

## Clinical and Genetic Features of Autosomal Dominant Alport Syndrome: A Cohort Study



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**Rationale & Objective:** Alport syndrome is a common genetic kidney disease accounting for approximately 2% of patients receiving kidney replacement therapy (KRT). It is caused by pathogenic variants in the gene COL4A3, COL4A4, or COL4A5. 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), whereas 133 harbored a pathogenic variant in COL4A4 (43 families). Digenic/complex inheritance was observed in 12 patients. Overall, the median kidney survival was 67 (95% CI, 58-73) years, without significant differences

across sex ( $P = 0.8$ ), causative genes ( $P = 0.6$ ), or type of variant ( $P = 0.9$ ). 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 estimated glomerular filtration rate change was  $-1.46$  ( $-1.66$  to  $-1.26$ ) mL/min/1.73 m<sup>2</sup> per year for the overall group, with no significant differences between ADAS genes ( $P = 0.2$ ).

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

**Conclusions:** Patients with ADAS have a wide spectrum of 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.

### Visual Abstract online

Complete author and article information provided before references.

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Alport syndrome was the first inherited kidney disease for which the genetic bases were identified.<sup>1</sup> It accounts for at least 1%-2% of all cases of patients undergoing kidney replacement therapy (KRT).<sup>2,3</sup>

Pathogenic variants in the COL4A3, COL4A4, and COL4A5 genes cause defective synthesis of the  $\alpha 3$ ,  $\alpha 4$ , and  $\alpha 5$  chains of collagen IV, preventing correct assembly of the glomerular basement membrane (GBM) collagen network and causing Alport syndrome.<sup>4,5</sup> Classical Alport syndrome is characterized by hematuria with progressive proteinuric kidney disease, GBM abnormalities, hearing loss, and ocular abnormalities.<sup>4,6,7</sup> This full-blown presentation is typically found in patients with autosomal recessive Alport syndrome (ARAS) and male patients with X-linked Alport syndrome. Women with X-linked Alport syndrome show a variable phenotype due to the X inactivation phenomenon.<sup>8</sup> Patients with heterozygous disease-causing variants in COL4A3 or COL4A4 display a wide spectrum of manifestations, ranging from asymptomatic to presentation with hematuria alone or with proteinuria and subsequent kidney failure in addition to hematuria. There is a lot of controversy as to how to designate the disease.<sup>9-16</sup> In cases in which there is only hematuria, the terms “familial benign hematuria” and “thin basement membrane disease” have been used.<sup>17-19</sup> However, these terms now seem outdated because: (1) familial benign hematuria implies a very mild disease, which is not

always the case; (2) thin basement membrane disease is a histopathologic finding associated with other histologic abnormalities in a minor, but not insignificant, percentage of patients; and (3) kidney disease may develop in “carriers of ARAS,” meaning they are not only carriers but patients themselves. The term autosomal dominant Alport syndrome (ADAS) has historically been reserved for patients with heterozygous disease-causing variants in COL4A3 or COL4A4 with kidney failure,<sup>10,11</sup> but there is increasing agreement that any patient harboring a heterozygous disease-causing variant in COL4A3 or COL4A4 should be considered as having ADAS.<sup>12,13,20-22</sup>

In recent years, the use of next-generation sequencing has allowed increased identification of disease-causing variants in COL4A3 and COL4A4. These have proven to be behind 40%-60% cases of familial hematuria<sup>23</sup> and most familial cases of focal segmental glomerulosclerosis (FSGS), but they are also found in sporadic FSGS or hematuria and chronic kidney disease (CKD).<sup>24-27</sup> Recently, Groopman et al found that, in a large adult cohort of patients with CKD, COL4A3, COL4A4, and COL4A5 were the most frequent causative genes of monogenic CKD after the polycystic kidney disease genes PKD1 and PKD2.<sup>28</sup> Although some attempts have been made to provide insights into the clinical features of patients with heterozygous disease-causing variants in COL4A3 and COL4A4, most studies

### 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 form, this entity is much less severe: most patients have hematuria but only a minority will experience 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 in whom kidney failure develops without an etiologic diagnosis.

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.<sup>27,29</sup> 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 complicates the disease phenotype.<sup>30</sup>

In this study, we provide an in-depth analysis of clinical features and types of variants in a large cohort of families with heterozygous disease-causing variants in COL4A3 and COL4A4.

## 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. Genetic tests were performed in these families in the case of: (1) families with autosomal dominant inherited microhematuria and proteinuria and/or CKD or (2) index patients with an abnormal GBM. Only 1 family with isolated microhematuria was included. A genetic diagnosis was made in this family to rule out X-linked Alport syndrome 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 participate 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 that included sex, date of

birth, age at diagnosis of kidney disease, age-related decrease in kidney function, repeated measurements of urinary protein-creatinine ratio, need for KRT, age at the time of KRT initiation, and description of genetic variants. Results of light microscopy of kidney biopsy specimens were classified into 4 groups: normal, FSGS, expansion of the mesangial matrix with positive immunofluorescence staining (unspecific immunoglobulin M and C3 deposits), and expansion of the mesangial matrix with negative immunofluorescence staining. If electron microscopy (EM) was available, the GBM findings were recorded.

