Am J Transl Res 2022;14(8):5746-5753
www.ajtr.org /ISSN:1943-8141/AJTR0141212

Case Report

A pedigree with *COL4A5* mutation presenting with Alport syndrome and focal segmental glomerulosclerosis lesions: a case report

Honghong Zou[1,2,3,4,5,6], Li Zhu[1,2,3,4,5], Rong Xu[1,2,3,4,5], Sufang Shi[1,2,3,4,5], Suxia Wang[1,2,3,4,5], Fude Zhou[1,2,3,4,5], Hong Zhang[1,2,3,4,5], Minghui Zhao[1,2,3,4,5]

[1]Renal Division, Department of Medicine, Peking University First Hospital, Beijing 100034, China; [2]Peking University Institute of Nephrology, Beijing 100034, China; [3]Key Laboratory of Renal Disease (Peking University), National Health Commission, Beijing 100034, China; [4]Key Laboratory of Chronic Kidney Disease Prevention and Treatment, Ministry of Education, Beijing 100034, China; [5]Research Units of Diagnosis and Treatment of Immune-mediated Kidney Diseases, Chinese Academy of Medical Sciences, Beijing 100034, China; [6]Department of Nephrology, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China

Received December 19, 2021; Accepted March 25, 2022; Epub August 15, 2022; Published August 30, 2022

Abstract: Alport syndrome (AS) is a heterogeneous hereditary nephropathy which can be caused by the *COL4A3/COL4A4/COL4A5* gene. Patients with AS present with many phenotypes associated with kidney defects, and commonly develop secondary focal segmental glomerulosclerosis (FSGS) late in the course of AS. Evidence supports the pathogenic role of *COL4A3/COL4A4/COL4A5* mutations in FSGS. We report a familial hematuria pedigree with two members that have AS and FSGS, respectively. The proband presented with microhematuria, proteinuria, renal dysfunction and sensorineural hearing loss. Pathological examination of his renal biopsy samples revealed FSGS lesions and massive foam cells by light microscopy, irregular GBM, and focal podocyte foot process effacement under electron microscopy, as well as negative α5 (IV) staining by immunofluorescence detection so he was diagnosed as AS. The proband's younger brother had only renal manifestations without obvious extrarenal lesions. Light microscopy examination of renal biopsy samples showed only FSGS lesion without foam cells. Electron microscopy and α5 (IV) staining were not performed and he was diagnosed with FSGS. Using whole-exome sequencing, we identified a novel *COL4A5* mutation (c.4456G>A:p.G1486S) in this pedigree, which affected two males (the proband and his brother) and three female family members. The three female family members were heterozygous or the *COL4A5* mutation and only presented with microhematuria. Our findings suggest importance of electron microscopy analysis and *COL4A3/COL4A4/COL4A5* mutation screening in patients with FSGS lesions under light microscopy.

Keywords: Alport syndrome, focal segmental glomerulosclerosis, *COL4A*

Introduction

The glomerular basement membrane (GBM) is an important component of the filtration barrier. The type IV collagen α3, α4, and α5 chains are major structural components of human GBM, encoded by *COL4A3*, *COL4A4* and *COL4A5* genes. Different from *COL4A3* and *COL4A4*, which located in chromosome 2, *COL4A5* gene is on the X chromosome [1]. Alport syndrome (AS), a hereditary nephropathy characterized by unevenly thickened GBM with typical splitting, lamellation, and basket weave-like appearance, usually presents with X-linked dominant inheritance pattern and is caused by *COL4A5* mutations [2]. Patients with AS present with many phenotypes associated with kidney defects, including hematuria, proteinuria, progressive renal failure, sensorineural hearing loss and ocular abnormalities [3]. Males are more severely and earlier affected than females, since males have only one X chromosome and females have two. Females are carriers with no symptoms or mild phenotypes. In addition, AS also has autosomal dominant/recessive inheritance due to pathogenic variants in *COL4A3* and *COL4A4* [4]. In structure, the type IV collagen α3, α4, and α5 chains form

