Author's response to reviews

Title: Search for Cardiac Calcium Cycling Gene Mutations in Familial Ventricular Arrhythmias Resembling Catecholaminergic Polymorphic Ventricular Tachycardia

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Version: 4  Date: 19 January 2009

Author's response to reviews: see over
January 19th, 2009

RE: Manuscript number 1051311299208940, a revised version

Dear Dr Norton,

Thank you for your letter of January 12th, 2009, implying that our manuscript entitled “Search for Cardiac Calcium Cycling Gene Mutations in Familial Ventricular Arrhythmias Resembling Catecholaminergic Polymorphic Ventricular Tachycardia” by Marjamaa et al. could be reconsidered for publication in the BMC Medical Genetics, provided an adequate revision can be carried out. We have very carefully revised the manuscript according to the comments raised by the Reviewers, and we feel that our manuscript has improved upon the revision.

We thank the Editors and Reviewers for their conscientious work and hope that our manuscript could be published in its present revised form in BMC Medical Genetics.

On behalf of the authors,

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Reviewer's report
Title: Search for Cardiac Calcium Cycling Gene Mutations in Familial Ventricular Arrhythmias Resembling Catecholaminergic Polymorphic Ventricular Tachycardia
Version: 3 Date: 22 December 2008
Reviewer: Christopher H George

Reviewer's report:
The inclusion of data relating to the exon 3 deletion is interesting and the paper has been slightly improved. It is mystifying why the paper's characterisation of the variant N3308S (which appears functionally benign) is presented in preference to functional data from the two novel mutations. I am wholly unconvinced by the authors' claim that the in vitro effects of S616L and R1051P need not be studied simply because the clinical phenotypes associated with these 'hot-spot' mutations are typical and thus any functional characterisation are unlikely to extend current knowledge. I disagree with this statement. If, for some reason the authors are unable to perform this characterisation of the two mutations, then it should be clearly stated as a limitation of the study.

Response:
We thank the Reviewers for the interest in our manuscript. The in vitro characterization of the RyR2 N3308S was performed since the variant was identified in a patient with atypical CPVT phenotype and because the family information was limited in order to evaluate the actual consequences of this gene defect. We fully agree with Reviewer that the lack of in vitro data regarding the RyR2 missense mutations S616L and R1051P is a limitation of the study. We have by no means intended to claim that the information on functional properties of these mutant channels would not be beneficial for the researchers in the field. Our reason for not including the single channel experiments of RyR2 S616L and R1051P in the present study was simply that we consider the data presented in the manuscript to be sufficient to declare that these mutations present causative mutations even without supporting in vitro data. We have added a note of this limitation to the Discussion (page 16, lines 4-6).

Minor essential revisions.
The quality of the figures is poor throughout- the new Figure 3 is illegible.

Response:
We have revised all the Figures, including Figure 3, for better resolution and clarity.

The authors should clearly state that they consider the S616L mutation to extend the N-terminal hot-spot region of RyR2.

Response:
We have added a note to the Discussion (page 15, lines 1-2) to clarify this.

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 interest

**Reviewer's report**
**Title:** Search for Cardiac Calcium Cycling Gene Mutations in Familial Ventricular Arrhythmias Resembling Catecholaminergic Polymorphic Ventricular Tachycardia
**Version:** 3  **Date:** 9 December 2008  **Reviewer:** Zahurul A. Bhuiyan

**Reviewer's report:**
Manuscript has been adequately revised.

**Level of interest:** An article of importance in its field

**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.

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**Reviewer's report**
**Title:** Search for Cardiac Calcium Cycling Gene Mutations in Familial Ventricular Arrhythmias Resembling Catecholaminergic Polymorphic Ventricular Tachycardia
**Version:** 3  **Date:** 9 January 2009  **Reviewer:** Jonathan G Seidman

**Reviewer's report:**
The manuscript is now suitable for publication, with the exception of Figure 4.

**Response:**
We have revised all the Figures in the manuscript, including Figure 4, for better clarity.

The manuscript would be considerably strengthened if segregation of phenotype and genotype could be assessed. What genotypes were observed in those individuals who have been clinically evaluated? This analysis is critical to the conclusions presented here.

**Response:**
In Families A, B, E, and D presenting CPVT phenotypes, the identified RyR2 mutations were fully co-segregated with the clinical phenotype. However, in Family C, the evidence of co-segregation is unconvincing owing to the only one clinically affected individual. We have made the following corrections to the manuscript in order to more precisely depict the phenotype-genotype segregation:

1) First, in Figure 3, we have revised the explanation of the affected/non-affected individuals to mutation carriers/non-carriers for further clarity.
2) Since mutation carriers in Families E and D also showed atrial fibrillation, which has previously been reported to associate with the RyR2 exon 3 deletion [1], we have added a symbol for this phenotype in Figure 3.
3) Similarly, sinusbradycardia was identified in three carriers of RyR2 exon 3 deletion, and this data, along with AV conduction abnormalities of a mutation carrier, is now incorporated into the Results section (page 12, lines 12-13,19).
4) The follow-up data of the index patient in Family E revealed increased trabeculation of the left ventricle suggestive of non-compaction cardiomyopathy and this data is now included as a note to the Results (page 12, lines 20-21).
5) We have further stressed the limited segregation data of RyR2 N3308S in the Discussion (page 15, lines 17-18).
A number of synonymous RyR2 variants and a previously reported common RyR2 polymorphism G2958R [2] were identified in the index patients, but since there is no evidence of the functionality of these variants, the clinically evaluated relatives were not specifically genotyped for these variants.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need