Reviewer’s report

Title: Exploring digenic inheritance in arrhythmogenic cardiomyopathy

Version: 0 Date: 16 Jun 2017

Reviewer: Jan Jongbloed

Reviewer's report:

Referee report MGTC-D-17-00055: "Exploring digenic inheritance in arrhythmogenic cardiomyopathy"

In this manuscript the authors describe their experiments using WES to identify variants additional to the PKP2 mutations already identified in two ARVC families. In the last decade in many papers it was hypothesised that such additional variants may explain why only part of mutation carriers develop disease while others do not. Of course, next to non-genetic factors being involved. After filtering for cardiac expressed genes and subsequently for two subpanels, ACM and PKP2 related genes respectively (and using knowledge on variants in non-carrying family members), and prioritizing identified variants within those panels, they further describe and discuss the two most highly ranked ones mainly and shortly address some other top candidates.

Major comments:

The author did chose a sound approach to filter their data, making use of the two gene panels. However, the underlying proof of the involvement is mainly speculation based and this should be extended. What the authors at least should do is to check whether other rare variants in FRZB can be identified in subsequent ARVC families/patients, both in those carrying desmosomal or other ARVC related mutations as well as in unsolved cases, as this gene was identified on the ARVD4 locus. Even when this does not result in identification of such variants, this should have been addressed. To evaluate whether an additional genetic component is the most contributing factor, the authors should provide more details on the clinical features of the affected carriers when compared to the unaffected carriers. Are major non-genetic affectors really excluded? This should be presented in more detail when possible and better discussed.

Of course, you may argue that variants in completely other pathways or cellular structures yet unknown to be related to ARVC could be involved, but underlying arguments are thus still lacking. However, this should be more clearly addressed in the discussion section.
Other comments:

Background:

As the authors indicate: the reported digenic inheritance often involves variants that on their own would not be pathogenic. Therefore it is better to use the term "variants" instead of "mutations". Moreover, it should be addressed that two scenarios apply: patients having a (likely) pathogenic mutation and in addition a putatively contributing VUS or those carrying two (or even more VUSses that both individually most likely not lead to disease.

Abstract:

- The FRZB and TTN variants are defined as "pathogenic", however not enough proof is currently available to conclude this; the authors should use other terminology. The same for these and other variants throughout the manuscript.

Methods:

- It is currently not clear how the additional family (female ACM affected and unaffected sister, both carrying a PKP2 mutation) data was used in filtering. This should be better explained.
- Please also indicate how "very rare"is defined (mentioned in supplement that <0.1% is considered rare).
- ACM gene set is not up to date with current knowledge. This should at least be complemented with the FLNC and CDK2 genes.

Results:

- Identification of candidate genes: this section starts with "the filtering strategy": please indicate what is meant with that.
- Please indicate which isoform was used to annotate variants, in particular the TTN variants being discussed.
- Is the healthy father of fam. 2 carrier of the TTN variant(s)? Please indicate (or indicate when this was not tested).
Methods, Results and Discussion:

- The authors mention variants that protect carriers; however they did not present a method to identify those (logically, as this is rather difficult with only a couple of families; can only be done in large patient groups vs healthy carriers); this should be removed from the manuscript.

Discussion:

- The authors claim that TTN mutations are associated with HCM. This is however under debate and should does be mentioned.

- Putative functional analyses discussion on iPSC too lengthy, while other options are not mentioned.

- See also major comments about non-genetic factors

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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