Digenic Alport Syndrome

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[1]Judy Savige, [2,3,4]Alessandra Renieri, [5]Elisabet Ars, [2,3]Sergio Daga, [4]Anna Maria Pinto, [6]Hansjorg Rothe, [7]Daniel P Gale, [8]Marina Aksenova, [9]Agne Cerkauskaite, [10]Olga Bielska, [10]Beata Lipska-Zietkiewicz and [1]Joel T Gibson

[1]The University of Melbourne Department of Medicine (Melbourne Health and Northern Health); [2]Medical Genetics, University of Siena, Siena, ITALY; [3]Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, University of Siena, Siena, Italy; [4]Genetics Medica, Azienda Ospedaliero-Universitaria Senese, Siena, Italy; [5]Molecular Biology Laboratory, Fundacio Puigvert, Instituto de Investigaciones Biomédicas Sant Pau (IIB-Sant Pau), Universitat Autonoma de Barcelona, Barcelona, SPAIN; [6]Centre for Nephrology and Metabolic Disorders, D-02943 Weisswasser, GERMANY; [7]Department of Renal Medicine, University College London, London, UNITED KINGDOM; [8]Veltischev Research and Clinical Institute for Pediatrics of the Pirogov Russian National Research Medical University, Moscow, RUSSIA; [9]Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University Vilnius, LITHUANIA; and [10]Centre for Rare Diseases, and Clinical Genetics Unit, Medical University of Gdansk, POLAND.

Address for Correspondence:
Prof Judy Savige
The University of Melbourne
Department of medicine (Melbourne Health and Northern Health)
Royal Melbourne Hospital
Parkville VIC 3050
AUSTRALIA

Abstract

Digenic Alport syndrome refers to the inheritance of pathogenic variants in *COL4A5* plus *COL4A3* or *COL4A4;* or in *COL4A3* plus *COL4A4.*

Where digenic Alport syndrome includes a pathogenic *COL4A5* variant, the consequences depend on the sex of the affected individual, *COL4A5* variant 'severity', and the nature of the *COL4A3* or *COL4A4* change. A male with a pathogenic *COL4A5* variant has all his collagen IV $\alpha 3\alpha 4\alpha 5$ heterotrimers affected, and an additional *COL4A3* or *COL4A4* variant may not worsen disease. A female with a pathogenic *COL4A5* variant has on average 50% of her heterotrimers affected which is increased to 75% with a further *COL4A3* or *COL4A4* variant, and associated with an increased risk of proteinuria.

In digenic Alport syndrome with pathogenic *COL4A3* and *COL4A4* variants, 75% of the heterotrimers are affected. The *COL4A3* and *COL4A4* genes occur head-to-head on chromosome 2, and inheritance is autosomal dominant when both variants affect the same chromosome (in cis) or recessive when they affect different chromosomes (in trans). This form of digenic disease results in increased proteinuria, and a median age of kidney failure intermediate between AD and AR Alport syndrome.

Previous guidelines have suggested that all Pathogenic or Likely Pathogenic digenic variants should be identified and reported. Affected family members should be identified, treated and discouraged from kidney donation. Inheritance within a family is easier to predict if the two variants are considered independently and if *COL4A3* and *COL4A4* variants are known to be inherited on the same or different chromosomes.

Text

Alport syndrome (OMIM#301050) is the second commonest cause of genetic kidney failure after polycystic kidney disease[1,2]. Inheritance is X-linked where there is a single pathogenic variant in the *COL4A5* gene, and autosomal recessive where there are pathogenic variants in both copies of the *COL4A3* or *COL4A4* genes. Autosomal dominant Alport syndrome is sometimes used where there is a heterozygous pathogenic *COL4A3* or *COL4A4* variant, but the term is incorrect since extrarenal or syndromic features are lacking. This condition is also known as Thin basement membrane nephropathy[3]. The three *COL4A3 – COL4A5* genes code for the collagen IV α3 – α5 chains which form the collagen IV $\alpha3\alpha4\alpha5$ heterotrimer[4]. A network of these trimers forms the backbone of the basement membranes in the kidney, ear and eye[4].