Kidney function was assessed by the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation for calculating estimated glomerular filtration rate (eGFR) in individuals with at least 3 creatinine values 3 years apart. Age at the time of KRT initiation was defined as the age at which the patient had a CKD-EPI-calculated eGFR <10 mL/min/1.73 m<sup>2</sup> or began KRT. Age at diagnosis of hearing loss, type of hearing loss, and ocular lesions were recorded.

### Genetic Testing

Genetic testing was performed in index cases by next-generation sequencing of a kidney disease gene panel containing COL4A3, COL4A4, and COL4A5 genes as previously reported.<sup>31</sup> 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 reports in the ClinVar database and Leiden Open Variation Database. 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),<sup>32</sup> and those variants classified as pathogenic or likely pathogenic were considered causative of disease (Table S1). Digenic/complex inheritance pattern was considered present when 2 disease-causing variants were identified in COL4A3 and COL4A4 in the index case.

Variants were classified into the following categories: in-frame insertion and deletion (indel) <5 amino acids, in-frame indel >5 amino acids, frame-shift indel (also referred to herein as a truncating indel because the frameshift variants we observed were predicted to lead to a truncated protein), missense, nonsense, and splicing. To study whether the type of disease-causing variant correlated with age at the time of KRT initiation, variants were classified into different groups (the 6 categories listed above or various combinations or subdivisions of these groups) according to their predicted severity.

## Statistical Analysis

For descriptive statistics, continuous variables are presented as mean  $\pm$  standard deviation or median (interquartile range [IQR]) according to their adherence to the Gaussian distribution, and categorical data are presented as frequencies and percentages. A Fisher exact test was used to compare categorical variables, whereas the Mann-Whitney test was used to compare continuous variables between sex and COL4A disease-causing variant.

The survival function as well as the median (95% CI) time to KRT initiation was estimated by the Kaplan-Meier method. We used the date of birth as the starting point, and the last point of the survival analysis was the age at KRT initiation or the last available observation. No patient died before reaching kidney failure (ie, no competing risks), and the number of at-risk patients is listed 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 with 95% CIs were calculated by means of the Cox model using the family as a cluster to account for intragroup correlation. Kidney function was assessed using a slope analysis by means of mixed models for repeated measurements, including time (age or time from diagnosis), participant 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 receiving KRT and the age at the start of KRT for patients who received a transplant or dialysis per family. The analysis was performed using SAS software (version 9.4; SAS Institute Inc), and the level of significance was established at the 0.05 level (2-sided).

## Results

### Clinical Features

#### Overview

Of the cohort of 252 patients, there were 142 women (56.3%), and the mean age at diagnosis was  $33.6 \pm 17.1$  years. No significant differences in kidney survival according to sex were identified ( $P = 0.8$ ; Fig S1; 14 patients were excluded from kidney survival analysis because of digenic/complex inheritance or lack of kidney function data). Thirty-four individuals 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 ADAS family ALP-281 (who had the same variant as family ALP-189), in which several individuals were undergoing KRT (Table S2).

#### Kidney Disease

Hematuria was present in 92.1% of patients (232 of 252). Of 20 patients without hematuria, 16 belonged to families in which someone had been diagnosed with ARAS and 4

were from families with ADAS. None of the patients without hematuria had exhibited proteinuria, but 2 had CKD glomerular filtration rate category 2 (CKD G2; ie,  $60\text{--}89\text{ mL/min/1.73 m}^2$ ) 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, of whom 157 (65.2%) had proteinuria. None of the patients without proteinuria needed KRT (only 1 patient without proteinuria presented with an  $\text{eGFR} < 45\text{ mL/min/1.73 m}^2$ ; this person had CKD G3b and was 70 years of age with a history of nephrectomy), but, among those with proteinuria, 61 of 157 (38.9%) received KRT. Data on the use of renin-angiotensin-aldosterone system (RAAS) inhibitors were not properly recorded.

