Author's response to reviews

Title: High Resolution Melt analysis for mutation screening in PKD1 and PKD2.

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Version: 2 Date: 10 July 2011

Author's response to reviews: see over
Dear editor,

Thank you for your interest in our manuscript called “High Resolution Melt analysis for mutation screening in *PKD1* and *PKD2*”. We were very interested in the comments made by the three reviewers. Please find in attached file a revised manuscript including asked modifications.

With regard to ethics, our experimental research was approved by ethics comitee. We included statement to this effect in the Methods section as asked: “This study was conducted in compliance with the Helsinki Declaration and was approved by the Comité de protection des personnes (CPP) Sud-Méditerranée 2.”.

Point-by-point responses to reviewer’s comments are given below.

Again, we are very grateful for considering publishing our manuscript in the journal and we hope our manuscript will be published in BMC Nephrology because of the importance of the article in the field as stated by reviewer 1 and 3.

Sincerely yours.

Professor Stéphane Burtey
Responses to reviewer’s comments

Reviewer: Sandro Rossetti

I suggest using the word “mutation” for pathogenic changes only and “sequence variant” as a general word for any change in the DNA sequence. Right now, the authors mention 440 “mutations” in the abstract (which are more properly “sequence variants”, and similar elsewhere), and introduce a third definition, “sequence variation”, in the 3 tables.

We agree with Dr Rossetti and we used the word “mutation” for pathogenic changes, the word “sequence variant” for any change in the DNA sequence and suppressed the word “sequence variation” in our revised manuscript.

It is unclear what exons are sequenced: PKD1 exons 1, 41 and 42 according to the discussion section, or PKD1 exons 1, 42 and 43 according to supplementary data 1 that describe primers and analysis conditions? This is important information for anyone wishing to adopt this approach. It is worth adding a couple of sentences explaining the technical issues that do not allow analysis of these exons by high resolution melt, this is again important information for anyone interested in evaluating such technology.

We thank Dr Rossetti for noticing the error made in discussion. The two exons that could not be screened with HRM were exons 42 and 43. As suggested, we added informations on technical issues that do not allow analysis of these exons in discussion.

A paper has been recently published (Hoefele J et al. Nephrol Dial Transplant. 2010, describing a similar work) that the authors may want to include in the discussion.

This interesting work has been included in discussion as suggested.

Reviewer: Jitka Stekrova

In „Methods“: There should be concise characteristics of the tested group – at least gender, age, family history, renal fiction.

Our study is clearly a technical study. We provided basic data as asked by Dr Strekrova and the establishment of relation between genotype and phenotype is beyond the scope of our work.

In „Methods“: There should be added the PKDB database in methods (paragraph “Sequence variation analysis and classification” https://portal.biobase-international.com/hgmd/pro/start.php

We are sorry we did not understand what was asked in this commentary. A link to the PKDB database has already been given in the Methods section: https:pkdb.mayo.edu. Please tell us if modifications are necessary.
In Results - paragraph 3: The number of new detected mutations must be corrected. Of these 28 variants, 9 (7 is not correct) were already described in literature. Of the 18 (20 was not correct) sequence variants.

These changes were made as recommended and in accordance with the comment below précising already described mutations.

In Results - paragraph 3: Mutations have to be described according to „Nomenclature for the description of sequence variants“ (http://www.hgvs.org/mutnomen/) Example: First sentence of the paragraph: „The first in frame deletion, p.Thr2337_Phe2338del (instead of p.del2337_2338ThrPhe),…. Second sentence of the paragraph: „The second deletion, p.Leu2433del (instead of p.del2433Leu)."

We agree with this comment and modified revised manuscript as required.

In „Results“ – paragraph 4, sentence: „Since leucine 2433 is a conserved residue (in chicken, frog and takifugu but not in mouse or rat),….“ the number of compared species should be enlarged (not only vertebræ).

As suggested by Dr Stekrova, we added a sentence explicating that in the article by Xu et al., the loss of Leu2433 is associated with a lack of expression of PC1 in primary cilia, which confirms the pathogenicity of this variant.

In „Results“ – paragraph 4, last sentence: „The two in frame deletions were not found in 100 control chromosomes tested.“ Control group should be enlarged- at least 100 individuals (200 chromosomes) and the description of the selection of control group should be added. The enlargement of kontrol group is necesery for the establishment of probably pathogenic mutations.

We added the characteristics of control group and are not able to include more relevant individuals related to the ethnic origin. The Leu2433 was previously described in literature as pathogenic. For the other deletion, is not present in the PKDB database as a polymorphism. So, this mutation is very unlikely to be a polymorphism.

In „Results“ - paragraph 5: the description of the mutation must be corrected according „Nomenclature for the description of sequence variants“ (http://www.hgvs.org/mutnomen/) Example: „The intronic sequence variation c.7210-5C>G (there is incorrect IVS18-5C>G) was predicted to alter splicing. “ All other splice-site mutations have to be corrected in manuscript and tables.

We agree with this comment and modified revised manuscript as required.

