Author’s response to reviews

Title: Non-collagen genes role in digenic Alport syndrome

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Author’s response to reviews:

To Editor in Chief:
Hayley Henderson

Siena, January 09, 2019

Dear Editor,

Answer to Reviewer #1

Major

1. Given the frequency if the observed variants we can’t indeed exclude that among Alport patients with an apparently dominant form of ATS there are much more patients with digenic
variants in both COL4 and LAMA5 genes and that this eventuality is underdiagnosed. As reported in the discussion there is likely a mutation type-dependent pattern of transmission based on which severely damaging COL4a3 and a4 chains mutations in heterozygous state are sufficient to determine an autosomal dominant ATS in all family members. The targeted panel approach preferred in this case won’t thus be sufficient to unveil the coexistence of a modulator gene such as LAMA5. Hypomorphis mutations instead need to cosegregate together or in association with variants in other genes of the extracellular matrix or podocyte cytoskeleton in order for a fully penetrant phenotype to develop and the inheritance either of one of them or of the two of them will give reason of a different clinical expressivity. An exome sequencing approach prompted by the unusual phenotypic expression evident only in a very large family, as the one described in our manuscript, will thus allow to unmask a digenic inheritance.

2. In this case, the impact of the LAMA5 mutation as modifier is confirmed by the occurrence of persistent microhematuria in the father harboring the same variant. The microhematuria in the mother is instead justified by the presence of the COL4A5 mutation.

3. In this case, the cosegregation of the multiorgan phenotype (microhematuria and bilateral hypoacusia) with the COL4 variant along with LAMA5 and NPHS2 variants, already reported as damaging, led us to conclude for a digenic pattern of transmission rather than ADAS. In the 50-year-old father it is still possible that an unknown gene could act as positive modulator for the onset of proteinuria, giving reason of the phenotypic heterogeneity often present in ATS families. Furthermore, we have previously estimated that, in individuals with a digenic ATS, phenotype worsen at a median age of 45-47 years (Mencarelli et al., 2015; Fallerini et al., 2017), so we cannot exclude a later-onset proteinuria in the proband as well as in the father.

4. The text was changed accordingly.

Minor

We have changed the p. of the variant in the Supplementary Figure 1 in accordance of what required.

Answer to Reviewer #2

Abstract

Line 44: The sentence was changed to underline this concept

Line 55: This issue was fixed.

Discussion

Line 285: The text was changed as suggested
Line 339: We agree that this is speculative; however, given the phenotypic expressivity, it is plausible to think that the proband likely shares the combination of the COL4A4 mutation with at least one of the other two variants.

Line 344: It was fixed.

General Comment: It has been inserted in the discussion at line 368-370.

Table 1

We have replaced the title of the first column (“Code”) with “family no. and individual pedigree ID”. The “Clinical folder” and “Relationship” column were removed.

Figure 1

We have corrected the Legend to Figure 1 in accordance of what required.

We added the personal ID to each pedigree in Figure 1.

We look forward to hearing from you.

Sincerely

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