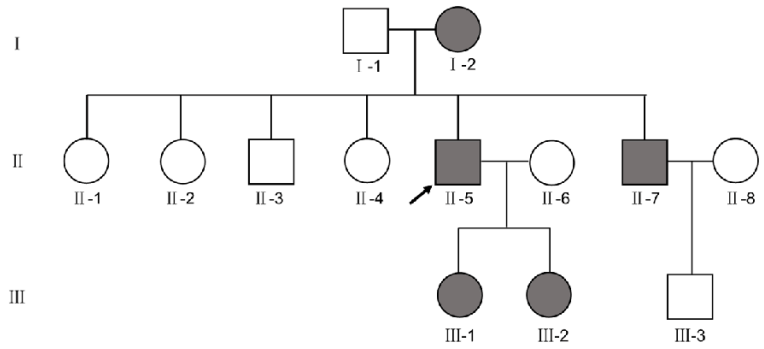

Figure 1. Pedigree structure of the family. There were 13 individuals in a Chinese three generation pedigree with no close relatives married. In this pedigree, 5 members had hematuria, and 2 of them were diagnosed with AS and FSGS, respectively. Circles indicate females, squares indicate males, arrow indicates proband, filled shapes indicates affected status, unfilled shaped indicate an unaffected individual.

a triple helix in GBM; therefore, mutation in any gene of *COL4A3/COL4A4/COL4A5* would affect GBM assembly and cause GBM lesions in AS.

In recent years, besides AS, evidence indicated a pathogenic role of *COL4A3/COL4A4/COL4A5* mutations in focal segmental glomerulosclerosis (FSGS). FSGS is a clinicopathologic diagnosis. It results from podocyte injury and is characterized by focal and segmental glomerular sclerosis, and foot process fusion or disappearance caused by podocyte degeneration [5]. Patients with FSGS typically present with proteinuria, frequently associated with nephrotic syndrome. It is known that the GBM, glomerular endothelial cells, and podocytes together constitute the glomerular filtration barrier. Podocytes as basic cells to maintain glomerular filtration barrier, can be injured by a variety of factors. For example, GBM deficiency may lead to secondary pathologic changes in podocytes, and then evolve into the FSGS lesions in the advanced stage of AS [5]. Based on this, Malone et al. screened diagnostic genetic variants in familial FSGS patients, and first reported rare variants in *COL4A3* and *COL4A4* in 10% of familial FSGS patients [5]. After that, multiple studies reported pathogenic variants of *COL4A5* in both familial and sporadic FSGS patients [5-7]. The latest guideline extended the indications for screening for pathogenic variants in the *COL4A5*, *COL4A3* and *COL4A4* genes to patients with FSGS [8].

Here we report a familial hematuria pedigree with *COL4A5* mutation. Our data reinforce the involvement of genetic change in *COL4A5* gene in AS and FSGS lesions.

Case presentation

The proband (II-5, **Figure 1**) was a 31 year old man, who was admitted to our hospital because urinalysis showed protein 3+ and occult blood 2+. Laboratory tests revealed proteinuria of 5.44 g/d, albumin (ALB) of 32.7 g/L, serum creatinine (Scr) of 162.0 umol/L, estimate glomerular filtration rate (eGFR) of 48.0 ml/min/1.73 m^2. The eGFR was calculated by the chronic kidney disease epidemiology collaboration creatinine equation [9]. The levels of blood immunoglobulin, complement, and anti-phospholipase A2 receptor antibody were normal. Ultrasound showed bilateral renal hyperechogenicity. Acoustic immittance and pure tone audiometry showed mild conductive hearing impairment. Ocular abnormalities were not detected. Renal biopsy was performed for the proband. Immunofluorescence showed all negative for immunoglobulins (Igs), C3, C1q, and fibrinogen-related antigen (FRA). Focal segmental glomerulosclerosis together with massive foam cells in the interstitium could be found by light microscopy (**Figure 2A-C**). Electron microscopy showed irregularity and multilayering of the GBM, and focal podocyte foot process effacement (**Figure 2D**). Indirect immunofluorescence detection of the renal basement membrane revealed negative α5 (IV) staining in the GBM, Bowman's capsule, and tubular basement membrane. This case was finally diagnosed as AS. The patient received oral valsartan capsules 80 mg/d. A week later, his proteinuria was reduced to 2.53 g/d, and Scr was reduced to 148.4 umol/L.