Table 1: The description of all mutations must be corrected according to „Nomenclature for the description of sequence variants“ (http://www.hgvs.org/mutnomen/)

We agree with this comment and modified revised manuscript as required.

Table 1: References should be changed in: mutation c.7108T>A - Ref. 11 (10 is incorrect); mutation c.9859_9861delCTC - Ref. 32 (30 is incorrect); mutation
c.11512C>T - Ref. 43 (31 is incorrect); mutation IVS44+2T>C (correction according to nomenclature) - Ref. 20 (22 is incorrect); mutation c.2599C>T of the PKD2 gene – reference Torra et al. Am J Kidney Dis. 36(4), 2000(32 is incorrect); References should be corrected in all manuscript and tables.

We agree with this comment and modified revised manuscript as required.

Table 1: Mutation c.7298_7300delTGC was already described: Xu et al. Am J Physiol Renal Physiol 292: F930-F945, 2007; Mutation c.11249G>A was already described: Ref. 30. The number of new mutations has to be corrected in all manuscript.

We apologize for these errors and modified references in all tables and manuscript as well as the number of new mutations.


We agree with this comment and modified revised manuscript as required.

In “Figures legends“ – Figure 1: you had better DNA change: „The three fragments with the red curves carry the same sequence variant p.Ala1555Ala (c.4665A>C), which is different from the fragment with the green curve p.Thr1558Thr (c.4674G>A).“

We agree with this comment and modified revised manuscript as required.

Reviewer: Celia Badenas

Results and discussion: The use of “mutation” both for any change detected and for mutation makes reading difficult. Authors should differentiate between variants, polymorphisms and mutations.

We appreciate this commentary that will make our paper easier to read. As already asked by Dr Rossetti (Reviewer 1), we made the changes in definitions.

Methods: DNA samples: The sentence “they had normal renal function or chronic renal failure or end-stage renal disease” does not add any information and should be removed. It should be included if informed consent from patients was obtained. Also if the study was approved by the ethics committee.

As asked by reviewer 2, we included more precise informations on patients included in the study. We apologize for forgetting to name the ethics committee and added it as also required by the editor.
**Methods** : Long Range PCR, first paragraph: exons amplified using this strategy should be stated here.

We agree with this comment and added this information in revised manuscript as required.

**Methods** : Real Time PCR and HRM conditions: Second paragraph: “and 15 s elongation at 72ºC for 25 s”. Please use only one elongation time.

The elongation time was 15 s. We corrected in the manuscript.

**Methods** : Third paragraph: “Exons that could not to be analyzed by HRM were sequenced”. This information has already been given in the primer section and should be removed.

We agree with this comment and added this information in revised manuscript as required.

**Methods** : Reverse transcription analysis: no information is given about RNA extraction and RT-PCR. Moreover name of the mutation studied could be included in the sentence instead of “the suspected splice mutation”. Also primers used by them from reference (20) should be given.

We agree with this comment and added required informations in revised manuscript.

**Methods** : Sequencing: technology used by subcontractor should be included.

We agree with this comment and added this information in revised manuscript as required.

**Results** : Paragraph 3 states that mutations “were identified in 27 patients”, when it should say 28. Also numbers through the paragraph do not make sense: 20 new sequence variants: in Table 1 there are 21, 14 novel mutations leading to a STOP codon when in Table there are 13. 5th paragraph: name of mutation should be included. Figure 4 should be stated instead of figure 3. Also commenting on that no follow-up could be made on this patient should be stated at the beginning of the paragraph.

We apologize for these errors and modified required elements through the paragraph 3.

**Results** : 6th paragraph: polymorphism IVS12+4G>A is detected in 4 individuals and IVS12+5G>A in 3 as stated in Table 2. Do all the patients with these changes carry a definite pathogenic mutation?

We precised in the manuscript: 3 patients out of four had already a pathogenic mutation.

**Results** : 7th paragraph: how many of all the 12 indeterminate variants were detected in mutation negative patients?

This is a very interesting question asked by Dr Badenas. 3 indeterminate variants were found in patient with no detected mutation. In addition, as we stated in manuscript, two of them are found in the same patient. We included this statement in the revised manuscript.
Discussion: 4th paragraph: large deletions or duplications represent approximately 4% of ADPKD patients, not 5%. Moreover, authors comment on the use of HRM to test for duplications and deletions but they have not tested them, why?

We corrected the 4% rate of large deletions or duplications in ADPKD. The student developing HRM technique had finished his research year and the hospital genomic facilities were not interested in developing molecular diagnosis of autosomal dominant polycystic kidney disease. Thus, we could not test HRM in conjunction with genomic Q-PCR for large duplications or deletions.

Table 1: As no mutation was detected in more than one individual, this fact could be reflected in the legend (and also in the text) and Nb column could be deleted.

We agree with this comment and deleted the Nb column and added the information in the text and legend.

Supplementary data: Primers used for PKD1 and PKD2 analysis: as detailed information is given for all primers used in PCR, sequences and references for LR-PCR primers not only for nested PCR could also be given.

The asked Primers were added to supplementary data.