Teresa Martinez-Larrad, Maria; Carmena, Rafael; Martin-Escudero, Juan; Rojo, Gemma; Chaves, Felipe

VERSION 1 – REVIEW

REVIEWER	Licinio manco University of Coimbra, Portugal
REVIEW RETURNED	15-Oct-2018

GENERAL COMMENTS	In this paper the authors analyse in three Spanish populations 48 SNPs from genes involved in Mitochondrial Respiratory Chain (MRC) pathway and their association with BMI and obesity risk. They found several significant associations in the individual populations and the associated SNPs were chosen for testing in the whole sample. The majority of loci associated with obesity have been discovered through GWAS in populations of European ancestry. Thus, studies testing for candidate loci performed in particular physiological pathways is a matter of interest. Some major points require the attention of the authors: 1. The authors claim in Abstract (Pg2, L10-14) and along the text that they study three different populations from Spain, suggesting that such differences could be important regarding findings of this association study. Because the three populations are similar in age (adults aged between 46 and 52 years-old), all of the same European ancestry (Spanish), the authors should clarify the significance of these differences. Moreover, this reviewer suggest to remove the term “difference” when referring for the three Spanish population samples. 2. In regards to the association results between SNPs and BMI or obesity risk depicted in Table 2, the model for SNPs rs683943 and rs1136224 seems to be inadequate since a genotype class in both SNPs is too low (only 4 and 2 subjects GG for rs683943 and less than 35 subjects GG for rs1136224). This may lead to false positive significant results, not reflecting true effects. Moreover, no significant association results were found for the whole population for these 2 SNPs; see Table 3. Thus, the association model for these 2 SNPs should be reconsidered such as: CC vs. CG/GG for rs683943 and AA vs. AG-GG for rs1136224.

	3. Most prior work in genetic association studies for obesity has observed overweight and obese individuals in a same group (cases) for comparison with normal weight subjects. Instead, the authors include overweight and lean subjects in the same group to compare with obese subjects. What is the reason for this option? 4. Pg12, L9-16: Why only two SNPs when testing for additive effects? And why the authors consider this additive effect as an epistatic interaction? 5. Finally, the manuscript is poorly written and needs to be revised. I recommend a professionally proofreading of all the document. Minor points The sentence concerning Results in Abstract (Pg3, L31-36) is confusing. Please rephrase. Rephrase the sentence in Introduction (pg3, L49-54): “However, it is also known that, due to limitations of GWAS, studies around 250 common variants with a similar effect to those described previously remain to be identified.[8]” Remove the term “studies”; Replace “Known” by “suggest / estimated”. Material and Methods, Pg5,L55: Include the classification for all weight groups (normal weight, overweight and obesity). Material and Methods, Pg7, L23-25: The authors state that “The Bonferroni correction cut off to assess significant associations was calculated for the 37 remaining SNPs”. However in table 2 and along the text the corrected p-value (P<0.0013) was never consider as cut off for significance (see Pg9, L14 or Table 2). Moreover, when reporting for p-values between 0.0013<P<0.05 the authors should refer for “nominal significance”. Pg11, L7-8: Clarify for the risk allele the sentence “On the other hand, rs4600063 and rs11205591 reduce obesity risk, while rs10891319 increases the risk (p<0.05)”.

REVIEWER	Habiba Alsafar Khalifa University, United Arab Emirates
REVIEW RETURNED	21-Oct-2018

GENERAL COMMENTS	The aim of this paper is to study the association between genes involved in MRC with BMI in Spanish population. the paper lack the following: 1. The name of the Ethical Committee who approved this study and the reference number 2. Power calculation (sample size calculation) 3. genes names should be italic 4. replucation studies in different population is missing 5. limitation of the study is missing 6. calculating for IBS is missing to check for any relatedness 7. calculating the homozygosity in the Quality control is missing 8. since some of these SNPs are within one gene, calculating a haplotype is highly recommanded

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

In this paper the authors analyse in three Spanish populations 48 SNPs from genes involved in Mitochondrial Respiratory Chain (MRC) pathway and their association with BMI and obesity risk. They found several significant associations in the individual populations and the associated SNPs were chosen for testing in the whole sample. The majority of loci associated with obesity have been discovered through GWAS in populations of European ancestry. Thus, studies testing for candidate loci performed in particular physiological pathways is a matter of interest.

