Heterozygous Urinary Abnormality–Causing Variants of COL4A3 and COL4A4 Affect Severity of Autosomal Recessive Alport Syndrome

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Abstract
Background Autosomal recessive Alport syndrome (ARAS) is an inherited renal disorder caused by homozygous and compound heterozygous mutations in COL4A3 or COL4A4, but the prognostic predictors for this disorder are not yet fully understood. Recently, the magnitude of the clinical spectrum of the COL4A3 and COL4A4 heterozygous state has attracted attention. This spectrum includes asymptomatic carriers of ARAS, benign familial hematuria, thin basement membrane disease, and autosomal dominant Alport syndrome.

Methods We retrospectively analyzed 49 patients with ARAS from 41 families with a median age of 19 years to examine the clinical features and prognostic factors of ARAS, including the associated genotypes.

Results The median age of patients with ARAS at ESKD onset was 27 years. There was no significant association between the presence or absence of hearing loss or truncating mutations and renal prognosis. However, there was a statistically significant correlation between renal prognosis and heterozygous variants that cause urinary abnormalities. Where the urinary abnormality–causing variant was absent or present in only one allele, the median age of ESKD onset was 45 years, whereas the same variant present on both alleles was associated with an age of onset of 15 years (P<0.001).

Conclusions This study was the first to demonstrate the clinical importance in ARAS of focusing on variants in COL4A3 or COL4A4 that cause urinary abnormalities in both the homozygous or heterozygous state. Although heterozygous mutation carriers of COL4A3 and COL4A4 comprise a broad clinical spectrum, clinical information regarding each variant is important for predicting ARAS prognosis.

Introduction
Alport syndrome is an inherited renal disorder accompanied by hearing loss and eye lesions. Autosomal recessive Alport syndrome (ARAS), in particular, is caused by either homozygous or compound heterozygous mutations in COL4A3 (NM: 000091) or COL4A4 (NM: 000092), and the proportion of patients with ARAS among the total number of patients with Alport syndrome is estimated to be as low as 15%. The prognostic predictors of ARAS have not yet been sufficiently clarified, whereas the phenotype-genotype correlation in male X-linked Alport syndrome is relatively established; missense mutations exhibit milder phenotypes compared with truncating mutations (1–7). Storey et al. (2) reported that patients with ARAS carrying truncating mutations on at least one allele tend to show early onset of ESKD compared with patients without truncating mutations. Savige et al. (8) reported that renal failure tended to occur at a younger age in patients with two truncating variants compared with no truncating variants. In a systematic review, Lee et al. (9) reported that the median age at onset of ESKD in patients without missense mutations was earlier compared with patients with at least one missense mutation. However, we previously analyzed 30 patients with ARAS and found no association between the presence of truncating mutations and the age at onset of ESKD (10). Patients with ARAS have been reported to develop ESKD on average in their early 20s, although some patients preserve their renal function until middle age (2,8,9,11,12). The development of genetic testing methods such as next generation sequencing (NGS) in recent years has increased opportunities for genetic diagnosis and thereby, increased the number of patients diagnosed with ARAS at earlier stages with mild symptoms. Therefore, to better manage this syndrome, it is necessary to elucidate the prognostic predictors of ARAS.

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Recently, the magnitude of the clinical spectrum of the 
COL4A3 and COL4A4 heterozygous state has attracted 
attention; some patients with heterozygous states are asymptom- 
tomatic (carriers of ARAS), some may have benign familial 
hematuria or thin basement membrane disease, and yet others have autosomal dominant Alport syndrome and develop ESKD (13–15). Moreover, it is known that COL4A3, COL4A4, and COL4A5 mutations can also affect the severity of concomitant kidney diseases caused by mutations in other podocyte-related genes such as NPHS2 and MYH9 (16–18). In this study, we focused on variants in COL4A3 and COL4A4 in patients with ARAS and their parents and determined whether these variants are associated with urina- 
ry abnormalities in the heterozygous state. Although heterozygous mutation carriers of COL4A3 and COL4A4 have a broad clinical spectrum, clinical information regarding 
these variants is potentially important in predicting the prognosis of patients with ARAS.

Materials and Methods
Ethical Considerations
All procedures involving human participants in this 
study were performed in accordance with the ethical stan-
dards of the Institutional Review Board of Kobe University 
School of Medicine and with the 1964 Helsinki Declaration 
and its later amendments or comparable ethical standards. 
Informed consent was obtained from patients and/or their 
parents.

