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Sporadic Case of Heterozygous X-Linked Alport Syndrome

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Abstract

Alport syndrome is a genetically and phenotypically heterogeneous disorder that can be transmitted in an X-linked, autosomal recessive, or autosomal dominant fashion and can affect glomerular, cochlear, and ocular basement membranes. The disorder results from mutations in the collagen IV genes *COL4A5* (X chromosome), *COL4A3*, and *COL4A4*. Alport patients are at lifetime risk for kidney failure, sensorineural deafness, and ocular abnormalities. Males with Alport syndrome typically present with severe phenotype with progression to end-stage kidney disease and/or sensorineural deafness and eye changes. Females generally having less severe presentation and diagnosis of X-linked Alport syndrome are generally not considered. Here, we report a case of a 3-year-old girl with gross hematuria, proteinuria, and chronic kidney disease who was found to have features of Alport syndrome on kidney biopsy and a sporadic heterozygous pathogenic *COL4A5* deletion on molecular testing. This case report emphasizes the importance of kidney biopsy and molecular testing in the work up of pediatric patients with hematuria, proteinuria, and/or chronic kidney disease. It is also a poignant illustration that females with heterozygous X-linked *COL4A5* mutations are often affected patients. It further illustrates the phenomenon of sporadic occurrence of genetic kidney disease in the absence of family history of kidney disease.

Case Presentation

A 3-year-old girl with no significant past medical history was referred to UCLA Children's Health Center for further evaluation of tea color urine for the prior 2 months. She was initially evaluated at her pediatrician office, and urinalysis revealed 3+ hematuria and proteinuria (random urine protein creatinine ratio around 0.6 mg/mg creatinine). Vital signs included normal blood pressure of 97/65 mm Hg, pulse of 110 beats/min, BMI of 14.94 kg/m^2 (63rd percentile for age). A physical examination was unremarkable. Her laboratory workup demonstrated normal kidney function (estimated glomerular filtration rate 113 mL/min/1.73 m^2) [1]. Urinalysis showed occult blood (3+), >210 RBCs/high-power field and a protein/creatinine ratio of 0.7 mg/mg creatinine. An extensive serological workup was unrevealing (Table 1). Due to concern for possible nutcracker syndrome, renal ultrasound with Doppler was obtained and it demonstrated no significant abnormalities. She had a febrile illness during the time of her work up with development of transient gross hematuria. No other history of fevers, cough, sore throat, rashes, urinary tract infections, flank pain, extremity, or facial swelling. The family history was significant for history of microscopic hematuria and gross hematuria in maternal grandmother and maternal great aunt but negative for end-stage kidney disease, hearing loss/deafness, autoimmune disease, or hypertension other than that in older age. Given concern for an underlying glomerular disease, a kidney biopsy was obtained.

Pathology Presentation

The kidney biopsy (shown in Fig. 1) was composed of cortex, corticomedullary junction, and medulla and contained 87 non-sclerotic glomeruli. Focally immature-

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Table 1. Patient laboratory findings at presentation

Hematology	
White blood cells	82,000/μL
Red blood cells	462,000 μL
Hemoglobin	13.0 g/dL
Hematocrit	38.10%
Platelets	347,000/μL
Blood chemistries and serology	
Blood urea nitrogen	15 mg/dL
Creatinine	0.25 mg/dL
Cystatin C	0.7 mg/L
Total protein	7 g/dL
Albumin	4.5 g/dL
Sodium	139 mmol/L
Potassium	4.2 mmol/L
Chloride	103 mmol/L
Calcium	9.9 mg/dL
AST	40 U/L
ALT	21 U/L
Alkaline phosphatase	228 U/L
C3	115 mg/dL
C4	21 mg/dL
ASO	<50 IU/L
ANCA screen	Negative
ANA screen	Negative
nDNA (Crithidia) Ab IFA	Negative
Myeloperoxidase antibody	<1
Proteinase 3 antibody	<1
Dnase B antibody	<95
Urinalysis	
pH	7
Color	Turbid
Specific gravity	1.017
Occult blood	3+
RBC	>210 cells/HPF
WBC	0 cells/HPF
Protein/creatinine	0.61 mg/mg creatinine