Overall kidney survival was 67 (95% CI, 58–76) years, whereas, for patients with proteinuria, it was 58 (95% CI, 56–57) years (Fig 1A). The presence of proteinuria significantly increased the risk of KRT in the time-varying survival analysis (hazard ratio, 5.57 [95% CI, 2.68–11.60];  $P < 0.001$ ; Fig 1B). In the whole group, 61 patients received KRT (24.2%) at mean and median ages of  $54.0 \pm 12.2$  and 55 (IQR, 49–58) years, respectively. Six patients needed KRT before 41 years of age. The slope of change in  $\text{eGFR}$ , which was  $-1.46$  (95% CI,  $-1.66$  to  $-1.26$ )  $\text{mL/min/1.73 m}^2$  per year for the overall group (Fig 2A), showed no significant differences between COL4A3 and COL4A4 ( $P = 0.2$ ). The slopes of change in  $\text{eGFR}$  for patients with versus without proteinuria were  $-1.78$  and  $-0.97\text{ mL/min/1.73 m}^2$  per year (difference,  $0.80$  [95% CI,  $0.45\text{--}1.18$ ];  $P < 0.001$ ; Fig 2B). A large intrafamilial variability with regard to age at initiation of KRT was also observed (Fig 3). The slope of the increase in urinary protein-creatinine ratio was  $11.34$  (95% CI,  $1.95\text{--}20.72$ )  $\text{mg/g}$  for each year of life.

#### Hearing Loss

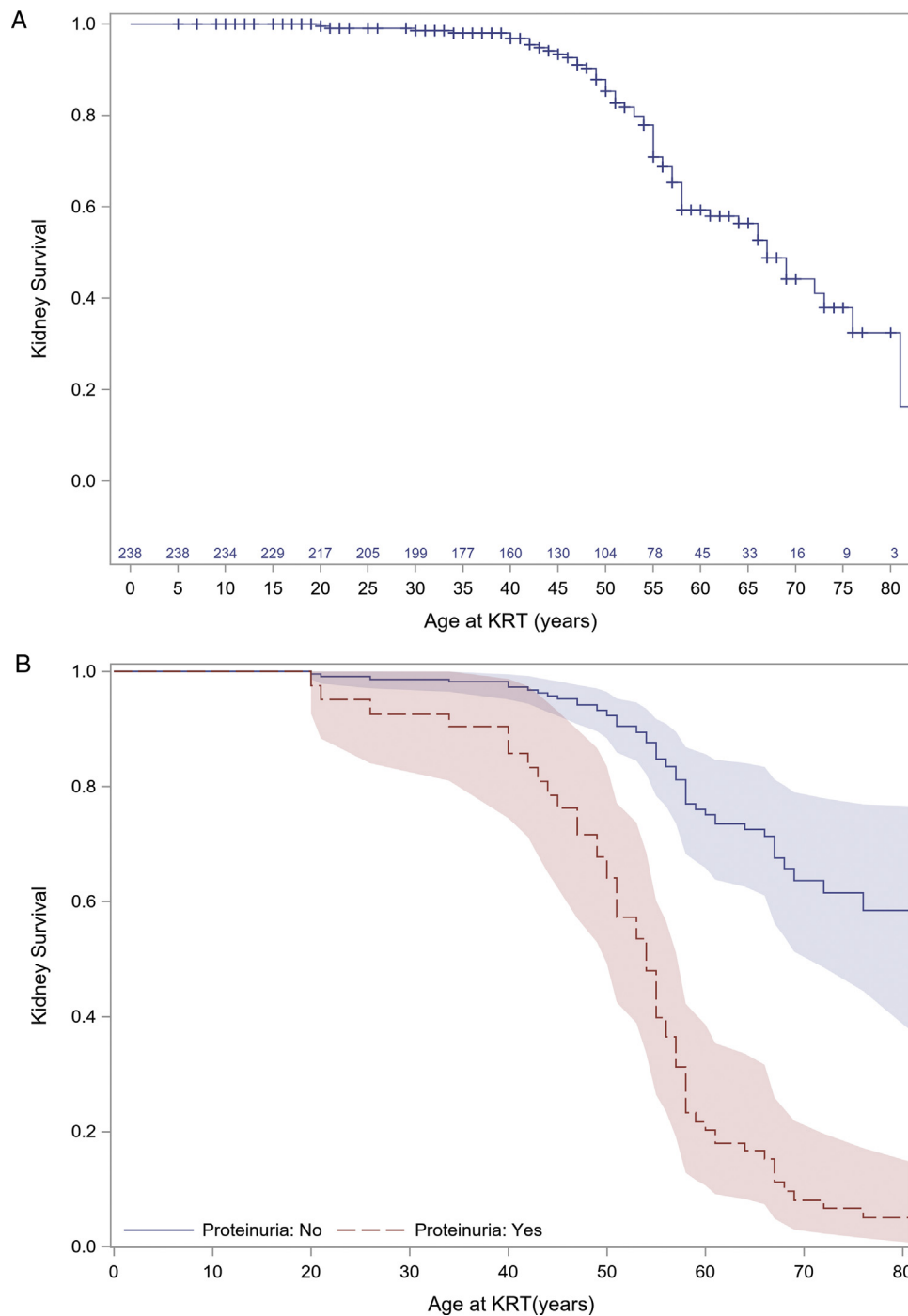
Among 131 patients with audiometry data recorded, 35.9% showed hearing loss, but only 11 patients (8.4%) showed high-tone bilateral sensorineural hearing loss. Only 1 patient with sensorineural hearing loss received KRT (at 67 years of age; Table S2).