Participants and Inclusion Criteria
Patients suspected of having Alport syndrome on the 
basis of their clinical symptoms, pathology finding, and/or 
family history were referred to Kobe University for 
genetic analysis between January 2006 and August 2019. 
After diagnosis, most patients were then followed in various 
local hospitals throughout Japan, but no further data were 
collected for the purpose of our study. For this study, we 
included all patients who were genetically defined as hav-
ing ARAS, comprising a total of 49 patients from 41 families 
with compound heterozygous or homozygous mutations in 
COL4A3 or COL4A4. Among these, our group previously 
reported 30 patients from 24 families (10). We added newly 
diagnosed patients to the previously cohort and also ana-
alyzed data from other individuals possessing the heterozy-
gous mutations of interest, such as the patients’ parents or 
patients reported in the literature. Only subjects who were 
found to have homozygous or compound heterozygous 
variants in either COL4A3 or COL4A4 were diagnosed with 
ARAS. The median age of the patients was 19 years. The 
men-to-women ratio was 20:29; 32 patients had COL4A3 
variants, and 17 patients had COL4A4 variants.

Clinical Information
All clinical information was collected with an information 
sheet that was sent with the blood samples for genetic 
analysis. We collected information regarding age, sex, 
height, body weight, serum Cr, urinary protein-creatinine 
ratio, history of dialysis or kidney transplantation (if applic-
able), the existence of hearing loss or eye lesions, and the 
results of kidney biopsy (if applicable). With regard to 
family history, we requested information on the presence or absence of renal disease, dialysis, hearing loss, eye 
lesions, and the age at which ESKD developed. We defined 
the age at ESKD onset as the time that dialysis was in-
troduced or kidney transplantation was performed. The 
eGFR (milliliters per minute per 1.73 m2) was calculated 
on the basis of serum creatinine using the equation reported by Uemura et al. (19) for patients aged 2–18 years and by 
Matsuo et al. (20) for patients aged 19 years and older.

Genetic Analyses
All patients were referred to our hospital for genetic 
testing between January 2006 and August 2019, and those 
confirmed to have ARAS were included in this study. Ge-
nomic DNA was isolated from patient peripheral blood 
leukocytes with the QuickGene-Mini80 system (Kurabo In-
dustries, Tokyo, Japan) according to the manufacturer’s 
instructions. Genetic testing was conducted either with 
Sanger sequencing of COL4A3 and COL4A4 genes (before 
NGS was available in our laboratory, January 2006 to No-
vember 2015) or with NGS using a targeted sequencing 
panel containing causative genes of inherited kidney disease 
(November 2015 to August of 2019). NGS analyses were 
conducted as described previously (21). Where heterozy-
gous mutations were not identified by direct sequencing, 
mRNA or multiplex ligation–dependent probe amplifica-
tion was performed for confirmation.

Variant Evaluation
Variants were evaluated to determine whether they could 
cause urinary abnormalities or were likely asymptomatic in 
the heterozygous state. This was performed by obtaining 
clinical information from the patients’ parents who were 
heterozygous variant carriers. Urinary abnormalities were 
considered to be present when at least hematuria was noted. 
To mitigate the fact that not all of the patients’ parents had 
undergone urinalysis or where there was a lack in uniform-
ity in the urinalysis procedure, we used variant informa-
tion from the HGMD variant database (https://portal.bio-
base-international.com/hgmd/pro/start.php) to determine 
which variants could cause urinary abnormalities (autosom-
al dominant Alport syndrome, benign familial hematuria, 
or thin basement membrane) in the heterozygous state.

Statistical Analyses
All calculations were performed using standard statistical 
software (JMP version 11 for Windows; SAS Institute, Cary, 
NC). The occurrence of events (age at ESKD onset) was 
analyzed with the Kaplan–Meier method. To calculate the 
P value, we used the log-rank test. For the comparison of 
eGFR, we used analysis of covariance after adjusting for age. 
We considered an association to be significant when the 
P value was 0.05.

