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Clinical and Genetic Features of Autosomal Dominant Alport Syndrome: A Case Series

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Clinical and Genetic Features of Autosomal Dominant Alport Syndrome: A Case Series

Settings & Participants

Retrospective cohort study

n = 252 patients
n = 82 families
Spanish hospitals

Heterozygous disease-causing variants in *COL4A3* & *COL4A4*

Observations

92.1% had microhematuria

Extrarenal features rare

38.9% of patients with proteinuria reached KRT (61/157)

Overall kidney survival: 67 (58–76) years

No patients without proteinuria developed CKD

No genotype-phenotype correlation was observed

CONCLUSION: ADAS patients present a wide spectrum of symptoms, regardless of the affected gene or disease-causing variants, making the diagnosis very challenging.

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Clinical and Genetic Features of Autosomal Dominant Alport Syndrome: A Case Series

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ABSTRACT

Rationale & Objective: Alport syndrome (AS) is a common genetic kidney disease accounting for approximately 2% of patients receiving kidney replacement therapy (KRT). It is caused by pathogenic variants in *COL4A3*, *COL4A4* or *COL4A5* genes. The aim of this study was to evaluate the clinical and genetic spectrum of patients with autosomal dominant Alport syndrome (ADAS).

Study design: Retrospective cohort study.

Setting & Participants: 82 families (252 patients) with ADAS were studied. Clinical, genetic, laboratory, and pathology data were collected.

Observations: A pathogenic DNA variant in *COL4A3* was identified in 107 patients (35 families) while 133 harbored a pathogenic variant in *COL4A4* (43 families). Digenic/complex inheritance was observed in 12 patients. Overall, the median kidney survival was 67 years (95% CI, 58–73), without significant differences across sex (*P*=0.79), causative genes *(P*=0.55) or types of variant (*P*=0.99). Microhematuria was the most common kidney manifestation (92.1%) and extrarenal features were rare. Findings on kidney biopsies ranged from normal to focal segmental glomerulosclerosis. The slope of eGFR decline was -1.46 mL/min/1.73 m^2 per year (-1.66 to -1.26) for the overall group, with no significant differences between ADAS genes (*P=0.24*).

Limitations: The relatively small size of this series from a single country, potentially limiting generalizability.

Conclusions: ADAS patients have a wide spectrum clinical presentations ranging from asymptomatic to kidney failure, a pattern not clearly related to the causative gene or type of variant. The diversity of ADAS phenotypes contributes to its underdiagnosis in clinical practice.

Keywords: Alport syndrome, autosomal dominant Alport syndrome, familial hematuria, inherited kidney disease, *COL4A3*, *COL4A4*

Supplementary key words

Genetic, genotype-phenotype correlation, hereditary kidney disease, thin basement membrane disease, familial benign hematuria, hearing loss

PLAIN LANGUAGE SUMMARY

Autosomal dominant Alport Syndrome is among the most underdiagnosed inherited kidney diseases and is not commonly diagnosed by most nephrologists. Compared with X-linked Alport syndrome or the autosomal recessive forms, this entity is much less severe: most patients have hematuria but only a minority will develop chronic kidney disease or extrarenal involvement. The relatively low prevalence of this condition, the paucity of clinical features in some patients, and a prominent role of environmental/genetic modifiers may account for its infrequent diagnosis. Raising awareness of autosomal dominant Alport syndrome will foster its diagnosis and reduce the number of patients developing kidney failure without an etiologic diagnosis.

INTRODUCTION

Alport syndrome (AS) was the first inherited kidney disease for which the genetic bases were identified.[1] It accounts for at least 1%–2% of all cases of patients on kidney replacement therapy (KRT). [2,3]

Pathogenic variants in *COL4A3*, *COL4A4* and *COL4A5* genes cause defective synthesis of the α3/α4/α5 chains, preventing correct assembly of collagen IV chains of the glomerular basement membrane (GBM) and causing AS.[4,5] Classical AS is characterized by hematuria with progressive proteinuric kidney disease, GBM abnormalities, hearing loss (HL) and ocular abnormalities.[4,6,7] This full-blown presentation is typically found in male patients with X-linked AS (XLAS) and patients with autosomal recessive AS (ARAS). Women with XLAS show a variable phenotype due to the X inactivation phenomenon.[8] Patients with heterozygous disease-causing variants in *COL4A3* or *COL4A4* genes display a wide spectrum of manifestations, ranging from asymptomatic to presentation with hematuria alone or with proteinuria and subsequent kidney failure on top of hematuria. There is a lot of controversy over how to designate the disease.[9–16] In cases where there is only hematuria, the terms “familial benign hematuria” and “thin basement membrane disease” have been used.[17–19] However, these terms now seem outdated because: (i) “familial benign hematuria” implies a very mild disease, which is not always the case, (ii) “thin basement membrane disease” is a histopathological finding associated with other histological abnormalities in a minor, but not insignificant, percentage of patients, and (iii) "carriers of ARAS" may develop kidney disease, being not only "carriers" but patients themselves. The term ADAS has historically been reserved for patients with heterozygous disease-causing variants in *COL4A3* or *COL4A4* with kidney failure[10,11] but there is increasing agreement that any patient harboring a heterozygous disease-causing variant in

COL4A3 or *COL4A4* should be considered as ADAS.[12,13,20–22]

In recent years, the use of next generation sequencing (NGS) has allowed increased identification of disease-causing variants in *COL4A3* and C*OL4A4.* These have proven to be behind 40%–60% cases of familial hematuria[23] and most familial cases of focal segmental glomerulosclerosis (FSGS) but are also found in sporadic FSGS or hematuria and chronic kidney disease (CKD).[24–27] Recently, *Groopman* et al. found that in a large adult cohort of patients with CKD, *COL4A3,* C*OL4A4*, and *COL4A5* were the most frequent causative genes of monogenic CKD after *PKD1* and *PKD2*.[28] Although some attempts have been made to provide insights into the clinical features of patients with heterozygous disease-causing variants in *COL4A3* and C*OL4A4,* most studies have included a low number of patients and/or have been subject to bias because patients were selected from a previously defined phenotype, such as isolated hematuria or FSGS, resulting in very mild or very severe phenotypes respectively.[27,29] There is also evidence for digenic inheritance (understood as patients carrying pathogenic variants in different *COL4A* genes) in collagen type IV nephropathies which, together with modifying genetic or environmental factors, further complicate the disease phenotype.[30]

In this study we provide an in-depth analysis of clinical features and type of variant in a large cohort of families with heterozygous disease-causing variants in *COL4A3* and C*OL4A4.*

METHODS

Patients

A total of 82 families (252 patients) were referred from Spanish hospitals between 2000 and 2019 and all had an index case with a heterozygous disease-causing variant in *COL4A3* or *COL4A4*, which was the inclusion criterion. The reason for performance of a genetic test in these families was as follows: (a) families with autosomal dominant inherited microhematuria and proteinuria and/or CKD, or (b) index patients with an abnormal GBM. Only one family with isolated microhematuria was included. The reason for performing a genetic diagnosis in this family was to rule out XLAS in the context of mother to son transmission.

The study was approved by the Fundació Puigvert institutional review board and all participants signed their informed consent to participation in the study.

Clinical data

Clinical data from patients and deceased family members were retrospectively obtained from medical records and were recorded in a database which included: sex, date of birth, age at diagnosis of kidney disease, age-related decline in kidney function, repeated measurements of protein–creatinine ratio, need for KRT, age at KRT and description of genetic variants. Results of kidney biopsy (KB), at light microscopy (LM), were classified into four groups: normal, FSGS, expansion of the mesangial matrix with positive immunofluorescence staining (unspecific IgM and C3 deposits) and expansion of the mesangial matrix with negative immunofluorescence staining. If electron microscopy (EM) was available, the GBM findings were recorded.

The kidney function was assessed by the estimated glomerular filtration rate (eGFR) CKD Epidemiology Collaboration (CKD-EPI) creatinine equation in individuals with at least three values 3 years apart. Age at KRT was defined as the age at which the patient had an eGFR CKD-EPI <10 mL/min/1.73 m^2 or initiated KRT.

Age at diagnosis of HL, type of HL and ocular lesions were recorded.

