Reviewer’s report

Title: Adverse events in families with hypertrophic or dilated cardiomyopathy due to mutations in the MYBPC3 gene

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Reviewer: Stephan Waldmueller

Reviewer’s report:

The manuscript describes the screening for mutations in the gene MYBPC3 in 87 patients with HCM and 71 patients with DCM. Using DGGE and capillary sequencing, 18 mutations were found, 7 of which were "novel". The causative nature of these mutations was deduced from their absence from 430 control individuals and, in one case, from familial cosegregation of the mutation and the cardiac phenotype. In one case, Arg272Cys, the authors refer to unpublished work showing functional consequences of the mutation. Novel evidence is presented that a mutation formerly classified as polymorphism, Gln1233X, indeed plays a role in HCM. Further mutations were excluded in a considerable fraction of known HCM-related genes, while most of the known DCM-related genes were not tested. Genetic testing of close relatives for the index mutations revealed a further 24 mutation carriers, most of which were then subjected to MRI or echocardiography. Among the living mutation carriers, a striking phenotypic diversity was observed. In one family, progression from HCM to DCM was found to be associated with a novel splice site mutation. Eleven out of 42 mutation carriers showed adverse events such as stroke or sudden death. In addition, a link between the MYBPC3 mutation and unexplained sudden death was suggested for 7 out of 12 families, though cardiac involvement was not documented and the carrier status of the deceased individuals remains unclear. The authors conclude that MYBPC3 mutations are more frequently associated with a poor prognosis than previously expected.

The manuscript is well written and it provides novel insight in the genetic causes of cardiomyopathies. It strengthens the notion that MYBPC3 mutations may indeed cause life-threatening events more frequently than assumed and it provides further basis for careful clinical risk stratification of genetically affected individuals. The article is of considerable interest for those working on closely related topics and it should therefore be considered for publication in BMC Medical Genetics.

Discretionary Revisions

1. Results, Phenotypic heterogeneity...: this section would be more clear if the observations would be grouped as follows: a) phenotypes observed in relatives of an index patient with HCM; b) phenotypes observed in relatives of an index patient with DCM.

2. As an attempt to identify novel genotype/phenotype correlations, the authors
may check whether in their HCM patients, missense mutations have a worse prognosis than mutations associated with a premature stop codon.

Minor Essential Revisions

1. Title and background (last sentence): "due to" should be replaced by "and".
2. Abstract, Methods: "positives" should be replaced by "and carriers of the mutation"
3. Abstract, Results, first sentence: "patient" must be replaced by "index patients".
4. Background, 1. paragraph, last sentence: please delete "generally"
5. Please verify "novelty" of the respective mutations again before resubmission.
6. p. 2: Remove one of the two colons after background. Insert "index" between "(2,8%)" and "patients". Add "myosin binding protein C" to the key words.
7. p. 3: delete "generally" in last sentence, 1. paragraph. Insert "encoding factors" between "those" and "involved" (last sentence).
8. p. 6: correct "therfore" to "therefore".
9. p. 7: use "asked to participate" instead of "asked for participation"
10. p 9, line 16: correct "an" to "and"
11. p 11, line 2: replace "thought" by "found".
12. p.12, line 7: please check whether it should rather call ", for which a different primary cause, e.g.....".
13. Discussion: "Most" must be removed from the first sentence in the 2. paragraph of p. 12.
14. p. 15, line 5: replace "are" by "denote".

Major Compulsory Revisions

1. Abstract, Results: Please provide the following figures: a) the total number of clinically characterized (confirmed) mutation carriers and the fraction of them with at least one adverse event (AE); b) the total number of isolated cases and the fraction with AE; c) the total number of mutation-positive families and the fraction of them with at least one AE (including sudden death).
2. Abstract, Results, entire manuscript: The term "sudden cardiac death" must only be used in case that cardiac involvement had been documented. Otherwise, the term "unexplained sudden death" should be used.
3. Methods: Please add details on: a) Echocardiography; b) the sensitivity of DGGE; c) both the fraction of mutation-positive and -negative individuals that underwent MRI, and the respective numbers for examination by echo.
4. Methods, Mutation screening: Please list the mutation/SNP libraries from which the identified "novel" mutations were found to be absent.
5. Methods, Family screening: Apparently, none of the mutation-negative family members were examined by either MRI or Echo. In this case, please state that
the presence of hypertrophy or dilatation can not be ruled out for these individuals.

6. Results, MYBPC3 mutations: The mutation nomenclature used should conform to the regulations by den Dunnen et al. (den Dunnen JT and Antonarakis SE (2000). Hum.Mutat. 15: 7-12; http://www.hgvs.org/mutnomen/examplesDNA.html#var). Please provide cDNA positions in addition to/instead of the genomic positions. The respective mRNA ID is : NM_000256.

7. Results,: Online programs such as Panther, Polyphen, SNP3D and Sift-Blink should be used to provide additional support for the causal role of the "novel" missense mutations. Whether or not at least one of these programs suggests a damaging effect of the substitution should be indicated in Table 1, column "Reference, comment". Alternatively, and preferentially, multiple alignments may be shown to illustrate evolutionary conservation of the amino acids affected by the novel mutations. Also, it should be discussed whether these mutations may affect functional protein domains. This may be done side-by-side with the discussion of the Arg272Cys data in the discussion part.

8. Results, Phenotypic .....; second paragraph, last sentence: please clarify for family 5: a) according to Figure 1, Ind. I:2 had a presumed or borderline disease phenotype. b) according to table 2, Ind. II:6 had marked trabeculations. Accordingly, it might be necessary to correct the first sentence on p. 12.

9. Results, Adverse clinical events: The numbers requested for the abstract (see above) should appear in this section, ideally with a differentiation of AE before (or at) age 35 and thereafter.

10. Discussion: The discussion could be significantly condensed by removing recurrent clinical (and other) details. As a suggestion, the part "These additional carriers......unless contraindicated" may be removed, except for the sentence "In ten of them...". Also, clinical details from the second paragraph on page 12 may be moved to the Results section ("The youngest.......are mutation carriers.").

11. Discussion: The data presented contrast previous findings that linked MYBPC3 mutations to a benign course of HCM. Beside referral bias, other possible reasons for this discrepancy should be discussed, such as (as a suggestion) the presence of additional mutations missed in this study or (i.e. present in genes not tested in this study). Alternatively, it could be stated that the reason for this discrepancy remains obscure.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests.