Reviewer's report

Title: Phenotype variability in a large Spanish Familily with Alport syndrome associated with novel mutations in COL4A3 gene

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Reviewer: Jens Michael Hertz

Reviewer's report:

The authors present a large Spanish family in which a COL4A3 sequence variant segregates together with a phenotype of hematuria (13 out of 13) and hearing loss (6 out of 13). One additional family member (IV.6) has hearing loss, but is not investigated for the sequence variant. According to HGMD, 225 different mutations in COL4A3 have so far been published, of which 130 are missense mutations. This paper adds another putative disease causing mutation together with two sequence variants of unknown significance.

It is stated that this is an autosomal dominant form of Alport syndrome, but it might as well be a family with TBMD and hearing loss in some family members. Hearing loss is quite frequent, and only 6 out of 13 with the mutation have hearing loss, and 3 of these have inherited another sequence variant from their mother also, and might have an autosomal recessive form of Alport syndrome. At least they do not fulfill the diagnostic criteria for Alport syndrome as suggested by Flinter et al. (1988), where three out of the following four criteria should be fulfilled:

A positive family history of hematuria with or without renal failure (ok)

Typical ultrastructural GBM changes (no: a kidney biopsy from only one patient showed typical TBMD changes and FSGS in a second biopsy)

High-tone sensorineural hearing loss (ok)

Characteristic ophthalomological changes (no)

No information regarding hearing loss and renal symptoms are provided about the family to the mothers of V.1 and V.2. Even though the two mothers (IV.2 and IV.4) are without hematuria, this might not be the case for their relatives? Have they been analyzed for hematuria only once?
Background:

Line 95: The correct locus for COL4A3 is 2q36.3

Line 110: Reference 17 is not the original reference to the information that 10 % of the COL4A5 mutations occur de novo. Use instead the reference to the original paper by H. Lemmink et al., 1997.

Subjects and methods:

Generally, this section contains results also presented in the result section. The description of the renal biopsy should be referred only in the result section

Line 135: The sentence: "Family history investigation…." is confusing and should be rephrased.

Line 140: An ultrastructural study can't be performed by light microscopy, only electron microscopy.

A cDNA reference sequence should be added.

Line 149: Immunofluorescence microscopy was negative, but for what? Alpha3-5(IV)?

Results:

The three sequence variants, p.G333E, p.P1461L, and p.S1492S, were not present in 100 healthy controls, but it could be relevant also to look in the 1000 genomes database and the ExAC database.

No mutations were detected in COL4A4, and the pedigree isn't in accordance with X-linked inheritance, but has COL4A5 been sequenced in any of the family members?

Line 221: "suffered" should probably be "received a"?

Line 223: "(IV.1 and IV.3)" should probably be changed to "(IV.2 and IV.4)"
Discussion:

Line 270: Reference 16 is not the correct reference?

Line 273: The two additional sequence variants in COL4A3, p.P1461L and p.S1492C, are definitely not polymorphisms. A locus is polymorphic if the rarer allele has a frequency of at least 1%. They are just sequence variants.

Line 275: A paper is referred (ref. 29) that suggest MYO1E or other non-COL4 podocyte genes should be screened if the clinical phenotype is more severe than expected. Have MYO1E or other relevant genes been screened? And if the answer is no - why not?

Table 1 and the figure (pedigree):

The table and the pedigree are not in accordance. IV.21 and V.16 both have hearing loss according to the pedigree, but no data according to table 1?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript
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Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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