appearing glomeruli were present, and glomeruli appeared small, consistent with the patient's age. There was mild mesangial hypercellularity, and glomerular capillary loops showed slightly weak silver staining. Rare red blood cell casts were present. There was no significant interstitial fibrosis, tubular atrophy, or arterio/arteriolosclerosis. There was no significant immunofluorescence staining. Electron microscopy demonstrated diffuse glomerular basement membrane structural abnormalities including segmental subepithelial scalloping, lamina dense splitting, and basket weave type remodeling. In areas without significant remodeling, glomerular basement membranes were diffusely thin (156 nm in thickness, standard deviation 58 nm, direct measurement, and arithmetic mean [2]). Some tubular basement membranes exhibited multi laminations. There was segmental mild podocyte foot process effacement (~20%). There was increased mesangial matrix. There were no tubuloreticular inclusions or any electron dense deposits.

A diagnosis of atypical glomerular basement membranes suggestive of Alport syndrome was rendered. Subsequent molecular testing was pursued via 385 gene panel next-generation sequencing assay which included evaluation of collagen IV alpha chains 3, 4, and 5 genes (*COL4A5*, *COL4A4*, *COL4A5*). This molecular assay was positive for a heterozygous likely pathogenic variant in the *COL4A5* gene (c. 485del(p.-Gly1618Valfs*35) diagnostic of X-linked Alport syndrome. No mutations in other *COL4A* genes were identified. A repeat single-gene analysis of the *COL4A5* gene was confirmatory. This sequence change creates a premature translational stop signal (p.Gly1618Valfs*35) in the *COL4A5* gene that is expected to disrupt the last 68 amino acids of the *COL4A5* protein. This variant is not present in the gnomAD population database and has not been reported in the literature in individual affected with *COL4A5*-related conditions. This variant disrupts a region of the *COL4A5* protein in which other variant(s) (p.Cys1678Tyr) have been determined to be pathogenic [3]. Thus, the variant present in this patient was likely to be disease causing.

Treatment and Follow-Up

The patient was evaluated in audiology and found to have normal hearing screen. She was also evaluated by pediatric ophthalmologist and had normal examination except for hyperopia. Both parents underwent targeted variant testing of the *COL4A5* gene, and the results of this test showed that the pathogenic variant in the *COL4A5* gene carried by the patient was de novo and not inherited from either parent. The patient continues to follow-up in pediatric nephrology every 6 months. Her kidney function and blood pressure have remained normal with a stable urine protein/creatinine ratio. She is not currently on angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker therapy. She will continue to be followed annually in audiology and ophthalmology clinics. Her eGFR has remained stable around 124 mL/min/1.73 m^2 based on CKiD U25 GFR equation [1].

