Peer Review File

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Responses and modifications according to the journal style requirements:

V1. Reviewer

Comment 1: It is suggested that the order of the pictures in the paper should be in the order of the results. In this paper, figure 1C appears in front of figure 1B, which is suggested to be modified.

Reply 1: Thank you for your advice, you're right, we should put the pictures of renal biopsy in front of the gene test report.

Changes in the text1: We have modified the citing.

Comment 2: In "Real-time PCR", references should be provided for the method used in the sentence "Relative quantitative target gene expression was performed using the $2^{-\Delta\Delta Ct}$ comparative method".

Reply 2: Your opinion is very rigorous. I have added the reference in the article.

Changes in the text2: In page 6, line 9, we added reference (11) after "$2^{-\Delta\Delta Ct}$ comparative method".

Comment 3: Many abbreviations should be given their full names when they first appear, such as PMSF, RIPA, PVDF, SDS-PAGE, SIFT and GBM.

Reply 3: We have added full names to each abbreviation you have proposed in the articular.

Changes in the text3: In page 6 line13, we added " phenylmethylsulfonyl fluoride" before "PMSF"; in page 6 line14, we added " radioimmunoprecipitation assay" before "RIPA"; in page 6 line17-18, we added "sodium dodecyl sulfate-polyacrylamide gel electrohoresis" before "SDS-PAGE"; in page 6 line 18, we added "polyvinylidene

fluoride ” before “PVDF”; in page 9 line2, we added “glomerular basement membrane” before “GBM”; in page 9 line14, we added “Sorts intolerant from tolerant” before “SIFT”; in page 9 line14, we added “Polymorphism phenotyping” before “PolyPhen”.

Comment 4: GAPDH is used as the internal reference in the methods, but beta-actin is used as the internal reference in the figures. The author lacks strictness in the process of writing this paper.

Reply 4: I’m so sorry for the mistake and have given the correct description in the paragraph.

Changes in the text4: In page 7, line 2-3, “beta-actin, mouse anti-β-Actin monoclonal antibody (Santa Cruz Biotechnology, USA) have been used with a 1:20000 dilution; goat anti-rabbit IgG-HRP (Jackson, UK) and goat anti-mouse IgG-HRP linked antibody” were instead of “GAPDH, goat anti-rabbit IgG-HRP linked antibody (Jackson, UK) and rabbit anti-goat IgG-HRP linked-antibody”.

Comment 5: It is suggested to provide a statistics histogram for the Western blotting results in Figure 3.

Reply 5: We have accepted your suggestion, scanned the gray value of the results of western blot and carried out statistical analysis, and the results have been added in Figure 3.

Changes in the text5: A histogram of gray value statistics of Western blot was added to Figure 3.

Comment 6: It seems that the fluorescence signal intensity of each group is not completely consistent with that described by the author. It is suggested to provide a statistical diagram of fluorescence intensity for figure 4-6, which is more intuitive.

Reply 6: Thank you for your valuable comments. It’s true that our description of the

immunofluorescence results is not very accurate, therefore, we reassessed and calculated the fluorescence intensity and redescribed it in the paper.

Changes in the text6: In page 10 line15-19, we added “COL4A5-normal group was significantly increased than the control and plasmid control groups, but the expression of COL4A5 significantly reduced than the normal group after transfection of the mutant plasmid. (Figure 4). Again, there was no significant difference in COL4A3 and COL4A1 protein immunofluorescence staining between the normal and mutant groups” instead of “COL4A5-mutant group was significantly reduced than the other groups, and the fluorescence intensity of COL4A5-normal group was the same as the control and plasmid control groups (Figure 4). Again, there was no significant difference in COL4A3 and COL4A1 protein immunofluorescence staining between all groups.”, and we added the statistical diagram of fluorescence intensity for figure 4-6 respectively.

Comment 7: Does the mutation affect the progression of the disease? It is suggested to add relevant contents in the discussion.

Reply 7: This mutation reduced the generation of a5 (IV), resulting in the inhibition of collagen a3a4a5 (IV) trimer binding, and finally leaded to GBM structure abnormalities. Large amounts of red blood cells and urine proteins are lost from the glomerular filtration barrier, eventually leading to a progressive decline in kidney function.

Changes in the text7: In page 13 line 1-4, we have added this part of discuss in the last paragraph. The addition is ”abnormal human GBM structure, so as to destroy the glomerular filtration barrier, leading to a large number of red blood cells and albumin leakage, progressive loss of kidney function, eventually to end-stage renal disease.”