Some major points require the attention of the authors:

REVIEWER 1 REQUEST 1- The authors claim in Abstract (Pg2, L10-14) and along the text that they study three different populations from Spain, suggesting that such differences could be important regarding findings of this association study. Because the three populations are similar in age (adults aged between 46 and 52 years-old), all of the same European ancestry (Spanish), the authors should clarify the significance of these differences. Moreover, this reviewer suggest to remove the term "difference" when referring for the three Spanish population samples.

RESPONSE: The term "different" referred to the different geographic origin of samples. However, we agree with the reviewer that this term may induce confusion and we have erased it from text. We have also specified:

• "This research was conducted in three open populations from different provinces of Spain". (Strengths and Limitations Section, Page 3)

• "This work studies three cross-sectional populations from Spain, representing three provinces: HORTEGA (Valladolid, Northwest/Center), SEGOVIA (Segovia, Northwest/center), and PIZARRA (Malaga,South)" (page 2, Abstract, Design sentence)

REVIEWER 1 REQUEST 2- In regards to the association results between SNPs and BMI or obesity risk depicted in Table 2, the model for SNPs rs683943 and rs1136224 seems to be inadequate since a genotype class in both SNPs is too low (only 4 and 2 subjects GG for rs683943 and less than 35 subjects GG for rs1136224). This may lead to false positive significant results, not reflecting true effects. Moreover, no significant association results were found for the whole population for these 2 SNPs; see Table 3. Thus, the association model for these 2 SNPs should be reconsidered such as: CC vs. CG/GG for rs683943 and AA vs. AG-GG for rs1136224.

RESPONSE: We agree with the referee. We have analyzed these two SNPs as suggested. We have not found any association between rs683943 and BMI or obesity risk in any of the three studied populations, and, consequently, this SNP has been erased from Table 2 and it is not mentioned in the text. (Table 2, page 10)

REVIEWER 1 REQUEST 3- Most prior work in genetic association studies for obesity has observed overweight and obese individuals in a same group (cases) for comparison with normal weight subjects. Instead, the authors include overweight and lean subjects in the same group to compare with obese subjects. What is the reason for this option?

RESPONSE: We are working with general population, and not with a case-control study. We are watching general effects and have chosen BMI>30Kg/m2 as a cut-off for obesity as indicated by WHO criteria.

REVIEWER 1 REQUEST 4- Pg12, L9-16: Why only two SNPs when testing for additive effects? And why the authors consider this additive effect as an epistatic interaction?

RESPONSE: We have studied all the possible additive effects between all the SNPs, being significant only the indicated (rs11205591 and rs10891319). We have clarify this point in the manuscript: "We studied additive effects for all these SNPs and an additive effect for rs11205591 and rs10891319 SNPs was found in the whole sample:…" (Page 12, second paragraph).

The "epistatic" term is erroneous. The correct one is "additive interaction". We regret for this mistake. We have corrected this:

• "In addition, rs11205591 and rs10891319 polymorphisms showed an additive interaction with BMI and obesity risk" (Page 2, Abstract, Results epigraph).

• "Furthermore, an additive interaction was observed between these two SNPs…" (Page 12, Discussion, second paragraph)

REVIEWER 1 REQUEST 5- Finally, the manuscript is poorly written and needs to be revised. I recommend a professionally proofreading of all the document.

RESPONSE: We have had the manuscript reviewed and edited by a professional

Minor points

MINOR POINT 1: The sentence concerning Results in Abstract (Pg3, L31-36) is confusing. Please rephrase.

RESPONSE: We have modified the indicated sentence:

"RESULTS: rs4600063 (SDHC), rs11205591 (NDUFS5) and rs10891319 (SDHD) SNPs were associated with BMI and obesity risk (p values for BMI were 0.04, 0.0011 and 0.0004, respectively, and for obesity risk, 0.0072, 0.039 and 0.0038). However, associations between rs4600063 and BMI, and between these 3 SNPs and obesity risk are not significant if Bonferroni correction is considered" (Page 2, Abstract, Results)

MINOR POINT 2: Rephrase the sentence in Introduction (pg3, L49-54): "However, it is also known that, due to limitations of GWAS, studies around 250 common variants with a similar effect to those described previously remain to be identified.[8]" Remove the term "studies"; Replace "Known" by "suggest / estimated".