Genetic testing

Genetic testing was performed in index cases by NGS of a kidney-disease gene panel containing *COL4A3*, *COL4A4* and *COL4A5* genes, as previously reported.[31] Filtering of the variants was performed by minor allele frequency <0.0005 in the Genome Aggregation Database and in our in-house database. Variant annotation and impact prediction were performed with the SnpEff 4.3 program. Missense variants were further evaluated using several pathogenic prediction algorithms included in the dbNSFP v4.0 missense variant database and using the VarSome tool. Splicing variants were evaluated using the Human Splicing Finder. All candidate disease-causing variants identified were checked for being reported in the ClinVar and LOVD databases. Validation and segregation analysis of the candidate disease-causing variants were performed by Sanger sequencing or by multiplex ligation-dependent probe amplification analysis. Variants were classified using the guidelines of the American College of Medical Genetics and Genomics (ACMG)[32] and those variants classified as pathogenic or likely pathogenic were considered disease causing (Table S1).

Digenic/complex inheritance pattern was considered present when two disease-causing variants were identified in *COL4A3* and *COL4A4* in the index case.

To study whether the type of disease-causing variant correlated with age at KRT, variants were classified into the following categories: indel <5 amino acids in frame, indel >5 amino acids in frame, truncating indel, missense, nonsense and splicing. In addition, missense variants were classified into different groups according to the degree of predicted damage to the protein: two groups for glycine and non-glycine variants, and also two groups to compare those patients with Gly substitutions by Arg, Glu, or Asp versus group 2 harboring a Gly substitution by non Arg, Glu, or Asp.

Statistical Analysis

For descriptive statistics, continuous variables are presented as mean ± standard deviation values or median (interquartile range [IQR], i.e., 25^{th}–75^{th} percentiles), according to their adherence to the Gaussian distribution and categorical data are presented as frequencies and percentages. Fisher's exact test was used to compare categorical variables whereas the Mann-Whitney test was used to compare continuous variables between gender and *COL4A* disease-causing variant. The survival function as well as the median (95% confidence interval [CI]) time to KRT was estimated by means of the Kaplan-Meier method. We used the date of birth as the starting point and the last point of the survival analysis was either the age at KRT or the last available observation. No patient died before reaching kidney failure (i.e., no competing risks) and the number of at-risk patients is described by time-point in the figures. We used the time-varying proteinuria status to assess the impact of proteinuria on KRT. Group comparisons and hazard ratios (HR) [95% CI] were calculated by means of the Cox model using the family as a cluster to account for intragroup correlation. The kidney function was assessed using a slope analysis by means of mixed models for repeated measurements, including time (either age or time from diagnosis), subject nested within family, and, when applicable, genetic data and the time-varying proteinuria status over time. The intrafamilial variability with regard to age at initiation of KRT was illustrated using a graphical procedure by plotting the present age of patients not on KRT and the age at starting KRT for transplanted or dialysis patients per family. The analysis was performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) and the level of significance was established at the 0.05 level (two-sided).

RESULTS

Clinical features

The cohort included 142 women (56.3%), and the mean age at diagnosis was 33.62±17.12 years. No significant differences in kidney survival according to gender were identified (*P*=0.79) (Figure S1). Two patients were excluded from kidney survival analysis due to lack of kidney function data. Thirty-four subjects were relatives of patients with ARAS. Some ADAS families had the same heterozygous disease-causing variant as ARAS families and showed a mild phenotype, an exception being the ADAS family ALP-281 (same variant as ALP-189*), with several individuals on KRT (Table S2).

Kidney disease. Hematuria was present in 92.1% (232/252) of patients. Out of 20 patients without hematuria, 16 belonged to families in which someone had been diagnosed with ARAS and four were from families with ADAS. None of the patients without hematuria had developed proteinuria although two of them showed CKD G2 at 76 and 61 years of age, seemingly due to reasons other than their *COL4A* disease-causing variant.

Data on proteinuria were available for 241 patients; 157 (65.15%) of these had proteinuria. None of the patients without proteinuria needed KRT (only one patient without proteinuria presented CKD G3b, at 70 years of age with a history of nephrectomy), but among those with proteinuria, 61/157 (38.9%) received KRT. Data on use of renin-angiotensin-aldosterone system (RAAS) inhibitors were not properly recorded.

Overall kidney survival was 67 years (95% CI, 58–76) while for patients with proteinuria it was 58 years (95% CI, 56-57) (Figure 1a). The presence of proteinuria significantly increased the risk of KRT in the time-varying survival analysis (HR [95%CI]: 5.57 [2.68–11.60], *P*<0.0001) (Figure 1b). In the whole group, 61 patients received KRT (24.2%) at a mean age of 54.0±12.2 years and median age of 55 years (IQR 49–58). Six patients needed KRT before 41 years of age.

The slope of decline in eGFR, which was -1.46 mL/min/1.73 m^2 per year (-1.66 to -1.26) for the overall group (Figure 2a), showed no significant differences between *COL4A3* and *COL4A4* *(P*=0.24*)*. The slope of decline in eGFR for patients with proteinuria was -1.78 compared to -0.97 mL/min/1.73 m^2 per year for patients without proteinuria (difference 0.80, 95%CI: 0.45–1.18, *P*<0.001) (Figure 2b). A huge intrafamilial variability with regard to age at initiation of KRT was also observed (Figure 3). The slope of the increase in the proteinuria/creatinine ratio was 11.34 mg/g (1.95–20.72) for each year of life.

Hearing loss. Among 131 patients with audiometry, 35.9% showed HL but only 11 patients (8.4%) showed high-tone bilateral sensorineural HL. Only one patient with sensorineural HL received KRT at 67 years of age (Table S2).

Ocular abnormalities. Seventy-five patients underwent a thorough ophthalmological examination and although 23% of them had abnormal findings only two had anomalies possibly related to AS: one had recurrent corneal erosions (not a contact lens wearer) and another had corneal dystrophy (Table S2).

Pathological changes

Forty-nine out of 157 patients with proteinuria underwent a KB while no patient with hematuria alone had a KB (Table S2).

Based on histology (Table S3), patients were classified as follows: normal at LM and negative immunostaining (Figure S2a): 32.65%; FSGS (Figure S2b): 32.65%; expansion of the mesangial matrix with unspecific positive immunostaining (Figure S2c): 18.37%; expansion of the mesangial matrix with negative immunofluorescence staining (Figure S2d): 16.32%. Of those with FSGS, 75% needed KRT.

EM was performed in 20 specimens, disclosing thinning +/- splitting and lamellation of the GBM in all patients and podocyte effacement in some.

The mean age at the time of the KB was 36.57±12.56 years (median age 35 years [IQR 26–51]) while mean eGFR was 79.13±28.94 ml/min/1.73 m^2. Magnitude of proteinuria at the time of the KB ranged from 0.10 to 6.8 g/day (mean 1.5±1.65 g/day), with 81% having proteinuria >300 mg/day.

Genetic testing

Genetic testing was performed in 215 patients; the remaining 37 had a history of kidney disease compatible with AS and family members with identified disease-causing variants but were not available for genetic testing.

A heterozygous disease-causing variant in *COL4A3* was identified in 107 patients (35 families) while 133 harbored a disease-causing variant in *COL4A4* (43 families) (Tables S1, S2). Digenic/complex inheritance was identified in 12 patients (Table S2).

Missense variants were the most common type of disease-causing variant (Figure 4). The amino acid distribution among the missense disease-causing variants with Gly substitution (90%) was as follows: 33% Arg, 21% Val, 19% Glu, 11% Ser, 4% Asp and 2% Cys. The percentage of families with truncating disease-causing variants was 31%.

Genotype–phenotype correlation

Kidney survival was assessed comparing the causative gene and, although there was not a statistically significant difference (P=0.55), the median age at KRT was 64 years [95% CI, 57–71] for *COL4A3* versus 69 years [95% CI, 58–81] for *COL4A4* (Figure 5a). Hence, to evaluate the remaining genotype–phenotype correlations we considered *COL4A3* and *COL4A4* variants altogether. When comparing patients with truncating and non-truncating variants, no significant differences regarding kidney survival were observed either *(P=0.52*; Figure 5b). Additionally, kidney survival was not influenced by variant properties, according to different classifications (Figure S3, S4 and S5).

Of six patients starting KRT before 41 years of age, three had a missense disease-causing variant involving Gly, one had a splicing disease-causing variant, and two had an indel disease-causing variant: one in frame involving more than five amino acids, and the other one truncating.