Results
Clinical Features
A total of 49 patients from 41 families were included in 
this study. The clinical and genetic information of the 
patients is shown in Table 1 and Supplemental Table 1. 
The median age of the patients when genetically diagnosed
Table 1. Patients’ clinical and allele information

| Patient Identification | Sex | Age, yr | ESKD Age (Creatinine-eGFR) | Urine Protein-Creatinine Ratio | Hearing Loss | Ocular Lesion | α5 Staining (Glomerular Basement Disease) | Gene | Truncating Allele | Urinary Findings Allele |
|------------------------|-----|---------|-----------------------------|-------------------------------|--------------|--------------|---------------------------------------------|------|----------------|------------------------|
| 108                    | Men | 16      | (107.0)                     | 1.2                           | –            | –            | Positive                                    | COL4A3 | 2             | 1                      |
| 115                    | Women | 19    | (57.8)                      | 0.12                          | +            | –            | Negative                                    | COL4A3 | 2             | 0                      |
| 155                    | Men | 36      | 19                          | ESKD                          | +            | –            | Negative                                    | COL4A3 | 2             | 0                      |
| 155–1                  | Women | 33    | 21                          | ESKD                          | +            | –            | Negative                                    | COL4A3 | 2             | 0                      |
| 143                    | Women | 2     | (122.1)                     | 0.57                          | –            | –            | Negative                                    | COL4A3 | 1             | 1                      |
| 165                    | Men | 6      | (167.8)                     | 0.30                          | –            | –            | Negative                                    | COL4A3 | 1             | 2                      |
| 166                    | Women | 18    | 18                          | ESKD                          | 0.80         | –            | Negative                                    | COL4A3 | 1             | 1                      |
| 169                    | Men | 19     | (107.8)                     | 6.3                           | +            | –            | Negative                                    | COL4A3 | 1             | 1                      |
| 170                    | Women | 7     | (119.5)                     | 0.35                          | +            | –            | Negative                                    | COL4A3 | 1             | 0                      |
| 415                    | Women | 17    | (107.7)                     | 0.35                          | +            | –            | Negative                                    | COL4A3 | 1             | 0                      |
| 525                    | Women | 11    | (129.8)                     | 0.35                          | +            | –            | Negative                                    | COL4A3 | 1             | 1                      |
| 570                    | Men | 45     | (53.8)                      | 2.2                           | –            | –            | Positive                                    | COL4A3 | 1             | 1                      |
| 570–1                  | Men | 47     | 31                          | ESKD                          | –            | –            | Negative                                    | COL4A3 | 1             | 1                      |
| 412                    | Women | 19    | (80.7)                      | 1.7                           | –            | –            | Negative                                    | COL4A3 | N/A           | 1                      |
| 114                    | Women | 17    | (64.1)                      | 0.24                          | –            | –            | Positive                                    | COL4A3 | 0             | 1                      |
| 125                    | Women | 22    | (138.1)                     | 0.46                          | –            | Retinal regeneration | Negative                                    | COL4A3 | 0             | 1                      |
| 125–1                  | Men | 21     | (8.9)                       | 2.4                           | –            | –            | Negative                                    | COL4A3 | 0             | 1                      |
| 125–2                  | Men | 11     | (126.2)                     | 0.14                          | –            | –            | Negative                                    | COL4A3 | 0             | 1                      |
| 130                    | Women | 16    | 15                          | ESKD                          | –            | Perimacular fleck | Negative                                    | COL4A3 | 0             | 2                      |
| 130–1                  | Women | 18    | 11                          | ESKD                          | –            | –            | Negative                                    | COL4A3 | 0             | 2                      |
| 137                    | Men | 20     | 13                          | ESKD                          | –            | –            | Negative                                    | COL4A3 | 0             | 1                      |
| 137–1                  | Women | 27    | 26                          | ESKD                          | –            | –            | Negative                                    | COL4A3 | 0             | 1                      |
| 167                    | Women | 21    | (159.7)                     | 2.1                           | +            | –            | Negative                                    | COL4A3 | 0             | 1                      |
| 168                    | Men | 19     | 19                          | ESKD                          | –            | –            | Negative                                    | COL4A3 | 0             | 2                      |
| 171                    | Men | 16     | 9                           | ESKD                          | +            | –            | Negative                                    | COL4A3 | 0             | 2                      |
| 171–1                  | Women | 11    | 11                          | ESKD                          | –            | –            | Negative                                    | COL4A3 | 0             | 2                      |
| 173                    | Men | 25     | 25                          | ESKD                          | –            | –            | Negative                                    | COL4A3 | 0             | 1                      |
| 179                    | Women | 45    | 45                          | ESKD                          | –            | –            | Positive                                    | COL4A3 | 0             | 1                      |
| 473                    | Women | 8     | (73.9)                      | 1.8                           | –            | –            | Negative                                    | COL4A3 | 0             | 2                      |
| 595                    | Women | 19    | 18                          | ESKD                          | –            | –            | Negative                                    | COL4A3 | 0             | 2                      |
| 309                    | Women | 2     | (126.7)                     | 0.78                          | –            | –            | Negative                                    | COL4A4 | 2             | 2                      |
| 471                    | Women | 41    | (29.5)                      | 2.9                           | +            | –            | Positive                                    | COL4A4 | 2             | 0                      |
| 156                    | Women | 7     | (136.6)                     | 0.43                          | –            | –            | Negative                                    | COL4A4 | 1             | 0                      |
| 172                    | Men | 16     | 14                          | ESKD                          | –            | –            | Positive                                    | COL4A4 | 1             | 2                      |
| 174                    | Men | 2      | (100.8)                     | 0.53                          | –            | –            | Negative                                    | COL4A4 | 1             | 1                      |
| 218                    | Men | 12     | (65.8)                      | 0.17                          | –            | Hyperopia | Negative                                    | COL4A4 | 1             | 2                      |
| 85                     | Women | 23    | (98.4)                      | 1.6                           | –            | –            | Negative                                    | COL4A4 | 0             | 2                      |
| 145                    | Women | 26    | (106.1)                     | 0.42                          | –            | –            | Positive                                    | COL4A4 | 0             | 0                      |
| 204                    | Men | 11     | (102.7)                     | 1.1                           | –            | –            | Negative                                    | COL4A4 | 0             | 1                      |
| 257                    | Women | 18    | (107.4)                     | 0.75                          | –            | –            | Negative                                    | COL4A4 | 0             | 1                      |
| 920                    | Women | 47    | (45.5)                      | 1.9                           | –            | –            | Positive                                    | COL4A4 | 0             | 1                      |
| 468                    | Women | 4     | (153.9)                     | 0.22                          | –            | –            | Negative                                    | COL4A4 | 0             | 1                      |
with ARAS was 19 (16–21) years. COL4A3 variants were detected in 32 patients from 25 families, and COL4A4 variants were detected in 17 patients from 16 families. Among these patients, 16 progressed to ESKD (14 patients had a COL4A3 mutation, and two patients had a COL4A4 mutation), and the median age for developing ESKD was 27 years (Figure 1). At the time of genetic testing, 18 patients had been diagnosed with hearing loss, 28 had not been diagnosed with hearing loss, and three had no information. The renal survival curves for patients with and without hearing loss at the time of genetic testing are shown in Figure 1. There was no statistically significant difference between these two groups. Only four patients had ocular lesions indicated at the time of genetic testing.

