

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Savige, J

Title:

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

Date:

2022-09-01

Citation:

Savige, J. (2022). 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 International Reports*, 7 (9), pp.1933-1938. <https://doi.org/10.1016/j.ekir.2022.06.001>.

Persistent Link:

<https://hdl.handle.net/11343/316746>

License:

CC BY-NC-ND

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



Judy Savige¹

¹Department of Medicine (Melbourne Health and Northern Health), Royal Melbourne Hospital, The University of Melbourne, Parkville, Victoria, Australia

The term “autosomal dominant (AD) Alport syndrome” is often used to describe the condition associated with heterozygous pathogenic *COL4A3* or *COL4A4* variants and has largely replaced “thin basement membrane nephropathy (TBMN).” AD Alport syndrome implies that affected individuals develop end-stage kidney failure (ESKF) as well as the typical Alport hearing loss and ocular abnormalities, but these features have been considered rare with TBMN.

Recent studies suggest that ESKF occurs in 14% to 30% of those with heterozygous pathogenic *COL4A3* or *COL4A4* variants but confirm that the hearing loss and ocular defects occur uncommonly if at all. Uncertainty over the risk of ESKF has persisted.

However all the cited studies of heterozygous pathogenic *COL4A3* or *COL4A4* variants and kidney failure are from hospital-based patients and thus biased toward more severe disease. Multiple unselected cohorts with ESKF have found heterozygous pathogenic variants in *COL4A3* and *COL4A4* occur about as often as *COL4A5* variants, which suggests that AD Alport syndrome causes ESKF as often as X-linked (XL) disease. In the normal population, heterozygous pathogenic *COL4A3* and *COL4A4* variants are present 20 times more often than *COL4A5* variants. Therefore, AD Alport syndrome is complicated by ESKF 20 times *less* often than XL disease and occurs in fewer than 3% of those with pathogenic *COL4A3* or *COL4A4* variants by the age of 60.

Nevertheless, individuals with heterozygous pathogenic *COL4A3* or *COL4A4* variants referred to a hospital are still more likely to develop impaired kidney function than those who remain at home undiagnosed.

Kidney Int Rep (2022) 7, 1933–1938; <https://doi.org/10.1016/j.ekir.2022.06.001>

KEYWORDS: AD Alport syndrome; Alport syndrome; *COL4A3*; *COL4A4*; *COL4A5*; kidney failure risk

© 2022 International Society of Nephrology. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Alport syndrome is an inherited disease characterized by hematuria, progressive kidney failure, hearing loss, and ocular abnormalities.¹ The use of the term AD Alport syndrome has been widely adopted for individuals with heterozygous pathogenic *COL4A3* or *COL4A4* variants.² However, it implies these individuals will develop ESKF, as well as the extra-renal features of hearing loss and ocular abnormalities. These risks have become a major concern for patients, clinicians, and genetic testing laboratories.

The risk of ESKF is especially important because AD Alport syndrome is very common. The new genetic classification that categorizes all heterozygous *COL4A3* or *COL4A4* variants as AD Alport syndrome means that this condition occurs in 1% of the population.² The previously-used diagnosis, TBMN was estimated to affect 1%,³ and examination of gnomAD have confirmed heterozygous pathogenic *COL4A3* or *COL4A4* variants in 1% of otherwise normal individuals.⁴

Much more is known about the clinical features of TBMN diagnosed on the basis of haematuria or a kidney biopsy than is known for AD Alport syndrome. ESKF was considered uncommon in many studies of TBMN.^{5–10} The hearing loss did not occur¹¹ and the ocular abnormalities such as lenticonus and central fleck retinopathy were not present.¹² Temporal retinal thinning also was not found.^{13,14} These observations were however made before widespread genetic testing

Correspondence: Judy Savige, Department of Medicine (Melbourne Health and Northern Health), The University of Melbourne, Parkville, Victoria 3050, Australia. E-mail: j.savige@unimelb.edu.au

Received 21 March 2022; revised 3 May 2022; accepted 1 June 2022; published online 7 June 2022