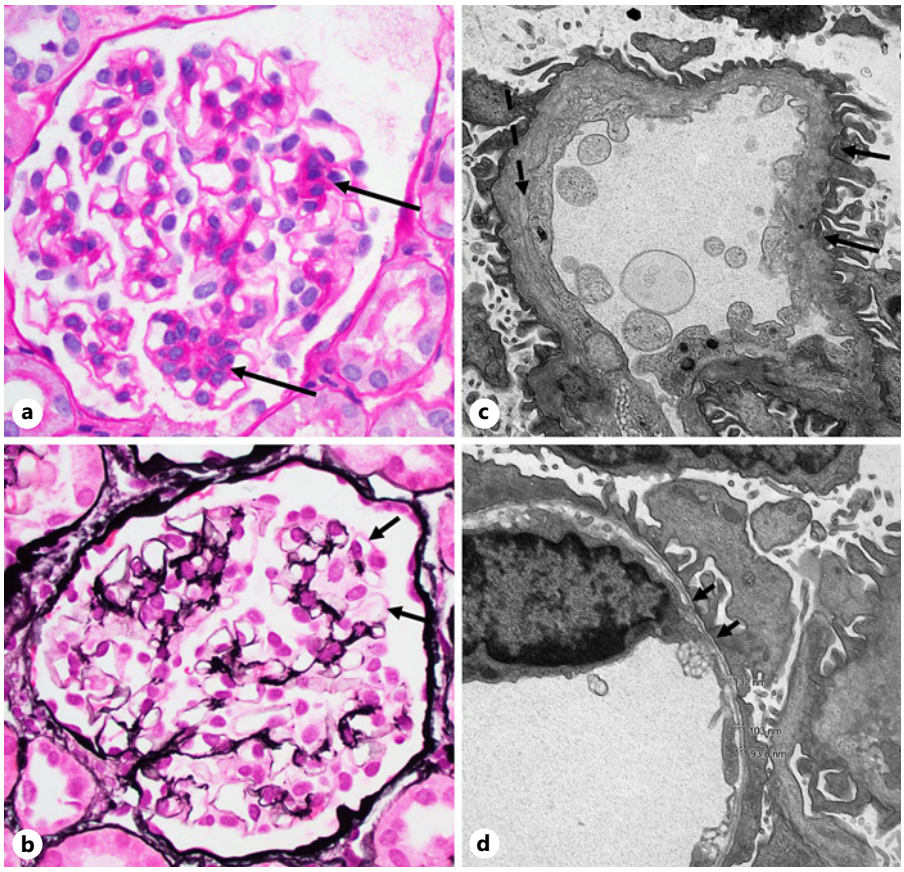

Fig. 1. Kidney biopsy findings. **a** Light microscopy demonstrated diffuse segmental mesangial hypercellularity (arrows); periodic acid Schiff stain; ×400. **b** Weakly silver-positive glomerular basement membranes (arrows) compared to strong staining of Bowman's capsule and tubular basement membranes; Jones silver stain; ×400. **c**–**d** Electron microscopy demonstrated glomerular basement membranes with atypical remodeling including subepithelial scalloping (solid arrow), lamination/splitting of the lamina densa in a basket weave appearance (dashed arrow), and areas of thinning (arrow heads); ×8,000.