The proband's 28 years old brother (II-7) presented with hematuria and proteinuria for more than three years, and hypertension and renal dysfunction for more than three months. Laboratory tests revealed protein of 3+, red blood cells of 20~30/HP, proteinuria of 0.96 g/d, ALB of 40.1 g/L, and Scr of 156.0 umol/L. Ultrasound showed the right kidney was small-

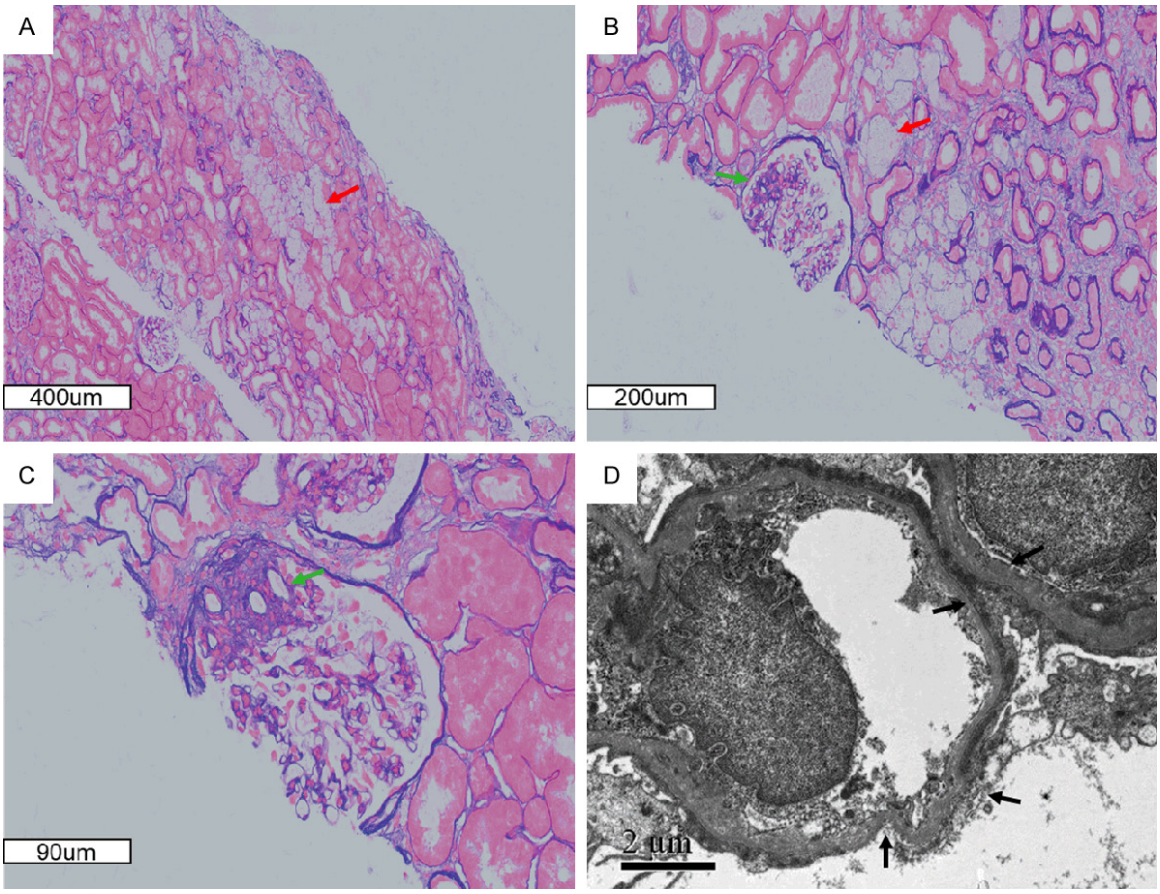

Figure 2. Renal pathologic features of the proband. A-C. PASM (40X, 100X, 200X) stained light micrograph showed focal segmental glomerulosclerosis together with massive foam cells in interstitium; D. Electron microscopy showed irregularity and multilayering of the GBM, and focal podocyte foot process effacement. The focal segmental glomerulosclerosis lesion, foam cells, and abnormal GBM are shown by a green arrowhead, red arrowhead, and black arrowhead, respectively.