Table 1. Relative numbers of pathogenic *COL4A5* and *COL4A3/COL4A4* variants in sequenced cohorts with kidney failure

Cohort	Pathogenic variants detected	Heterozygous <i>COL4A3/ COL4A4</i> variant	X-linked Alport syndrome (<i>COL4A5</i> variants)	<i>COL4A3/ COL4A4:COL4A5</i>	Reference
Transplant series, 1972–2014, excluding 32, where no variant found (<i>n</i> = 73)	<i>n</i> = 73	<i>n</i> = 15	<i>n</i> = 57	15:57 = 0.3	Gillion <i>et al.</i> ¹⁹
CKD, awaiting transplant not considered genetic (<i>n</i> = 57)	<i>n</i> = 6	<i>n</i> = 1	<i>n</i> = 2	1:2 = 0.5	Ottlewski, 2018 ²⁰
ESKF with glomerular disease (Supplementary Table S3)	<i>n</i> = 21	<i>n</i> = 8	<i>n</i> = 3	8:3 = 2.7	Bullich <i>et al.</i> ²¹
CKD including unknown cause, known familial disease or hypertension (<i>n</i> = 92)	<i>n</i> = 22	<i>n</i> = 1	<i>n</i> = 5	1:5 = 0.2	Lata <i>et al.</i> ²²
CKD (<i>n</i> = 3037 and 2144 with CKD)	<i>n</i> = 307	<i>n</i> = 27 + 21 = 48	<i>n</i> = 44	48:44 = 1.1	Groopman <i>et al.</i> ²³
CKD in pediatric kidney transplant recipients (<i>n</i> = 104)	<i>n</i> = 34	<i>n</i> = 0	<i>n</i> = 1	0:1	Mann <i>et al.</i> ²⁴
CKD in adults from families, or with extra-renal features, or 20 with neither (<i>n</i> = 114)	<i>n</i> = 42	<i>n</i> = 2	<i>n</i> = 5	2:5 = 0.4	Connaughton <i>et al.</i> ²⁵

CKD, chronic kidney disease; ESKF, end-stage kidney failure.

and where the causative pathogenic *COL4A3* or *COL4A4* variant was not confirmed nor a *COL4A5* variant excluded. These cohorts may have also included some individuals with glomerular basement membrane (GBM) thinning from other causes.³

Several recent studies have helped to clarify the risks of hearing loss and ocular defects with pathogenic heterozygous *COL4A3* or *COL4A4* variants. A systematic review of 777 affected individuals from 258 families in 48 publications found a hearing loss in 101 (16%) and ocular abnormalities in 16 (3%).¹⁵ Data from a further single center cohort with 240 individuals from 78 families confirmed the typical hearing loss in 11 patients (8%) and ocular features in 2 (1%).¹⁶ Therefore, both the Alport-specific hearing loss and ocular abnormalities are uncommon with heterozygous pathogenic *COL4A3* or *COL4A4* variants. They could result, in the case of the hearing loss, from another cause and some of the ocular abnormalities included cataracts which are not typical of Alport syndrome and were probably coincidental. These other features may have resulted from an undetected second *COL4A3* or *COL4A4* variant, and a likely diagnosis of autosomal recessive disease.

Nevertheless, these same studies further suggested that 199 of 691 (29%) and 61 of 240 (24%) individuals with AD Alport syndrome developed ESKF.^{15,16} There are other reports of 14% to 20% of cohorts of *COL4A3* and *COL4A4* heterozygotes with kidney failure.^{16–18} However, it has become apparent that AD and even XL Alport syndrome are underdiagnosed in the community⁴ and these same studies have all been hospital-based series, which were likely to be biased toward more severe disease.

We now have the ability to determine the risk of ESKF in unbiased series of patients with AD Alport

syndrome. This can be achieved by considering whether the ratio of *COL4A3/COL4A4* to *COL4A5* variants is the same in hospital series of kidney failure as in the normal population.

Many recently-published studies have examined the relative frequency of pathogenic *COL4A3/COL4A4* and *COL4A5* variants in hospital-based, mainly adult cohorts with inherited or sporadic kidney failure who have undergone massively parallel DNA sequencing. Most studies have found that heterozygous pathogenic *COL4A3* and *COL4A4* variants occur about as often as pathogenic *COL4A5* variants (median 0.4, range 0.1–2.7) (Table 1^{19–25}).

