Author’s response to reviews

**Title:** Functional Effects of the TMEM43 Ser358Leu Mutation in the Pathogenesis of Arrhythmogenic Right Ventricular Cardiomyopathy

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**Version:** 3  **Date:** 20 February 2012

Author’s response to reviews:

UPMC Heart and Vascular Institute Part of
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February 10, 2012
Tim Sands, PhD
In-House Editor
BMC Medical Genetics
Re: Submission of Revised Manuscript # 1780456973608661, “Functional Effects of the TMEM43 Ser358Leu Mutation in the Pathogenesis of Arrhythmogenic Right Ventricular Cardiomyopathy”

Dear Dr. Sands:

We greatly appreciate your invitation to submit this revised manuscript as a candidate for publication as a short report in the BMC Medical Genetics. We also greatly appreciate the reviewer’s comments, which are reproduced below in italics, followed by responses describing corresponding revisions to the manuscript.

The present study is original in aiming to investigate the function of a TMEM43 mutation (S358L) and mechanism by which this mutation causes ARVC; however the value added to the present study by reporting genetic screening results of desmosomal genes in a small cohort of patients remains unclear. The authors should stress further the link between these mutations and the experimental investigation described on the present study.

The overall goal of this study was to investigate the role of TMEM43 mutations generally and the reported S358L mutation specifically in ARVC. Given the novelty of the reported mutation in TMEM43 in the context of previously reported mutations that were in genes encoding desmosomal proteins, we intended (1) to
determine the prevalence of TMEM43 mutations in a small cohort of ARVC probands available to us; and (2) to determine the mechanistic role of S358L TMEM43 mutation in ARVC. While some probands were found to have mutations in desmosomal genes, no mutations were observed in TMEM43, suggesting that mutations in this gene are a rare cause of ARVC. Since common mechanisms often underlie each cardiomyopathy, even in the setting of genetic heterogeneity, we performed in vitro experiments to determine whether the functional abnormalities caused by the TMEM43 S358L parallel those found in the setting of desmosomal mutations. We have reworded the manuscript to explain the connection between these two interlinked objectives.

“Furthermore, mutant TMEM43 did not alter the expression of genes that are suggested to be associated with cardiomyopathy.” (page5, 13) The above mentioned statement of the authors implies that genes for a range of cardiomyopathies has been investigated. It should be more appropriate to refer only to “lamin-cardiomyopathies or laminopathies”.

We have clarified this statement as suggested at multiple points in the manuscript.

Please follow the MIQE guidelines (Bustin SA et al., Clinical Chemistry, 2009;55:611-622) to report your qPCR experiments. Experimental procedures of gene expression analysis typically include sampling-processing steps, quality and quantity assessment, as well as reverse transcription steps. Consequently, sequence information for primers should be provided, how many biological and technical replicants were included and so on.

As suggested, we have included in the Methods section a more detailed description of the QPCR experiment performed in this study, including RNA isolation, quantity assessment, cDNA synthesis, QPCR reaction using SYBR Green, data analysis, etc. A reference has also been included for details of the primer sequences.

Moreover, refer to the description of genes (location and reason why investigated) targeted by qPCR experiments in the method section and not in the result section....Please move the description of genes (location and reason why investigated) targeted by qPCR experiments in the method section and not in the result section. (page14 lanes 1,2)

In the revised manuscript, the reason for selecting the four misexpressed genes described by Merner and colleagues is now discussed in the Methods section and only briefly recapitulated in the Results section.

Please refer to the HGVS guidelines regarding mutation nomenclature (i.e. page 11, Thr412AsnfsX2).

We apologize for not using current nomenclature guidelines. This oversight has been corrected in the revised manuscript.

Please note, that the plasmid ratio and protein extraction method should be described in methods section and not in results section as well as the method to obviated solubility effects of TMEM43 in Triton-X100 (page 13 lanes 10,11).

These methodologies are now described in the Methods section.
Figure 1 should be correctly resized.

Figure 1 has been resized and all images have been edited to conform with the journal’s requirements.

Figure 2A, the blot for DSP is not proper. Please provide a better picture of this blot.

Unfortunately, despite repeated attempts, we have not been able to acquire a clearer image. However, we feel that the blot clearly shows a similar trend among the soluble and insoluble fractions, indicating no change in DSP localization pattern for both wildtype and mutant TMEM43.

Figure 2A and 2B. Pictures from the immunoblot of a reference protein are lacking.

Since these blots represent different protein fractions, a reference protein, such as GAPDH, cannot be used to quantify protein across different fractions. For this reason, instead of a single reference protein, we have used Coomassie blue staining of replicate gels to document global protein quantities loaded for any given fraction.

Compound/digenic heterozygosity in ARVC patients that leads to a more severe disease phenotype has been extensively described by Xu et al., JACC, 2010 and Bauce et al., Heart Rhythm, 2010.

We thank the reviewer for these suggestions, and have included these additional references.

Thank you for considering our revised manuscript.

Sincerely yours,
Ferhaan Ahmad, MD, PhD, FRCPC