#### Ocular Abnormalities

Seventy-five patients underwent a thorough ophthalmologic examination, and, even though 23% of them had abnormal findings, only 2 had anomalies possibly related to Alport syndrome: one had recurrent corneal erosions (this individual did wear contact lenses) and another had corneal dystrophy (Table S2).

#### Pathologic Changes

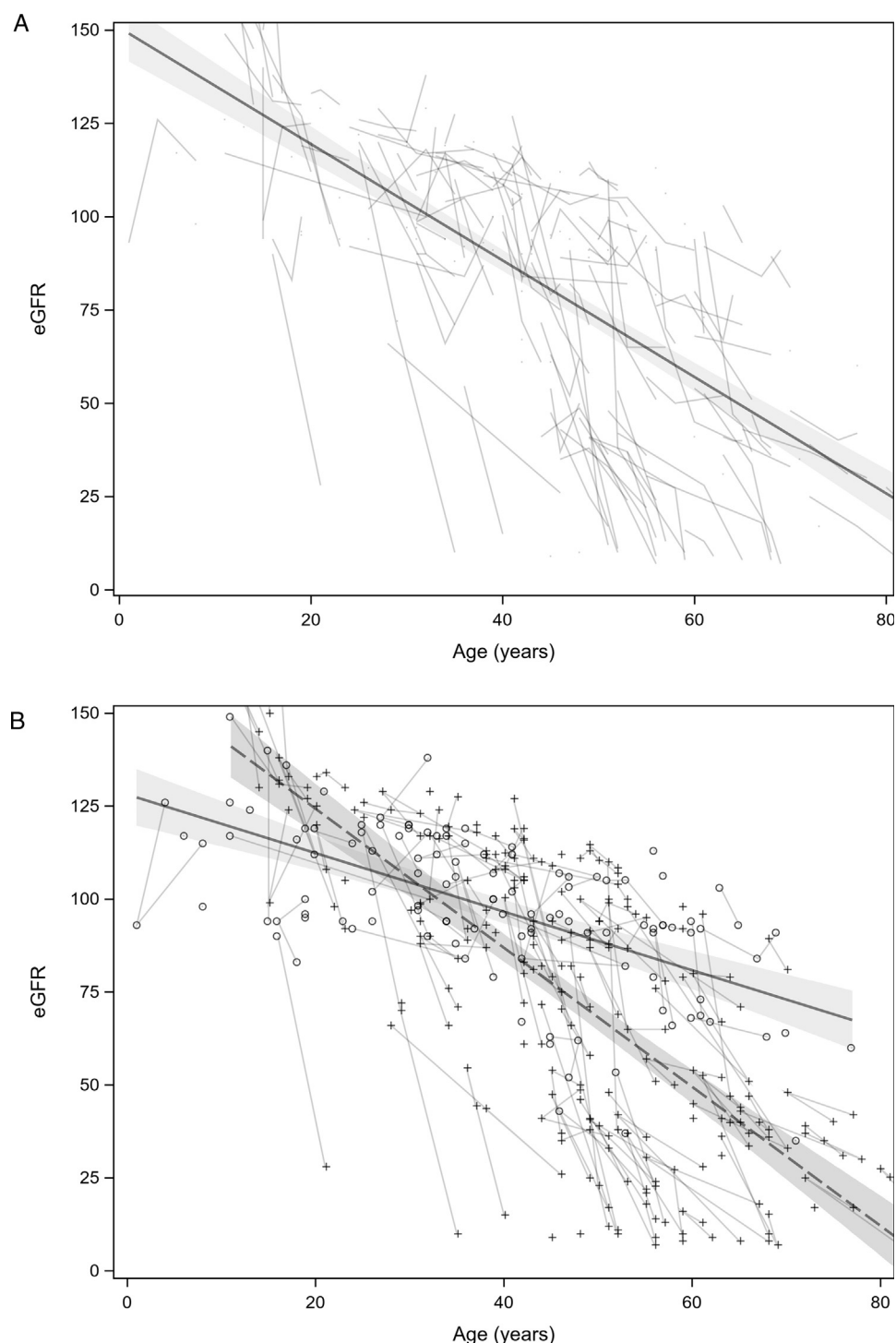
Forty-nine of 157 patients with proteinuria underwent a kidney biopsy, whereas no patient with hematuria alone had a kidney biopsy (Table S2). Based on histology



**Figure 1.** Probability of reaching KRT. (A) Kaplan-Meier plot for the overall cohort. (B) Cox survival function with proteinuria status as time-varying covariate (hazard ratio for incident KRT, 5.57 [95% CI, 2.68-11.60] in those with vs without proteinuria;  $P < 0.001$ ).