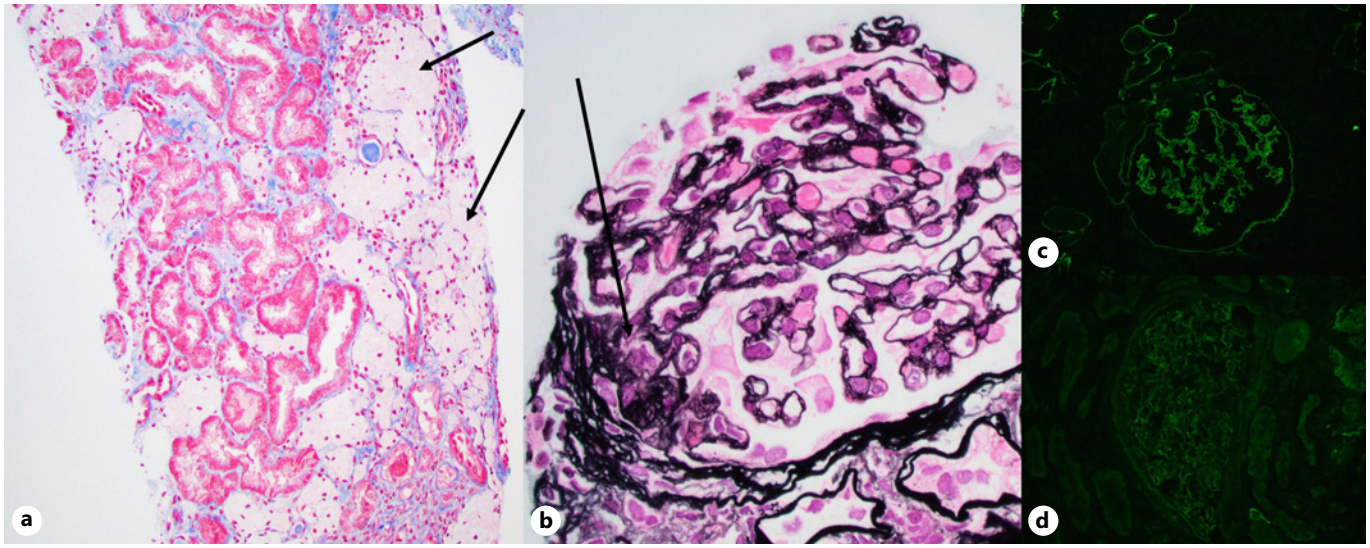

Fig. 2. Additional biopsy findings found in patients with Alport syndrome. **a** Interstitial foam cells (arrows); trichrome stain; ×200. **b** Segmental glomerulosclerosis (arrow); Jones silver stain; ×400. **c** Normal pattern of collagen IV alpha 5 chain immunofluorescence; ×400. **d** Loss of collagen IV alpha 5 chain immunofluorescence staining in glomerular capillary walls, Bowman's capsule, and distal tubules in a different case of X-linked Alport syndrome; ×400.

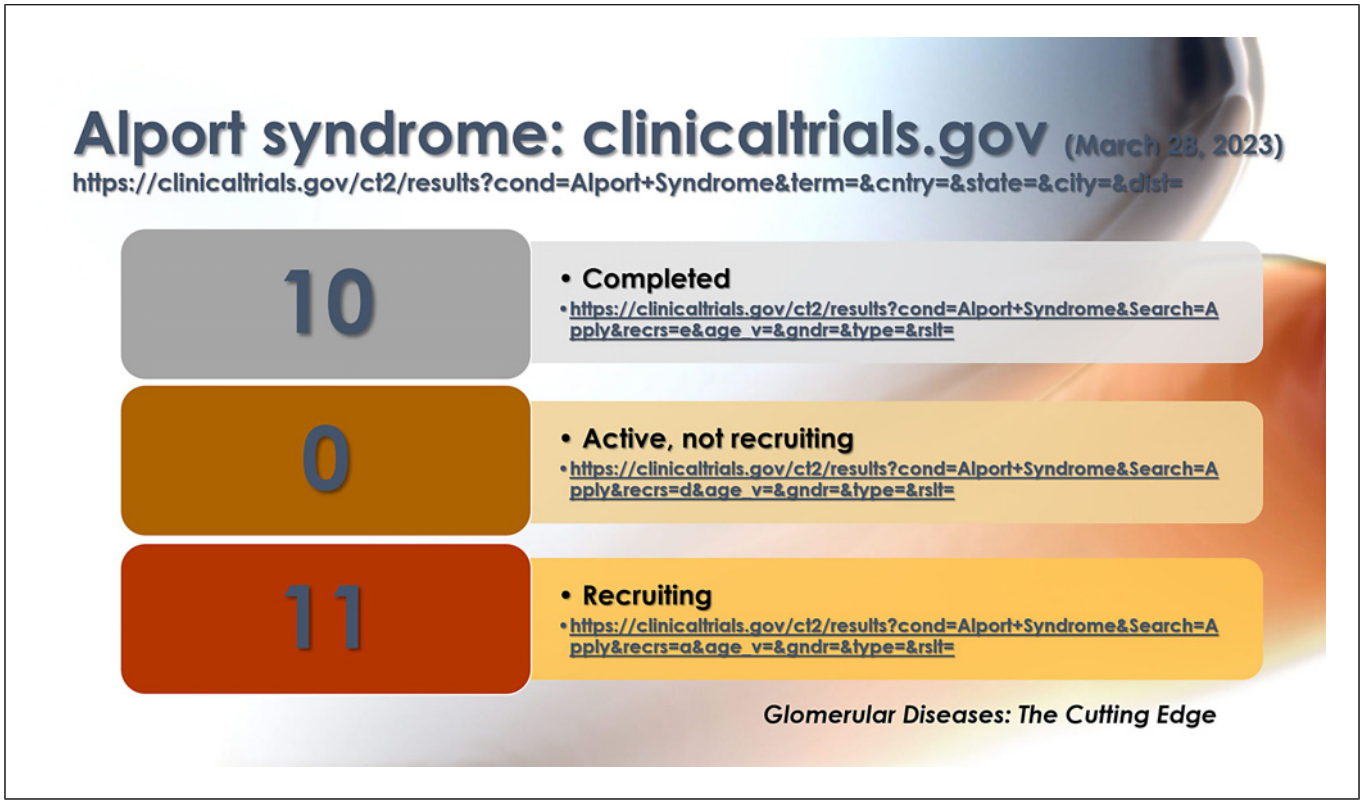

Fig. 3. Current active, enrolling, and completed clinical trials in Alport syndrome.