er. Renal biopsy was performed. Immunofluorescence microscopy was negative for IgA, IgG, C3, C1q and FRA. Only IgM (+) was deposited in mesangial areas. Light microscopy showed segmental sclerosis in one glomerulus, and no foam cells could be found. Unfortunately, electron microscope and α5 (IV) staining was not performed. This patient was diagnosed with FSGS. After maximally tolerated dose of renin-angiotensin system (RAS) inhibition therapy for over 3 months, he still had proteinuria >1.00 g/d.

In addition, the proband's mother (I-2) and two daughters (III-1 and III-2) were found to have microscopic hematuria. Renal manifestations were not found in other family members of this pedigree (**Figure 1**; **Table 1**).

Genetic analysis

For molecular genetic diagnosis of this pedigree, whole exome sequencing (WES) was at first performed in 7 individuals (I-1, I-2, II-2, II-3, II-4, II-5, II-7) across the pedigree using hybridization-based enrichments and next-generation sequencing. For the identification of diagnostic variants, 625 nephropathy-associated genes were selected according to a previous publication [10]. Subsequently, all variants in the 625 nephropathy-associated genes were evaluated following the American College of Medical Genetics and Genomics guidelines [11]. "Pathogenic" and "likely pathogenic" variants were stringently filtered and took into consideration for the patients' nephropathy. In total, six "pathogenic" or "likely pathogenic" variants (in

Table 1. Clinical characteristics of the family members in the pedigree

Individual ID	Sex	Age (years)	Hematuria	Proteinuria	Renal dysfunction	Hearing loss	Ocular lesions	Renal pathology	Gene mutation
I-1	M	65	No	No	No	No	No	None	G
I-2	F	62	Yes	No	No	No	No	None	AG
II-1	F	43	NA	NA	NA	No	No	None	NA
II-2	F	39	No	No	No	No	No	None	GG
II-3	M	35	No	No	No	No	No	None	G
II-4	F	33	No	No	No	No	No	None	GG
II-5	M	31	Yes	Yes	Yes	Yes	Yes	AS	A
II-6	F	27	No	No	No	No	No	None	GG
II-7	M	29	Yes	Yes	Yes	No	No	FSGS	A
II-8	F	27	NA	NA	NA	No	No	None	NA
II-1	F	6	Yes	No	No	No	No	None	AG
II-2	F	3	Yes	No	No	No	No	None	AG
III-3	M	1	NA	NA	NA	No	No	None	NA

Abbreviation: M, male; F, female; AS, Alport syndrome; FSGS, Focal segmental glomerulosclerosis; NA, not applicable. Gene mutation: the genotype in the mutation site of *COL4A5* (c.4456G>A).

Table 2. Candidate pathogenic variants identified in this pedigree

Position	Gene	Function change	Variants
Chr10: 99361747	*HOGA1*	synonymous	exon2:c.G345T:p.A115A
Chr10: 99361748	*HOGA1*	Splicing	exon2:c.345+1G>T
Chr14: 76543036	*IFT43*	Splicing	exon4:c.310+2T>C
Chr15: 89816607	*FANC1*	Splicing	exon11:c.883-1G>A
Chr17: 61574674	*ACE*	missense	exon25:c.C3868T:p.R1290W
ChrX: 107930870	*COL4A5*	missense	exon47:c.G4456A:p.G1486S

genes *FANCI*, *IFT43*, *HOGA1*, *COL4A5*, *ACE*) in this pedigree were detected (**Table 2**). Among these variants, only a novel variant in *COL4A5* (c.4456G>A) co-segregated with the nephropathy phenotype in the pedigree. This variant was located at exon 47 of *COL4A5* gene, and resulted in glycine at position 1486 being substituted by serine.