RESPONSE: We have removed the indicated terms:

"However, it is also suggested that, due to limitations of GWAS, there are around 250 common variants with a similar effect to those described previously remain to be identified.[8]" (Page 4, first line)

MINOR POINT 3: Material and Methods, Pg5,L55: Include the classification for all weight groups (normal weight, overweight and obesity).

RESPONSE: We have included the classification as suggested: "Briefly, obesity was diagnosed with a BMI >30 kg/m2, overweight as BMI between 25.0 and 29.9 kg/m2, and normal weight as BMI<24.9 kg/m2." (Page 6, first paragraph"

MINOR POINT 4: Material and Methods, Pg7, L23-25: The authors state that "The Bonferroni correction cut off to assess significant associations was calculated for the 37 remaining SNPs".

However in table 2 and along the text the corrected p-value (P<0.0013) was never consider as cut off for significance (see Pg9, L14 or Table 2). Moreover, when reporting for p-values between 0.0013

RESPONSE: After performing the first analysis looking for associations between all the SNPs analyzed and BMI and obesity risk in the 3 independent populations, we have chosen as cut off the nominal p value (0.05) to select SNPs for a more detailed analysis. We regret because this has not been correctly explained in the text, and have clarified this point:

• "Those polymorphisms associated in at least one of the three populations and showing the same tendency in the remaining ones, or results with p-values near the nominal cut off point in the three studies (p<0.05)..." (Page 7, Materials and Methods, Statistical Analysis, second paragraph).

• "Table 2 shows results for those SNPS with differences in at least one of the three populations and a similar trend in the remaining ones, or that had p-values near the nominal cut-off point (p<0.05)in the three studies,…" (Page 9, Results, "Association between MRC genes SNPs and obesity", first paragraph).

MINOR POINT 5: Pg11, L7-8: Clarify for the risk allele the sentence "On the other hand, rs4600063 and rs11205591 reduce obesity risk, while rs10891319 increases the risk (p<0.05)".

RESPONSE: We regret for this omission and have corrected the mistake:

"On the other hand, rs4600063 (genotypes AG and GG) and rs11205591 (GG genotype) reduced obesity risk, while rs10891319 (AG and GG genotypes) and rs1136224 (AG and GG genotypes) increased the risk (p<0.05), but they did not reach the Bonferroni cut-off point" (Page 11, above Table 3).

Reviewer: 2

The paper lack the following:

REVIEWER 2 REQUEST 1- The name of the Ethical Committee who approved this study and the reference number

RESPONSE: We have used three populations, HORTEGA, PIZARRA and SEGOVIA, from 3 different provinces. This work has been approved by the Research and Ethics Committee from the University Clinical Hospital and INCLIVA (reference number 2010/013). We have specified this:

"The study was approved by the Research and Ethics Committee from the Valencia University Clinical Hospital and INCLIVA (reference number 2010/013)" (Page 5, Materials and Methods section, Sample Population, first paragraph)

REVIEWER 2 REQUEST 2- Power calculation (sample size calculation)

RESPONSE: We regret for this mistake. We have added (page 6, second paragraph):

"We have calculated the statistical power for our three samples independently (minor allele frequency (MAF) >0.10, genotype relative risk (1.5)), number of obese and non-obese and prevalence of obesity in each of the populations. Furthermore, the statistical power was over 85% for this conditions in all populations and it increases for increased allele frequency (http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html)"

REVIEWER 2 REQUEST 3- genes names should be italic

RESPONSE: We regret for this mistake and have formatted gene names

REVIEWER 2 REQUEST 4- replication studies in different population is missing

RESPONSE: We have performed the analysis in independent studies. Each population cited in this work is different, with a different geographic origin in Spain: Valladolid (North Center), Segovia (center) and Andalucia (South). All of them show the same trend for the SNPs where we have found an association. Therefore, we assume that these studies can be considered a replication study. In this way, other analysis from different populations should be done. We have specified this fact in the "Strengths and limitations" and "Discussion" sections:

• "Results from this study are promising and should be validated by larger sample sizes" (Strenghts and Limitations)

• "Further functional studies and association analyses in larger samples and other populations should be carried out to confirm our results" (page 14, last paragraph of Discussion)

REVIEWER 2 REQUEST 5- limitation of the study is missing

RESPONSE: We regret for this mistake.

