Reviewer’s report

Title: Non-collagen genes role in digenic Alport syndrome

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Reviewer: Kandai Nozu

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The authors have reported the possibility of LAMA5 gene variants can influence the severity in autosomal dominant Alport syndrome (ADAS) cases. They showed 3 familial cases and discussed about the correlation between severity of ADAS and existence of LAMA5 variants. They call coexistence of the variants in COL4 and LAMA5 genes as digenic that might mean these two variants equally contributed to the pathogenesis. Although it has been doubted modifier genes can influence the severity in ADAS, no reports have systematically proven the fact. Therefore, this study is very important to detect gene variants other than COL4 genes at the same time. However, I have serious concerns regarding this paper as follows.

Major

1. In Family 1, c.5149C>T in LAMA5 gene was detected. However, as the authors described, this variant allele frequency in European population is about 7%. According to the gnomAD browser, the frequency was as follows.

   European (Finissh) 9.5%
   European (non-Finissh) 9.0%
   Other 7.9%

   There is no clear evidence; however, it is said at least 1% of the population have pathogenic variants in either COL4A3 or COL4A4 (Savige, PMID 30450445). It means there would be much more patients with digenic variants in both COL4 and c.5149C>T in LAMA5.

2. The proband case in Family 2. He is currently 26 yo with moderate proteinuria without kidney dysfunction. He had a hemizygous missense mutation in COL4A5 gene and diagnosed with X-linked AS (XLAS). I couldn’t find this case showing severe phenotype but just showing a typical clinical course for male XLAS. So, I’m wondering LAMA5 gene variant really works as a modifier gene?

3. Family 3. The father had only hematuria although he is 50 and having digenic variants. We can’t say this digenic inheritance led him to ADAS but only hematuria right now.
4. The variant, c.2321C>T in LAMA5 gene. The authors described this mutation can have an impact in alteration of the splicing site (p.11, line 23). I checked this variant by Alamut software and Human Splice Finder. As a result, only SpliceSiteFinder-like showed the possibility of alteration of the splice donor site. Human Splicing Finder showed the possibility of alteration of ESS and ESE sites not the alteration of the donor site. From these variable in silico analysis results, to prove the possibility, mRNA analysis should be conducted. Otherwise the description about the splicing abnormality should be deleted.

Minor

Supplementary Figure 1. P.(His171Tyr) should be 1717.

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