Author’s response to reviews

Title: Whole exome sequencing reveals novel COL4A3 and COL4A4 mutations and resolves diagnosis in Chinese families with kidney disease

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Author’s response to reviews: see over
Dear Sir/Madam,

We are very grateful for you for review of our manuscript entitled “Whole exome sequencing reveals novel COL4A3 and COL4A4 mutations and resolves diagnosis in Chinese families with kidney disease.”

We would also like to thank the reviewers for their comments and we have revised the manuscript accordingly. We hope that the revisions we have made have addressed the points raised and will meet the requirements of the reviewers and the journal.

Below is our point-by-point response to the reviewers.

Reviewer 1:

A. Major changes:

1. Most Pro to Leu changes are NOT pathogenic so there needs to be more vigorous discussion of this point.

   We searched the LOVD database (//grenada.lumc.nl/LOVD2/COL4A/). Of the 15 unique COL4A4 missense mutations involving proline that are present in the LOVD database (//grenada.lumc.nl/LOVD2/COL4A/), only the p.Pro1572Leu and one other, p.Pro1132Leu, are considered to be likely pathogenic. However, several pathogenic proline substitutions (both in the collagenous and non-collagenous domains) have been reported in COL4A5, establishing that this type of mutation can disrupt Type IV collagen. Considering that the COL4A4 p.Pro1572Leu mutation lies in the non-collagenous domain at a residue that is highly conserved across all human Type IV collagen α chains and has previously been described in two separate ARAS cases, we come to the conclusion that COL4A4 p.Pro1572Leu is likely a pathogenic variant.
We have now inserted a discussion of this important point in the Results section of Family 1.

2. There should be some more discussion of the ability of this technique to pick up compound heterozygotes e.g. COL4A5/NPHS2.

We agree – unbiased screening of large numbers of genes using whole exome sequencing allows epistatic effects to be detected, such as the recognized association of NPHS2 variant p.R229Q with proteinuria and renal impairment in TBMN. We have added a discussion of this issue to the discussion section.

3. It would also good to have some discussion of comparison of WES with a 'panel' capture assay.

We have added a discussion of this issue to the discussion section.

B. Minor changes:

1. The EM is not very supportive of the diagnosis of Alport syndrome.

We have discussed the interpretation of the EM result in the results section of Family 1.

2. What is the significance of the CUBN variant?

Mutation of CUBN, encoding cubulin, can cause nephrotic syndrome. Therefore we included CUBN in the 32 prioritized genes known to be associated with non-syndromic familial nephropathy and/or kidney disease for variants filtering. Using WES we found a rare heterozygous CUBN missense mutation [c.C9206T (p.Thr3069Ile)] with MAF of 0.003 in the index patient of family 3. But the CUBN variant was absent in some affected family members and was also present in two unaffected family members. Therefore it cannot explain the familial kidney disease we observed and may have no functional effect on the protein.

We have included a discussion this CUBN variant in the results section of Family 3 and we also have removed the CUBN variant from Figure 4 to avoid any confusion.

Reviewer 2:
Comments: No major compulsory revisions. More discussion surrounding the use of WES or targeted gene panels in the clinical or research setting should be provided along side reference to current guidelines in AS testing and the detection rates based on testing criteria.

We have now summarized reports of detection rates for mutations using different techniques (Sanger sequencing, targeted panel and WES) in suspected AS in the
background section. We have also included more detailed discussion of issues surrounding the use of next generation sequencing in the diagnosis of AS in the first paragraph of the discussion section of the revised manuscript.

Yours sincerely,

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