The distinguishing features of all forms of Alport syndrome are persistent haematuria and a family history of haematuria and sometimes kidney failure together with basement membrane thinning or lamellation[5]. Proteinuria and focal and segmental glomerulosclerosis are recognised increasingly[6]. Hearing loss, lenticonus and fleck retinopathy are also common but the hearing loss may be coincidental and the ocular abnormalities may not be recognised.

Males with X-linked Alport syndrome and males and females with autosomal recessive disease have a similar phenotype with kidney failure, hearing loss, lenticonus and a fleck retinopathy. Ninety % of males with X-linked disease have kidney failure by the age of 40 years[7]. Fifteen – 30% of females with X-linked disease have kidney failure by the age of 60, and hearing loss and fleck retinopathy are common but lenticonus does not occur[8]. Most individuals with AD Alport syndrome have haematuria only, and while a few develop proteinuria and kidney failure, hearing loss and ocular abnormalities are very rare.[9, 10]

Two further forms of inheritance are now recognised, and definitions differ, but the following interpretations have been adopted for Alport syndrome[3]. 'Digenic' disease refers to two pathogenic variants in different *COL4A3 – COL4A5* genes[11]. This differs from 'modifying' variants where a heterozygous pathogenic *COL4A3- COL4A5* variant occurs together with a pathogenic change in a gene encoding another podocyte or glomerular filtration barrier protein[12, 13].

Some geneticists prefer to use 'double heterozygotes' rather than digenic variants where the pathogenic changes affect genes that on their own cause disease. However digenic disease is used here to be consistent with the recent classification of Alport syndrome and previous reports[3, 11, 14-16]. Digenic inheritance refers to variants at two loci that explain the phenotype of some affected individuals better than a variant at one locus alone[17]. Both loci may be equally important, but one may also increase the disease risk or severity [17, 18]. The outcome depends on whether the affected individual is a male with a *COL4A5* change in which case all heterotrimers are already affected. The second variant may also determine who develops clinical features, or has disease at a younger age. This is because the second variant usually increases the proportion of heterotrimers that are defective. While digenic disease contributes to the variability of clinical features in some families, this is not a common finding.

Types of digenic inheritance

Digenic Alport syndrome is due to a pathogenic variant in *COL4A5* plus one in *COL4A3* or *COL4A4* or due to a pathogenic variant in *COL4A3* plus one in *COL4A4*. These will be considered separately because their population frequencies, mode of inheritance, and molecular and clinical consequences are different. For digenic variants affecting *COL4A5* plus *COL4A3* or *COL4A4*, many of these features depend on whether the *COL4A5* variant affects a male or female, and in all cases, variant 'severity' also contributes to the phenotype[19]

Pathogenic variants in *COL4A5* plus *COL4A3* or *COL4A4* in males or females

The number of published reports of digenic Alport syndrome has increased with the widespread adoption of Massively Parallel Sequencing for routine genetic testing. Massively Parallel Sequencing has dramatically increased the ability to detect multiple variants in different genes contemporaneously in a single individual. Previous practice was to sequence the *COL4A3 – COL4A5* genes where there were the characteristic features of X-linked or autosomal recessive Alport syndrome, that is, kidney failure, hearing loss, and ocular abnormalities. Once a pathogenic variant was found the laboratory ceased to look for a further change. This meant that few digenic variants were identified, most included a pathogenic *COL4A5* change, and only some of the digenic variants identified as such in a publication.

Here we have reviewed all the published digenic variants found in a literature search and after requests to testing laboratories attending the 2021 International Workshop on Alport syndrome[20], and where at least some clinical data (age, sex, proteinuria, age at kidney failure) were also available. This strategy identified 43 *COL4A5* plus *COL4A3/COL4A4* variants and 32 *COL4A3* plus *COL4A4* variants.