Is this consistent with the relative frequency of *COL4A3/COL4A4* and *COL4A5* variants in the normal population? We recently published the relative proportions of predicted pathogenic *COL4A3/ COL4A4* and *COL4A5* variants in the gnomAD database, an unselected dataset of participants without known genetic kidney disease.⁴ Predicted pathogenic variants were chosen to include truncating and splicing variants, and missense variants that affected critical amino acids, typically position 1 glycine (Gly) residues, in the collagen IV α chains. The accuracy of this approach was confirmed in a normal control subset (gnomAD), independent variant datasets (Exome Variant Server and TOPMed), and by comparison with the known frequency of TBMN in normal donor kidney biopsies.⁵

This analysis predicted heterozygous pathogenic *COL4A3* or *COL4A4* variants occurred in about 1 in 106 of the population and pathogenic *COL4A5* variants in 1 in 2300.⁴ Thus, pathogenic *COL4A3* and *COL4A4* variants were found about 20 times as often as pathogenic *COL4A5* variants in the normal

population, and if each of these is disease-causing, then AD Alport syndrome occurs about 20 times as often as XL Alport syndrome.

If pathogenic *COL4A3* and *COL4A4* variants were just as likely to cause ESKF as *COL4A5* variants, they would then be present 20 times as often in cohorts with kidney failure. However, they occur approximately as often, which means that heterozygous pathogenic variants in *COL4A3* and *COL4A4* are 20 times *less* likely to cause ESKF than *COL4A5* variants.

How Often Pathogenic *COL4A5* Variants Result in ESKF in XL Alport Syndrome

Not everyone with a pathogenic *COL4A5* variant develops ESKF. Some males have hypomorphic variants that are associated with late-onset disease or impaired kidney function only. Such variants are recognized increasingly.²⁶ In addition, women have XL Alport syndrome twice as often as men because of their 2 X-chromosomes but are less likely to develop kidney failure.²⁷ Nevertheless, the strategy that we used to determine the relative proportions of *COL4A3* and *COL4A4* to *COL4A5* variants only examined more severe variants, namely truncating and splicing variants, and position 1 Gly substitutions in the collagenous domain.⁴ It did not consider non-Gly substitutions which probably have milder consequences.⁴ This same strategy was used for all the genes, *COL4A3*, *COL4A4*, and *COL4A5*.

Our current understanding is that 90% of men with pathogenic *COL4A5* variants develop kidney failure by the age of 40 years and that this is still about 90% at the age of 60.^{28,29} However two-thirds of the population with pathogenic *COL4A5* variants are female, and only 20% of women with a *COL4A5* variant have kidney failure by 60 years.⁹ The risk of a person with a pathogenic *COL4A5* variant developing ESKF by 60 years of age can be calculated as follows.

Because one-third of people with a pathogenic *COL4A5* variant are men with a 90% risk of kidney failure by the age of 60, the overall contribution from men to kidney failure risk is $1/3 \times 90\%$ (30%). Similarly, the risk from women with a pathogenic *COL4A5* variant is $2/3 \times 20\%$ (13%). Therefore, the risk of a person with a pathogenic *COL4A5* variant developing ESKF by the age of 60 is the sum of these risks, or 43%.

The risk of kidney failure by 60 from pathogenic *COL4A3* or *COL4A4* variants is about one-twentieth that from a *COL4A5* variant or $1/20 \times 43\%$, which is 2%. Therefore, the risk of ESKF by the age of 60 years from pathogenic *COL4A3* or *COL4A4* variants is <3%, and slightly >3% by the age of 80.