Patients with a digenic/complex inheritance were not included in the survival analysis due to the low number of patients but are described in Table S2. While some of them have an early phenotype, others have a very mild disease.

DISCUSSION

The present article provides clinical and genetic information for a Spanish cohort of 252 patients with disease-causing variants in *COL4A3* and *COL4A4.*

In recent years, the increased number of disease-causing variants identified in *COL4A3*, *COL4A4*, and *COL4A5* genes has led to an expansion in our understanding of the phenotypic spectrum of AS[14,26,33,34] and has increased the estimated prevalence of ADAS from being "very rare" to accounting for at least 20% of cases of AS.[20,21,35] The scientific community can either choose to keep defining AS as a full-blown syndrome[6] or redefine it based on genetic findings.[12–14,36,37] The latter implies that a patient may be diagnosed with AS when they have only microhematuria or are even asymptomatic due to incomplete penetrance. If this option becomes accepted, nephrologists, genetic counselors and patients will need to be educated on the wide spectrum of the disease. Also, the number of people carrying one of these mutated alleles may be high and found frequently by NGS.[28] A precise genetic diagnosis of AS prevents changes in the clinical diagnosis based on the clinical features that occur during life or the assignment of diverse diagnoses to different members of a single family. In this discussion we will use the term ADAS for patients with disease-causing variants in *COL4A3* or *COL4A4*, while accepting that there is as yet no international consensus.

The present cohort of patients with ADAS is the largest published to date. Furthermore, based on the inclusion of patients with very diverse severity of the disease, there may be less ascertainment bias than in other series in which patients were recruited from familial hematuria, FSGS or CKD cohorts.[21,27,28] The present data support previous studies in which progression to KRT has been observed in some patients while others remain with isolated hematuria throughout their life, thus showing a huge inter- and intrafamilial variability. Median kidney survival in the present cohort was 67 years (95% CI, 58–76), which is definitively longer than in the X-linked and recessive forms of AS. The name AS suggests severity and early age at KRT, but, as evidenced in this series, the autosomal dominant form is much milder. This is in line with most autosomal dominant kidney diseases when compared to X-linked or autosomal recessive forms. Digenic/complex inheritance has been proposed as a seminal explanation for severe phenotypes, but none of the patients who needed KRT before 41 years of age in the present cohort showed digenic/complex inheritance. The huge inter-/intrafamilial variability suggests a role for genetic modifiers and epigenetic or environmental factors.[38]

The usual late age of onset of the disease together with the finding of *COL4A3* and *COL4A4* disease-causing variants in a significant percentage of patients with CKD[28,34] may suggest that anomalies in the triple helix of collagen IV may be sufficient to cause severe kidney damage in some patients, but it cannot be ruled out that *COL4A3* and *COL4A4* disease-causing variants represent a harmful genetic background for other kidney conditions such as hypertensive or diabetic kidney disease.

Hematuria is the most prevalent feature in this study, but in 7.9% of patients it was repeatedly absent; most of these patients were relatives of patients with ARAS, as supported by the literature.[22,39,40] This implies an incomplete penetrance, which is not uncommon in genetic kidney diseases.[14,41] There is longstanding evidence that the same disease-causing variant can cause very different phenotypes.[19,22]

In ADAS, clinical findings beyond hematuria are highly variable and age dependent, proteinuria being the next most frequent finding. Although many patients may have only hematuria during their lifetime, some develop proteinuria, the reported frequency of which varies among studies. The pathogenesis of proteinuria is not well understood, but altered permselectivity of the GBM, abnormal cell–matrix interactions, and defective trafficking of GBM matrix components by the podocyte are probably all mechanisms of the disease. In this study 65.15% of patients developed proteinuria and 38.9% of these needed KRT. No patient without proteinuria developed CKD, except a 70-year-old patient with a single kidney, confirming the natural history of ADAS: microhematuria, proteinuria, and CKD. Only three patients developed nephrotic range proteinuria. In one of them (DNA 17-142), the immunological tests were negative and the KB revealed unspecific deposits of IgM with expansion of the mesangial matrix. Another (DNA 14-216) presented with CKD-G5 and KB was not feasible, while the third patient (DNA 16-133) had only one kidney and KB was rejected. Due to its rarity, and because other glomerular diseases may co-exist, we recommend performance of KB, even with proven disease-causing variants in the *COL4* genes, if the patient develops sudden nephrotic range proteinuria. There is an increasing body of evidence supporting the efficacy of RAAS inhibitors in delaying CKD in AS[13,42], which could not be proved in the present study due to lack of adequate retrospective data on this matter. Large series on the use of RAAS inhibitors in ADAS are lacking, although, as per good clinical practice, patients with any degree of proteinuria should be under treatment with RAAS inhibitors.

As reported in all series, the pathological changes in the present cohort were unspecific. In early stages, thinning of GBM at EM is the only finding, although no patient with only microhematuria was biopsied. FSGS develops over time and its presence depends on the stage of the disease at which the biopsy is performed. Even in patients with a certain degree of proteinuria, 32.65% of kidney biopsies were normal at LM, while unspecific changes were identified in the remainder. Twenty patients underwent EM analysis showing thinning +/- splitting and lamellation of the GBM as well as podocyte effacement in some cases. As EM is not performed as regular clinical practice, it is clear from these findings that AS will hardly ever be suspected based on a KB with only LM study. Consequently, when suspicion of AS arises, nowadays it seems more reasonable to request a genetic test than a KB.[10]

Most families had private variants, although 14 different disease-causing variants were found in two or more unrelated families. No differences in kidney survival according to the causative gene were observed (although there was a tendency toward better kidney survival in *COL4A3* patients), and among the 61 patients who received KRT, the proportion with *COL4A3* or *COL4A4* variants was similar. In contrast to the previously reported genotype–phenotype correlation in males with XLAS and patients with ARAS[43–45] or ADAS[27], kidney survival did not correlate with type of variant in the present cohort. Discrepancies in genotype–phenotype correlations may be due to a relatively small cohort size, as demonstrated in other autosomal dominant diseases, such as autosomal dominant polycystic kidney disease, where large series have succeeded in showing excellent genotype–phenotype correlation while smaller ones ruled out any genotype–phenotype correlation.[46,47]

Extrarenal features were cardinal for the diagnosis of AS before the genomic era; a proteinuric kidney disease with HL was highly suggestive of AS, while nowadays patients are usually diagnosed on the basis of genetic testing and do not all show the full-blown syndrome. Extrarenal involvement seems to be infrequent in ADAS [22,23,27,48]; only 9% of patients from the present cohort had sensorineural HL while only two had ophthalmologic findings compatible with, though not pathognomonic for AS. This highlights the need not to attribute to AS those common eye or ear abnormalities that are usually related to age.

In summary, the diagnostic process for the disease is very challenging due to its wide spectrum, which makes ADAS a very underdiagnosed genetic kidney disease. Based on all the available evidence, we can infer that ADAS may be behind many cases of CKD of unknown cause, especially if there are affected relatives and/or presence of hematuria. A proper diagnosis of ADAS will allow genetic counselling for families, avoidance of KB and treatment advice. Given the extremely wide phenotypic expression of the disease, predictors of rapid progression for ADAS are absolutely needed in order to facilitate appropriate prescription of approved drugs and recruitment of patients for clinical trials.

Supplementary Material

Table S1: Variant classification according to the guidelines of the *American College of Medical Genetics and Genomics* (ACMG).

Table S2: Clinical and genetic data of patients with disease-causing variants in *COL4A3* and *COL4A4*.

Table S3: Patient characteristics stratified by type of lesion at kidney biopsy.

Figure S1: Probability of reaching kidney failure with replacement therapy in patients with heterozygous disease-causing variants in *COL4A3* and *COL4A4* by gender *(P=0.79).* Twelve of these patients had digenic/complex inheritance and were not included for survival analysis, and data on kidney function were unavailable for two patients at the time of the study.

Figure S2: Light microscopy microphotographs of the four types of lesion found in kidney biopsies of autosomal dominant Alport syndrome: a) no glomerular abnormality (hematoxylin and eosin stain ×20); b) focal segmental distribution of glomerulosclerotic lesions (hematoxylin and eosin stain ×40); c) expansion of the mesangial matrix with unspecific IgM immunostaining (immunofluorescence ×100); d) expansion of the mesangial matrix with negative immunofluorescence staining (periodic acid–Schiff ×20).