It is however possible to deduce the relative population frequencies of digenic variants more accurately from recently published data for pathogenic variants in *COL4A5* of about one in 2,000, and for *COL4A3* or *COL4A4* of about one in 100[1]. Thus, digenic inheritance of a *COL4A5* variant plus a *COL4A3* or *COL4A4* variant occurs in about 1/2000 x 1/100 = 1/200,000, if both occur independently, and in about one % of all individuals with X-linked Alport syndrome. This was confirmed in a study of 417 mainly children with suspected Alport syndrome where 6 had pathogenic variants in both *COL4A5* and *COL4A3* or *COL4A4*[21].

Inheritance. With *COL4A5* plus *COL4A3/COL4A4* variants, the variants are inherited independently from one parent or from both. In these cases, inheritance of the individual *COL4A5* plus *COL4A3/COL4A4* variants each resemble the inheritance pattern of the underlying variant, namely X-linked or autosomal dominant. Inheritance in an individual family is easier to understand if the inheritance of each variant is considered separately.

Thus, for a male with a pathogenic *COL4A5* variant none of his sons will inherit the *COL4A5* variant, but all of his daughters will, and half his sons and half his daughters will inherit the *COL4A3* or *COL4A4* variant. Thus none of his sons have digenic disease but half his daughters will.

For a female with a pathogenic *COL4A5* variant as well as a pathogenic *COL4A3 or COL4A4* variant, half her sons and half her daughters will inherit the pathogenic *COL4A5* variant, and, as an independent event, half her sons and half her daughters will inherit the pathogenic *COL4A3* or *COL4A4* variant. Thus, if she had four children, on average, one has a *COL4A5* variant plus a *COL4A3* or *COL4A4* variant (and digenic disease), one has a *COL4A5* variant only, one a *COL4A3* or *COL4A4* variant only, and one has none.

The clinical consequences also depend on the effect of the variants on the collagen IV α chains and whether they affect the interaction of the impaired chains.

Disease pathogenesis. In order to understand the effects of an additional pathogenic variant it is important to understand why some pathogenic *COL4A5* variants result in more severe disease and more pronounced proteinuria or earlier onset kidney failure.

Pathogenic *COL4A5* variants that result in severe disease include truncating variants, major rearrangements, large deletions, and splicing variants that induce exon skipping or intronic retention[7, 19]. Premature stop codons typically result in nonsense mediated decay of the mRNA transcript and absence of the collagen IV $\alpha3\alpha4\alpha5$ heterotrimer from affected membranes[22]. 'Severe' *COL4A5* variants result in severe disease with early onset kidney failure, hearing loss, and often lenticonus and central fleck retinopathy. In contrast, missense variants typically produce less disruption of the collagen IV α chains which may be partly retained within the endoplasmic reticulum resulting in increased associated stress. However the collagen IV α chains also persist in smaller amounts within the affected membranes[23, 24]. These variants are associated with milder disease with later onset kidney failure, hearing loss, and less often with lenticonus. Hypomorphic variants also result in mild disease with late onset proteinuria, and kidney failure in the sixth or seventh decade often without hearing loss, lenticonus or the central fleck retinopathy[25, 26]. These variants include Gly substitutions that are adjacent to a non-collagenous sequence or a non-Gly substitution. The p.Gly624Asp substitution is a common hypomorphic *COL4A5* variant in people of European ancestry[26].

On their own, heterozygous pathogenic *COL4A3* and *COL4A4* variants typically have a less severe effect than pathogenic heterozygous *COL4A5* variants. They usually result in haematuria, and sometimes proteinuria, but kidney failure is less likely, and hearing loss, lenticonus and fleck retinopathy are very rare if they occur at all[9, 10]. Sometimes there is no haematuria or proteinuria.