Caveats

There are caveats to these estimates. Sometimes, heterozygous pathogenic *COL4A3* or *COL4A4* variants result in ESKF. The cohorts with kidney failure cited here probably included more individuals with recognisably “typical” Alport features who were more likely to have XL disease and undergo genetic testing. This would have resulted in a relative increase in the number of people with pathogenic *COL4A5* variants and fewer with heterozygous *COL4A3* or *COL4A4* variants. Conversely, hypomorphic or milder *COL4A5* variants are now recognized more often and calculations based on 20-year-old data may have overestimated the corresponding ESKF risk.^{28,29} Recent analyses suggest that pathogenic *COL4A5* variants from the last 5 years are milder and associated with a later kidney failure onset.

Risk of Impaired Kidney Function but Not Kidney Failure

While these calculations may be correct for the likelihood of heterozygous pathogenic *COL4A3* or *COL4A4* variants causing ESKF, they do not consider the risks of lesser degrees of impaired kidney function and its comorbidities.

About half of all the heterozygous variants in the *COL4A3* and *COL4A4* genes are severe and have an increased risk of proteinuria, which itself represents a risk for impaired kidney function and kidney failure.²⁸ Comorbidities include cardiovascular disease and hospitalizations.^{30–32} Individuals with pathogenic heterozygous *COL4A3* or *COL4A4* variants may develop proteinuria, hypertension, and kidney impairment and must still be identified and monitored.³³ Indeed, individuals with a heterozygous *COL4A3* or *COL4A4* variant who are referred to the renal clinic for a specialist medical opinion may be those at greatest risk of ESKF.

Explanation of the Reduced Risk of ESKF and Extra-renal Features With Pathogenic Heterozygous *COL4A3* and *COL4A4* Variants

The *COL4A3*, *COL4A4*, and *COL4A5* genes code for the collagen IV $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains that normally form the collagen IV $\alpha 3\alpha 4\alpha 5$ heterotrimer and network, and represent the major component of the basement membranes in the glomerulus, cochlea, and eye.³⁴

About half of all the pathogenic variants in *COL4A3* and *COL4A4* are severe (truncating variants, many splicing variants), which result in the loss of the corresponding α chains by nonsense-mediated decay.^{35,36} To date, 80% of reported missense variants are Gly substitutions and the other 20% are substitutions of non-Gly residues. More disease-

causing non-Gly substitutions are likely but have been more difficult to categorize as pathogenic because they are often hypomorphic.

Heterozygous pathogenic *COL4A3* or *COL4A4* variants usually result in about half the $\alpha3\alpha4\alpha5$ heterotrimers being abnormal.³⁷ This means that 50% of the heterotrimers are absent from affected membranes with severe variants, and 50% of heterotrimers are defective with missense variants. Nevertheless, immunohistochemistry has confirmed that there is less of all the collagen IV $\alpha3$, $\alpha4$, and $\alpha5$ chains, even though only one gene is affected. Pathogenic heterozygous variants of the *COL4A3* or *COL4A4* genes result in a thinned GBM because of the reduced amount of the collagen IV $\alpha3\alpha4\alpha5$ network.³⁸ There is a compensatory increase in the collagen IV $\alpha1\alpha1\alpha2$ heterotrimer, which is more susceptible to proteolysis,³⁹ and may not be sufficiently strong to prevent disease.

The abnormal GBM in TBMN is associated with the loss of the overlying podocytes⁴⁰ and the development of proteinuria. Podocyte loss may be the mechanism underlying focal segmental glomerulosclerosis and progressive kidney failure. In addition, a canine model of AD Alport syndrome suggests that a reduction in nephron number also contributes to impaired kidney function.⁴¹

The median age of individuals with AD Alport syndrome who developed ESKF was 67 years (95% CI, range 58–73 years) in one cohort, and the mean age was 53 years (range 21–84 years) in a review of reported cases.^{15,16} Seventy-five percent of pathogenic *COL4A5* variants in men have a consistent age at kidney failure,²⁸ which can largely be predicted from the genotype.^{28,36,42–44} This is also true for autosomal recessive Alport syndrome.⁴² Women with pathogenic *COL4A5* variants demonstrate a less consistent genotype-phenotype correlation, which has been attributed to lyonization.²⁹ The variation in age at kidney failure for pathogenic heterozygous *COL4A3* or *COL4A4* variants suggests a smaller genotype effect and that other determinants are important. Some evidence now reveals that variant severity correlates with proteinuria, which is itself a risk factor for disease progression and kidney failure. Poorly controlled hypertension and coincidental diabetes, obesity, or acute kidney injury from another cause may contribute.⁴⁵

