Author's response to reviews

Title: Protective effect of KCNH2 single nucleotide polymorphism K897T in an LQTS family and identification of novel KCNQ1 and KCNH2 mutations

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Author's response to reviews: see over
Dear Dr. Manning:

Thank you for the opportunity to submit the revised version of manuscript entitled “Identification of novel KCNQ1 and KCNH2 mutations and the protective effect of KCNH2 SNP K897T in Long QT Syndrome families” to BMC Medical Genetics. In the revised manuscript, we addressed the concerns and comments from reviewers. The point-by-point response and revisions made are listed below:

Referee 1 Edmund Lee

Minor critics:
1. Abstract, methods: the sentence “In this study, we characterized a cohort of LQTS families and patients, for the two large families with LQTS, linkage analysis with markers spanning known LQTS genes was carried out to identify the specific gene for mutational analysis, in a cohort of LQTS patients,” is grammatically incorrect and needs to be restructured. The mutational analysis of the KCNQ1 gene (in the newly-recruited families/patients) should be mentioned here.

   The grammatical error was corrected. The mutational analysis of KCNQ1 was added.

2. Abstract, conclusion, last sentence: “VT” should be defined as “ventricular tachycardia” as the term is mentioned here for the first time.

   The recommended change was made.

3. Page 3, line 4: “channelopathy” should be spelt as “channelopathy”.

   The recommended change was made.

4. Page 3, line 7: “especially under physical, emotional stress or taking QT prolonging drugs.” should be replaced with “when subjected to physical or emotional stress, or upon exposure to QT prolonging drugs.”

   The recommended change was made.

5. Page 3, line 10, “…in many cases, the first symptom is sudden death.” should be replaced with “…sudden death may present as the first symptom in many cases.”

   The recommended change was made.
6. Page 3, last line from bottom: The typographical errors in amino acid residue designations should be corrected. The sentence should read “…and six known mutations A490T, A561T, D609N, A614V, N629S, and R366X in KCNH2.” Also, references should be cited for these reported mutations.

The recommended changes were made.

7. Page 5, mutational analysis: The names (KCNQ1, KCNH2) and region (coding) of the genes should be stated here.

The recommended changes were made.

8. Page 6, 3rd line from bottom: “As shown in Fig. 1C, all patients in the family…” the word “patients” should be replaced with “affected members”.

The recommended change was made.

9. Page 7, line 9: “Among mutation carriers, 58% of (18/31) had normal to borderline prolonged QTc…” The word “of” should be deleted.

The recommended change was made.

10. Page 7, line 11: “Mean QTc was significantly prolonged during exercise in gene carriers,….” The word “was” should be inserted.

The recommended change was made.

11. Page 8, line 12: “There was no - family history of cardiac arrest and sudden cardiac death.” The “-“ should be deleted.

The recommended change was made.

12. Page 9, discussion: The authors should discuss briefly the possible consequences of L187P in KCNQ1

As recommended, we have discussed briefly the possible consequence of KCNQ1 mutation L187P.

13. Page 10, line 4: “Functional studies revealed that the mutant channel showed the reduced current density by 39% compared to the wild type channel.” The word “the” should be deleted.

The recommended change was made.
14. Page 10, line 6: The sentence should read “…none of the seven mutation carriers with A490T and K897T showed a QTc > 0.48 s.”

The recommended change was made.

15. Page 10, line 7: “The difference of QTc between A490T alone and combination of both A490T and K897T was notable.” The word “of” should be substituted with “in”.

The recommended change was made.

16. Page 11, 2nd last line from bottom: “The association of SNP K897T with shorter QTc was not without controversy.” The word “shorter” should be inserted.

The recommended change was made.

17. Page 12, line 3: “(0.465 s vs. 0.447 s)” The closing bracket is missing.

The closing bracket was inserted.

18. Page 12, line 10: “The cis-localization between the mutation A490T and SNP K897T in our family vs. trans-localization in the study by Crotti et al. may be one of the potential causes for the discrepancy.” “A490T” should be inserted.

“A490T” has been inserted

19. Page 12, line 13: The sentence should read “Overall, our results are more consistent with the finding that the minor allele T of SNP K897T plays a protective role against QTc lengthening.”

“role” has been inserted

20. Page 13, line 2: The sentence should read “…DNA sequence analysis in the cohort of LQTS families…”

The recommended change was made.