Pathogenic variants in *COL4A5* plus *COL4A3* or *COL4A4* in males. The major consequences of digenic variants where one affects *COL4A5* depends on the sex of the individual and the variant severity. In males, a *COL4A5* variant affects all the collagen IV $\alpha3\alpha4\alpha5$ heterotrimers **(Figure 1a).** If the *COL4A5* variant is already 'severe' (a large rearrangement, truncating or splice variant) the heterotrimers are often absent and kidney failure is likely before the age of 30[7]. Even the addition of even a severe *COL4A3* or *COL4A4* variant may have no consequences since the collagen IV $\alpha3$ and $\alpha4$ chains are destroyed rather than being expressed in basement membranes. However, if the *COL4A5* variant is a missense variant resulting in milder or hypomorphic disease then the collagen IV $\alpha3\alpha4\alpha5$ heterotrimer is

abnormal and the additional *COL4A3* or *COL4A4* variant may further disrupt half of the already abnormal heterotrimers **(Figure 1a).**

While these effects are likely, it is still difficult to confirm that the combination of a *COL4A5* and *COL4A3* or *COL4A4* variant results in worse disease because so few individuals with digenic disease have been described, many affected children are too young to have kidney failure, and GBM lamellation, hearing and ocular defects may not be known. Common clinical data is often sparse, and rarely includes the age at which proteinuria was first detected, which is often more relevant than age at kidney failure.

In addition, some individuals reported with a *COL4A5* variant may have an undetected pathogenic variant in *COL4A3* or *COL4A4.* In addition, *o*ther extra-renal factors are also important in the risk of kidney failure such as hypertension control, and avoiding diabetes and becoming overweight[27, 28]. Furthermore, the age at kidney failure has been further delayed with the widespread use of ACE inhibitor treatment[29].

A review of all reported digenic *COL4A5* variants in 16 males with a median age of 16 years (5 – 55) found that the *COL4A5* variant was severe in 5 (31%) and the additional variant was severe in only 2 (13%)[16, 21, 30-32] **(Table 1)**. Digenic disease was associated with proteinuria in 12 (12/13, 92%) and kidney failure in 5 (5/15, 33%) which developed at a median age of 27 (range 23 – 47), as well as hearing loss (6/10, 60%) and ocular abnormalities (3/6, 50%). In one study there were more affected boys with proteinuria[21] but there was no difference in the median age at kidney failure of 24 years compared with 23 years without the additional *COL4A3 COL4A4* variant (p=0.75, comparison with LOVD results) **(Figure 1b)(S Table 1).** Digenic Alport syndrome with a hypomorphic *COL4A5* and a further pathogenic *COL4A3* or *COL4A4* variant may be associated with more severe disease with earlier onset proteinuria and a younger age at kidney failure, but there is no evidence for this currently.

Pathogenic variants in *COL4A5* plus *COL4A3* or *COL4A4* in females. Twice as many females inherit a pathogenic *COL4A5* variant because they have two X chromosomes[27]. This means that they are therefore twice as likely to have digenic disease with a pathogenic *COL4A5* variant plus a pathogenic *COL4A3* or *COL4A4* variant.

Overall, in such females the pathogenic *COL4A5* variant affects half the heterotrimers and the additional *COL4A3* or *COL4A4* variant means then that 75% of the heterotrimers are defective **(Figure 2a)**[16]. However, the situation is more complicated because inheritance is different for *COL4A5* where there is X chromosome inactivation, and for *COL4A3* or *COL4A4* variants. A severe *COL4A5* variant results in the loss of collagen IV $\alpha3\alpha4\alpha5$ heterotrimer from on average half the cells, whereas a severe *COL4A3* or *COL4A4* variant results in half the collagen IV $\alpha3\alpha4\alpha5$ heterotrimers in each cell. For a mild *COL4A5* variant, on average half the cells have a defective heterotrimer, and for a mild *COL4A3* or *COL4A4* variant, half the heterotrimers in each cell are defective.