While pathogenic heterozygous *COL4A3* and *COL4A4* variants commonly predispose to kidney cysts these are generally too few and too small to significantly affect function.^{17,46,47} However pathogenic heterozygous variants in *COL4A3* or *COL4A4* are also associated with glomerular immune deposits, which may worsen kidney function.^{48,49} IgA glomerulonephritis and

heterozygous pathogenic variants occur together too often to be coincidental and the thinned GBM may facilitate glomerular immune complex deposition.⁵⁰

Inappropriateness of “AD Alport Syndrome” as a Diagnosis

These observations that pathogenic heterozygous *COL4A3* or *COL4A4* variants are uncommonly associated with ESKF, hearing loss, and ocular abnormalities suggest that the term “AD Alport syndrome” itself is not appropriate since ‘syndrome’ implies the presence of extra-renal features.

Why TBMN Is Also Not an Appropriate Name

For many years, TBMN was used for individuals with a pathogenic heterozygous *COL4A3* or *COL4A4* variant. This was a histologic diagnosis where the GBM width was less than the normal range determined within an individual laboratory. However not all individuals with a heterozygous pathogenic *COL4A3* or *COL4A4* variant have had a kidney biopsy, and a genetic diagnosis is more accurate and more consistent.

Renaming Alport Syndrome

Heterozygous pathogenic *COL4A3* or *COL4A4* variants are the most common finding in individuals with familial hematuria, but affected individuals are unlikely to develop ESKF, or have a hearing loss or ocular abnormalities. Therefore, there are efforts to rename the diseases referred to as “Alport syndrome.” Renaming requires consultation with patient groups, clinicians, geneticists, and testing laboratories. The new name must be accurate, informative, acceptable to patients, as well as easy to pronounce and to remember, including for those who use a language other than English. The new name must also conform with any proposed new naming system for genetic kidney diseases, and preferably include the gene name and mode of inheritance.

Population Frequencies of Different Modes of Inheritance of Alport Syndrome

A further unrelated consequence of better understanding the population frequencies of *COL4A5* and *COL4A3* or *COL4A4* variants⁴ is that, it is no longer correct that the XL disease occurs in 85% of families with Alport syndrome, and autosomal recessive disease in 15% of families.⁵¹ These frequencies were first reported 40 years ago but are still often cited.⁵¹ Because pathogenic *COL4A5* variants affect 1 in 2300 individuals and AD Alport syndrome 1 in 100, the ratio of XL to AD disease is about 1:20. Autosomal recessive inheritance and digenic disease are much rarer so that XL to AD disease affects the population in a ratio of

5:95 with autosomal recessive and digenic Alport syndrome each accounting for <1%.

Conclusion

Individuals with a heterozygous pathogenic *COL4A3* or *COL4A4* variant have only a slightly increased risk of ESKF compared with the normal population, and do not develop the typical Alport features of hearing loss or ocular abnormalities. In these regards, the diagnosis of AD Alport syndrome is widely used and understood but incorrect because ESKF and the syndromic or extra-renal features are rare. It remains nevertheless important to identify *COL4A3* or *COL4A4* heterozygotes and monitor them for the possible development of proteinuria, hypertension, and kidney impairment.³³

DISCLOSURE

The author declared no competing interests.

