Author's response to reviews

Title: A Follow-Up Study for Left Ventricular Mass on Chromosome 12p11 Identifies Potential Candidate Genes

Authors:

David Della-Morte (ddellamorte@med.miami.edu)
Ashley Beecham (ABeecham@med.miami.edu)
Tatjana Rundek (Trundeked@med.miami.edu)
Liyong Wang (lwang1@med.miami.edu)
Mark S McClendon (MMcClendon2@med.miami.edu)
Susan Slifer (SSlifer@med.miami.edu)
Susan H Blanton (SBlanton@med.miami.edu)
Marco R Di Tullio (md42@columbia.edu)
Ralph L Sacco (rsacco@med.miami.edu)

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Author's response to reviews: see over
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**Dr. Jigisha Patel, MRCP, PhD**  
Series Editors - Medicine  
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Dear Dr. Patel,

We are submitting our revised manuscript (No. MS: 1860612615208250) entitled “A Follow-Up Study for Left Ventricular Mass on Chromosome 12p11 Identifies Potential Candidate Genes” to *BMC Medical Genetics*.

The authors are: David Della-Morte, Ashley Beecham, Tatjana Rundek, Liyong Wang, Mark S. McClendon, Susan Slifer, Susan H. Blanton, Marco R. Di Tullio, and Ralph L. Sacco.

We have substantially revised the manuscript based on the suggestions of the referees. All changes have been marked in the manuscript. The work is original and has not been published elsewhere. It will not be submitted to another journal until a decision concerning publication has been reached by the *BMC Medical Genetics* journal.

Thank you for considering our manuscript for publication.

Sincerely yours,

Ralph L. Sacco, MD, MS

**Answers to the critiques**

We would like to thank reviewers for their insightful and constructive criticisms. We have attempted to answer each comment to the best of our abilities.

**Reviewer 1**

This paper reports an interesting investigation that identifies potential candidate genes for left ventricular mass on chromosome 12p11 in a follow-up study. The introduction, the aim of the study, the methods, the results and the conclusions are rational and appropriate. I have no concerns.

**Reviewer 2**

The manuscript of Della-Morte et al about the potential association between LVM and candidate genes located in Chr 12p11 in a particular population of Caribbean Hispanics is on an issue of scientific interest and public Health impact. There are, however, several methodological and design concerns and this are major compulsory revisions.

**Comment 1:** Genotyping was performed by GW Human SNP 6.0 chip from Affimatrix. As stated by the manufacturer, “the new Affymetrix Genome-Wide Human SNP Array 6.0 features 1.8 million genetic markers, including more than 906,600 single nucleotide polymorphisms (SNPs) and more than 946,000 probes for the detection of copy number variation”. So, this reviewer feels that the total tests performed a priori is much higher than the restricted numbers (7085 or 4334 SNPs used for multiple testing correction and, then, for defining the statistical significance threshold, even admitting that the strategy of using a less conservative approach such as SimpleM is correct) and the authors have made
a post-hoc analysis for the region of chr12p11. Taking into account the above consideration there are non significant results to be described.

**Answer:** We thank the review for this comment. While we genotyped 906,600 SNPs on the Affymetrix Genome-Wide Human SNP Array 6.0 in 1137 individuals, this was done primarily for the purpose of a different study evaluating subclinical brain phenotypes. For the study of left ventricular mass, we have used this available data source as a convenience sample for follow-up of our previously described linkage peak on chromosome 12. Therefore, we have not studied all 906,600 SNPs for association with left ventricular mass and have restricted our analysis to the SNPs under the linkage peak because of our limited sample size and therefore limited power. We do appreciate and agree with the reviewer’s suggestion to consider the remaining markers across the genome for association with left ventricular mass, but that would be the subject of a different more conventional GWAS approach. The purpose of the current paper was only to follow up on our previous linkage findings in the family study. However, we are planning for genotyping of additional individuals beyond the 1137, who have left ventricular mass measured in our NOMAS population. We hope to further analyze the genetic impact on this phenotype in a larger sample from NOMAS as part of a separate analysis.

**Comment 2:** For the association test, the adjustment for covariates using SBP is not appropriate, because 64% of the subjects are hypertensive and they probably medicated. It seems that no effort has been made for controlling this problem. Even worse, the population has a mean age of 70.8 years meaning that there is a potential bias for studying “survivors”, in particular for a risk factor for CVD. At best, the results of the study are exclusively limited to this particular population subset.

**Answer:** We thank the reviewer for these observations. In agreement with reviewer, we have now removed SBP as a covariate from our model and instead used hypertension as an indicator variable. Hypertension is defined as having an SBP \( \geq 140 \), DBP \( \geq 90 \), history of hypertension, or being on hypertensive medications (page 5, paragraph 3; and page 6, paragraph 1; Table 2, and 3). Our results were not changed drastically, with SNPs in or near our most notable genes remaining the most significant for both the overall association and interaction analyses.