(Table S3), patients were classified as follows: normal by light microscopy and with negative immunostaining (Fig S2A), 32.7%; FSGS (Fig S2B), 32.7%; expansion of the mesangial matrix with unspecific positive immunostaining (Fig S2C), 18.4%; and expansion of the mesangial matrix with negative immunofluorescence staining (Fig S2D), 16.3%. Of those with FSGS, 75% needed KRT.

EM was performed in 20 specimens, disclosing thinning with or without splitting and lamellation of the GBM in all patients and podocyte effacement in some. The mean age at the time of kidney biopsy was  $36.6 \pm 12.6$  (median, 35 [IQR, 26–51]) years, and mean eGFR was  $79.1 \pm 28.9$  mL/min/1.73 m<sup>2</sup>. Magnitude of proteinuria at the time of kidney biopsy ranged from 0.10 to 6.8 (mean,  $1.5 \pm 1.65$ ) g/d, with 81% having proteinuria  $>300$  mg/d.



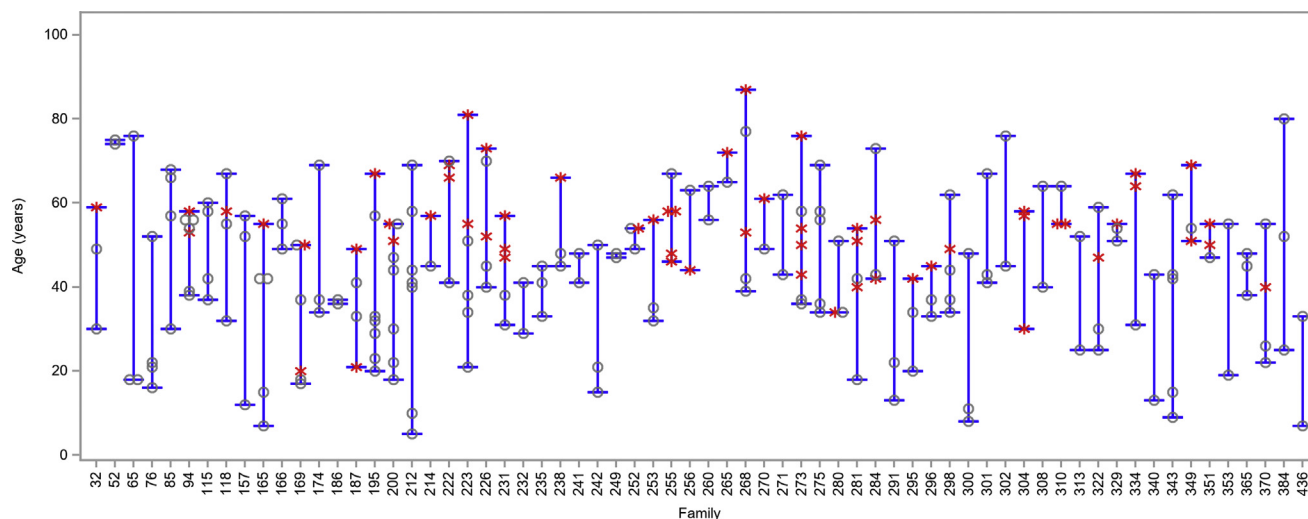
**Figure 2.** eGFR decrease trajectories of patients with heterozygous disease-causing variants in *COL4A3* and *COL4A4*. The x axis shows the age of the patients, and the y axis shows eGFR in mL/min/1.73 m<sup>2</sup>. The dots are 2 or more eGFR data derived from creatinine measurement; the black line is the estimated trajectory. Results are shown for the overall cohort (A) and (B) using proteinuria status as time-varying covariate (slope difference,  $-0.81$  [ $-1.18$  to  $-0.45$ ];  $P < 0.001$ ).

### Genetic Testing

Genetic testing was performed in 216 patients; the remaining 36 had a history of kidney disease compatible with Alport syndrome 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 35 families (95 patients genetically analyzed and 12 inferred), and a disease-causing variant

