Author’s response to reviews

Title: Genetic relevance and determinants of mitral leaflet size in hypertrophic cardiomyopathy

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Version: 1 Date: 25 Sep 2019

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Comments from the Editors and Reviewers:

Our thanks to the reviewers for thoughtful critiques of our manuscript. We have tried to adopt all of the suggestions as follows. We think that the manuscript has been greatly improved by these revisions and we hope that you will now find it suitable for publication in Cardiovascular Ultrasound. Our point-by-point responses to comments are detailed on the following pages.
Review 1:

Comment #1. I have only a minor suggestion: please add the number of patients of each group in the figure 3.

Reply) Thank you for your kind comments. We added the number of patients of each group in the figure 3.

Reviewer 2:

Comment #1. DNA preparation - What was the quality of DNA?

Reply) Thank you for your kind comments. We added a mention in the Method.

Change) gDNA concentration and purity were assessed by a Nanodrop 1000 spectrometer (Thermo Scientific, Waltham, MA, USA). Mean gDNA yield and A260/280 values were 87.0 ng/μL and 1.8, respectively. (Additional file 1, page: 1, line: 4 -6)

Comment #2. Data analysis of the HCM gene panel -

Comment #2.1. What was the Q score?

Reply) Thank you for your valuable comments. We added a mention in the Method.

Change) Only bases meeting the minimum base quality (≥ 20) from reads are considered. (page: 6, line: 53-54)

Comment #2.2. Were pathogenetic variants retained if they were revealed to be damaging by at least one of prediction programs?

Reply) We appreciate the reviewer’s insightful comment. We assessed the pathogenicity of variants based on the American College of Medical Genetics and Genomics standards and guidelines. According to ACMG guideline, damaging by computational and predictive programs were used as a piece of ‘supporting evidence (PP3)’ for classification of pathogenic or likely pathogenic variants. And the results of in silico tools were insufficient to determine a clinical assertion. Therefore, we used the results of prediction programs as a part of the criteria for interpretation of sequence variants.
We used multiple software programs for sequence variant interpretation including Align GVGD, SIFT, PolyPhen and MutationTaster. When majority of computational evidence support a deleterious effect, we added “PP3” evidence in interpretation of sequence variants. We added a mention in the Method.

Change) The impact of missense change was predicted with Align GVGD, SIFT, PolyPhen and MutationTaster. (page: 6, line: 59-60)

Comment #2.3. Were the variants validated by Sanger Sequencing?

Reply) Confirmatory analysis by Sanger sequencing of SNVs detected via capture-based NGS testing that meets appropriate quality thresholds is unnecessarily redundant. (J Mol Diagn. 2015 Jul;17(4):456-61.) Sikkema-Raddatz et al. validated targeted NGS panel for diagnostic testing of patients with hereditary cardiomyopathies. They reported the sensitivity and specificity of targeted NGS are equal to those of Sanger sequencing. Additionally, they proposed that variants with more than 30x coverage, a minimum number of four reads for a call and altered allele frequency >20% could be reported without Sanger confirmation. When compared with Sanger sequencing, the sensitivity of 99.96% for a heterozygote in NGS assay. (Hum Mutat, 34 (2013), pp. 1035-1042)

We performed the validation and optimization of workflow for NGS assay with the HCM gene panel using reference materials and sequencing confirmed materials. We obtained 100% of sensitivity and specificity with candidate variants (altered allele frequency >30% and >50x coverage). And we performed confirmatory studies for all detecting VUSs and pathogenic variants in a clinical setting. However, in a study for research purpose, we didn’t carry out sequencing for pathogenic variants with allele frequency >30%, >50x coverage and their visual inspection using the Integrative Genomics Viewer (IGV) were acceptable (Brief Bioinform. 2013 Mar; 14 (2):178-92). We performed Sanger confirmation of pathogenic variants which showed unacceptable quality metrics. We added a mention in the Method.

Change) Visual inspection of candidate variants were performed using the Integrative Genomics Viewer (IGV) (13) and we performed Sanger confirmation of pathogenic variants which showed unacceptable quality metrics. (page: 6, line: 60-62)

Comment #3. Additional file 1 and file 2 are reversed.
Reply) Thank you for your valuable comments. We corrected the name of Additional files.

In addition, there were some incorrect descriptions due to our mistake. 1) In the result, we reported MYBPC3 as 32 cases and TTN mutation as 1 case in sarcomere mutations. However, MYBPC3 was 33 cases and no TTN mutations have been found. We corrected this. (page: 8, line: 123-124). 2) In the result, we reported 50 (7%) had mitochondria-related nDNA variants. However, we mistyped 15 (7%) as 50 (7%). We corrected this. (page: 9, line: 127). We apologize for our mistake. Thank you.