**Table 1.** (Continued)

| Patient Identification | Sex | Age, yr | ESKD Age, yr | Urinary Protein:Creatinine Ratio | Truncating Allele | Ocular Lesion | Hearing Loss | Urinary Findings Allele |
|------------------------|-----|---------|--------------|-------------------------------|------------------|--------------|-------------|------------------------|
| 476                    | Women | 21      | (125.0)      | 0.37                          | COL4A4/44        | Positive     | 0           | 2                      |
| 601                    | Men   | 22      | (82.1)       | 4.1                           | COL4A4/44        | Positive     | 0           | 02                     |
| 678                    | Women | 37      | (102.9)      | 8.1                           | COL4A4/44        | N/A          | 1           | 02                     |
| 738                    | Women | 33      | N/A           | N/A                           | COL4A4/44        | N/A          | 1           | 02                     |

| Gene | Truncating Allele | Ocular Lesion | Hearing Loss |
|------|-------------------|--------------|-------------|
| COL4A3 | COL4A4        | Positive | 0           |
| COL4A4 | COL4A4        | Positive | 0           |

- No indication at the time of genetic testing. +, it was pointed out at the time of genetic testing. The number of alleles with truncating mutation.

**Figure 1.** Probability of developing ESKD was not associated with hearing loss. The solid line indicates all patients (n=49). The median age at ESKD onset was 27 years. The dashed line indicates patients with hearing loss at the time of genetic diagnosis (n=18). Their median age at ESKD onset was 27 years. The dashed-dotted line indicates patients without hearing loss at the time of genetic diagnosis (n=28). This group had not reached the median age of ESKD onset even at 40 years. NS, not significant.

- No indication at the time of genetic testing. +, it was pointed out at the time of genetic testing. The number of alleles with truncating mutation.