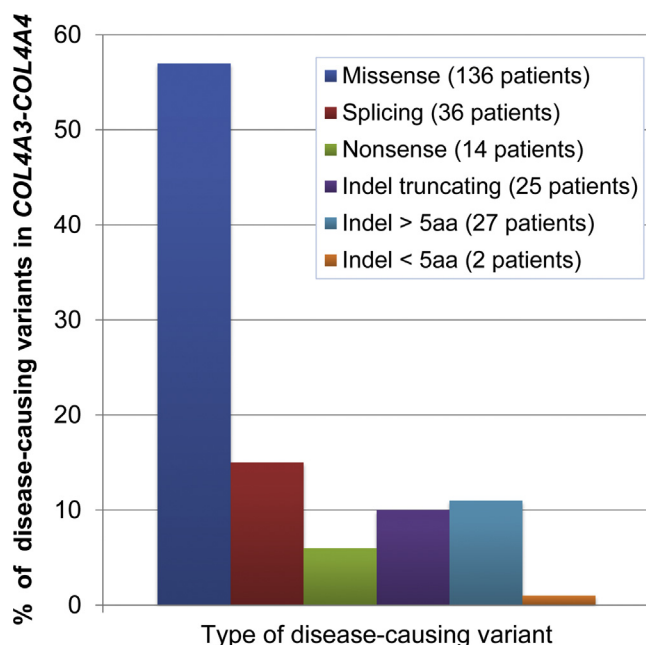




**Figure 3.** Intrafamilial variability of ADAS. Circles indicate present age of patients who are not undergoing KRT, and red symbols represent age at the start of KRT for transplant or dialysis recipients.

in COL4A4 was identified in 43 families (109 patients genetically analyzed and 24 inferred) (Tables S1-S2). Digenic/complex inheritance was identified in 12 patients (Table S2).

Missense variants were the most common type of disease-causing variant (Fig 4). The amino acid distribution among the missense disease-causing variants in which there was substitution of glycine (91.9%) was as follows: 35.2% arginine, 29.6% valine, 20% glutamate, 9.6% serine, 1.6% aspartate, and 4% cysteine. The percentage of patients with truncating disease-causing variants was 31%.



**Figure 4.** Percentage of patients according to the type of variant in COL4A3 and COL4A4. Abbreviations: aa, amino acid; indel, insertion and deletion.

### Genotype–Phenotype Correlation

Although there was not a statistically significant difference in kidney survival when comparing causative genes ( $P = 0.6$ ), the median age at KRT was 64 (95% CI, 57–71) years for COL4A3, versus 69 (95% CI, 58–81) years for COL4A4 (Fig 5A). Hence, to evaluate the remaining genotype-phenotype correlations, we considered COL4A3 and COL4A4 variants together. When comparing patients with truncating and nontruncating variants, no significant differences regarding kidney survival were observed ( $P = 0.5$ ; Fig 5B). Additionally, kidney survival did not vary by other classifications based on the predicted severity of the variant type (Figs S3–S5).

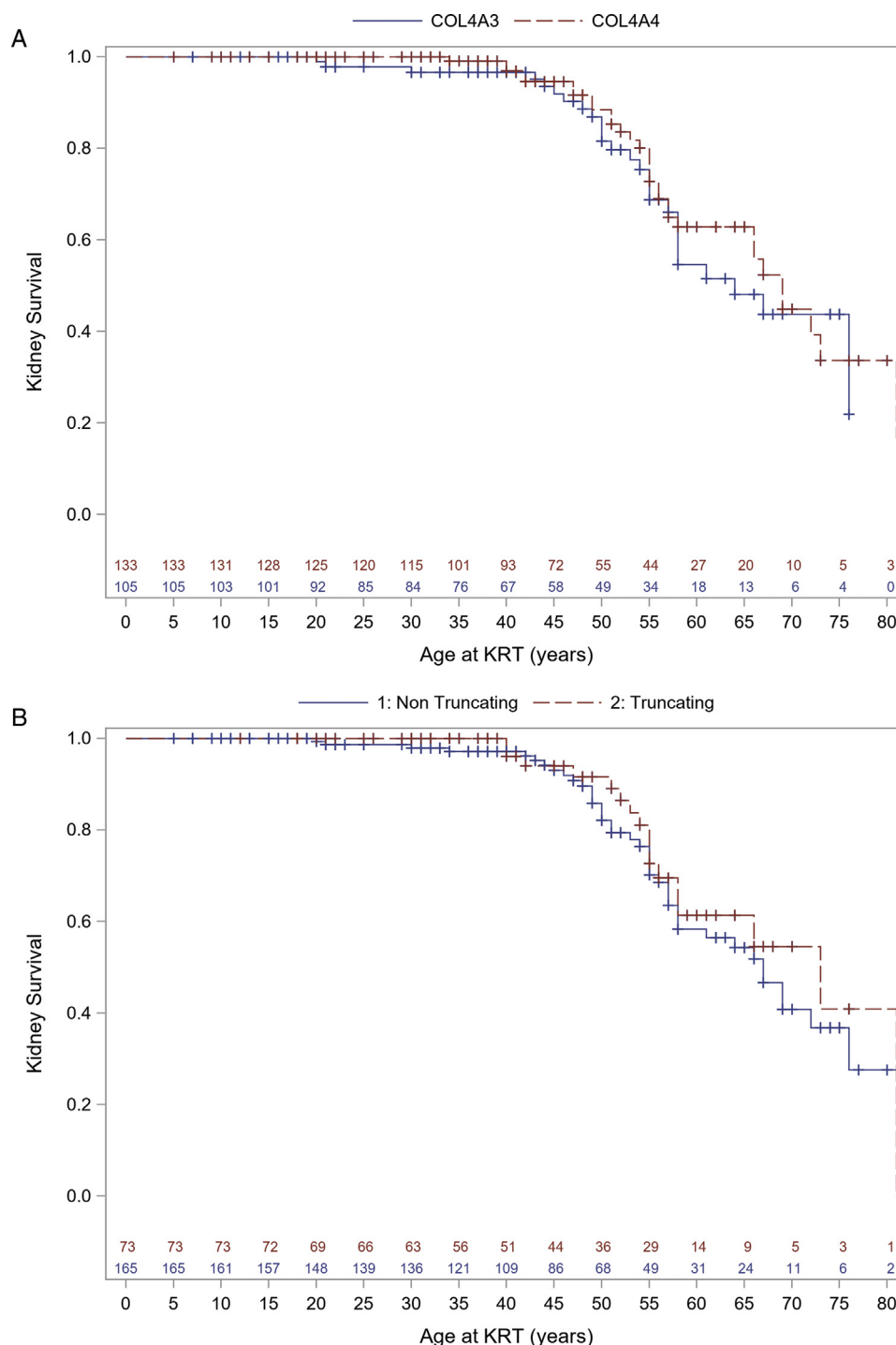
Of 6 patients starting KRT before 41 years of age, 3 had a missense disease-causing variant involving substitution of glycine, 1 had a splicing disease-causing variant, and 2 had an indel disease-causing variant: one in-frame indel involving more than 5 amino acids, and the other a truncating indel due to a frameshift variant.