Data were available from 26 females with a pathogenic *COL4A5* variant and a *COL4A3* or *COL4A4* variant and with a median age of 13 years (range 3 – 55), **(Table 2)**[15, 16, 21, 30, 33-37]. In 7 cases (27%) the *COL4A5* variant was severe. Sixteen (62%) had proteinuria which had developed at a median of 22 years (range 3 – 45) and only two had kidney failure which

developed at 40 and 44 years, respectively. Three (15%) had a hearing loss and one (7%) had unspecified ocular abnormalities. There was GBM lamellation in 5 of 6 examined (83%) and GBM thinning in one of 6 (17%). At present, too few women have been described with digenic variants that include a *COL4A5* variant, to demonstrate an earlier age at kidney failure. However when compared with women with severe *COL4A5* variants[37], women with pathogenic digenic changes demonstrated a trend to proteinuria at a younger age (p=0.09) **(Figure 2b)**.

Thus, women with digenic disease that includes a *COL4A5* variant have an increased risk of developing proteinuria, which also increases their risk of kidney failure[7]. This suggests that women with digenic disease that includes a pathogenic *COL4A5* variant have a worse prognosis that those with only a pathogenic variant in *COL4A5*.

Course. The clinical course depends on the sex of the affected individual and the severity of both the *COL4A5* and the *COL4A3* or *COL4A4* variant and whether both variants are individually associated with severe disease.

Management. Previous guidelines for the management of Alport syndrome have addressed digenic forms of the disease[5, 14, 38, 39]. These are summarised here.

All individuals who undergo testing for Alport syndrome should be examined for variants in all the *COL4A3, COL4A4* and *COL4A5* genes since additional pathogenic variants in these genes may worsen clinical features.

Males with digenic variants that include a pathogenic *COL4A5* variant should typically be treated from the time of diagnosis with renin-angiotensin- aldosterone blockade[38]. Females with a digenic variants that include a pathogenic *COL4A5* variant should be treated from the time of diagnosis because of their increased risk of proteinuria and kidney failure[38]. These recommendations should be applied less stringently in children because of the risk of severe dehydration with vomiting and diarrhoea that are more common in a paediatric age group.

All first degree family members should undergo genetic testing since they may have both pathogenic variants or only one, and half the sons of an affected women may themselves develop kidney failure with only the *COL4A5* variant[39].

Individuals with digenic disease should be discouraged from kidney donation because of their own risk of kidney failure[14, 39].

Inheritance is complicated and easier to understand if the variants are considered separately. Advice about the disease in future generations will be different in males and females with a pathogenic *COL4A5* variant.

Pathogenic heterozygous variants in *COL4A3* plus *COL4A4*

Population frequency. Based on population studies, digenic disease with a pathogenic variant in *COL4A3* plus a pathogenic variant in *COL4A4* is more common than a pathogenic variant in *COL4A5* plus one in *COL4A3* or *COL4A4* because pathogenic *COL4A3* and *COL4A4* variants are more common[1]. This form of digenic disease is predicted to affect about one in

200 (for the approximate frequency of a pathogenic *COL4A3* variant) x one in 200 (for the *COL4A4* variant) = one in 40,000 of the population, or one in 200 of those with suspected familial haematuria (where the index case has haematuria and is likely to have a pathogenic heterozygous (COL4A3 or COL4A4 variant). About 0.5% of all *COL4A3* or *COL4A4* variants have a second variant since digenic disease must include a second variant in the other gene.This is about 5 times as often as the combination of a pathogenic variants in *COL4A5* and another in *COL4A3* or *COL4A4*.