Figure S3: Probability of reaching kidney failure with replacement therapy comparing two groups: Group 1 (insertions, deletions, nonsense, splicing and substitutions of Gly for Arg, Glu, or Asp) versus Group 2 (substitutions of Gly for non Arg, Glu or Asp) *(P=0.52).*

Figure S4: Probability of reaching kidney failure with replacement therapy comparing: 1) non-truncating disease-causing variants, Gly substitutions, 2) non-truncating disease-causing variants, non-Gly substitutions and 3) truncating disease-causing variants (*P*=0.31).

Figure S5: Probability of reaching kidney failure with replacement therapy comparing the type of variant (missense, nonsense, splicing, frameshift, indel >5 amino acids (aa) in frame and indel <5 amino acids in frame) (*P*=0.99).

Article information

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REFERENCES

1. Barker DF, Hostikka SL, Zhou J, et al. Identification of mutations in the *COL4A5* collagen gene in Alport syndrome. *Science.* 1990; 248(4960):1224-7.doi:10.1126/science.2349482
2. Gretz N, Broyer M, Brunner FP, et al. Alport's syndrome as a cause of renal failure in Europe. *Pediatr Nephrol.* 1987;1(3):411-415. doi:10.1007/bf00849245
3. Mallett A, Tang W, Clayton PA, et al. End-stage kidney disease due to alport syndrome:

Outcomes in 296 consecutive Australia and New Zealand dialysis and transplant registry cases. *Nephrol Dial Transplant*. 2014;29(12):2277-2286. doi:10.1093/ndt/gfu254

4. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport's Syndrome, Goodpasture's Syndrome, and Type IV Collagen. *N Engl J Med.* 2003; 348(25):2543-56. doi: 10.1056/NEJMra022296
5. Miner JH. Pathology vs. molecular genetics: (Re)defining the spectrum of Alport syndrome. *Kidney Int*. 2014;86(6):1081-1083. doi:10.1038/ki.2014.326
6. Alport AC. Hereditary Familial Congenital Haemorrhagic Nephritis. *Br Med J*. 1927;1(3454):504-506. doi:10.1136/bmj.1.3454.504
7. Kruegel J, Rubel D, Gross O. Alport syndrome - Insights from basic and clinical research. *Nat Rev Nephrol*. 2013;9(3):170-178. doi:10.1038/nrneph.2012.259
8. 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(10):2603-2610. doi:10.1097/01.ASN.0000090034.71205.74
9. Torra R, Tazón-Vega B, Ars E, Ballarín J. Collagen type IV (α3-α4) nephropathy: From isolated haematuria to renal failure. *Nephrol Dial Transplant*. 2004;19(10):2429-2432. doi:10.1093/ndt/gfh435
10. Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F. Expert guidelines for the management of alport syndrome and thin basement membrane nephropathy. *J Am Soc Nephrol*. 2013;24(3):364-375. doi:10.1681/ASN.2012020148
11. Savige J, Ariani F, Mari F, et al. Expert consensus guidelines for the genetic diagnosis of Alport syndrome. *Pediatr Nephrol*. 2019;34(7):1175-1189. doi:10.1007/s00467-018-

3985-4

12. Kashtan CE, Ding J, Garosi G, 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):1045-1051. doi:10.1016/j.kint.2017.12.018
13. Torra R, Furlano M. New therapeutic options for Alport syndrome. *Nephrol Dial Transplant*. 2019;34(8):1272-1279. doi:10.1093/ndt/gfz131
14. Stokman MF, Renkema KY, Giles RH, Schaefer F, Knoers N, Van Eerde AM. The expanding phenotypic spectra of kidney diseases: Insights from genetic studies. *Nat Rev Nephrol*. 2016;12(8):472-483. doi:10.1038/nrneph.2016.87
15. 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):271-280. doi:10.1159/000435789
16. Gross O, Kashtan CE, Rheault MN, et al. Advances and unmet needs in genetic, basic and clinical science in Alport syndrome: Report from the 2015 International Workshop on Alport Syndrome. *Nephrol Dial Transplant*. 2017;32(6):916-924. doi:10.1093/ndt/gfw095
17. Lemmink HH, Nillesen WN, Mochizuki T, et al. Benign familial hematuria due to mutation of the type IV collagen alpha4 gene. *J Clin Invest*. 1996;98(5):1114-1118. doi:10.1172/JCI118893
18. 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(9):2721-2729. doi:10.1093/ndt/gfp158

19. Badenas C, Praga M, Tazón B, et al. Mutations in the *COL4A4* and *COL4A3* genes cause familial benign hematuria. *J Am Soc Nephrol*. 2002;13(5):1248-1254.
20. Fallerini C, Dosa L, Tita R, et al. Unbiased next generation sequencing analysis confirms the existence of autosomal dominant Alport syndrome in a relevant fraction of cases. *Clin Genet*. 2014;86(3):252-257. doi:10.1111/cge.12258
21. Morinière V, Dahan K, Hilbert P, et al. Improving mutation screening in familial hematuric nephropathies through next generation sequencing. *J Am Soc Nephrol*. 2014;25(12):2740-2751. doi:10.1681/ASN.2013080912
22. Kamiyoshi N, Nozu K, Fu XJ, et al. Genetic, clinical, and pathologic backgrounds of patients with autosomal dominant alport syndrome. *Clin J Am Soc Nephrol*. 2016;11(8):1441-1449. doi:10.2215/CJN.01000116
23. Papazachariou L, Demosthenous P, Pieri M, et al. Frequency of *COL4A3/COL4A4* Mutations amongst families segregating glomerular microscopic hematuria and evidence for activation of the unfolded protein response. Focal and segmental glomerulosclerosis is a frequent development during ageing. *PLoS One*. 2014;9(12). doi:10.1371/journal.pone.0115015
24. Gast C, Pengelly RJ, Lyon M, et al. Collagen (*COL4A*) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. *Nephrol Dial Transplant*. 2016;31(6):961-970. doi:10.1093/ndt/gfv325
25. Xie J, Wu X, Ren H, et al. *COL4A3* mutations cause focal segmental glomerulosclerosis. *J Mol Cell Biol*. 2014;6(6):498-505. doi:10.1093/jmcb/mju040

26. Wang M, Chun J, Genovese G, et al. Contributions of Rare Gene Variants to Familial and Sporadic FSGS. *J Am Soc Nephrol*. 2019;30(9):1625-1640. doi:10.1681/asn.2019020152

27. Malone AF, Phelan PJ, Hall G, et al. Rare hereditary *COL4A3/COL4A4* variants may be mistaken for familial focal segmental glomerulosclerosis. *Kidney Int*. 2014;86(6):1253-1259. doi:10.1038/ki.2014.305

28. Groopman EE, Marasa M, Cameron-Christie S, et al. Diagnostic Utility of exome sequencing for kidney disease. *N Engl J Med*. 2019;380(2):142-151. doi:10.1056/NEJMoa1806891

29. 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. *Nephrol Dial Transplant*. 2009;24(9):2721-2729. doi:10.1093/ndt/gfp158

30. Mencarelli MA, Heidet L, Storey H, et al. Evidence of digenic inheritance in alport syndrome. *J Med Genet*. 2015;52(3):163-174. doi:10.1136/jmedgenet-2014-102822

31. 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(2):363-371. doi:10.1016/j.kint.2018.02.027

32. Richards S, Aziz N, Bale S, 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):405-424. doi:10.1038/gim.2015.30

33. Ars E, Torra R. Rare diseases, rare presentations: Recognizing atypical inherited kidney

disease phenotypes in the age of genomics. *Clin Kidney J*. 2017; 10(5):586-593. doi: 10.1093/ckj/sfx051

34. Cameron-Christie S, Wolock CJ, Groopman E, et al. Exome-based rare-variant analyses in CKD. *J Am Soc Nephrol*. 2019;30(6):1109-1122. doi:10.1681/ASN.2018090909

35. 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(2):363-371. doi:10.1016/j.kint.2018.02.027

36. Pescucci C, Mari F, Longo I, et al. Autosomal-dominant Alport syndrome: Natural history of a disease due to *COL4A3* or *COL4A4* gene. In: *Kidney International*. 2004 May;65(5):1598-603. doi:10.1111/j.1523-1755.2004.00560.x

37. Pescucci C, Longo I, Bruttini M, Mari F, Renieri A. Type-IV collagen related diseases. *J Nephrol*. 2003 Mar-Apr;16(2):314-6.