Two in silico functional evaluation tools, Polyphen2 and Combined Annotation Dependent Depletion (CADD), were used to predict the functional significance of novel variants. Polyphen2 is a tool that predicts possible impact of an amino acid substitution on the structure and function of a human protein based on the HumanVar database for monogenic genetic diseases. CADD is a tool for scoring the deleteriousness of single nucleotide variants and insertion/deletions variants. According to Polyphen2 and CADD, *COL4A5*:c.4456G>A was consistently predicted as the damaged variant to induced impaired protein function. Hence, it was identified as the diagnostic variant for this pedigree. The other 5 variants were did not explain the glomerular disease phenotypes in this pedigree.

Next, polymerase chain reaction (**Table 3**) and Sanger sequencing was applied to all family members with available DNA samples for the identification or verification of the diagnostic variant in *COL4A5* (**Figure 3**). Among the detected ten familial members, five individuals, including I-2, II-5, II-7, III-1 and III-2 had this variant (**Figure 3**). I-2 was the proband's mother with hematuria phenotype; she was a heterozygote for *COL4A5:*c.4456G>A; II-5 and II-7 were hemizygous, and diagnosed with AS and FSGS, respectively; III-1 and III-2 were the proband's daughters. At present, both have a hematuria phenotype with normal renal function and renal biopsy was not done. The other 5 family members (I-1, II-2, II-3, II-4 and II-6) did not carry *COL4A5:*c.4456G>A, and none of them had an abnormal urine test.

Diagnostic criteria and prognosis of AS

The diagnosis of AS can be difficult and should rely on integration of the family history, clinical manifestations, skin and/or renal biopsy, tissue immunofluorescence and electron microscopy. The proband was diagnosed with AS according

Table 3. Primers and annealing temperatures for sequencing of identified mutation in *COL4A5* gene

Amplified targets	Primers' sequence (5'-3')	Annealing temperature
COL4A5	Forward: CAAATGAGGTCATAATGTTTTGTCA	55°C
c.4456G>A:p.G1486S	Reverse: GGCCAAGGCTACTCTAGAACC	