Inheritance. Because *COL4A3* and *COL4A4* occur back-to-back on chromosome 2 with a short intervening sequence[40], the two pathogenic variants in *COL4A3* and *COL4A4* may occur on the same (*in cis*) or different (*in trans*) chromosomes. These different patterns affect the mode of inheritance and whether disease occurs in successive generations. Where both variants occur on the same chromosome, they are inherited together in consecutive generations and the pattern mimics autosomal dominant inheritance. This occurs in about half the cases, and only one parent is affected, with a digenic phenotype and typically haematuria.

Thus for an affected male or female with pathogenic variants in both *COL4A3* and *COL4A4*, half their children inherit both pathogenic variants and have digenic disease. Successive generations with the digenic variants will pass these on to half their children in each generation.

In contrast, when the abnormal *COL4A3* and *COL4A4* genes affect different chromosomes, which also occurs in half the cases, both parents are affected typically with haematuria, and the inheritance pattern mimics that of autosomal recessive disease.

Thus for an affected male or female with pathogenic variants in both *COL4A3* and *COL4A4* on different chromosomes, on average half their children have the pathogenic *COL4A3* or *COL4A4* variant, but none have digenic disease.

There may, however, be an ascertainment bias in the reporting of families with these two inheritance patterns, since individuals with variants on different chromosome are likely to have more severe disease and to undergo genetic testing.

Pathogenesis. A single pathogenic variant in *COL4A3* or *COL4A4* in both males and females results in 50% of the collagen IV $\alpha3\alpha4\alpha5$ heterotrimers being defective, but two pathogenic variants in these genes result in 75% of the heterotrimers being defective[16] **(Figure 3a)**.

Clinical features. The phenotype of a single pathogenic heterozygous variant is typically haematuria, while proteinuria is less common, and kidney failure is uncommon, and hearing loss and ocular abnormalities are rare. Hearing loss occurs from many other causes but any ocular abnormalities in an individual with a single pathogenic variant should undergo a rigorous examination for a further pathogenic variant in the *COL4A3 – COL4A5* genes.

The cohort of 32 individuals reported with digenic pathogenic variants in *COL4A3* plus *COL4A4* [9, 15, 16, 36, 41, 42] included 10 males and 15 females, with a median age of 34 years (1 to 66), where there was no severe variant in 15 (47%), one in 12 (38%) and two in 5 (16%)

individuals **(Table 3, S Table 3)**. Nineteen (66%) had proteinuria which was the same as for AD disease of a hospital-based cohort (65%), and the median age at kidney failure was 54 years (n=8) which was later than for autosomal recessive Alport syndrome[43] (23, range 10 – 38, , $p=10^{-6}$) and younger than for autosomal dominant Alport syndrome [9](66, range 58 – 73, p=0.01) **(Figure 3b).** In addition, four (27%) had a hearing loss which was more often than for autosomal dominant Alport syndrome but less often than for autosomal recessive disease (23/35, 67%). None had an ocular abnormality. One individual had GBM lamellation, and two had membrane thinning. Thus, the risk of kidney failure for a pathogenic heterozygous *COL4A3* or *COL4A4* variant on its own is very small but the addition of a further pathogenic change may increase this risk.

Course. Again the clinical course depends largely on the nature of the underlying pathogenic variants, but it appears that the risk of proteinuria is increased with digenic variants in *COL4A3* and *COL4A4* compared with that of pathogenic heterozygous variants.

Management. Again these recommendations appear in various Expert guidelines[5, 14, 38, 39] and they are consistent with the recommendations for digenic disease due to a pathogenic *COL4A5* plus a *COL4A3* or *COL4A4* variant. Individuals with digenic variants should be treated from the time of diagnosis[38]. As for other forms of Alport syndrome it is important to minimise the amount of proteinuria and to control the blood pressure[5, 14]. At risk first degree family members should undergo genetic testing[14]. First degree family members should be strongly discouraged from kidney donation[14]. Inheritance is complicated and easiest to understand if variants are considered separately.

