Peer Review File

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Response to the reviewers

Comment1:In “Abstract”, the background part is too simple. It is suggested to further supplement the relevant content.

Response 1: Agree. We added some contents in the abstract (see Page3, Line3-4)

Changes in the text： Background: Bicuspid aortic valve (BAV) is a common congenital heart defect (0.5–2.0% in the adult), potentially an onset factor of aortic stenosis. BAV is a heritable trait, but the genetic basis underlying this cardiac malformation remains poorly understood.

Comment2: In figure 1B and C, the position of the lesion or the position to be observed should be marked with arrows or other marks in the picture.

Response 2: Agree. We added arrows in the picture.

Changes in the text: See figure 1B and C for details.

Comment3: In figure 3C, it is suggested to supplement the protein test results of Mybpc3.

Response 3: Agree.

Peripheral blood of the proband and her parents were denatured at high temperatures (100℃, 5 minutes). The proteins were resolved on a sodium dodecyl sulfate 10% polyacrylamide gel and transferred onto polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA) and incubated with primary antibodies (1:1000 dilution) against Mybpc3 (Abcam Company, USA) at 4℃ overnight. The blots were washed with phosphate buffer saline (TBST) for three times and then incubated with horseradish peroxidase-conjugated secondary antibodies for another 1 hour at room temperature. After washing, the blots were visualized by using chemiluminescent substrate (ECL).

The densities of immunoblot bands were analyzed using a scanning densitometer (model GS-800, Bio-Rad Laboratories, Hercules, CA, USA) coupled with Bio-Rad personal computer analysis software.

Results: The expression of Mybpc3 protein in the proband and her father was reduced in comparison to proband's mother. Furthermore, Western blot results exhibited that relative Mybpc3 protein levels in the proband and her father were almost half of proband's mother (Figure 3E).

Changes in the text：(see Page9, Line13-14 and Page13, Line3-4)

Comment4: Many abbreviations should be given their full names when they first appear, such as GATK, BAM, HGMD, OMIM and VCF

Response 4: Agree. We had modified our text as advised.

Changes in the text:

GATK（Genome Analysis Toolkit）(see Page5,Line12)

BAM（Binary Alignment/Map）(see Page5,Line13)

HGMD (Human Gene Mutation Database) (see Page8,Line3)

OMIM (Online Mendelian Inheritance in Man, https://omim.org/) (see Page8,Line3)

VCF (Variant Call Format）(see Page5,Line13)

GERP (Genomic evolutionary rate profiling) (see Page8,Line5)

SIFT (Sorts intolerant from tolerant) (see Page8,Line4)

MAF (Minor Allele Frequency) (see Page8,Line12)

Comment5: In figure 4, the description of figure legends is not clear enough. It is recommended to modify it again. The authors showed "A-B. Wild type and mutant of Mybpc3 protein structure". Whether A is wild type of Mybpc3 protein structure or mutant of MYBPC3 protein structure should be described clearly.

Response 5: Agree. The description of figure legends was supplemented in the revised manuscript. (see Page22,Line8-9).

Changes in the text：

Figure 4. Prediction of the mutation on its functional

A. Wild type of Mybpc3 protein structure.

B. Mutant of Mybpc3 protein structure.

Comment6: Does the mutation affect the progression of the disease? It is suggested to add relevant contents in the discussion

Response 6: Agree. We added some contents in the text (see Page14,Line8-17)

Changes in the text： Loss of Mybpc3 phosphorylation may cause a primary increase in calcium sensitivity.An increase in calcium transients may also have effects on calcium dependent enzymes such as calcineurin, calmodulin dependent kinase, and protein kinase C all of which have been shown to be important for the initiation of myocardial hypertrophy. Many Idiopathic dilated cardiomyopathy (DCM) probands also have congenital defects, including two with bicuspid aortic valve with aortic regurgitation. Family history and genetic information have potential roles on individuals with aortic regurgitation. Rare variants in the MYBPC3 gene have been reported in several cases of DCM .However, the precise molecular mechanism of how down-regulated Mybpc3 expression affects this system needs further explore.

Comment7: If MYBPC3 heterozygous mutation has potential help for the treatment of the disease in the future, please add relevant content in the discussion.

Response7: Agree. We added some contents in the text (see Page15,Line6-9)

Changes in the text： Determining the genetic origins of BAV is essential to improve the clinical care of patients as well as to develop tailored therapeutic strategies for monitoring disease progression and preventing related aortopathy.