Reviewer’s report

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

Version: 1 Date: 18 June 2011

Reviewer: celia Badenas

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The article reports the screening in the PKD1 and PKD2 genes of 37 proband ADPKD individuals. They use high resolution melt technology and detect 440 different variations, of which 28 are mutations. Their mutation detection rate is of 75%, which is similar to already used technologies (direct sequencing).

The data provide a useful contribution in mutation detection in ADPKD, and are worth publishing. However, the paper needs some revision before it can be acceptable.

Results and discussion are very difficult to follow, so they should be completely re-written in order to make them more comprehensive.

HRM technology is only compared to dHPLC. There is another new technology (next generation sequencing) which has been used for PKD1 mutation detection and it is not even mentioned in the text.

There is no follow-up of any patient. In cases where indeterminate variants have been detected family study could help to clarify results.

• Major Compulsory Revisions

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.

Minor Essential Revisions

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.

- Long Range PCR, first paragraph: exons amplified using this strategy should be stated here

- Real Time PCR and HRM conditions:

Second paragraph: “and 15 s elongation at 72°C for 25 s”. Please use only one elongation time.
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.

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.

Sequencing: technology used by subcontractor should be included.

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.

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?

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

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?

Tables

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.

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.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

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