We do recognize that the mean age of our population may be a limitation and have now mentioned this in the discussion (page 12, paragraph 2). This underscores the importance of following up on these findings in other populations. It is however worthwhile to note that our families used for the original linkage screen are younger (46.1 ± 17.3) and also points to evidence for a genetic signal on chromosome 12p.

**Comment 3:** In addition, this reviewer feels that there is not a clear cutoff for the effect of WC on CVD or LVH risk and the effect of WC is, as for other traits, continuous. Therefore, it would have more power and be more logical to include WC as a continuous covariate in the model, instead of BMI (of course these are two very, but not perfectly, related variables).

**Answer:** We agree with the reviewer that it would more logical to include WC as a continuous covariate in the model, and so we have now added this analysis (page 5, paragraph 3; Table 2, and 3). However, we have left BMI in the model as well as it is additionally associated with left ventricular mass beyond the contribution of WC. Our results were not changed drastically, with SNPs in or near our most notable genes remaining the most significant for both the overall association and interaction analyses.

**Minor Essential Revisions**

**Comment 1:** Finally, it would be important, though not strictly essential, to replicate the findings in an independent sample because the number of the sample is rather modest.

**Answer:** We do agree regarding the importance of replication and plan to do it. As mentioned previously, there are more NOMAS individuals with left ventricular mass measured that can be genotyped given the funds in the future. We also have an interest in doing further genotyping in our family study in order to perform family based associations under the peak. However, we feel that it is important to publish these findings now so that other investigators may replicate independently, particularly in light of some interesting and novel candidate genes reported in our study.

**Comment 2:** The discussion at light of concerns described above should be more conservative and easily shortened.

**Answer:** We thank the reviewer for this suggestion and have made the discussion of our findings more conservative (page 10, paragraph 1; page 13, paragraph 1) as well as shorter.

**Reviewer 3**

In the current study, Della-Morte et al. conducted association analyses of SNPs in the 1 LOD critical region on chromosome 12p11, which was identified to be linked to Left ventricular mass (LVM) in Dominican families, with LVM in an independent sample of 895 Caribbean Hispanics. They found that rs10743465, downstream of the SOX5 gene, was significantly associated with LVM even after correction for multiple testing. Also, some clues of waist
circumference (WC)-SNP interaction were observed, although no SNP reached the peak-wide significance.
In general, I think the paper is very well written and it provides additional insight into the genetic determinants underlie LVM. I only have some minor concerns about the paper:

Comment 1: In a previous linkage study, the authors observed that the linkage to LVM was significantly increased in a subset of families with the high average WC, and they speculated that WC might provide additional information to BMI in the analysis of LVM. Then why didn't they include WC into the multivariate regression analysis as a covariate instead of BMI in the current study? Whether the significance of association would be changed remarkably after taking WC into consideration?
Answer: We thank the review for this useful comment. Our intent was to replicate as closely as we could what was done in the linkage study, where WC was not used as a covariate in the model since it did not come in as significant in the stepwise regression. Using Ordered Subset Analysis, we did observe a very significant interaction of WC with genotype in association with left ventricular mass even without a significant main effect of WC.
We do recognize that using linkage analysis followed by OSA in a family study is methodologically different than association testing in a cohort. After considering this further, we agree that it is important to include WC as a covariate in the model, particularly when studying the interaction of WC with genotype. Therefore, we have now added WC as a covariate in the multivariate regression model (page 5, paragraph 3; Table 2, and 3). We did leave BMI in the model as well as it explains variation in left ventricular mass beyond that of WC. The results however have not changed remarkably after this consideration.

Comment 2: Given that no SNP reached peak-wide significance in the analysis of WC-SNP interaction, the authors may calculate the statistical power they had. Also a discussion on this may be needed.
Answer: We thank the reviewer for the suggestion and have now added a power calculation for the WC-SNP interaction. Assuming independence of individuals, MAF of 0.20, an additive genetic effect, a population mean of 5.22 and standard deviation of 0.27 for the natural logarithm of LVM, a two-sided alpha of 0.001, and a population prevalence of 55% for high WC; we had over 80% power to detect a difference in beta coefficients of 0.125 between the high and low WC subsets. As the reviewer predicted, most of our effect sizes for the interaction were less than 0.125. This has now been added to the paper (page 7, paragraph 2).

Comment 3: The authors may stratify the subjects by quartiles of WC and see if there would be some changes.
Answer: When stratifying WC into two, we took sex into consideration instead of looking at the 50th percentile in our sample and dividing the data. High WC was defined as ≥40 inches in men and ≥35 inches in women according to the Third Report of the National Cholesterol Education Program - Adult Treatment Panel III. This is a more standardized division and so it is easier to argue its biological relevance. Also because our sample size is small, the more numerous and smaller the strata get, the less power we have to see an effect in each subset even if there were one there. In addition we wanted to as closely as possible follow the methodology that we used for our linkage-study. There we saw an increase in the genetic signal for a subset of families having high average waist circumference, essentially only dividing the data into two subsets (Wang L. et al., BMC Medical Genetics 2009, 10:74).