21. Page 13, line 4: “LZ, SR, RB, CO, GV performed the clinical characterization of the patients.” “Perform” should be in past tense.

The recommended change was made.

22. Page 13, line 8: The typographical errors should be corrected. “We are grateful to the family members for their enthusiastic participation in this study.”

The typographical errors were corrected.
Discretionary Revisions (which the author can choose to ignore)

1. The authors may wish to comment on some limitations of the study: (1) The comparison of the mean QTc among carriers with A490T, A490P, and A490T/K897T was made in individuals of different geographical origins under different clinical settings. The accuracy of the analysis might be compromised as the ethnic and environmental influences have not been accounted for. (2) Only the two major LQTS-associated genes were screened in the study population; the effect of the presence of genetic variations in other candidate genes e.g. SCN5A, KCNE1, KCNE2 etc has not been taken into consideration.

We included the first comment in Discussion.

For the families reported here, we also screened SCN5A, KCNE1 and KCNE2, but no mutation was identified.

2. The authors may wish to discuss the findings from the following papers on K897T in KCNH2.

We focused our discussion on genetics on SNP K897T.

3. An in vitro functional study to examine the electrophysiological effects of the mutations / combinations of mutations may provide an insight into the molecular mechanism underlying the pathogenesis of LQTS.

This comment has been added as one limitation (see page 13).

Referee 2 Tomas Novotny

Major compulsory revisions:
1. While it is obvious these days that LQT syndrome is genetically very heterogenous disease, identification of some new mutations in LQT related genes is not surprising. Therefore such finding cannot be the leading theme of present-day article (unfortunately, since in all centers we have some yet undescribed mutations...). In my opinion the most
important message of presented manuscript is the finding of mutation-SNP interaction in KCNH2 gene and its effect on QT interval. I strictly recommend to make this finding the leading theme of the article – from the title on throughout all the manuscript. Then, new mutations can be mentioned as a byproduct of the study.

**We completely agree with the Reviewer and have revised the manuscript to ensure the leading theme of the paper to be the modifying effect of SNP K897T.**

2. Abstract: In Background paragraph there is no information on the aim of the study. The Methods paragraph is one long sentence – probably some punctuation is missing, therefore the sense is confused. Information on number of investigated individuals is missing. Results paragraph, line 5: „....interacts with mutation A490T in cis.“ The proper term is „cis orientation“. This should be corrected and unified throughout all the manuscript - the meaning of cis vs. trans orientation should be later briefly explained.

**We added the aim in Background. The method section was revised for grammatical errors. “cis” has been changed to “cis orientation” and “trans” has been changed to “trans-orientation”**

3. The last 10 lines of „Background“ contains rather results and conclusions. Therefore these statements should be omitted here and should be moved to corresponding sections of the manuscript.

**We shortened this section to two sentences.**

4. The „Methods“ section should be organized as follows: Study subjects, clinical examination, DNA isolation, linkage, mutational and SSCP analyses.

**As recommend, we re-organized the methods section.**

5. Clinical diagnosis of LQTS is based on diagnostic score (Schwartz et al, Circulation 1993;88:782-4.). According to this score in a particular individual the diagnosis of LQT can be present with low, intermediate or high probability. Therefore it is not clear how the authors considered a particular individual as affected or non-affected. T wave morphology assessment is mentioned but it is not specified which classification was used.

**Clinical diagnosis of LQTS was described in detail and referenced. We did not use the Schwartz method.**

6. ECG recording sweep and voltage should be specified. Since QT interval values are presented with precision of 10 ms the supposed sweep must be at least 50 mm/s. Otherwise the precision of the measurement should be corrected.

**In North America the standard 12-lead ECG is callipered to 25 mm/s for paper speed and 10 mm/mV for voltage. Since only standard ECG tracings were available**
for this study, all the QT intervals were measured at paper speed of 25 mm/s and it has been indicated in the methods section.

7. In the „Methods“ there is no information on exercise test protocol and equipment and Holter ECG devices. It is also not clear if ECGs were evaluated by more than one physician (cardiologist?) and if he or she was aware of the diagnosis.

For the purpose of LQTS diagnosis, a stationary bike (ergometer) exercise protocol as described previously (Vincent GM, Jaiswal D, Timothy KW. Effects of exercise on heart rate, QT, QTc and QT/QS2 in the Romano-Ward inherited long QT syndrome. Am J Cardiol. 1991 Aug 15;68(5):498-503.) was used for this study. Superior to treadmill tests, ergometer tests can eliminate the upper body movement to reduce ECG artifacts. An accurate QT measurement therefore can be achieved for exercise ECG recordings.

