Author’s response to reviews

Title: Non-Familial Cardiomyopathies in Lebanon: Exome Sequencing Results for Five Idiopathic Cases

Authors:

Marwan Refaat (mr48@aub.edu.lb)
Sylvana Hassanieh (shassanieh@mgh.harvard.edu)
jad ballout (jad.ballout1@gmail.com)
Patrick Zakka (pzakka@gmail.com)
Mostafa Hotait (mah58@mail.aub.edu)
Athar Khalil (aak67@mail.aub.edu)
Fadi Bitar (fbitar@aub.edu.lb)
Mariam Arabi (ma81@aub.edu.lb)
Samir Arnaout (sarnaout@aub.edu.lb)
Hadi Skouri (hs13@aub.edu.lb)
Antoine Abchee (aa14@aub.edu.lb)
Bernard Abi-Saleh (ba47@aub.edu.lb)
Maurice Khoury (mk04@aub.edu.lb)
Andreas Massouras (andreas.massouras@saphetor.com)
Georges Nemer (gn08@aub.edu.lb)

Version: 2 Date: 13 Nov 2018

Author’s response to reviews:

POINT-BY-POINT RESPONSE

We thank the reviewers for their positive feedback and constructive comments and critiques and provide a point-by-point response to the comments and an explanation of how they were
addressed in the revised manuscript. We hope that the manuscript in its modified version finds its way for acceptance and publication in your esteemed journal.

Reviewer 1

Major concerns

1. Overall, the paper can be shortened further.

We did further shorten the manuscript as suggested.

2. Authors keep using "likely pathogenic" for nonclinical genes. These variants are uncertain significance in genes of uncertain significance (GUS) and should not be called 'likely pathogenic' or 'diagnosed' and is very confusing. Use HGVS nomenclature for all variant descriptions.

We removed all the attributes from the uncertain significant genes’ variants, and we only kept those attributes to the 84 cardiomyopathy genes filtered in the first round as per the HGVS nomenclature.

3. Abstract Conclusion: It's not convincing that exome is needed to identify novel variants. It should reflect what's in the discussion. How does clinical management change based on molecular diagnosis for cardiomyopathies in Lebanon?

Corrected to read as follows: Our results unravel novel mutations in known genes implicated in cardiomyopathies in Lebanon. Changes in clinical management however, require genetic profiling of a larger cohort of patients.

4. page 4 line 48 "The average total number of single nucleotide variants (SNV) and Indels in all samples was around 20000 and 5500 respectively (Figure 1). Amongst these, around 8500 SNVs are nonsynonymous, and 450 Indels are in the coding regions" : Is it concerning that less than 10% of indels are in the coding region?

Most of the Indels would be in repetitive regions usually in the intronic, promoter, 5’UTR and 3’UTR regions.

5. Not sure why MR22 is in section 3.2 while it sounds like there's no clear molecular diagnosis made.

We removed patient MR22 since the case has additionally syndromic features that include mental retardation.
Minor concerns:

1. page 4 line 29 sheered > sheared produce small fragments please put average size of the fragments

Thank you corrected. The size fragments as assessed on the Bioanalyzer varies between 150 to 200bp (added to the text on page 4).

2. page 4 line 38 as previously described what was previously described? Ref paper missing?

Our whole exome work on pulmonary hypertension added as reference (Abou Hassan O, et al 2018)

3. page 4 line 48 What does ACGS stand for?

ACGS (Association for Clinical Genetic Science) was added to the material and methods section

4. page 5 line 13: ambrygen repeated

Thank you, we removed the repetition

5. page 5 line 51: nonsense variants are not necessarily disease causing and since these are nonclinical genes, ExAC pLI score will have to be used to determine if nonsense variants are likely to be causal.

Indeed we check the pLI scores for the genes that were selected and we indicated that they didn’t seem to have a causal effect.

6. page 6 line 25: leading to a missense mutation p.P1112L, previously reported in patients with HCM or DCM: reference needed

Thank you, we added the appropriate references.

7. page 8 line 29: would panel sequence introns?

Yes, panel sequence intronic variants, but again some of these do not fit into the ACMG and ACGS guidelines.
8. Table 4 and table 2 are the same

Sorry for the mistake in uploading the files, we make sure to have the correct ones uploaded.