REFERENCES

- Gubler M, Levy M, Broyer M, et al. Alport's syndrome. A report of 58 cases and a review of the literature. *Am J Med.* 1981;70:493–505. [https://doi.org/10.1016/0002-9343\(81\)90571-4](https://doi.org/10.1016/0002-9343(81)90571-4)
- Kashtan CE, Ding J, Garosi G, et al. Alport syndrome: a unified classification of genetic disorders of collagen IV alpha345: a position paper of the Alport Syndrome Classification Working Group. *Kidney Int.* 2018;93:1045–1051. <https://doi.org/10.1016/j.kint.2017.12.018>
- Savage J, Rana K, Tonna S, et al. Thin basement membrane nephropathy. *Kidney Int.* 2003;64:1169–1178. <https://doi.org/10.1046/j.1523-1755.2003.00234.x>
- Gibson J, Fieldhouse R, Chan MMY, Sadheghi-Alavijeh O, Burnett L, Izzi V, et al. Prevalence estimates of predicted pathogenic *COL4A3* - *COL4A5* variants in a population sequencing database and their implications for Alport syndrome. *J Am Soc Nephrol.* 2021;32:2273–2290. <https://doi.org/10.1681/ASN.2020071065>
- Dische FE, Anderson VE, Keane SJ, et al. Incidence of thin membrane nephropathy: morphometric investigation of a population sample. *J Clin Pathol.* 1990;43:457–460. <https://doi.org/10.1136/jcp.43.6.457>
- Perry GJ, George CR, Field MJ, et al. Thin-membrane nephropathy—a common cause of glomerular haematuria. *Med J Aust.* 1989;151:638–642. <https://doi.org/10.5694/j.1326-5377.1989.tb139637.x>
- Nieuwhof CM, de Heer F, de Leeuw P, van Breda Vriesman PJ. Thin GBM nephropathy: premature glomerular obsolescence is associated with hypertension and late onset renal failure. *Kidney Int.* 1997;51:1596–1601. <https://doi.org/10.1038/ki.1997.219>
- Abe S, Amagasaki Y, Iyori S, et al. Thin basement membrane syndrome in adults. *J Clin Pathol.* 1987;40:318–322. <https://doi.org/10.1136/jcp.40.3.318>
- Goel S, Davenport A, Goode NP, et al. Clinical features and outcome of patients with thin and ultrathin glomerular membranes. *Q J M.* 1995;88:785–793.
- van Paassen P, van Breda Vriesman PJ, van Rie H, Tervaert JW. Signs and symptoms of thin basement membrane nephropathy: a prospective regional study on primary glomerular disease—the Limburg Renal Registry. *Kidney Int.* 2004;66:909–913. <https://doi.org/10.1111/j.1523-1755.2004.00835.x>
- Aarons I, Smith PS, Davies RA, et al. Thin membrane nephropathy: a clinico-pathological study. *Clin Nephrol.* 1989;32:151–158.
- Colville D, Savage J, Branley P, Wilson D. Ocular abnormalities in thin basement membrane disease. *Br J Ophthalmol.* 1997;81:373–377. <https://doi.org/10.1136/bjo.81.5.373>
- Chen Y, Colville D, Ierino F, et al. Temporal retinal thinning and the diagnosis of Alport syndrome and Thin basement membrane nephropathy. *Ophthalm Genet.* 2018;39:208–214. <https://doi.org/10.1080/13816810.2017.1401088>
- Savage J, Sheth S, Leys A, et al. Ocular features in Alport syndrome: pathogenesis and clinical significance. *Clin J Am Soc Nephrol.* 2015;10:703–709. <https://doi.org/10.2215/CJN.10581014>
- Matthaiou A, Poulli T, Deltas C. Prevalence of clinical, pathological and molecular features of glomerular basement membrane nephropathy caused by *COL4A3* or *COL4A4* mutations: a systematic review. *Clin Kidney J.* 2020;13:1025–1036. <https://doi.org/10.1093/ckj/sfz176>
- Furlano M, Martinez V, Pybus M, et al. Clinical and genetic features of autosomal dominant Alport syndrome: a cohort study. *Am J Kidney Dis.* 2021;78:560–570.e1. <https://doi.org/10.1053/j.ajkd.2021.02.326>
- Pierides A, Voskarides K, Athanasiou Y, et al. Clinico-pathological correlations in 127 patients in 11 large pedigrees, segregating one of three heterozygous mutations in the *COL4A3/COL4A4* genes associated with familial haematuria and significant late progression to proteinuria and chronic kidney disease from focal segmental glomerulosclerosis. *Nephrol Dial Transplant.* 2009;24:2721–2729. <https://doi.org/10.1093/ndt/gfp158>
- Voskarides K, Damianou L, Neocleous V, et al. *COL4A3/COL4A4* mutations producing focal segmental glomerulosclerosis and renal failure in thin basement membrane nephropathy. *J Am Soc Nephrol.* 2007;18:3004–3016. <https://doi.org/10.1681/ASN.2007040444>
- Gillion V, Dahan K, Cosyns JP, et al. Genotype and outcome after kidney transplantation in Alport syndrome. *Kidney Int Rep.* 2018;3:652–660. <https://doi.org/10.1016/j.ekir.2018.01.008>
- Ottlewski I, Munch J, Wagner T, et al. Value of renal gene panel diagnostics in adults waiting for kidney transplantation due to undetermined end-stage renal disease. *Kidney Int.* 2019;96:222–230. <https://doi.org/10.1016/j.kint.2019.01.038>
- Bullich G, Domingo-Gallego A, Vargas I, et al. A kidney-disease gene panel allows a comprehensive genetic diagnosis of cystic and glomerular inherited kidney diseases. *Kidney Int.* 2018;94:363–371. <https://doi.org/10.1016/j.kint.2018.02.027>
- Lata S, Marasa M, Li Y, et al. Whole-exome sequencing in adults with chronic kidney disease: A pilot study. *Ann Intern Med.* 2018;168:100–109. <https://doi.org/10.7326/M17-1319>
- Groopman EE, Marasa M, Cameron-Christie S, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl*