Conclusion

In conclusion, digenic variants are rare but recognised increasingly, their inheritance is complicated and, except for X-linked Alport syndrome in males, where the phenotype is already severe, are probably associated with increased proteinuria and an earlier age at kidney failure. Individuals with digenic variants should be treated early, their affected family members identified and also treated, and all those with digenic variants dissuaded from kidney donation.

Many digenic variants are probably overlooked especially where the second variant is hypomorphic. There are also occasional reports of digenic variants being associated with secondary FSGS and with cystic kidney disease as happens with other *COL4A3 – COL4A5* variants.

There are still few data on GBM lamellation, hearing loss and ocular abnormalities in digenic disease with pathogenic *COL4A3* and *COL4A4* variants but the data on age at proteinuria suggest that the phenotype is intermediate between AD and AR Alport syndrome.

Conflicts of Interest

The authors have no Conflicts of Interest to declare.

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List of Tables and Figures

Table 1: Clinical features in males with digenic Alport syndrome where one variant affects *COL4A5* compared with features with one pathogenic *COL4A5* variant only

	X-linked Alport syndrome in males (n=315, Jais 2000)	**X-linked Alport syndrome in males after ACE treatment (n=282, Yamamura 2020)**	**Digenic Alport syndrome in males including *COL4A5* variant (n=16)**
Age (median, range, years)	Not provided	13 (0 – 73)	16 (5 – 55)
***COL4A5* – severe variant**	75/195 families (38%)	125 (47%)	5 (31%)
p.G624D in *COL4A5*	Not specified	0	4
COL4A3	Not applicable	Not applicable	11
COL4A4	Not applicable	Not applicable	5
Additional variant is severe	Not applicable	Not applicable	2/16
GBM lamellation, splitting or basket-weave pattern	88/98 (90%) at a mean age of 12.5 years	Not specified	5/5
GBM thinning	12/98 (12%) at mean age of 11 years	Not specified	Not specified
Abnormal collagen IV α3α4α5 immuno-histochemistry	14/16 (88%)	64/146 (44%)	Not specified
Haematuria	191/193 (99%)	262/264 (99%)	14/14 (100%)
Proteinuria	207/218 (95%)	238 (91%)	12/13 (92%)
Number with kidney failure	282/360 (78%)	61/282 (22%)	5/15 (33%)
Age at kidney failure (median, years)	25 (9 - >41)	35 (32 – 40)	25 (23 to 47)
Hearing loss	239/303 (79%)	77 (32%)	6/10 (60%)
Ocular abnormalities	57/162 (35%)	13 (6%)	3/6 (50%)

'Severe' *COL4A5* variants include major rearrangements, truncating and splice site variants; GBM glomerular basement membrane. Precise numbers were not always provided but have been deduced from % or figures in manuscript.

Table 2: Clinical features in females with digenic Alport syndrome where one variant affects *COL4A5* compared with features with one pathogenic *COL4A5* variant only

	X-linked Alport syndrome in females (n=349, Jais 2003)	**X-linked Alport syndrome in females (n=275, Yamamura, 2017)**	**X-linked Alport syndrome in females (n=24, Mastrangelo, 2020)**	**Digenic Alport syndrome in females including *COL4A5* variant (n=26)**
Age (median, range, years)	Not provided	24 (0-92)	8 (1 – 41)	13 (3-55)
***COL4A5* – severe variant**	191/315 (61%)	138 (50%)	14/24 (58%)	7 (27%)
COL4A3	Not applicable	Not applicable	Not applicable	15
COL4A4	Not applicable	Not applicable	Not applicable	11
Additional variant is severe	Not applicable	Not applicable	Not applicable	7/26 (27%)
GBM lamellation, splitting or basket-weave pattern	20/28 (71%)	Not specified	16/16 (basket-weave in 8/16)	5/6 (83%)
GBM thinning	6/28 (21%)	Not specified	16/16	1/6 (17%)
Abnormal collagen IV α3α4α5 immuno-histochemistry	7/9 (78%)	Not specified	Not specified	Not specified
Haematuria	309 (96%)	232 (98%)		23/23 (100%)
Proteinuria	176/234 (75%)	175 (73%)	10/24 (42%)	16/26 (62%)
Age at proteinuria onset (median, years)	Not specified	7	13 (5 – 30)	12 (3 – 45)
Number with kidney failure (n,%)	51/288 (18%)	33 (12%)	1/24 (4%)	2/24 (8%)
Age at kidney failure (median, years)	38 (18 – 70)	65	40	40, 44
Hearing loss (n,%)	239/303 (79%)	15 (6%)	4/24 (17%)	3/20 (15%)
Ocular abnormalities	57/162 (35%)	4 (2%)	0/24 (0%)	1/14 (7%)

