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Case Report

A novel mutation in a Chinese family with autosomal recessive Alport syndrome: a case report

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Abstract: Alport syndrome (AS) is a familial hereditary nephropathy which is characterized by molecular abnormalities in Collagen IV a345. As more gene mutations are discovered, it has been reported that autosomal recessive disease accounts for a smaller proportion (about 4%) of AS patients than previously recognized. We report here a novel mutation in *COL4A4* in a Chinese family with autosomal recessive AS. Patient 1 was a 24-year-old Chinese man. He and his brother (patient 2) had a history of proteinuria and hematuria with renal dysfunction and sensorineural deafness. Pathologic findings were consistent with Alport syndrome, and genetic analysis revealed that both patients had two heterozygous mutations, c.1423 G>T (p.Gly475Cys) in EX21/CDS20 and c.735 G>A (p.Pro245Pro) in EX12/CDS11, and that each mutation originated from their mother or father who were carriers for one of these two mutations. Both patients showed similar results by laboratory examination and histopathologic assessment. Patient 1 received ACEI treatment and ran a stable clinical course, whereas patient 2 refused ACEI treatment and had progressive deterioration of renal function. This is the first report of a novel mutation in the collagen domain of *COL4A4* gene. The results add to the spectrum of mutations in *COL4A4* of Alport syndrome.

Keywords: Alport syndrome, autosomal recessive, *COL4A4* mutation

Introduction

Alport syndrome (AS) is a familial hereditary nephropathy, characterized by molecular abnormalities in Collagen IV a345, the main constituent of glomerular basal collagen. Mutations commonly occur in *COL4A3, COL4A4*, and *COL4A5* genes, and are clinically accompanied by hematuria, sensorineural deafness, and ocular anomalies. Most patients with Alport syndrome develop renal failure [1]. X-linked genetic changes account for about 85% of all Alport syndrome, with mutations involving the COL4A5 collagen type IV a5 chain. About 15% of cases of Alport syndrome have autosomal recessive inheritance caused by two mutations in COL4A3 or COL4A4. The frequency of COL4A5 gene mutation has been estimated at 1:5000 and that of autosomal recessive Alport syndrome is 1:50,000 [2, 3]. With high-throughput next generation sequencing (NGS) technology, more gene mutations are being discovered and it has been reported that autosomal recessive disease accounts for a smaller proportion (about 4%) of Alport syndrome patients than previously recognized [4-6]. Here, we report two hepatitis B virus carrier patients with novel COL4A4 mutation autosomal recessive Alport syndrome.

Case presentation

Patient 1 and patient 2 had renal biopsies. All were stained with H&E, PAS, PASM, and Masson, and observed by light microscopy. Immunofluorescence (IF) for fibrin, IgA, IgG, IgM, C1q, Kappa, and Lambda was done. Electron microscopy (EM) was also performed. COL4A3, COL4A4, COL4A5 gene detection method was based on the standardization of the target area capture high-throughput sequencing (BGI Genomics, BGI-Shenzhen, Shenzhen, China). Data analysis: application of high-throughput sequencing data analysis process BGIv.0.1.0, human genome reference: UCSC hgl9Feb.2009; Comparison software: BWA 0.6.2-r126; Mutation detection application software: SOAPsnp software2.0, SAMtools vI.4; The variation annotation applied the common frequency [dbsNP(snp137); 1000 Genome (phase I); HapMap

(combined data from Phases II and IID)] and the own database (BGI-DB, HGVD). The data interpretation rules are based on guidelines from the American College of Medical Genetics and Genomics (ACMG). Variables named according to the rules of HGVS (http://www.hgvs.org/mutnomen/) are given.