38. Deltas C, Pierides A, Voskarides K. Molecular genetics of familial hematuric diseases. *Nephrol Dial Transplant*. 2013;28(12):2946-2960. doi:10.1093/ndt/gft253

39. Heidet L, Arrondel C, Forestier L, et al. Structure of the human type IV collagen gene *COL4A3* and mutations in autosomal Alport syndrome. *J Am Soc Nephrol*. 2001;12(1):97-106.

40. Boye E, Mollet G, Forestier L, et al. Determination of the Genomic Structure of the *COL4A4* Gene and of Novel Mutations Causing Autosomal Recessive Alport Syndrome. *Am J Hum Genet.* 1998;63(5):1329-40.

41. Ayasreh N, Bullich G, Miquel R, Furlano M, Ruiz P, Lorente L, Valero O, García-González MA, Arhda N, Garin I, Martínez V, Pérez-Gómez V, Fulladosa X, Arroyo D, Martínez-Vea A, Espinosa M, Ballarín J, Ars E, Torra R. Autosomal Dominant Tubulointerstitial Kidney Disease: Clinical Presentation of Patients With ADTKD-UMOD and ADTKD-MUC1. Am J

Kidney Dis. 2018;72(3):411-418. doi: 10.1053/j.ajkd.2018.03.019.

42. Temme J, Peters F, Lange K, et al. Incidence of renal failure and nephroprotection by RAAS inhibition in heterozygous carriers of X-chromosomal and autosomal recessive Alport mutations. *Kidney Int*. 2012;81(8):779-783. doi:10.1038/ki.2011.452

43. 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(4):649-57.

44. Bekheirnia MR, Reed B, Gregory MC, et al. Genotype-phenotype correlation in X-linked Alport syndrome. *J Am Soc Nephrol*. 2010;21(5):876-883. doi:10.1681/ASN.2009070784

45. Storey H, Savige J, Sivakumar V, Abbs S, Flinter FA. *COL4A3/COL4A4* mutations and features in individuals with autosomal recessive alport syndrome. *J Am Soc Nephrol*. 2013;24(12):1945-1954. doi:10.1681/ASN.2012100985

46. Rossetti S, Burton S, Strmecki L, et al. The position of the polycystic kidney disease 1 (*PKD1*) gene mutation correlates with the severity of renal disease. *J Am Soc Nephrol*. 2002;13(5):1230-1237. doi:10.1097/01.asn.0000013300.11876.37

47. Gall EC Le, Audrézet MP, Chen JM, et al. Type of *PKD1* mutation influences renal outcome in ADPKD. *J Am Soc Nephrol*. 2013;24(6):1006-1013. doi:10.1681/ASN.2012070650

48. Marcocci E, Uliana V, Bruttini M, et al. Autosomal dominant Alport syndrome: Molecular analysis of the *COL4A4* gene and clinical outcome. *Nephrol Dial Transplant*. 2009;24(5):1464-1471. doi:10.1093/ndt/gfn681

FIGURES AND LEGENDS

Figure 1: Probability of reaching kidney replacement therapy. a) Kaplan-Meier plot for the overall cohort. b) Cox survival function with proteinuria status as time-varying covariate (HR [95%CI]: 5.57 [2.68–11.60] (*P*<0.0001).

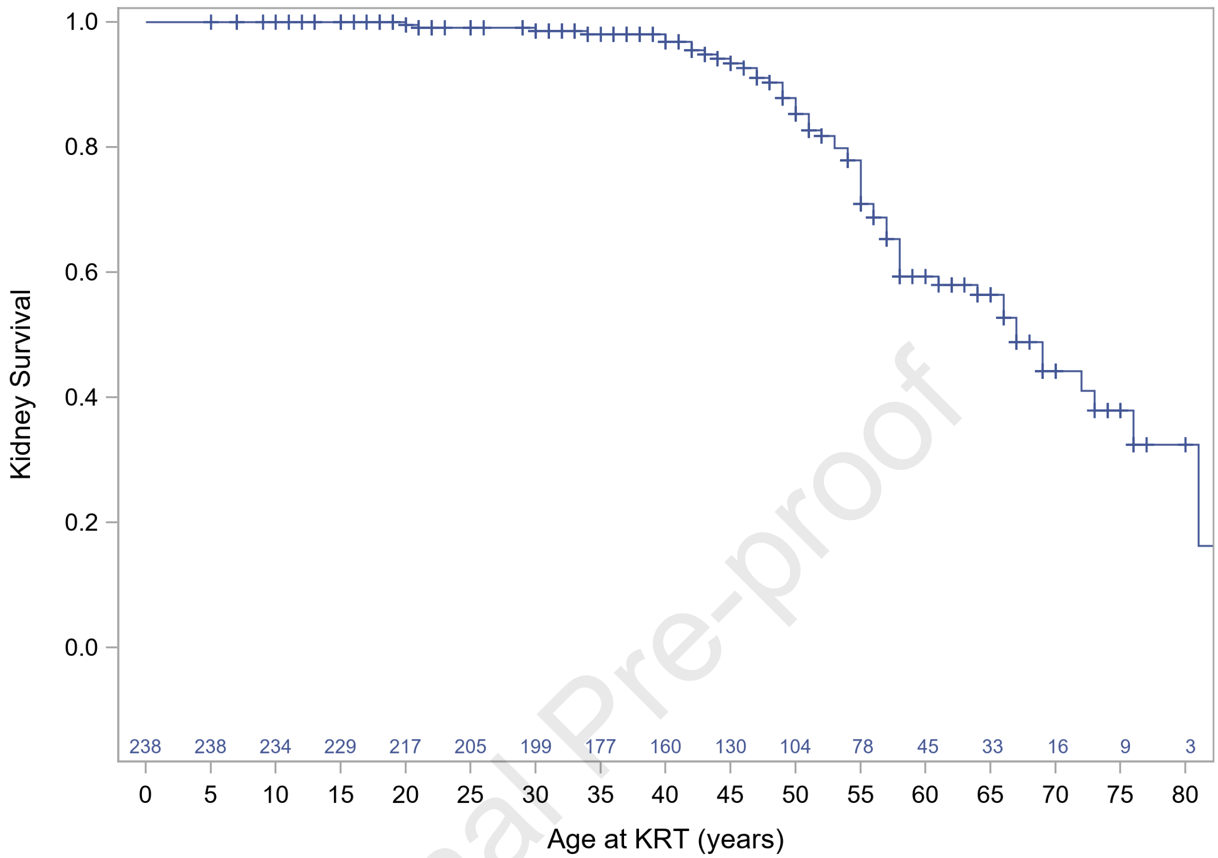
Figure 2: Progressive eGFR (estimated glomerular filtration rate) decline trajectories of patients with heterozygous disease-causing variants in *COL4A3* and *COL4A4*. The x-axis shows the age of the patients and the y-axis shows eGFR in mL/min/1.73 m^2. The dots are two or more eGFR data derived from creatinine measurement; the black line is the estimated trajectory. a) For overall cohort; b) using proteinuria status as time-varying covariate (slope difference: -0.81 [-1.18 to -0.45] (*P*<0.0001).

Figure 3: Intrafamilial variability of autosomal dominant Alport syndrome. Circles indicate present age of patients who are not on kidney replacement therapy (KRT) and red symbols represent age at starting KRT for transplanted or dialysis patients.