Patients with a digenic/complex inheritance were not included in the survival analysis because of the low number of patients but are described in Table S2. Whereas some of them had an early phenotype, others had 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 has led to an expansion in our understanding of the phenotypic spectrum of AS<sup>14,26,33,34</sup> and has increased the estimated prevalence of ADAS from being “very rare” to accounting for at least 20% of cases of Alport syndrome.<sup>20,21,35</sup> The



**Figure 5.** Probability of reaching KRT (A) in patients with heterozygous disease-causing variants in *COL4A3* or *COL4A4* ( $P = 0.6$ ) and (B) comparing truncating versus nontruncating disease-causing variants ( $P = 0.5$ ). Truncating disease-causing variants included 14 nonsense variants, 23 frameshift indels resulting in premature termination, and 36 splicing variants. Analysis limited to individuals available to assess kidney survival.

scientific community can choose to keep defining Alport syndrome as a full-blown syndrome<sup>6</sup> or redefine it based on genetic findings.<sup>12–14,36,37</sup> The latter implies that patients may be diagnosed with Alport syndrome even when they have only microhematuria or even when they are asymptomatic as a result of incomplete penetrance. If this

option becomes accepted, nephrologists, genetic counselors, and patients will need to be educated on the wide spectrum of the disease.<sup>38</sup> Also, the number of people carrying one of these variant alleles may be high, and they may be identified frequently by next-generation sequencing.<sup>28</sup> A precise genetic diagnosis of Alport

syndrome 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, to our knowledge, 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.<sup>21,27,28</sup> The present data support previous studies in which progression to KRT has been observed in some patients whereas others remain with isolated hematuria throughout their life, thus showing huge inter- and intrafamilial variability. Median kidney survival in the present cohort was 67 (95% CI, 58–76) years, which is longer than in the X-linked and recessive forms of Alport syndrome. The name Alport syndrome suggests severity and early age at KRT initiation, but, as evidenced in this series, the autosomal dominant form is much milder. This is in line with most autosomal dominant kidney diseases compared with X-linked or autosomal recessive forms.

Digenic/complex inheritance has been proposed as an 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 large inter- and intrafamilial variability suggests a role for genetic modifiers and epigenetic or environmental factors.<sup>39</sup>

The usual late age of onset of the disease, together with the finding of COL4A3 and COL4A4 disease-causing variants in a substantial percentage of patients with CKD,<sup>28,34</sup> 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.<sup>22,40,41</sup> This implies an incomplete penetrance, which is not uncommon in genetic kidney diseases.<sup>14,42</sup> There is longstanding evidence that the same disease-causing variant can cause very different phenotypes.<sup>19,22</sup>