We have completed the last paragraph of Discussion: "One limitation of this study is the reduced size of the analyzed populations. However, the statistical power is sufficient for the number of analyzed SNPs. On the other hand, we have not studied all the possible SNPs present in these MRC genes" (page 14, last Discussion paragraph)

REVIEWER 2 REQUEST 6- calculating for IBS is missing to check for any relatedness

RESPONSE: We are working with known open populations. Spanish population is known as a wide open Caucasian population. In Spain there are only some regions in traditionally geographically isolated small areas that could be considered as isolated populations, but none of them are included in this study.

REVIEWER 2 REQUEST 7- calculating the homozygosity in the Quality control is missing

RESPONSE: We have tested the possible excess of homozygosity in our population by Hardy-Weimberg test, but we have not found any deviation in the SNPs finally analyzed. We have clarified this point:

"Hardy-Weimberg test indicated no loss of heterozigosity in the analyzed populations for these SNPs" (page 7, Statistical analysis, second paragraph)

REVIEWER 2 REQUEST 8- since some of these SNPs are within one gene, calculating a haplotype is highly recommended

RESPONSE: We agree with the referee. In fact, we calculated haplotypes but we did not obtain any result. We have specified this fact in text:

"On the other hand, some of the SNPs were within the gene. We calculated possible haplotypes, with negative results" (Page 12, Results, last paragraph)

FORMATTING AMENDMENTS (if any)

Required amendments will be listed here; please include these changes in your revised version:

- Please provide better qualities figures, ensuring the figures are not pixelated when zoomed in on.

Figures can be supplied in TIFF or JPG format (figures in PDF, DOCUMENT, EXCEL or POWERPOINT format will not be accepted), we also request that they have a resolution of at least 300 dpi and 90mm x 90mm of width.

RESPONSE: We have tried to supply figures as TIFF or JPG as requested, without success. We are able to upload those TIFF or JPEG files, but platform shows the following error message: “File will not display in proof”. Formatting guidelines from the Authors Center specifies “Figures should be submitted in TIFF, EPS, JPEG or PDF formats”. If we upload them as pdf files, we do not obtain any error message. Due to this problem, we have uploaded figures as pdf files again. We regret for this, and we will supply files in the correct format as soon you indicate how we can solve this problem

VERSION 2 – REVIEW

REVIEWER	Licinio Manco, PhD University of Coimbra, Portugal
REVIEW RETURNED	28-Nov-2018

GENERAL COMMENTS	The authors have attended all the reviewer suggestions. I have no further comments.

REVIEWER	Habiba alsafar Khalifa University, United Arab Emirates
REVIEW RETURNED	19-Dec-2018

GENERAL COMMENTS	The study aim, objective and results are presented very clearly. my only concerns is the age of the cohort there is not younger generation and the cohort are all overwieght no normal or under weight cases.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

QUESTION: The study aim, objective and results are presented very clearly. my only concerns is the age of the cohort there is not younger generation and the cohort are all overwieght no normal or under weight cases.

RESPONSE: Obesity is increasing in Western Societies, becoming a very important health problem. This is the current situation in Spain, a Western Society. Our samples are representative for general Spanish population, and, therefore, are showing this trend towards an increment in obesity. Due to this reason, the weight means are around the overweight values.

In the same way, our population is getting older, but it has a very high life expectancy. Therefore, the age mean of our sample shows this fact, mainly because we have included people older than 18 years, the minimal legal age to consent to participate in this type of studies.

We have added the sentence: “Increased means of age correspond to the age structure of our population. BMI means correspond to overweight values, what is in agreement with the fact that obesity is increasing in Western Societies (WHO)”. (Page 8, Results, Characteristics of Studied populations, First paragraph)”