Conclusion

Alport syndrome is a genetically and phenotypically heterogeneous disorder that can be transmitted as an X-linked, autosomal recessive, or autosomal dominant fashion and can affect glomerular, cochlear, and ocular basement membranes. The disorder results from mutations in the collagen IV genes *COL4A5* (X chromosome), *COL4A3*, and *COL4A4*. Alport patients are at lifetime risk for kidney failure, sensorineural deafness, and ocular abnormalities [4].

The diagnosis and classification of Alport syndrome has undergone a recent paradigm shifts that recognize the heterogeneity in the genetics and presentation of this disorder [5]. This new paradigm emphasizes the role of genetically defined disease rather than specific kidney or extra-renal signs/symptoms. Important conceptual paradigm shifts include the concept that females with heterozygous variants in *COL4A5* are at risk for progressive kidney disease [6–8]. Random X inactivation (the process known as lyonization) accounts for the variable clinical course of the disease in female patients [6]. The risk of developing ESRD before the age of 40 years is 12% in girls and women versus 90% in boys and men, but the risk of progression in women appears to increase after the age of 60 years [7]. This case also illustrates that in some cases, heterozygous X-linked Alport syndrome may have a severe and early phenotype.

Similarly, patients with thin basement membrane lesion alone and any variant in *COL4A3*, *4*, or *5* genes may also be at risk for disease progression. Thus, the terms "thin basement membrane nephropathy" and "benign familial hematuria" are best avoided [9–12]. For an initial pathology report, the term "thin basement membrane lesion" or a descriptive diagnosis of "diffusely or segmentally thin glomerular basement membranes" should be used. Subsequent genetic evaluation and clinical/pathologic correlation can then be used to risk stratify and appropriate classify patients with such biopsy findings. There is controversy regarding the consensus nomenclature and classification of Alport syndrome and thin basement membrane lesions which is beyond the scope of this brief review [13].

It is also important to be aware of the limitations of genetic studies as up to 10% of cases' clinical and pathological findings' essential diagnosis for Alport syndrome may have genetic changes not identified by next-generation sequencing panels or even whole-exome

sequencing [14]. Additionally, some gene variants identified will be of uncertain significance, especially in patients whose family geographic background is not well represented in reference gene data sets [15]. Genetic counseling is also an important aspect of this work-up, especially as nephrologist may have limited experience or training in the interpretation of molecular pathology reports. Recommendations for genetic testing are beyond the scope of this manuscript; we refer the reader to this comprehensive work by Savige et al. [16] for recommendations.

This case also highlights how family history of kidney disease (or lack thereof) may be misleading in the diagnosis of Alport syndrome or other genetic kidney diseases. In this case, despite some history of familial hematuria, the patient's mutation was sporadic and not shared by either parent. Such disease-causing sporadic mutations have been characterized to occur; however, there are limited data on the frequency of such events [17, 18].

Kidney biopsy can be extremely helpful in the diagnosis of Alport syndrome, especially when the disease is renal limited (or initially renal limited) and there is no definitive family history. Kidney biopsies should include a full light, routine immunofluorescence, and electron microscopic workup. The light microscopic findings are typically non-specific (see Fig. 2) and can range from near normal morphology to extensive interstitial fibrosis/tubular atrophy and global glomerulosclerosis with or without segmental glomerulosclerosis. Mesangial widening and hypercellularity has been noted in some cases [19, 20]. Some cases show prominent interstitial foam cells. The routine immunofluorescence panel typically shows no immune complex deposits. Electron microscopic studies are heterogenous and can show thin glomerular basement membrane lesion alone or variable amounts of atypical glomerular basement membrane remodeling (e.g., lamina densa splitting, basket weave pattern lamina densa remolding, and subepithelial scalloping) [21]. The presence of any of these electron microscopic findings should trigger considerations for genetic studies.

