Reviewer’s report

Title: Non-collagen genes role in digenic Alport syndrome

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Reviewer: Jens Michael Hertz

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The authors present three families with an autosomal dominant form of Alport syndrome in which affected family members have a type-IV collagen gene mutation in addition to a non-collagen gene mutation (LAMA5/NPHS2), and a more severe phenotype than should be expected based on the type-IV collagen mutation alone.

In general, the paper is well written, and raises some important issues and the importance of using a NGS approach including extracellular matrix or podocyte cytoskeleton genes in the evaluation of patients with Alport syndrome.

I have some comments and corrections to the text, figure and table, and a more general comment.

Abstract:

Line 44: The disease has not always been linked to COL4A3/COL4A4/COL4A5, but only since the three genes have been found to cause the diseases. Alport syndrome was described many decades before the first gene was associated with the disease in 1990 (COL4A5).

Line 55: No pathogenic COL4A3 mutation co-inherited with a LAMA5 mutation have been presented; only COL4A4 and COL4A5 mutations have.

Discussion

Line 285: A clinical criteria for ATS is not "pedigree analysis" but a positive family history of hematuria with or without chronic renal failure, according to Flinter et al. (1988).

Line 339: There is no supportive data presented that the LAMA5 sequence variant in family 3 should contribute to the phenotype. The probands brother has only the LAMA5 sequence variant and is healthy and without hematuria? It is speculative to postulate that the probands paternal uncle in family 3 also have all three sequence variants, since he hasn't been analyzed. These three sequence variants segregate independently in the family.

Line 344: I;2, I;3; I;4 and I;5 should be corrected to: II;2, II;3; II;4 and II;5
A more general comment: It should be emphasized that a casuistic presentation of three families doesn't prove an additive effect of two mutations in different genes. Only a larger study powered to compare two matched groups of patients, one with only a COL4A3-5 mutation and one with a COL4A3-5 mutation together with a non-collagen (LAMA5) sequence variant, will be able to answer the question.

Table 1:

First column should refer to the family no. and the individual pedigree ID. The "code" no. doesn't provide the reader with any information of interest. The "Clinical folder" and "Relationship" column could be omitted.

Figure 1:

Personal ID referred to in the text (eg. II;2 in family 1) is missing in all three pedigrees. Legend to figure: The pedigrees are not characterized at the transcriptional level, but at the phenotypic level.

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