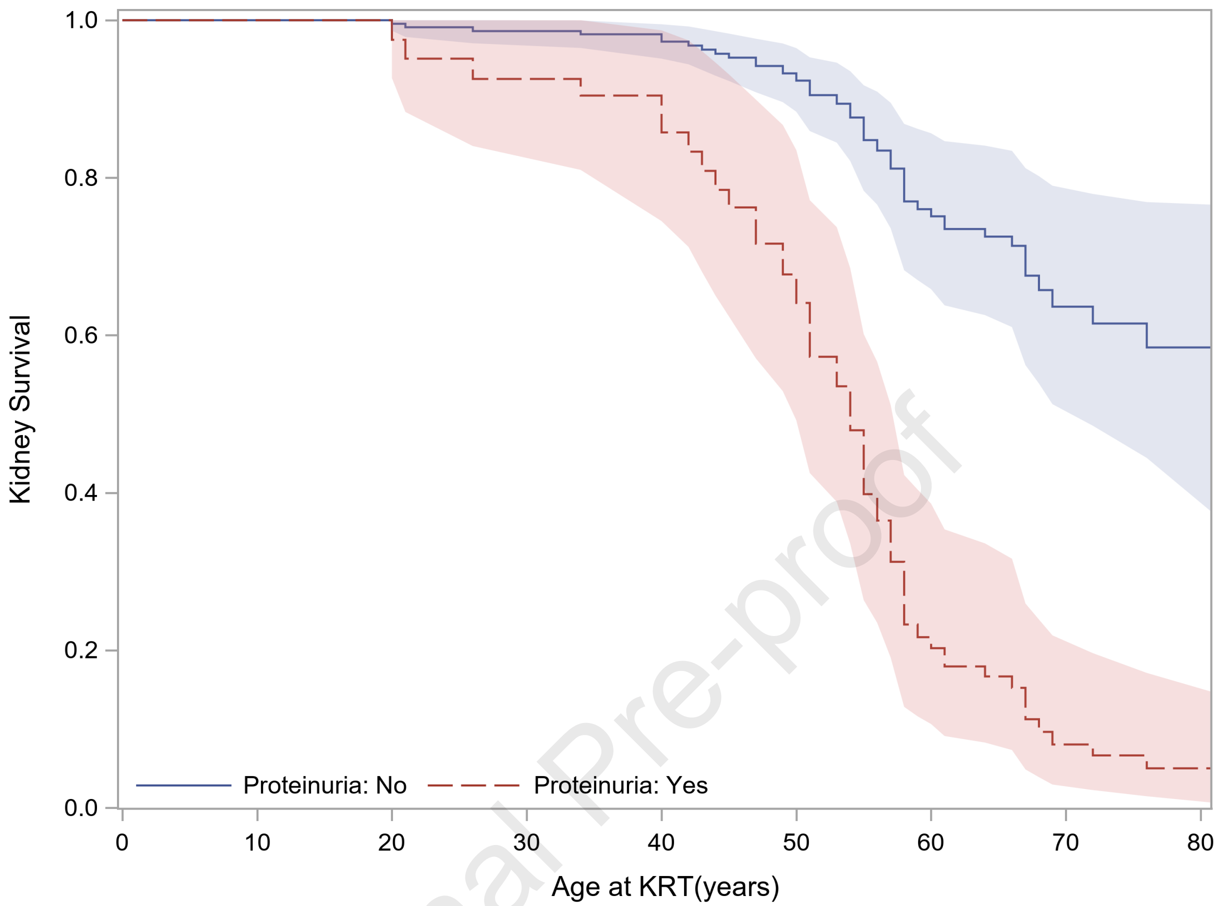
Figure 4: Percentage of families according to the type of variant in *COL4A3* and *COL4A4.*

Figure 5a: Probability of reaching kidney replacement therapy in patients with heterozygous disease-causing variants in *COL4A3* and *COL4A4* (*P*=0.55).

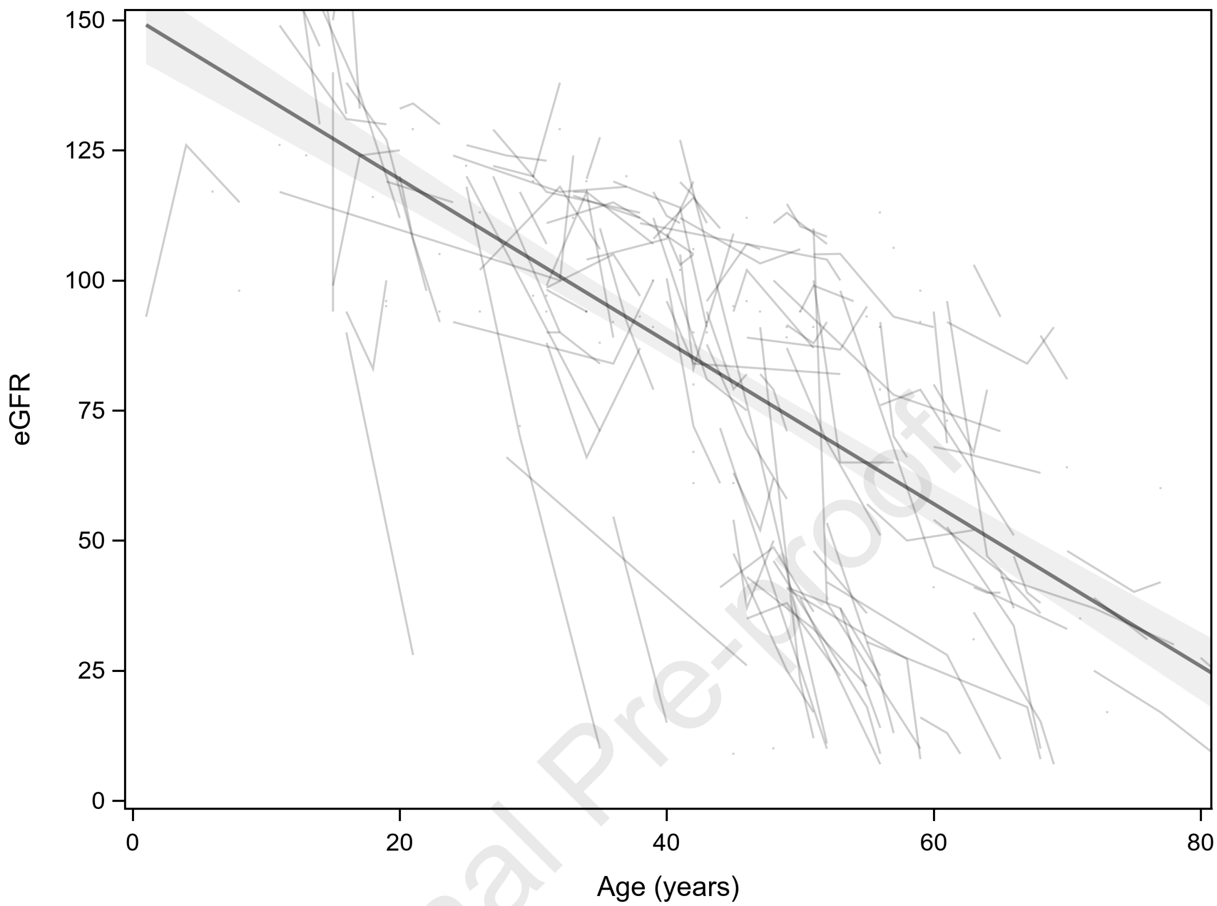
Figure 5b: Probability of reaching kidney replacement therapy comparing truncating versus non-truncating disease-causing variants (*P*=0.52).