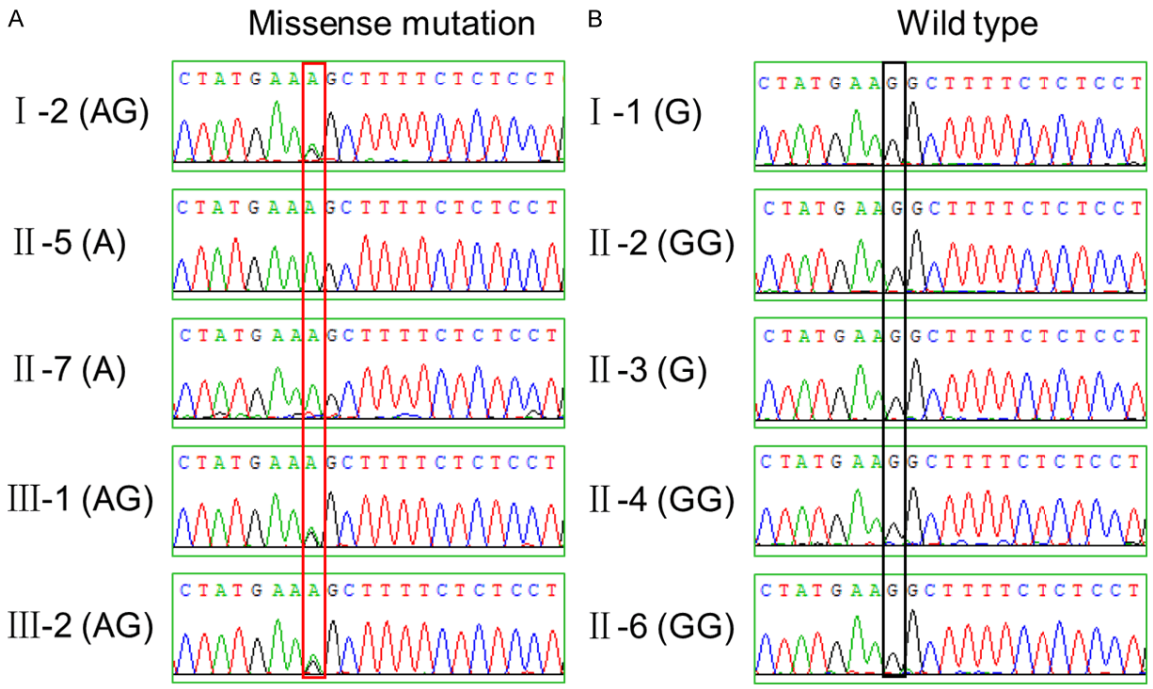

Figure 3. Verification of the identified *COL4A5* mutation (c.4456G>A:p.G1486S) by Sanger sequencing. A. The proband (II-5), his brother (II-7) and his two daughters (III-1 and III-2) inherited c.4456G>A:p.G1486S mutation from his mother (I-2). B. The proband's father (I-1) and other relatives (II-2, II-3, II-4, II-6) were normal phenotype and did not carry the c.4456G>A:p.G1486S mutation. Red box points to the missense mutation. Black box points to the wild type.

to the following diagnostic criteria, while the other male patient was diagnosed with FSGS based on the lesions under light microscopy. Five diagnostic criteria for AS were used in the present study with at least three required to establish the diagnosis [12, 13]: 1) Complete or partial expression of type IV collagen α3, α4, and α5 chains observed in renal and/or skin biopsy; 2) Electron microscopy of GBM showed extensive abnormities, especially thickening, thinning and stratification; 3) A family member as proband whose diagnosis was based on evidence of electron microscopy and/or type IV collagen in renal/skin biopsy, or on a detailed family history of autosomal recessive/X-linked dominant inheritance (defined as hematuria both parents or consanguineous parents; 4) Evidence of binaural high-tone deafness, with or without clinical deafness; 5) Evidence of lens abnormalities (anterior or posterior lenticonus) and/or flecks in the peripheral or midperipheral retinal area.

The goal of treatment in AS is to delay progression to end-stage renal disease as long as possible. Treatment regimen is based on reduction of proteinuria and renal fibrosis through the use of angiotensin-converting enzyme inhibitors [14]. The two male patients were impaired in renal function and in the renal functional decompensatory period. Their renal function improved slightly after intervention by RAS inhibition.

Discussion

Pathogenic variants in *COL4* genes (*COL4A3*, *COL4A4* and *COL4A5*) are reported to be associated with several kidney diseases, including AS and FSGS. In this study, using WES, we reported a novel *COL4A5* mutation, c.4456G>A,

as the pathogenic factor for a three-generation pedigree with familial hematuria.

COL4A5 gene encodes type IV collagen α5 chain, which combines with type IV collagen α3 and α4 chains to form triple helix structure in GBM. Type IV collagen α5 chain is composed of an intermediate collagenous domain and two terminal non-collagenous domains [15]. The *COL4A5* mutation we identified in this pedigree, was located in exon 47 in the C-terminal non-collagenous domain. This mutation caused the glycine at position 1486 substituted by serine and disrupted the Gly-X-Y triplets in type IV collagen α5 chain. Glycine, with low molecular weight, is the only amino acid that has no side chain substituents and can be bent to fill the triple helix structure, which is involved in the formation of disulfide bonds between the α chains. It is crucial for the formation of the triple helical structure. Glycine substitutions, especially in the collagenous domain, are generally considered as pathogenic, which could lead to weaker GBM and increased fragility of podocyte cytoskeleton [16]. Accordingly, glycine substitution within the Gly-X-Y repeat sequence is one of the most common types of pathogenic variants found in AS [17]. Among the more than 1000 mutations in *COL4A5* gene included in Human Gene Mutation Database, nearly 50% of them are glycine substitutions. Furthermore, at the same codon site of our identified mutation, mutations resulting in different amino acid changes had previously been identified as pathogenic in families with AS. The studies showed that glycine at position 1486 was substituted by alanine or aspartic acid, resulting in the Gly-X-Y repeat sequence interruptions, and patients with those mutations presented with hearing loss, or end-stage renal disease and other features [11, 18]. Hence, we inferred that the *COL4A5* mutation (c.4456G>A:p.G1486S) we identified in this pedigree was pathogenic.

