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

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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.

Keywords: AD Alport syndrome; Alport syndrome; COL4A3; COL4A4; COL4A5; kidney failure risk

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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 occur,¹¹ and the ocular abnormalities such as lenticous 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
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%).5 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 community7 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 119–25).

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.3 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.3

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.

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

| Cohort | Pathogenic variants detected | Heterozygous COL4A3/ COL4A4 variant | X-linked Alport syndrome (COL4A5 variants) | COL4A3/COL4A4:COL4A5 | Reference |
|--------|-------------------------------|-------------------------------------|------------------------------------------|-----------------------|-----------|
| Transplant series, 1972–2014, excluding 32, where no variant found (n = 73) | n = 73 | n = 15 | n = 57 | COL4A3/ COL4A4:COL4A5 | 15.57:0.3 Gillion et al.19 |
| CKD, awaiting transplant not considered genetic (n = 57) | n = 6 | n = 1 | n = 2 | COL4A3/ COL4A4:COL4A5 | 1:2:0.5 Ottlevski, 201820 |
| ESKF with glomerular disease (Supplementary Table S3) | n = 21 | n = 8 | n = 3 | COL4A3/ COL4A4:COL4A5 | 8.3:2.7 Bullich et al.21 |
| CKD including unknown cause, known familial disease or hypertension (n = 92) | n = 22 | n = 1 | n = 5 | COL4A3/ COL4A4:COL4A5 | 1.5:0.2 Lata et al.22 |
| CKD (n = 3037 and 2144 with CKD) | n = 307 | n = 27 | n = 44 | COL4A3/ COL4A4:COL4A5 | 48.44:1.1 Groopman et al.23 |
| CKD in pediatric kidney transplant recipients (n = 104) | n = 34 | n = 0 | n = 1 | COL4A3/ COL4A4:COL4A5 | 0:1 Mann et al.24 |
| CKD in adults from families, with extra-renal features, or 20 with neither (n = 114) | n = 42 | n = 2 | n = 5 | COL4A3/ COL4A4:COL4A5 | 2.5:0.4 Connaughton et al.25 |

CKD, chronic kidney disease; ESKF, end-stage kidney failure.
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. In addition, women have XL Alport kidney function only. How Often Pathogenic variants in COL4A3 and COL4A4 often, which means that heterozygous pathogenic variants in COL4A5 and COL4A4 are 20 times less likely to cause ESKF than COL4A5 variants.

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.

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. Comorbidities include cardiovascular disease and hospitalizations. Individuals with pathogenic heterozygous COL4A3 or COL4A4 variants may develop proteinuria, hypertension, and kidney impairment and must still be identified and monitored. 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 α3, α4, and α5 chains that normally form the collagen IV α3α4α5 heterotrimer and network, and represent the major component of the basement membranes in the glomerulus, cochlea, and eye.

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. 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 $\alpha_3 \alpha_4 \alpha_5$ heterotrimers being abnormal.\textsuperscript{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 $\alpha_3$, $\alpha_4$, and $\alpha_5$ chains, even though only one gene is affected. Pathogenic heterozygous variants of the $\text{COL4A3}$ or $\text{COL4A4}$ genes result in a thinned GBM because of the reduced amount of the collagen IV $\alpha_3 \alpha_4 \alpha_5$ network.\textsuperscript{38} There is a compensatory increase in the collagen IV $\alpha_1 \alpha_2 \alpha_3$ heterotrimer, which is more susceptible to proteolysis,\textsuperscript{29} and may not be sufficiently strong to prevent disease.

The abnormal GBM in TBMN is associated with the loss of the overlying podocytes\textsuperscript{40} 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.\textsuperscript{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.\textsuperscript{15,16} Seventy-five percent of pathogenic $\text{COL4A5}$ variants in men have a consistent age at kidney failure,\textsuperscript{28} which can largely be predicted from the genotype.\textsuperscript{28,36,42–44} This is also true for autosomal recessive Alport syndrome.\textsuperscript{32} Women with pathogenic $\text{COL4A5}$ variants demonstrate a less consistent genotype-phenotype correlation, which has been attributed to lyonization.\textsuperscript{29} The variation in age at kidney failure for pathogenic heterozygous $\text{COL4A3}$ or $\text{COL4A4}$ 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.\textsuperscript{45}

While pathogenic heterozygous $\text{COL4A3}$ and $\text{COL4A4}$ variants commonly predispose to kidney cysts these are generally too few and too small to significantly affect function.\textsuperscript{15,46,47} However pathogenic heterozygous variants in $\text{COL4A3}$ or $\text{COL4A4}$ are also associated with glomerular immune deposits, which may worsen kidney function.\textsuperscript{38,49} IgA glomerulonephritis and heterozygous pathogenic variants occur together too often to be coincidental and the thinned GBM may facilitate glomerular immune complex deposition.\textsuperscript{50}

Inappropriateness of “AD Alport Syndrome” as a Diagnosis

These observations that pathogenic heterozygous $\text{COL4A3}$ or $\text{COL4A4}$ 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 $\text{COL4A3}$ or $\text{COL4A4}$ 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 $\text{COL4A3}$ or $\text{COL4A4}$ variant have had a kidney biopsy, and a genetic diagnosis is more accurate and more consistent.

Renaming Alport Syndrome

Heterozygous pathogenic $\text{COL4A3}$ or $\text{COL4A4}$ 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 $\text{COL4A5}$ and $\text{COL4A3}$ or $\text{COL4A4}$ variants\textsuperscript{4} 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.\textsuperscript{51} These frequencies were first reported 40 years ago but are still often cited.\textsuperscript{51} Because pathogenic $\text{COL4A5}$ 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.

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