Lifecard CF (Renolds Medical) 24-h Holter ECG monitoring was used in the study.

LQTS phenotyping including ECG measurement was performed by G.M.V. and R.B. prior to the genetic testing.

8. Results, Identification of a novel KCNQ1 mutation. Numbers of investigated individuals are presented in confusing manner. It is not clear, how many family members were really examined by investigators. Results, paragraph 1, line 2: Five affected members had a history of syncope.“ Is this number related to over 300 family members...” mentioned in the line 1 of this paragraph or to forty-two family members...“ two lines below?

It is related to over 300 people. To clarify this issue, we moved the last sentence to the 2\textsuperscript{nd} paragraph.

Clinical characteristics of the investigated individuals is scattered throughout all the 4 paragraphs of this section. Some table would be helpful. In one case only abolute number are stated, in another case only percentage, while both numerical information are important. Results, paragraph 4, lines 3 and 4; „....(age 33±22 yrs, 13 F)...“ – yrs means probably years, F – females – but these should be spelled out.

To clarify the issue, we changed “thirty-one family members” to “31 of 42 family members”. Yrs and F were spelled out.

Results, paragraph 4, lines 7-10: while investigating 24 carriers vs. 4 non-carriers, it is highly problematic to compare statistically QTc in these very different groups. On the other side I wonder in how many mutation carriers the initially normal QTc did reach pathologic values during exercise test.

Because this is a human study, we could not consent all carriers and non-carriers to participate. It is even more challenging to consent non-carriers as they feel normal
and are reluctant to do any test. All mutation carrier with a normal or borderline prolonged QT interval reached QTc of 0.48 sec or greater during exercise tests.

9. Results, Identification of two co-segregating variant.. this should be the leading theme of the article to be presented in the first place.

We agree with the reviewer. We have moved this section as the first section of Results. We also changed the title of manuscript to emphasize this point.

But again: Immediately in the first line a family QW2648 is mentioned, but no other information on numbers or clinical characteristics are provided.

We added two sentences: Family QW2648 is a LQTS family with 11 family members (Fig. 1A). Two family members, I:1 and II:2, had QTc of 0.47 s or higher (Fig. 1A).

In the next paragraph, line 5, it is stated that „....QTc was significantly prolonged (P=0.0002). “ Compared to whom? No information on exercise testing in non-carriers is provided ..

We added “compared to QTc before testing”.

Bradycardia was seen in five of seven carriers...“ Under which conditions? Resting ECG or else? Holter monitoring is mentioned in only 2 individuals showing bradycardias. There were no Holters in the other family members? If this is true then information on Holters should be omitted because when performed only in 2 family members it brings no experimentally relevant data, just 2 case reports.

Bradycardia were defined in the text now. The diagnosis was based on resting ECG. Holter monitoring was done in two carriers. We feel it provides valuable important information to confirm the diagnosis by resting ECG, thus we kept this information in the manuscript.

10. Results, Identification of a novel 2-bp insertion mutation Author are describing a patient with depressed left ventricle function and left bundle branch block, who presented with VT (the term is not spelled out) with the need of cardioversion. Information on VT morphology is not provided (if it was monomorphic, then it is definitely not related to LQT syndrome).

The patient had polymorphic VT which led to multiple syncopal episodes. “Polymorphic” was added. See page 9.

During Holter monitoring polymorphic ventricular ectopics were present and also runs (how long?) of polymorphic ventricular tachycardia (now the term is spelled out while an
abbreviation could have been used yet). In my opinion in this patient a high probability of coronary artery disease is present, or dilated cardiomyopathy if the former is excluded. But this possibility was not excluded and even not mentioned (troponin level?, coronary angio is obligatory in such patient). I wonder how the authors did avoid an artificial QT prolongation in an individual with intraventricular conduction prolongation, QRS duration is not mentioned. In conclusion, if presented in this manner, the association of the described mutation to LQT syndrome is highly problematic. A membrane electrophysiogy study is not available, is it?

Cardiac catheterization showed mild luminal irregularities without obstructing lesions, and mild global systolic left ventricular dysfunction consistent with hypertensive cardiomyopathy.

[Troponin]=0.01 ng/ml, which is in the normal range (0.00-1.30), [CK MB]=4.8 ng/ml (0.26-5.16). QRS=122 ms.