In view of the fact that the *COL4A5* gene encoded the type IV collagen α5 chain, the pathogenic *COL4A5* mutation (c.4456G>A:p.G1486S) altered the structure of type IV collagen, which in turn might lead to weakened GBM and increased fragility of podocyte cytoskeleton. Altered permselectivity of the GBM and defective expression or trafficking of GBM matrix components (eg. GBM with thickness unevenness, typical splitting, lamellation, and basket weave-like appearance) and abnormal matrix-podocyte interactions could induce the pathologic diagnosis of AS and might lead to secondary pathologic changes of podocytes, then cause proteinuria and evolve into the secondary FSGS lesion in the advanced stage of AS [5]. This may also explain why both male patients had proteinuria and presented with focal segmental glomerulosclerosis under light microscopy.

Five family members in the pedigree had this mutation, but their phenotypes were highly variable. Since *COL4A5* gene is located in Xq22, female family members were heterozygotes, while the male individuals were hemizygotes [19]. All three female mutation carriers in this pedigree only presented with mild hematuria, but both male family members with this mutation got more severe kidney phenotypes (hematuria, proteinuria and mild impairment of renal function) which required renal biopsy for further diagnosis. However, although the two male siblings had the same *COL4A5* mutation, their pathologic diagnoses were not the same. Both male patients presented with focal segmental glomerulosclerosis under light microscopy. Electron microscopy analysis was only performed in the proband, in which significant GBM lesions were found, including the uneven and layered GBM. The identification of GBM lesions under electron microscopy induced the pathologic diagnosis of AS, while the other male patient was diagnosed with FSGS based on the lesions under light microscopy. In previous study, Yao et al. reported that FSGS was the most frequent misdiagnosis in female X-linked AS (fXLAS) patients [20]. This evidence suggested that electron microscopy analysis and genetic screening for *COL4A3/COL4A4/COL4A5* mutations were important tools to provide supportive information for patients with primary FSGS.

In summary, we report a familial hematuria pedigree with glycine substitution mutation in *COL4A5* gene. Two male siblings had the same mutation and diagnosed as AS and FSGS, respectively. Our findings suggest importance of electron microscopy analysis and *COL4A3/COL4A4/COL4A5* mutation screening in patients with FSGS lesion under light microscopy.

Acknowledgements

This project was supported by grants from the National Key Research and Development Program of China (2020YFC2005003), National Science Foundation of China (81922013, 81970598, 82070733), National Science Foundation of Beijing (7202206), Youth development project form Peking University Health Science Center (BMU2021PY004) and CAMS Innovation Fund for Medical Sciences (2019-I2M-5-046).

Disclosure of conflict of interest

None.

Address correspondence to: Li Zhu and Rong Xu, Renal Division, Department of Medicine, Peking University First Hospital, Beijing 100034, China. Tel: +86-10-66551122 Ext. 2388; Fax: +86-10-66551055; E-mail: funnyzhuli@bjmu.edu.cn (LZ); xrailzz@sina.com (RX)

References

[1] Gatseva A, Sin YY, Brezzo G and Van Agtmael T. Basement membrane collagens and disease mechanisms. Essays Biochem 2019; 63: 297-312.

[2] Kashtan CE. Familial hematuria. Pediatr Nephrol 2009; 24: 1951-1958.

[3] Savige J, Gregory M, Gross O, Kashtan C, Ding J and Flinter F. Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. J Am Soc Nephrol 2013; 24: 364-375.

[4] Kashtan CE. Alport syndrome. An inherited disorder of renal, ocular, and cochlear basement membranes. Medicine (Baltimore) 1999; 78: 338-360.

[5] Malone AF, Phelan PJ, Hall G, Cetincelik U, Homstad A, Alonso AS, Jiang R, Lindsey TB, Wu G, Sparks MA, Smith SR, Webb NJ, Kalra PA, Adeyemo AA, Shaw AS, Conlon PJ, Jennette JC, Howell DN, Winn MP and Gbadegesin RA. Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis. Kidney Int 2014; 86: 1253-1259.

[6] Gast C, Pengelly RJ, Lyon M, Bunyan DJ, Seaby EG, Graham N, Venkat-Raman G and Ennis S. Collagen (COL4A) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. Nephrol Dial Transplant 2016; 31: 961-970.