- J Med.* 2019;380:142–151. <https://doi.org/10.1056/NEJMoa1806891>
24. Mann N, Braun DA, Amann K, et al. Whole-exome sequencing enables a precision medicine approach for kidney transplant recipients. *J Am Soc Nephrol.* 2019;30:201–215. <https://doi.org/10.1681/ASN.2018060575>
 25. Connaughton DM, Kennedy C, Shril S, et al. Monogenic causes of chronic kidney disease in adults. *Kidney Int.* 2019;95:914–928. <https://doi.org/10.1016/j.kint.2018.10.031>
 26. Zurowska AM, Bielska O, Dacza-Roszak P, et al. Mild X-linked Alport syndrome due to the COL4A5 G624D variant originating in the Middle Ages is predominant in Central/East Europe and causes kidney failure in midlife. *Kidney Int.* 2021;99:1451–1458. <https://doi.org/10.1016/j.kint.2020.10.040>
 27. Savage J, Colville D, Rheault M, et al. Alport syndrome in women and girls. *Clin J Am Soc Nephrol.* 2016;11:1713–1720. <https://doi.org/10.2215/CJN.00580116>
 28. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history in 195 families and genotype-phenotype correlations in males. *J Am Soc Nephrol.* 2000;11:649–657. <https://doi.org/10.1681/ASN.V114649>
 29. Jais JP, Knebelmann B, Giatras I, 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;14:2603–2610. <https://doi.org/10.1097/01.asn.0000090034.71205.74>
 30. Khan SS, Kazmi WH, Abichandani R, et al. Health care utilization among patients with chronic kidney disease. *Kidney Int.* 2002;62:229–236. <https://doi.org/10.1046/j.1523-1755.2002.00432.x>
 31. Go AS, Chertow GM, Fan D, et al. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med.* 2004;351:1296–1305. <https://doi.org/10.1056/NEJMoa041031>
 32. Matsushita K, van der Velde M, Astor BC, et al. Chronic kidney disease prognosis C. *Lancet.* 2010;375:2073–2081. [https://doi.org/10.1016/S0140-6736\(10\)60674-5](https://doi.org/10.1016/S0140-6736(10)60674-5)
 33. Savage J, Storey H, Watson E, et al. 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. <https://doi.org/10.1038/s41431-021-00858-1>
 34. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport’s syndrome, Goodpasture’s syndrome, and type IV collagen. *N Engl J Med.* 2003;348:2543–2556. <https://doi.org/10.1056/NEJMra022296>
 35. Bateman JF, Boot-Handford RP, Lamande SR. Genetic diseases of connective tissues: cellular and extracellular effects of ECM mutations. *Nat Rev Genet.* 2009;10:173–183. <https://doi.org/10.1038/nrg2520>
 36. Savage J, Storey H, Il Cheong H, et al. X-linked and autosomal recessive Alport syndrome: pathogenic variant features and further genotype-phenotype correlations. *PLoS One.* 2016;11:e0161802. <https://doi.org/10.1371/journal.pone.0161802>
 37. Mencarelli MA, Heidet L, Storey H, et al. Evidence of digenic inheritance in Alport syndrome. *J Med Genet.* 2015;52:163–174. <https://doi.org/10.1136/jmedgenet-2014-102822>