In ADAS, clinical findings beyond hematuria are highly variable and age-dependent, with proteinuria being the next most frequent finding. Although many patients may have only hematuria during their lifetime, some exhibit 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 the present study, 65.2% of patients exhibited proteinuria and 38.9% of these needed KRT. CKD developed in only 1 patient without proteinuria, a 70-year-old patient with a single kidney, confirming the natural history of ADAS (microhematuria, proteinuria, and CKD). Nephrotic-range proteinuria developed in only 3 patients. In one of them (DNA 17-142), immunologic test results were negative and kidney biopsy revealed unspecific deposits of immunoglobulin M with expansion of the mesangial matrix. Another (DNA 14-216) presented with CKD G5 and kidney biopsy was not feasible, and the third patient (DNA 16-133) had only 1 kidney and a kidney biopsy was decided against. Because of the rarity of this condition and because other glomerular diseases may coexist, we recommend kidney biopsy be performed, even with proven disease-causing variants in the collagen type IV genes, if the patient exhibits sudden nephrotic-range proteinuria. There is an increasing body of evidence supporting the efficacy of RAAS inhibitors in delaying CKD in Alport syndrome,<sup>13,43</sup> which could not be assessed in the present study because of a lack of adequate retrospective data on this matter. Large series on the use of RAAS inhibitors in ADAS are lacking, although, per good clinical practice, patients with any degree of proteinuria should be receiving treatment with RAAS inhibitors.

As reported in all series, the pathologic changes in the present cohort were unspecific. In early stages, thinning of GBM on EM is the only finding, although no patient with only microhematuria underwent a biopsy. 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, 32.7% of kidney biopsy results were normal on light microscopy, whereas unspecific changes were identified in the remainder. Twenty patients underwent EM analysis, which showed thinning with or without splitting and lamellation of the GBM, as well as podocyte effacement in some cases. As EM is not usually performed in clinical practice, it is clear from these findings that Alport syndrome will hardly ever be suspected based on a kidney biopsy with only light microscopy studies. Consequently, when suspicion of Alport syndrome arises, at present, it seems more reasonable to request a genetic test than a kidney biopsy.<sup>10</sup>

Most families had private variants, although 14 different disease-causing variants were found in 2 or more unrelated families. No differences in kidney survival according to the causative gene were observed (although there was a nonsignificant tendency toward better kidney survival in patients with COL4A3 variants), and, among the 61 patients who received KRT, the proportions with COL4A3 and COL4A4 variants were similar. In contrast to the previously reported genotype–phenotype correlation in male patients with X-linked Alport syndrome and patients with ARAS<sup>44–46</sup> or ADAS,<sup>27</sup> 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, in which large series have succeeded in showing excellent genotype-phenotype correlation whereas smaller ones ruled out any genotype-phenotype correlation.<sup>47,48</sup>

Extrarenal features were cardinal for the diagnosis of Alport syndrome before the genomic era; a proteinuric kidney disease with hearing loss was highly suggestive of Alport syndrome, whereas, at present, 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<sup>22,23,27,49</sup>; only 9% of patients in the present cohort had sensorineural hearing loss, and only 2 had ophthalmologic findings compatible with (though not pathognomonic for) Alport syndrome. This highlights the need not to attribute to Alport syndrome those common eye or ear abnormalities that are usually related to age.

In summary, the diagnostic process for ADAS is very challenging because of 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 counseling for families, avoidance of kidney biopsy, and treatment advice. Given the extremely wide phenotypic expression of the disease, predictors of rapid progression for ADAS are absolutely needed to facilitate appropriate prescription of approved drugs and recruitment of patients for clinical trials.

## Supplementary Material

### Supplementary File (PDF)

**Figure S1:** Probability of incident KRT by sex.

**Figure S2:** Light microscopy images of the 4 lesion types found in kidney biopsies of ADAS.

**Figure S3:** Probability of incident KRT comparing (1) insertions, deletions, nonsense, splicing, and missense variants involving substitution of glycine by arginine, glutamate, or aspartate versus (2) missense variants involving substitution of glycine by an amino acid other than arginine, glutamate, or aspartate.

**Figure S4:** Probability of incident KRT comparing (1) nontruncating disease-causing missense variants involving a glycine substitution versus (2) nontruncating disease-causing missense variants not involving a glycine substitution versus (3) truncating disease-causing variants.

**Figure S5:** Probability of incident KRT comparing the type of variant: missense, nonsense, splicing, frameshift indel, in-frame indel >5 amino acids, and in-frame indel <5 amino acids.

**Table S1:** Variant classification according to ACMG guidelines.

**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 on kidney biopsy.

## Article Information

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

Settings & Participants	Observations		
<p>Retrospective cohort study</p>   <p>n = 252 patients n = 82 families Spanish hospitals</p>  <p>Heterozygous disease-causing variants in COL4A3 &amp; COL4A4</p>	 <p>92.1% had microhematuria</p>  <p>38.9% of patients with proteinuria reached KRT (61/157)</p>  <p>No patients without proteinuria developed CKD</p>	 <p>Extrarenal features rare</p>  <p>Overall kidney survival: 67 (58–76) years</p>  <p>No genotype-phenotype correlation was observed</p>	

**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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