New information has been added into the text. See page 10. Echocardiographic data was discussed in the text and no dilated cardiomyopathy was detected (page 10).

11. Results. Identification of five other mutations…: I do not understand, why in patient with R366X mutation the clinical data are provided, while not in the others.

The LQTS phenotype in other patients was not remarkable, thus we did not discuss them in detail. We added one sentence in the text: “Patients with mutations A561T, D609N, A614V, N629S all had a typical diagnosis of LQTS.”

The patient with R366X had more clinical phenotype, thus we discussed it in detail.

12. In all cases information on concomitant therapy is completely missing, concomitant diagnosis is described in only one case.

Information on therapy was not complete or not remarkable, thus we did not include the information.

13. The „Discussion“ section is interesting, clearly written and well supported by data from literature. It clearly shows that the authors are aware of the most important message of their manuscript. Nevertheless I still have some comments: Page 10, line 11. It is not clear how „bradycardia phenotype“ was defined. Page 10, line 12: The provided data do not clarify why authors consider residue A490 a mutation hotspot. Page 11, line 4: If advantage of family based approach is stressed it is obligatory to consider also limitations.

Bradycardia were defined in the text now. The diagnosis was based on resting ECG. The diagnosis was confirmed in two cases using Holter monitoring.

We deleted the sentence about mutation hotspot: “Interestingly, the amino acid residue A490 of KCNH2 is a mutation hotspot”.
The limitation of this family-based study is now discussed. See page 14.

14. No limitation of the presented study are mentioned throughout all the article

**Limitations are added (see page 14).**

15. Figure 1: „Individuals with uncertain LQTS diagnosis or without clinical data are shown in gray symbols.“ This should be distinguished to avoid confusion.

To distinguish the two, we denoted individuals without clinical data with a question mark (?).

Minor compulsory revisions:
1. In title no abbreviations should be present, therefore „SNP“ should be spelled out.

**We spelled out SNP.**

2. In the abstract there should be „results“ instead of „result“.

**“Result” was changed to “Results”**

3. Background, paragraph 2, line 2: The correct name of the gene is ANK2, not ANKB (it encodes a protein called ankyrin B).

**“ANKB” has been changed to ANK2**

4. P values should be expressed uniformly throughout the manuscript: either 0,01 or 1x10-2.

**The recommended changes were made.**

5. Measurement of ECG should be expressed in either seconds or miliseconds –this should be unified.

**The recommended change was made.**

**Reviewer3 Pascale Guicheney**

It is known that other variants in ionic channels, NOSAP1 influence QTc length. Other potent modifiers may be present in addition in these families and it is thus difficult to be certain about the specific role of the T897 allele in these families. The presence of other known variants should be studied to compare the families and their influence on severity. These important limitations should be introduced in the article. The potential differential effect of a cis or trans variant on a LQTS mutation is an interesting point which should be answered by other methods.
We have discussed the limitations of this study. We included it in Page 14:

Lack of in vitro functional studies has been included as one limitation. See page 14.

Reviewer4 Jonathan Skinner

The authors present the results of a lot of work with large families, and in particular report two novel LQTS mutations. While these are nicely presented, I find that it detracts from the most interesting part of the paper, the cosegregation of the SNP K897T in KCNH2. The result is rather a lack of data on this most interesting part- I'd like to read more about the separataion of these SNP carriers and non-carriers- symptoms as well as QT intervals. At present the only data of interest is summarised in figure 3D. The paper reads like a list of findings which are not really connected. Its all very good work, and the authors are to be congratulated on that, but I'd suggest separating these aspects out-providing a paper which focuses on the SNP aspect. If they wish to keep some of the other data in, then I suggest tabulating it, but keep most of it out of the text which I found hard to read. Lets hear more about this particular family, which is fascinating.

Major compulsory revisions
Basically summarised above, I think the paper should be re-written, with much data taken out, and focus purely on the mutation A490T and its cosegregating SNP, with more data about this part. The rest really follows with that, so I won't go into further details.

As recommended, we have re-organized the manuscript so that the finding of the modifying effect of KCNH2 K897T is the major theme. Specifically, we changed the title to reflect this. We also present the K897T finding first in Results. We also started Discussion with the K897T finding, and identification of two novel mutations was included as a by-product of this study.

We really appreciate the constructive comments from all reviewers that make this manuscript better.

Yours sincerely,

Qing Kenneth Wang