Measurements of glomerular basement membranes should also be interpreted in the context of the age and sex of the patient and normal reference range for the renal pathology laboratory. We recommend using direct measurement of glomerular basement membrane thickness – distance from endothelial to podocyte plasma membrane; 10 peripheral capillary loops and at least 30 measurements – and determination of the arithmetic mean of such measurements as it is straightforward and convenient with modern digital cameras [2]. Furthermore, it avoids pitfalls of measuring tangential-sectioned areas or other processing artifacts which may skew the orthogonal intercept/mean harmonic thickness method. Measurement using simple direct measurement produces thinner measurements than the orthogonal intercept/mean harmonic thickness method.

Immunofluorescence studies to demonstrate loss of the normal collagen IV alpha 5 and alpha 3 staining pattern can also be helpful in diagnosis and provide prognostic information, however, may be equivocal or normal in a high percentage of cases [22]. Typically, we defer directly to genetic studies with any suspicious biopsy findings as this will generally provide a definitive diagnosis with the most prognostic information. Furthermore, genetic studies also are important to rule out the rare biopsy mimics of Alport syndrome including, *LMX1B*, *LAMB2*, and *WT-1* mutations [21].

The progression of Alport nephropathy follows a consistent pattern including isolated hematuria with subsequent mild then severe proteinuria and decline in glomerular filtration rate [23]. There is marked heterogeneity of progression rate among patients influenced predominantly by *COL4A* genotype and sex [7]. Affected male patients with X-linked *COL4A5* mutation have a very high probability of developing end-stage kidney disease by 30 years of age [7]. The risk of developing hearing defects and ocular changes like lenticonus is also dependent of *COL4A* genotype and deletion of gene. Hearing and ocular defects are more common with deletion and missense mutations in *COL4A5* genes in males with X-linked Alport syndrome [24].

The current goals of therapy are to halt or decrease the rate of disease progression. The mainstay of therapy is ACE inhibitor treatment, optimally initiated prior to any GFR decline and in all patients with overt proteinuria [14, 25, 26]. Initiation of therapy can be tailored to patient sex and severity of mutation in cases of mild to moderate proteinuria. There are currently no KDIGO guidelines for Alport syndrome treatment. Clinical trials are underway to understand the role of ACE inhibitor therapy with mild to moderate proteinuria or hematuria alone. A variety of clinical trials of novel therapies, therapeutic strategies, and biomarker discover for Alport disease are underway (Fig. 3). Kidney transplant is a treatment often pursued in patients who develop end-stage kidney disease from Alport syndrome. Unfortunately, it is not often definitive, especially in young people given the high risk of allograft loss over decades.

Statement of Ethics

Written informed consent was obtained from the parent/legal guardian of the patient for publication of the details of their medical case and any accompanying images. IRB approval is not required for this study based on UCLA guidelines.

Conflict of Interest Statement

J.E.Z. has been a paid consultant for Leica Biosystem and PathAI as well as an Editorial Board Member of PathologyOutlines.com.

Author Contributions

J.E.Z. and R.S. wrote the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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References