**Figure 2.** Probability of developing ESKD was not associated with truncating mutations. The solid line indicates patients with two alleles containing a truncating mutation (n=5), the dashed line indicates those with one such allele (n=13), and the dashed-dotted line indicates those with no such alleles (n=28). The median ages at ESKD onset of these three groups were 21, 31, and 26 years, respectively. NS, not significant.
In this study, in contrast to XLAS, there was no association between the presence of truncating mutations and the renal prognosis of the patients with ARAS, which is consistent with the report by Oka et al. (10) but contradictory to the results reported by Storey et al. (2), Savige et al. (8), and Lee et al. (9). A common feature among these previous reports is a bias toward identified variants that may be due to a “founder effect.” Because of this bias and the small number of patients included in these reports, it is likely that the characteristics of the individual variants affected the results. Although our report only included a small number of Japanese patients due to the rarity of the disease, it did not include hot spots in the Japanese population, and therefore, we believe that there was no extreme bias; however, patients with a family history may lead to an early genetic diagnosis, which may have introduced some bias. Moreover, as an unavoidable limitation of any genotype-phenotype investigation, this study does not provide evidence of whether the heterozygous mutations of interest caused the phenotype or were simply associated with it. Additionally, the relationship as “variants that may be associated urinary abnormalities in the heterozygous state.” Nine patients possessed no alleles with mutations that could cause urinary abnormalities in the heterozygous state, whereas 26 patients possessed one such allele, and 14 patients possessed two such alleles (Table 3). The renal survival curves of these three groups are shown in Figure 3A. The median age at ESKD onset for the three groups was as follows: one allele, 31 years; two alleles, 15 years; and overall, 27 years. In the group with no alleles of interest, seven of nine patients had not developed ESKD at the time of genetic diagnosis, which may reflect a better prognosis; however, we could not compare the different prognoses statistically. Thus, we next divided all of the patients into two groups instead: a zero-allele and one-allele group (n=35) versus the two-allele group (n=14). The median ages of ESKD onset for these two groups were 45 and 15 years, respectively (Figure 3B). Age at onset of ESKD differed significantly between these two groups (P<0.001). The decrease of the eGFR tended to be faster (P=0.08) in the two-allele group (n=14) compared with the combined zero-allele and one-allele group (n=34).

Discussion
This is our second report of a patient series of patients with ARAS; concurrently, it is the study performed that pays attention to the clinical information of individuals with heterozygous mutations.

Urinary Abnormalities Associated with Heterozygous Variants
Participants were classified according to the number of COL4A3 and COL4A4 alleles that they had that may be associated with urinary abnormalities in the heterozygous state. As indicated in previous reports, even in cases where the patients’ parents have not been found to have an abnormal urinalysis result, the heterozygous patient may have urinary abnormalities (10,22,23). Such variants were classified as “variants that may be associated urinary abnormalities in the heterozygous state.” Nine patients possessed no alleles with mutations that could cause urinary abnormalities in the heterozygous state, whereas 26 patients possessed one such allele, and 14 patients possessed two such alleles (Table 3). The renal survival curves of these three groups are shown in Figure 3A. The median age at ESKD onset for the three groups was as follows: one allele, 31 years; two alleles, 15 years; and overall, 27 years. In the group with no alleles of interest, seven of nine patients had not developed ESKD at the time of genetic diagnosis, which may reflect a better prognosis; however, we could not compare the different prognoses statistically. Thus, we next divided all of the patients into two groups instead: a zero-allele and one-allele group (n=35) versus the two-allele group (n=14). The median ages of ESKD onset for these two groups were 45 and 15 years, respectively (Figure 3B). Age at onset of ESKD differed significantly between these two groups (P<0.001). The decrease of the eGFR tended to be faster (P=0.08) in the two-allele group (n=14) compared with the combined zero-allele and one-allele group (n=34).

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### Table 2. Distribution of patients on the basis of the number of alleles containing truncating mutations

| No. of Alleles with Truncating Mutation | Patients with ESKD (Median ESKD Age, yr) | Patients without ESKD |
|----------------------------------------|------------------------------------------|-----------------------|
| 0                                      | 10 (26)                                  | 18                    |
| 1                                      | 3 (31)                                   | 10                    |
| 2                                      | 2 (21)                                   | 3                     |
| Total                                  | 15 (31)                                  | 31                    |

Table 2. Distribution of patients on the basis of the number of alleles containing truncating mutations

| No. of Alleles That Can Cause Urinary Findings with Heterozygous Mutation | Patients with ESKD (Median ESKD Age, yr) | Patients without ESKD |
|--------------------------------------------------------------------------|------------------------------------------|-----------------------|
| 0                                                                        | 2 (N/A)                                  | 7                     |
| 1                                                                        | 7 (31)                                   | 19                    |
| 2                                                                        | 7 (15)                                   | 7                     |
| Total                                                                    | 16 (27)                                  | 33                    |

