Author's response to reviews

Title: Gene expression network analysis reveals new transcriptional regulators as novel factors in human ischemic cardiomyopathy

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Author's response to reviews: see over
Dear Tim Sands PhD
Executive Editor
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BioMed Central
Floor 6, 236 Gray's Inn Road
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Valencia 30th, December 2014

Dear Editor:

Enclosed please find the revised version of the manuscript entitled "Gene expression network analysis reveals new transcriptional regulators as novel factors in human ischemic cardiomyopathy" (MS: 1116143175131242), by Isabel Herrera, Esther Roselló-Lletí, Ana Ortega, Estefanía Tarazón, María Micaela Molina-Navarro, Juan Carlos Triviño, Luis Martinez-Dolz, Luis Almenar, Francisca Lago, Ignacio Sanchez-Lázaro, Jose Ramón González-Juanatey, Antonio Salvador, Manuel Portolés and Miguel Rivera. We want to thank the associate editor and the reviewer for their constructive criticism and suggestions that substantially have improved the manuscript.

Please find below our comments that we hope satisfactorily address the suggestions of Reviewer #1, including a second review of the manuscript by a professional translator that is also a native English-speaker.

Sincerely,

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Response to REVIEWER # 1:

First of all, we sincerely thank Reviewer # 1 for the interest in improving our manuscript. We apologize for not adequately responding to some of the previous comments.

(1) The usage of 'replicates'/‘biological replicates' is wrong. Samples from different patients with the same phenotype cannot be regarded as biological replicates!

As suggested by Reviewer 1, we have removed the term “biological replicate.” We hope that we have clarified our strategy in the revised manuscript, which was intended to eliminate individual variability while allowing us to explore common intra-group expression and to identify statistical differences between groups.

(2) Line 319 (previous version). The original '5 normalized transcript reads' was changed into '5 normalized transcript read counts' which is not much clearer. From the context the reader may assume that '5 normalized read counts per gene/transcript' is meant, which is low for long transcripts.

We agree with Reviewer # 1 that 5 normalized read counts/ gene/transcript is low for the expression inference; however, the differential expression method applied in this study is based on a negative binomial distribution and has a different overexpression inference for the number of reads. Importantly, we found that in spite of the low expression, the levels increased significantly in patients with the disease, and many patients had the sample phenotype (Control and Disease). We also found that the statistical inference has a good statistical fit.
We mean to say that any biological sample was eliminated from this study using this criterion. In the present study we applied an initial exploratory analysis for the identification of possible bias in the transcriptomic expression of all samples, and we choose Pearson correlation as a measure of similarity distance between samples. We considered that two samples with a Pearson coefficient of 0.85 are very close, indicating the absence of transcriptome bias. We would also like to apologize because this value was misspelled in the original version (Pearson 0.85, not Pearson 0.9). We corrected this term to 0.85.

Fig1 'Correlation coefficient scores showed that Pearson correlation between samples was 0.85' has not been changed. From the response I got that the authors mean that the minimal correlation between two samples is 0.85. The heat scale of the figure does not cover that range.

We agree with Reviewer #1. The heat scale of the figure is automatically generated, with a scale ranging from a maximum value of 1 for the Pearson Correlation to a minimum value of 0.85.

We selected differentially expressed genes with a p-value < 0.05 and a fold change of at least 1.5. That is explained in the line 234.
RESPONSE: This does not answer my question. I did not ask how DEGs were selected but how and how many relationship between expression levels were tested.

Thank you for giving us the opportunity to clarify this answer. In the analysis of the relationship of ventricular function with mRNA levels, all data for all genes were used.

(6) Line 367 (previous version). Sorry, I still do not understand the meaning of the sentence 'we highlight the powerful features of the transcriptional regulatory networks that this bioinformatics tool identity by the enrichment analysis'.

We agree with Reviewer 1. The sentence was not clear, and we have removed it from the new version of our manuscript.

(7) Line 78 (previous version). It is still not clear (to me) what 'ejection fraction (EF)' means.

We apologize for not adequately explaining this term. From a technical point of view, EF is calculated by subtracting the left end diastolic volume from the left ventricular end-systolic volume, and dividing the result by the end-diastolic left ventricular volume. This number then is multiplied by 100. The equation is written as follows: EF = [(LVEDV-LVESV)/(LVEDV)] x100

EF value is normally above 50%. If the value is less than 50%, this is indicative of LV systolic dysfunction. In HF, the value can be less than 20%, sometimes as low as 10%. There is a known relationship between LV ejection fraction and functional capacity. Lower ejection fraction is linked to difficulties in carrying out the tasks of a normal everyday life. From this point of view, it makes sense
to compare the expression of the new transcripts with the patient's ventricular systolic function and to correlate that with functional impairment.

We again apologize for sufficiently explaining this term, which is a classical indicator of ventricular function and one of the main technical factors used to indicate whether a particular patient should receive a heart transplant. We have decide not include this explanation in the manuscript since this is not our main focus. If Reviewer #1 believes it is needed, we will be happy to add the full explanation to the latest version of the manuscript.

(8) Line 371 (previous version). CRITICISM: Please clarify that RNA-seq does not provide information on 'gene expression' but quantifies steady state transcript levels only. AUTHORS' RESPONSE: Enrichment analysis gives information about the transcription factors, as protein functions. And taking advantage of the results and explaining which TF have been found, we provided information about RNA-Seq data to emphasize that both results are in accordance. We have added gene expression levels to clarify the sentence in line 371. REVIEWER'S RESPONSE: Sorry, I do not understand the answer. My point is that by determining transcript levels by RNA-seq and/or qRT-PCR you do not measure gene expression as such, which would require determination of protein levels.

We agree with Reviewer # 1 and in the text we have changed this original sentence: “Next, we used qRT-PCR to validate differential gene expression between ICM and CNT samples of the 4 following genes: *SP100, CITED2, CEBPD, and BCL3*. ” The new sentence is: “We used qRT-PCR to validate the RNA-seq data indicating differences between ICM and CNT samples in the
mRNA levels of *SP100, CITED2, CEBPD, and BCL3*” (lines 307-309, new manuscript)

(9) Line 397 (previous version). CRITICISM: SP100 must be given here as gene symbol. AUTHORS’ RESPONSE: We have checked carefully the term, but we are talking about the protein function. So, it should be written as a protein symbol. REVIEWER'S RESPONSE: Sorry, I cannot follow your logic.

We apologize for the confusion. We have followed the suggestion of Reviewer #1. *SP100* is now indicated as a gene symbol in this new version of the manuscript.

(10) Line 509 (previous version). Sorry, I still not get what you are mean by 'long-term activation of hypoxia stimulus underlies to their response failing'.

We agree that the sentence it is unclear. The original sentence, “But long-term activation of hypoxia stimulus underlies to their response failing, specifically it was determined that chronic activation of the HIF pathway in ischemic heart disease, is maladaptive and detrimental [50],” has been changed. The new sentence is: “Lei et al. [50], for example, suggested that chronic activation of the HIF pathway in ischemic hearts is maladaptive and contributes to cardiac degeneration and progression to heart failure.” (lines 438-440, new manuscript)

(11) Quality of written English: Not suitable for publication unless extensively edited.

We agree with Reviewer #1 that the successive changes in the text have deteriorated the quality of the written English in our "not native hands."

Therefore, the manuscript has been rechecked by a professional editing service.

Once again, we thank Reviewer # 1 for the relevant and useful comments.