Table 3: Clinical features in digenic Alport syndrome where pathogenic variants affect *COL4A3* and *COL4A4* compared with AR or AD Alport syndrome

	Digenic Alport syndrome (n=32)	Autosomal dominant Alport syndrome (n= 252, Furlano, 2021)	Autosomal recessive Alport syndrome (n=40, Storey, 2013)
Age (median, range, years)	34 (1 -66)	48 (5 – 87)	31 (6 -54)
COL4A3	32	107 (35 families)	20/40 (50%)
COL4A4	32	133 (43 families)	20/40 (50%)
No severe variant	15 (47%)	136 (57%)	8 (20%)
One severe variant	12 (38%)	104 (43%)	12 (30%)
Two severe variants	5 (16%)	NA	20 (50%)
GBM lamellation or splitting	1/3 (33%)	Not specified	34/36 (94%)
GBM thinning	2/3 (67%)	Not specified	2/36 (6%) but biopsy performed as a child
Abnormal collagen IV α3α4α5 immuno-histochemistry	Not specified	Not specified	Not specified
Haematuria	Not specified	**232/252 (92%)**	Not specified
Proteinuria	**19/29 (66%)**	**157/241 (65%)**	Not specified
Age at proteinuria onset (median, years)	**43 (5 – 59)* taken from age at proteinuria recorded**	**Not specified**	Not specified
Number with kidney failure	**8 (25%)**	**61 (24%)**	**20/34 (59%)**
Age at kidney failure (median, years)	**54 (n=8)**	**67 (58-73)**	**22.5 (10-38)**
Hearing loss	**4/15 (27%)**	**11/131 (8%)**	**23/35 (67%)**
Ocular abnormalities	0/7	2/75 (2%)	10/18 (56%)

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Supplementary Materials

Supplementary Methods

Individuals provided informed consent to the use of their deidentified clinical data at the time of testing or their data in publications or databases were considered to be in the public domain.

Kaplan-Meier plots of kidney survival were constructed as follows.
Males with X-linked Alport syndrome and a pathogenic *COL4A5* variant were identified from LOVD (https://databases.lovd.nl/shared/genes/COL4A5) and age at kidney failure extracted. Age at kidney failure was the age at the diagnosis of kidney failure, or where this was not available, the commencement of dialysis, or at first kidney transplant. Affected male family members were included separately. Where only median or mean age at kidney failure was reported for a family, this was used instead. Where a male had not yet progressed to kidney failure, his age at most recent examination was included as a censored data point. Kidney survival curves were compared using the log-rank test.

Survival analysis was performed in R (version 3.6.2) using the *survival* and *survminer* packages [1,2,3].

Supplementary Tables

Supplementary Table 1: Genotype-phenotype correlation in males with digenic Alport syndrome including a *COL4A5* variant

Supplementary Table 2: Genotype-phenotype correlation in females with digenic Alport syndrome including a *COL4A5* variant

Supplementary Table 3: Genotype-phenotype correlation in males and females with digenic Alport syndrome with a *COL4A3* and *COL4A4* variant

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