1 Pierce CB, Muñoz A, Ng DK, Warady BA, Furth SL, Schwartz GJ. Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease. Kidney Int. 2021;99(4):948–56.
2 Haas M. Alport syndrome and thin glomerular basement membrane nephropathy: a practical approach to diagnosis. Arch Pathol Lab Med. 2009;133(2):224–32.
3 Morinière V, Dahan K, Hilbert P, Lison M, Lebbah S, Topa A, et al. Improving mutation screening in familial hematuric nephropathies through next generation sequencing. J Am Soc Nephrol. 2014;25(12):2740–51.
4 Warady BA, Agarwal R, Bangalore S, Chapman A, Levin A, Stenvinkel P, et al. Alport syndrome classification and management. Kidney Med. 2020;2(5):639–49.
5 Kashtan CE, Ding J, Garosi G, Heidet L, Massella L, Nakanishi K, et al. Alport syndrome: a unified classification of genetic disorders of collagen IV α345: a position paper of the Alport Syndrome Classification Working Group. Kidney Int. 2018;93(5):1045–51.
6 Savige J, Colville D, Rheault M, Gear S, Lennon R, Lagas S, et al. Alport syndrome in women and girls. Clin J Am Soc Nephrol. 2016;11(9):1713–20.
7 Jais JP, Knebelmann B, Giatras I, De Marchi M, Rizzoni G, Renieri A, et al. X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a "European community Alport syndrome concerted action" study. J Am Soc Nephrol. 2003;14(10):2603–10.
8 Rheault MN. Women and Alport syndrome. Pediatr Nephrol. 2012;27(1):41–6.
9 Savige J. Heterozygous pathogenic COL4A3 and COL4A4 variants (autosomal dominant Alport syndrome) are common, and not typically associated with end-stage kidney failure, hearing loss, or ocular abnormalities. Kidney Int Rep. 2022;7(9):1933–8.
10 Savige J, Harraka P. Pathogenic variants in the genes affected in Alport syndrome (COL4A3–COL4A5) and their association with other kidney conditions: a review. Am J Kidney Dis. 2021;78(6):857–64.
11 Deltas C, Savva I, Voskarides K, Papazachariou L, Pierides A. Carriers of autosomal recessive Alport syndrome with thin basement membrane nephropathy presenting as focal segmental glomerulosclerosis in later life. Nephron. 2015;130(4):271–80.
12 Gast C, Pengelly RJ, Lyon M, Bunyan DJ, Seaby EG, Graham N, et al. Collagen (COL4A) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. Nephrol Dial Transplant. 2016;31(6):961–70.
13 Savige J. Should we diagnose autosomal dominant Alport syndrome when there is a pathogenic heterozygous COL4A3 or COL4A4 variant? Kidney Int Rep. 2018;3(6):1239–41.
14 Kashtan CE. Alport syndrome: achieving early diagnosis and treatment. Am J Kidney Dis. 2021;77(2):272–9.
15 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. 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(5):405–24.
16 Savige J, Lipska-Zietkiewicz BS, Watson E, Hertz JM, Deltas C, Mari F, et al. Guidelines for genetic testing and management of Alport syndrome. Clin J Am Soc Nephrol. 2022;17(1):143–54.
17 Ermisch B, Gross O, Netzer KO, Weber M, Brandis M, Zimmerhackl LB. Sporadic case of X-chromosomal Alport syndrome in a consanguineous family. Pediatr Nephrol. 2000;14(8–9):758–61.
18 Gribouval O, Boyer O, Hummel A, Dantal J, Martinez F, Sberro-Soussan R, et al. Identification of genetic causes for sporadic steroid-resistant nephrotic syndrome in adults. Kidney Int. 2018;94(5):1013–22.
19 Heidet L, Gubler MC. The renal lesions of Alport syndrome. J Am Soc Nephrol. 2009;20(6):1210–5.
20 Barua M, John R, Stella L, Li W, Roslin NM, Sharif B, et al. X-linked glomerulopathy due to COL4A5 founder variant. Am J Kidney Dis. 2018;71(3):441–5.
21 Lusco MA, Fogo AB. Hereditary nephritis and thin glomerular basement membrane lesion. Glomerular Dis. 2021. 1(3):135–144.
22 Yoshioka K, Hino S, Takemura T, Maki S, Wieslander J, Takekoshi Y, et al. Type IV collagen alpha 5 chain. Normal distribution and abnormalities in X-linked Alport syndrome revealed by monoclonal antibody. Am J Pathol. 1994;144:986–96.
23 Kashtan C. Long-term management of Alport syndrome in pediatric patients. Pediatr Health Med Ther. 2013;41:41.
24 Kashtan CE. Alport syndromes: phenotypic heterogeneity of progressive hereditary nephritis. Pediatr Nephrol. 2000;14(6):502–12.
25 Gross O, Beirowski B, Koepke M-L, Kuck J, Reiner M, Addicks K, et al. Preemptive ramipril therapy delays renal failure and reduces renal fibrosis in COL4A3-knockout mice with Alport syndrome. Kidney Int. 2003;63(2):438–46.
26 Gross O, Licht C, Anders HJ, Hoppe B, Beck B, Tönshoff B, et al. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. Kidney Int. 2012;81(5):494–501.