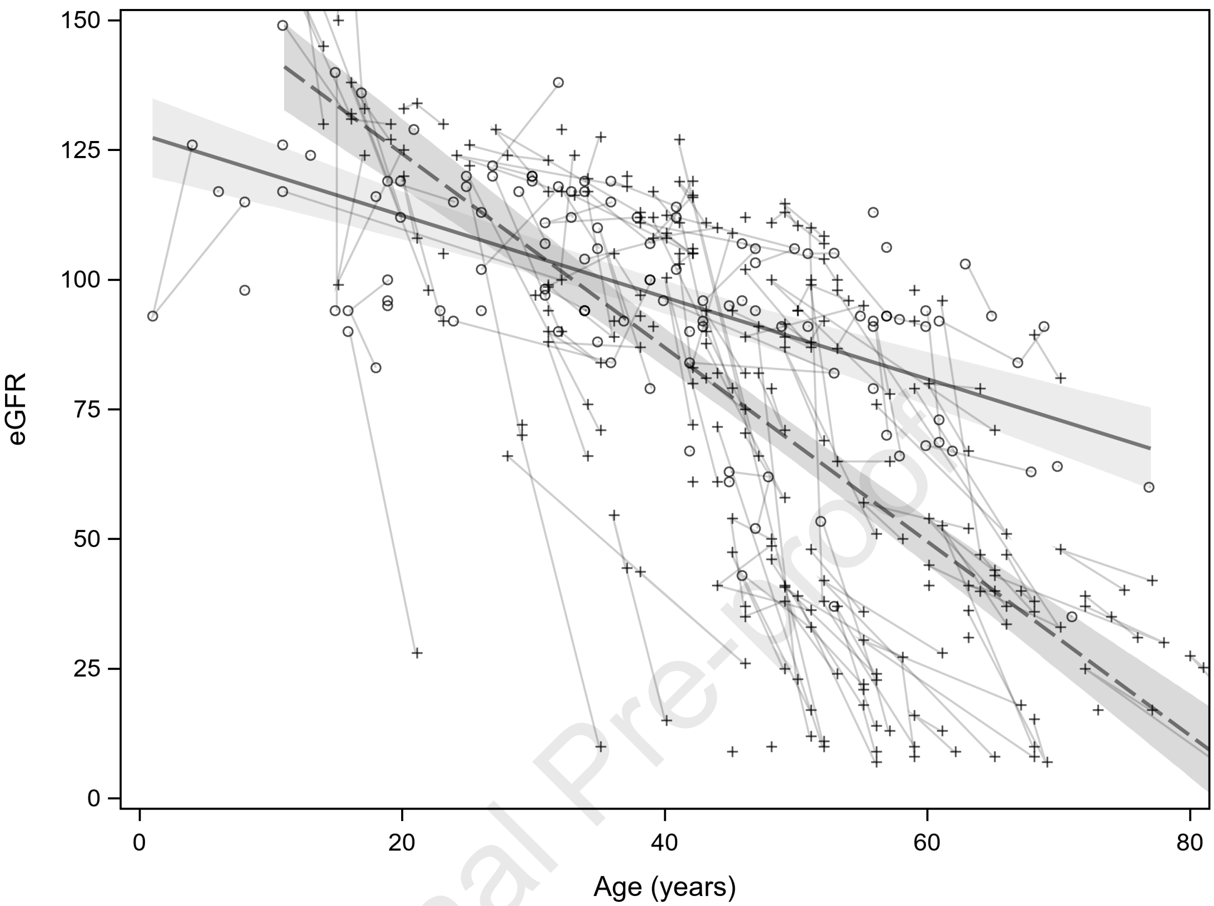
Kidney Survival
1.0
0.8
0.6
0.4
0.2
0.0
238 238 234 229 217 205 199 177 160 130 104 78 45 33 16 9 3
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Age at KRT (years)

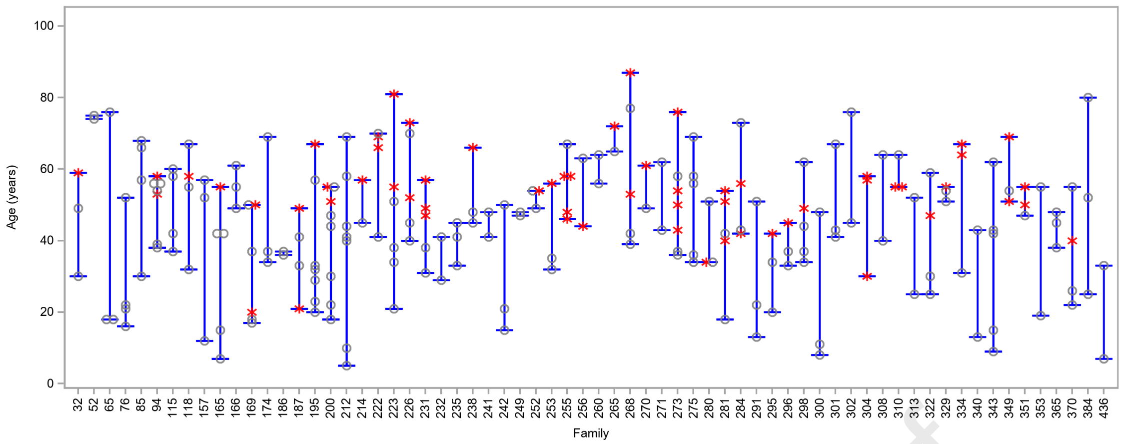


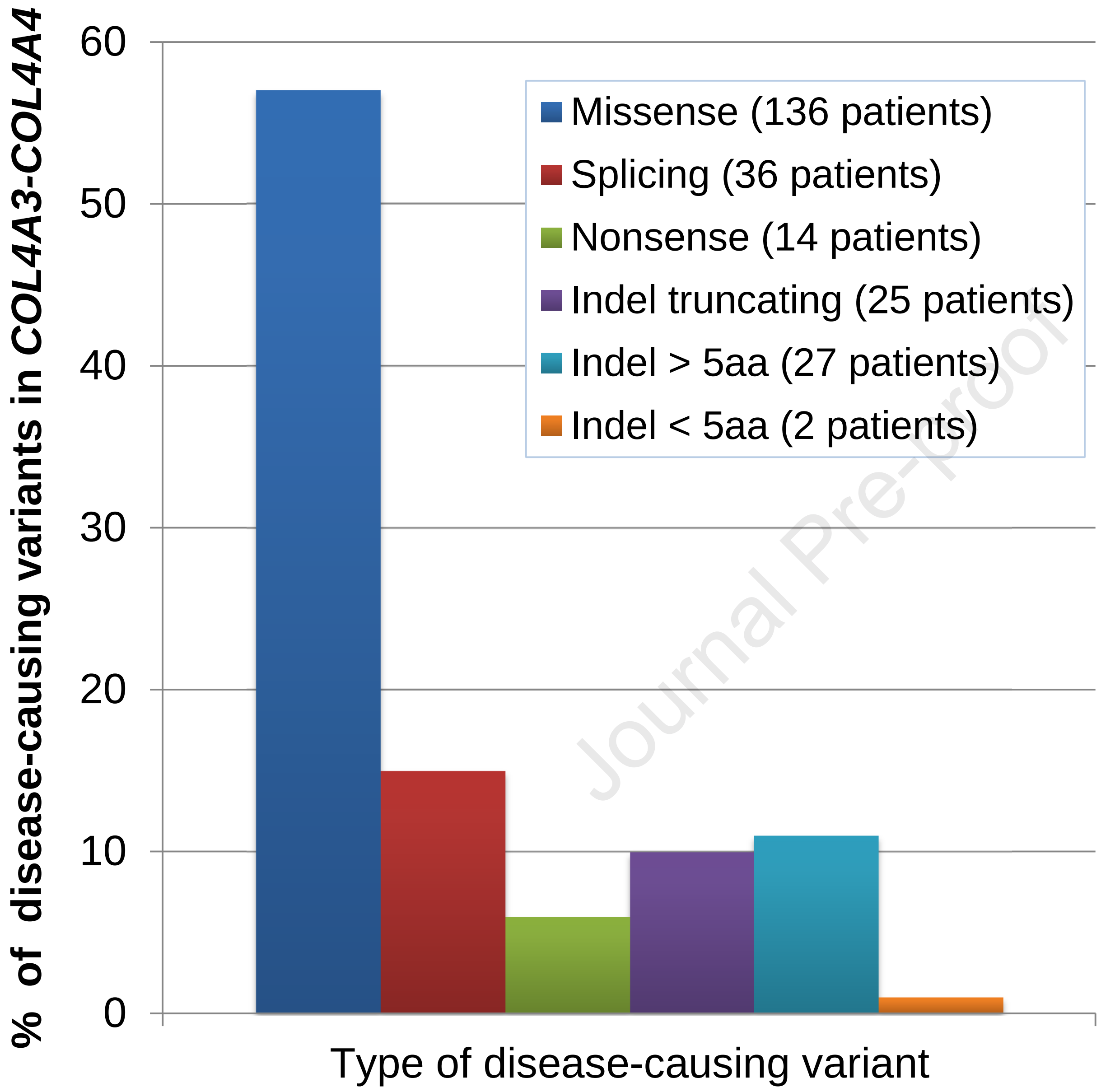
Kidney Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
Age at KRT(years)
Proteinuria: No
Proteinuria: Yes