Patient 1 was a 26-year-old Chinese man with two years' history of microscopic hematuria and proteinuria. He had clinically detectable hearing loss but no ocular abnormalities. Urinalysis showed 2+ proteinuria and 3+ hematuria, with 24 h urinary protein quantification 14,566 mg/24 h and a urinary protein/creatinine ratio (P/Cr) of 0.19 g/gCr. His serum creatinine level was 122 mg/dL, serum total protein level was 55.2 g/L, and albumin level was 32.5 g/L. He was also serologically positive for HBsAg, HBeAb, and HBcAb. Entecavir was used for treatment of his chronic hepatitis B. He took oral prednisone, cyclosporine A, tripterygium glycosides, and perindopril before his kidney disease diagnosis. Recently, perindopril (4 mg, daily) and traditional Chinese medicine were administrated orally. The patient's first younger brother (patient 2) was a 25-year-old, who also had proteinuria (3+) and hematuria (1+) and Cr 135 μmol/L, and also was serologically positive for HBsAg, HBeAb, and HBcAb. He had clinically detectable hearing loss but no ocular abnormalities. He took oral prednisone before diagnosis. Recently, he was taking oral perindopril (4 mg, daily) and traditional Chinese medicine.

The renal biopsies of both patients are shown in **Figure 1A-N**. In patient 1, 14 glomeruli were observed. One glomerulus showed mild mesangial hyperplasia; all 13 remaining glomeruli showed normal cellularity and intact capillary tuft architecture. Foamy changes in tubular cells were accompanied by visible tubular atrophy and interstitial infiltration of inflammatory cells, accounting for about 30% of the lesion. Protein casts were seen in the renal tubules. The lesions of interlobular arteries and glomerular arterioles were not readily visible. IF showed that alpha 4 chain of type IV collagen was absent in glomerular basement membrane (GBM) and Bowman's capsule (**Figure 1G**). EM demonstrated markedly irregular subendothelial GBM surface with splitting and multilamellation ("basket weaving") as well as "bread crumbs" in the GBM. The epithelial cells were activated, and they exhibited extensive or universal effacement or fusion of their foot processes, associated with prominent microvilli formation (**Figure 1K**, **1L**). In patient 2, seven glomeruli were examined by light microscopy. Of these, one was obsolescent and the other six showed segmental sclerosis. There was significant inflammatory cell infiltration, foam cells aggregates and interstitial fibrosis, accounting for about 30% of the lesion. IF and EM features were similar to patient 1 (**Figure 1I**, **1M**, **1N**).

Sequencing of *COL4A3, COL4A4*, and *COL4A5* genes in the index patients and their family members was performed using the chip capture high-throughput sequencing method. No known pathogenic mutations or suspected pathogenic mutations were found in any of the tested subjects by any of the genetic tests. The frequency of the site in each database, the principal clinical description of the subject combined with the morbidity of the disease, and the following clinically meaningful mutations were detected (**Table 1** and **Figure 1O**, **1P**). (1) *COL4A4*; NM_000092; c.1423 G>T; p.Gly475Cys; EX21; CDS20: Missense mutation. This variation has not been reported in the literature. In the gnomAD database, the frequency is 0, the SIFT prediction result is 0 deleterious, and the PolyPhen prediction result is 0.987 SIFT and PolyPhen were used to predict the protein function. The effect is harmful, and the rate of occurrence in the normal population was extremely low. Comprehensive analysis indicated unknown clinical significance, and pathogenicity cannot be excluded. (2) *COL4A4*; NM_000092; c.735 G>A; p.Pro245Pro|p.P245P; EX12; CDS11: synonymous mutations. This variation also has been reported in the literature. In the gnomAD database, the frequency is extremely low (0.000007217). Because they are synonymous mutations, SIFT and PolyPhen could not be used to predict the deleterious effects of any specific amino acid substitution. This mutation was located in the last base of exon 12 of the *COL4A4* gene, thus this variation could affect the structure and function of proteins by affecting the structure of mRNA. The predicted most probable result of this mutation was interference with splicing with HSF. Comprehensive analysis indicated clinically unknown significance, and unknown variation, and pathogenicity could not be excluded.

Both patients 1 and 2 had two heterozygous and clinically unknown mutations in the *COL-*