[7] Frese J, Kettwig M, Zappel H, Hofer J, Grone HJ, Nagel M, Sunder-Plassmann G, Kain R, Neuweiler J and Gross O. Kidney injury by variants in the COL4A5 gene aggravated by polymorphisms in slit diaphragm genes causes focal segmental glomerulosclerosis. Int J Mol Sci 2019; 20: 519.

[8] Savige J, Storey H, Watson E, Hertz JM, Deltas C, Renieri A, Mari F, Hilbert P, Plevova P, Byers P, Cerkauskaite A, Gregory M, Cerkauskiene R, Ljubanovic DG, Becherucci F, Errichiello C, Massella L, Aiello V, Lennon R, Hopkinson L, Koziell A, Lungu A, Rothe HM, Hoefele J, Zacchia M, Martic TN, Gupta A, van Eerde A, Gear S, Landini S, Palazzo V, Al-Rabadi L, Claes K, Corveleyn A, Van Hoof E, van Geel M, Williams M, Ashton E, Belge H, Ars E, Bierzynska A, Gangemi C and Lipska-Ziętkiewicz BS. Consensus statement on standards and guidelines for the molecular diagnostics of Alport syndrome: refining the ACMG criteria. Eur J Hum Genet 2021; 29: 1186-1197.

[9] Levey AS and Stevens LA. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. Am J Kidney Dis 2010; 55: 622-627.

[10] Groopman E, Goldstein D and Gharavi A. Diagnostic utility of exome sequencing for kidney disease. Reply. N Engl J Med 2019; 380: 2080-2081.

[11] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K and Rehm HL. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-424.

[12] Flinter FA, Cameron JS, Chantler C, Houston I and Bobrow M. Genetics of classic Alport's syndrome. Lancet 1988; 2: 1005-1007.

[13] Savige J, Ariani F, Mari F, Bruttini M, Renieri A, Gross O, Deltas C, Flinter F, Ding J, Gale DP, Nagel M, Yau M, Shagam L, Torra R, Ars E, Hoefele J, Garosi G and Storey H. Expert consensus guidelines for the genetic diagnosis of Alport syndrome. Pediatr Nephrol 2019; 34: 1175-1189.

[14] Kashtan CE. Alport syndrome: achieving early diagnosis and treatment. Am J Kidney Dis 2021; 77: 272-279.

[15] Kawai S, Nomura S, Harano T, Harano K, Fukushima T and Osawa G. The COL4A5 gene in Japanese Alport syndrome patients: spectrum of mutations of all exons. The Japanese Alport Network. Kidney Int 1996; 49: 814-822.

[16] Fidler AL, Vanacore RM, Chetyrkin SV, Pedchenko VK, Bhave G, Yin VP, Stothers CL, Rose KL, McDonald WH, Clark TA, Borza DB, Steele

RE, Ivy MT, Hudson JK and Hudson BG. A unique covalent bond in basement membrane is a primordial innovation for tissue evolution. Proc Natl Acad Sci U S A 2014; 111: 331-336.
[17] Hashimura Y, Nozu K, Kaito H, Nakanishi K, Fu XJ, Ohtsubo H, Hashimoto F, Oka M, Ninchoji T, Ishimori S, Morisada N, Matsunoshita N, Kamiyoshi N, Yoshikawa N and Iijima K. Milder clinical aspects of X-linked Alport syndrome in men positive for the collagen IV alpha5 chain. Kidney Int 2014; 85: 1208-1213.
[18] Plant KE, Green PM, Vetrie D and Flinter FA. Detection of mutations in COL4A5 in patients with Alport syndrome. Hum Mutat 1999; 13: 124-132.
[19] Naito I, Kawai S, Nomura S, Sado Y and Osawa G. Relationship between COL4A5 gene mutation and distribution of type IV collagen in male X-linked Alport syndrome. Japanese Alport Network. Kidney Int 1996; 50: 304-311.
[20] Yao XD, Chen X, Huang GY, Yu YT, Xu ST, Hu YL, Wang QW, Chen HP, Zeng CH, Ji DX, Hu WX, Tang Z and Liu ZH. Challenge in pathologic diagnosis of Alport syndrome: evidence from correction of previous misdiagnosis. Orphanet J Rare Dis 2012; 7: 100.