 38. Pettersson E, Tornroth T, Wieslander J. Abnormally thin glomerular basement membrane and the Goodpasture epitope. *Clin Nephrol.* 1990;33:105–109.
 39. Kalluri R, Shield CF, Todd P, et al. Isoform switching of type IV collagen is developmentally arrested in X-linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. *J Clin Invest.* 1997;99:2470–2478. <https://doi.org/10.1172/JCI119431>
 40. Wickman L, Hodgin JB, Wang SQ, et al. Podocyte depletion in thin GBM and Alport syndrome. *PLoS One.* 2016;11:e0155255. <https://doi.org/10.1371/journal.pone.0155255>
 41. Hood JC, Dowling J, Bertram JF, et al. Correlation of histopathological features and renal impairment in autosomal dominant Alport syndrome in Bull terriers. *Nephrol Dial Transplant.* 2002;17:1897–1908. <https://doi.org/10.1093/ndt/17.11.1897>
 42. Gross O, Netzer KO, Lambrecht R, et al. Meta-analysis of genotype-phenotype correlation in X-linked Alport syndrome: impact on clinical counselling. *Nephrol Dial Transplant.* 2002;17:1218–1227. <https://doi.org/10.1093/ndt/17.7.1218>
 43. Storey H, Savage J, Sivakumar V, et al. COL4A3/COL4A4 mutations and features in individuals with autosomal recessive Alport syndrome. *J Am Soc Nephrol.* 2013;24:1945–1954. <https://doi.org/10.1681/ASN.2012100985>
 44. Bekheirnia MR, Reed B, Gregory MC, et al. Genotype-phenotype correlation in X-linked Alport syndrome. *J Am Soc Nephrol.* 2010;21:876–883. <https://doi.org/10.1681/ASN.2009070784>
 45. Smith JD, Colville D, Lyttle N, et al. Increased microvascular disease in X-linked and autosomal recessive Alport syndrome: a case control cross sectional observational study. *Ophthalmol Genet.* 2019;40:129–134. <https://doi.org/10.1080/13816810.2019.1589528>
 46. Gulati A, Sevillano AM, Praga M, et al. Collagen IV gene mutations in adults with bilateral renal cysts and CKD. *Kidney Int Rep.* 2020;5:103–108. <https://doi.org/10.1016/j.ekir.2019.09.004>
 47. Sevillano AM, Gutierrez E, Morales E, et al. Multiple kidney cysts in thin basement membrane disease with proteinuria and kidney function impairment. *Clin Kidney J.* 2014;7:251–256. <https://doi.org/10.1093/ckj/sfu033>
 48. Monga G, Mazzucco G, Roccatello D. The association of IgA glomerulonephritis and thin glomerular basement membrane disease in a hematuric patient: light and electron microscopic and immunofluorescence investigation. *Am J Kidney Dis.* 1991;18:409–412. [https://doi.org/10.1016/s0272-6386\(12\)80106-5](https://doi.org/10.1016/s0272-6386(12)80106-5)
 49. Li Y, Groopman EE, D’Agati V, et al. Type IV collagen mutations in familial IgA nephropathy. *Kidney Int Rep.* 2020;5:1075–1078. <https://doi.org/10.1016/j.ekir.2020.04.011>
 50. Savage 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;78:857–864. <https://doi.org/10.1053/j.ajkd.2021.04.017>
 51. Feingold J, Bois E, Chompret A, et al. Genetic heterogeneity of Alport syndrome. *Kidney Int.* 1985;27:672–677. <https://doi.org/10.1038/ki.1985.63>