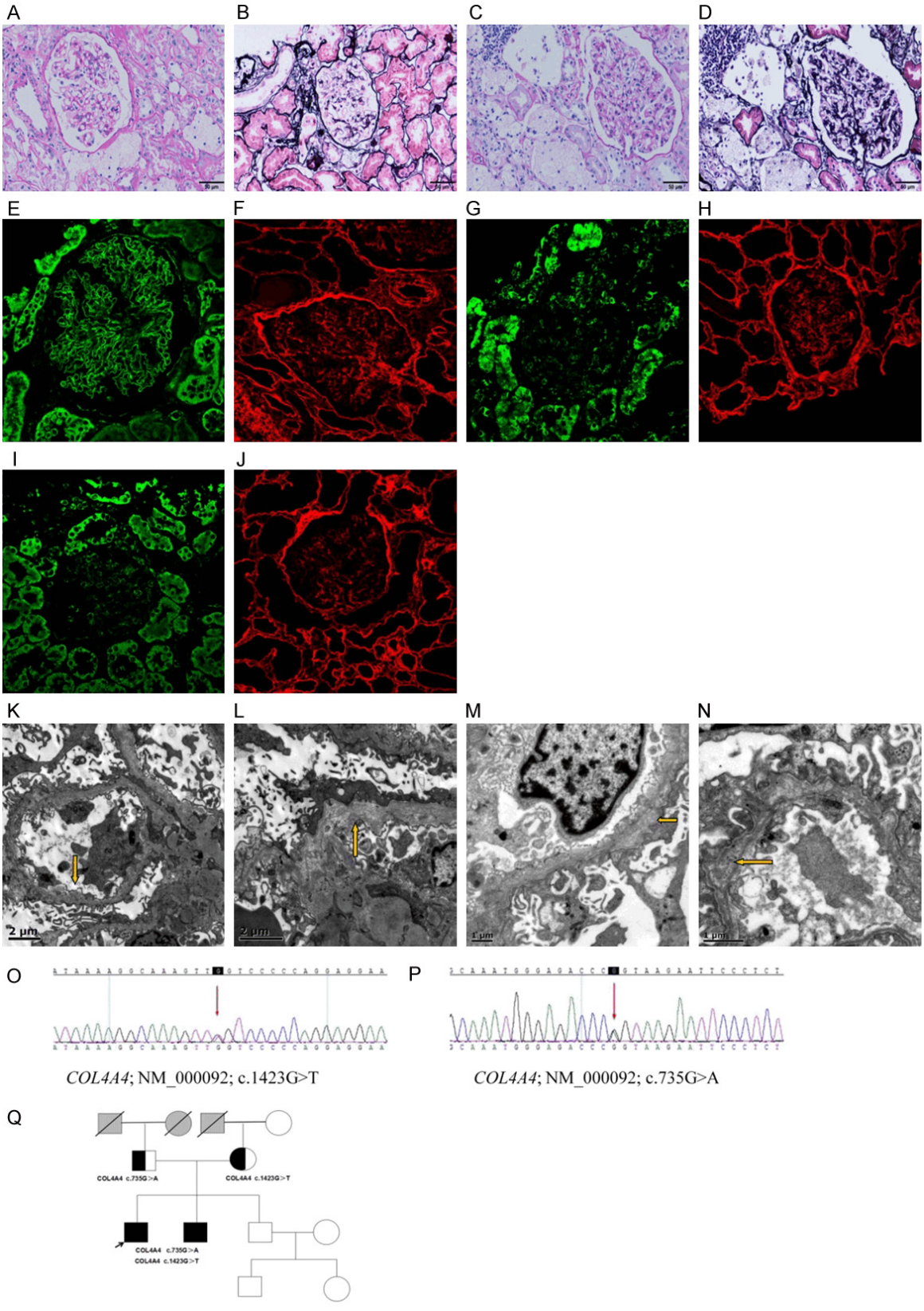

Figure 1. Light microscopy of renal biopsy in patient 1 (A, B) and patient 2 (C, D). Periodic acid-Schiff staining (A) shows almost normal glomerular histology with foamy changes of tubular cells. Periodic acid-silver methenamine staining (B) shows weakened staining in GBM and mesangial matrix. Patient 2 showed almost the same staining