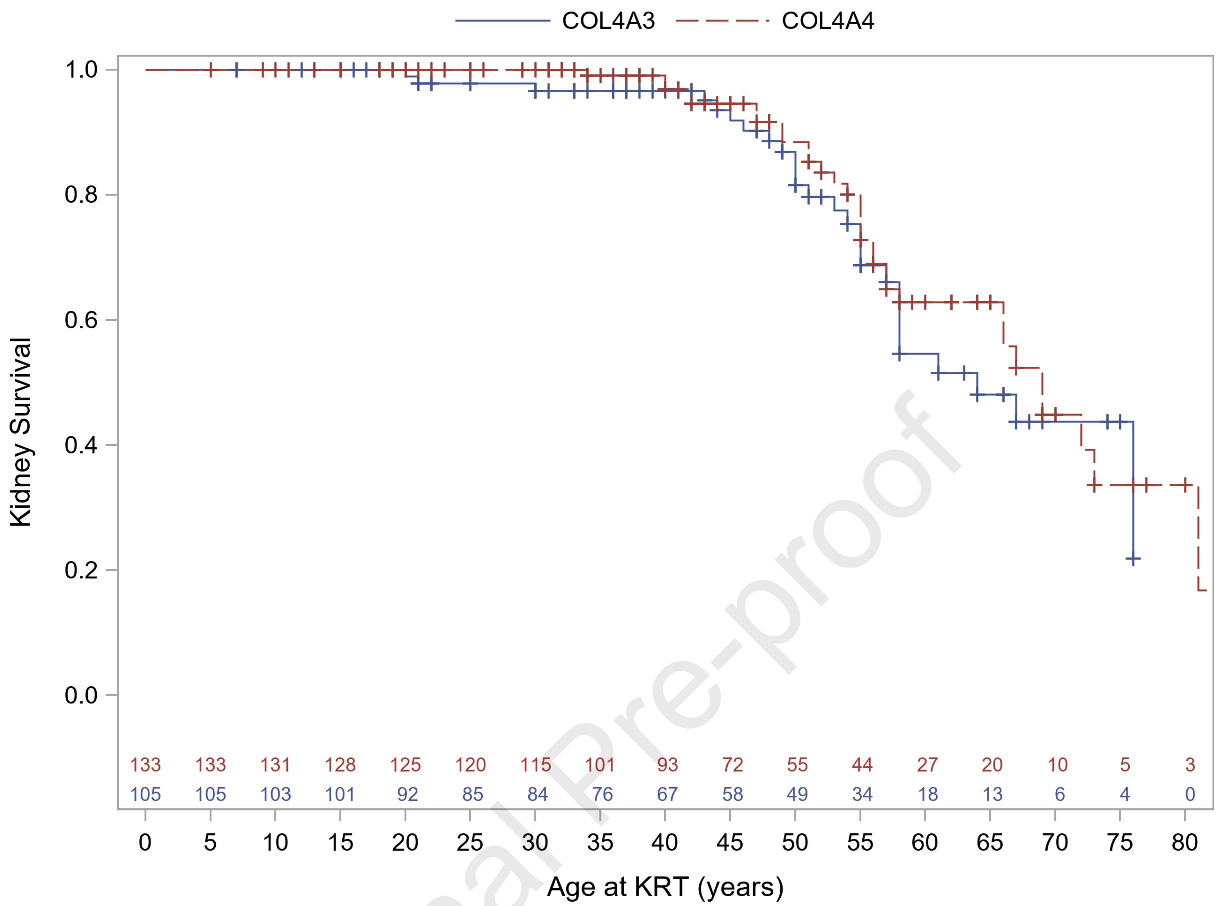


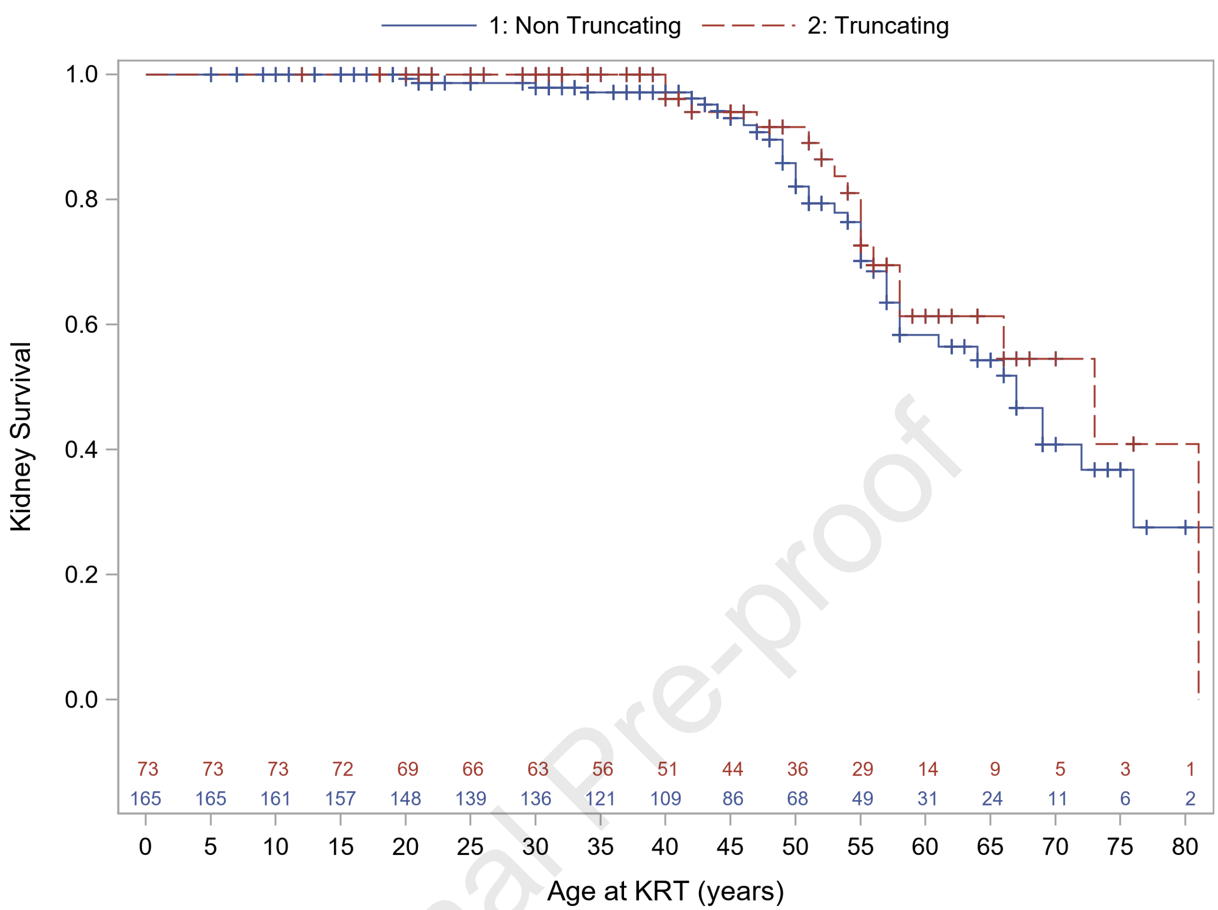
150
125
100
75
50
25
0
eGFR
0
20
40
60
80
Age (years)


150
125
100
75
50
25
0
eGFR
0
20
40
60
80
Age (years)


Age (years)
100
80
60
40
20
0
Family
32 52 65 76 85 94 115 118 157 165 166 169 174 186 187 195 200 212 214 222 223 226 231 232 235 238 241 242 249 252 253 255 256 260 265 268 270 271 273 275 280 281 284 291 295 296 298 300 301 302 304 308 310 313 322 329 334 340 343 349 351 353 365 370 384 436


% of disease-causing variants in *COL4A3-COL4A4*
60
50
40
30
20
10
0
Missense (136 patients)
Splicing (36 patients)
Nonsense (14 patients)
Indel truncating (25 patients)
Indel > 5aa (27 patients)
Indel < 5aa (2 patients)
Type of disease-causing variant


COL4A3
COL4A4
Kidney Survival
1.0
0.8
0.6
0.4
0.2
0.0
133 133 131 128 125 120 115 101 93 72 55 44 27 20 10 5 3
105 105 103 101 92 85 84 76 67 58 49 34 18 13 6 4 0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Age at KRT (years)


1: Non Truncating
2: Truncating
Kidney Survival
1.0
0.8
0.6
0.4
0.2
0.0
73 73 73 72 69 66 63 56 51 44 36 29 14 9 5 3 1
165 165 161 157 148 139 136 121 109 86 68 49 31 24 11 6 2
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Age at KRT (years)