pattern as patient 1 but increased inflammatory cell infiltration (C, D). (E and F) shows a normal pattern of the alpha 4 chain (green) and the alpha 1/2/3/4/5/6 chain (red) of collagen IV in the GBM. (G, H) Patient 1 shows a segmental weakened staining in the GBM of the alpha 4 chain of collagen IV in the GBM and the absence of staining of Bowman's capsule but a normal pattern of alpha 1/2/3/4/5/6 chain (red) in patient 1. (I, J) Patient 2 has the same pattern as patient 1 in immunofluorescent staining of alpha 4 chain (green) and alpha 1/2/3/4/5/6 chain (red). Electron microscopy (EM) demonstrated a markedly irregular subendothelial GBM surface with splitting and multilamellation ("basket waving") in patient 1 (K), and the epithelial cells are activated and exhibit extensive or universal effacement or fusion of their foot processes, associated with prominent microvillous formation (L). Patient 2 showed the characteristic features of GBM outpouching (M) and "bread crumbs" (N). No obvious electron-dense deposits were observed. Both patients showed the same sequencing results, with c.1423 G>T and c.735 G>A mutation of the COL4A4 gene. (O) c.1423 G>T mutation at 21st exon of COL4A4 gene. (P) c.735 G>A mutation at 12th exon of the COL4A4 gene. (Q) Pedigree analysis confirmed that these two mutations originated from their parents, and it could be concluded that these two mutations were likely to constitute the etiology of the patients.

Table 1. Site details and detection result

Gene	Reference sequence	Nucleotide changes	Amino acid change	Gene subregion	Heterozygosity	Chromosomal position	Variation type
COL4A4	NM_000092	c.1423 G>T	p.Gly475Cys	EX21/CDS20	Het	chr2:227954620	VUS
COL4A4	NM_000092	c.735 G>A	p.Pro245Pro	EX12/CDS11	Het	chr2:227973297	VUS

4A4 gene. The patient's father and mother each carried one mutation site, c.735 G>A (p.Pro245Pro) and c.1423 G>T (p.Gly475Cys), respectively. Patient 1's younger brother is married, has one son and one daughter, and showed no genetic mutations upon testing. Pedigree analysis (**Figure 1Q**) confirmed that these two mutations originated from patient 1's parents. Combined with the patient's phenotype analysis, it could be concluded that these two mutations were likely pathogenic.

Discussion

NGS is an efficient and appropriate form of genetic testing for Alport syndrome [7]. In the current cases, two heterozygous variants, c.1423 G>T (p.Gly475Cys) and c.735 G>A (p.Pro245Pro), were detected in the coding region of the *COL4A4* gene related to Alport syndrome. c.1423 G>T is a missense mutation of the *COL4A4* gene, resulting in the conversion of glycine to cysteine residue at the 475th amino acid residue. c.735 G>A is a synonymous mutation of the *COL4 A4* gene located near the shear site. Human splice finder software predicts that the variation will affect shearing of mRNA. Both mutations have not been reported in the literature, and were not found in the normal population genome sequencing database (thousands of Asian databases, the ESP6500 database and Huada internal database). In general, the nucleotide locus and the base sites were generally conserved in vertebrates. As Alport syndrome is an autosomal recessive genetic disease related to COL4A4 gene, homozygous or compound heterozygous pathogenic mutations in alleles may lead to disease. Two heterozygous mutations of the COL4A4 gene detected in the patients were derived from their parents and formed a compound heterozygote. It was possible to infer a patient's etiology from the genetic pattern. Thus, it is seen that a variation of c.1423 G>T (p.Gly475Cys) and c.735 G>A (p.Pro245Pro) of the *COL4A4* gene may be related to the prevalence of the disease.

One of the special clinical manifestations of the gene mutation that occurred in both patients was that they all had hearing abnormalities and no visual abnormalities, which is different from other Alport syndromes. It may also be a unique clinical manifestation of the new mutation site's effect on the function of the COL4A4 gene. Unlike other reported cases, both our patients were also carriers of the hepatitis B virus, while their parents were not. It was not clear whether there was a link between the presence of the virus and the onset of the disease. ACEI is effective in controlling proteinuria in Alport syndrome patients [8]. A large, study of 79 cases showed early, long-term ACEI and ARB treatments in children with AS to be effective and well-tolerated [9]. Currently, COL4A4 heterozygous Alport syndrome patients with proteinuria are candidates for angiotensin blockade [10, 11], thus early and accurate diagnosis and monitoring is crucial [12]. The treatment efficacies for the current two patients were also different. Patient 1 underwent ACEI

treatment, and had normal renal function. Patient 2 declined the ACEI regimen and developed increased creatinine level and significantly impaired hearing.

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Disclosure of conflict of interest

None.

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