N/A, not available.
Figure 3. Probability of developing ESKD was associated with heterozygous urinary abnormality-causing mutations. (A) Probability of patients with autosomal recessive Alport syndrome developing ESKD on the basis of the number of alleles containing heterozygous mutations associated with urinary abnormalities. The solid line indicates patients with two such alleles (n=14), the dashed-dotted line indicates those with one such allele (n=26), and the dashed line indicates those with no such alleles (n=9). The median ages at ESKD onset of these three groups were 15, 31 years, and undetermined, respectively. The group with no alleles had not reached the median age of ESKD onset even at 40 years. NS, nNot significant nor significant. (B) Combined probability of patients with autosomal recessive Alport syndrome developing ESKD on the basis of the number of alleles containing heterozygous mutations associated with urinary abnormalities. The solid line indicates patients with two such alleles (n=14), and the dashed-dotted line indicates those with one or no such allele (n=35). The median ages at ESKD onset were 15 and 45 years, respectively (P<0.001).

It may become clear if protein prediction modeling can be carried out, instead of simply distinguishing from truncating and nontruncating.

Here, we compiled the first report on ARAS containing clinical information regarding heterozygous variant carriers such as patients’ parents or by including data extracted from a database of previously reported variants. Our study showed that patients with ARAS have more severe phenotypes when they carry certain heterozygous variants associated with urinary abnormalities, and it indicates the necessity of trio analysis combined with parent urinalysis for early diagnosis and improved patient management. It is evident that further cases of ARAS with genetic diagnosis and the clinical information regarding the presence of heterozygous variants are needed to confirm the prediction of the prognosis of ARAS.

It has been reported that heterozygous mutations in the COL4A3 and COL4A4 genes act as disease modifiers (18). Recently, common variants of UMOD, the causative gene of autosomal dominant nodular interstitial kidney disease, was found to be a susceptibility gene for CKD, hypertension, and renal calculi in the general population (24,25). In the same way, considering the broad spectrum of COL4A3 and COL4A4, these two genes may be disease susceptibility genes for certain renal diseases.

In conclusion, this study showed the clinical importance of noting the urinary findings of heterozygous mutation carriers, such as the parents of patients with ARAS. Notably, our results indicate that patients with ARAS tend to have a poorer prognosis when they carry more mutations associated with urinary abnormalities, even where these are present in the heterozygous state.

Disclosures
K. Iijima and K. Nozu have filed a patent application on the development of antisense nucleotides for exon skipping therapy in Alport syndrome. K. Nozu has received lecture fees from Novartis Pharmaceuticals and corporation and consulting fees from Kyowa Kirin Co., Ltd. All remaining authors have nothing to disclose.

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Author Contributions
T. Horinouchi, K. Iijima, K. Nakanishi, and K. Nozu conceptualized the study; Y. Aoto, T. Horinouchi, K. Iijima, S. Ishiko, C. Nagaro, K. Nozu, R. Rossanti, N. Sakakibara, and T. Yamamura were responsible for data curation; T. Horinouchi, C. Nagaro, K. Nakanishi, K. Nozu, N. Sakakibara, and Y. Shima were responsible for investigation; T. Horinouchi, K. Iijima, K. Nakanishi, K. Nozu, Y. Shima, and T. Yamamura were responsible for methodology; K. Iijima, N. Morisada, K. Nakanishi, K. Nozu, Y. Shima, and T. Yamamura were responsible for validation; T. Horinouchi wrote the original draft; K. Iijima and K. Nozu were responsible for funding acquisition; K. Iijima and K. Nozu provided supervision; K. Nozu was responsible for project administration; K. Nozu was responsible for resources; and T. Horinouchi reviewed and edited the manuscript.
Supplemental Material

This article contains the following supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.0000372019/-/DCSupplemental.

Supplemental Table 1. Patient clinical and genetic information.

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Heterozygous urinary abnormality-causing variants of \textit{COL4A3} and \textit{COL4A4} affect severity of autosomal recessive Alport syndrome

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| Patient ID | Gender | Age | ESRD age (Cr-eGFR) | Hearing loss | Ocular lesion | Gene | Nucleotide change | Amino acid change | Urinary/Pathological findings or diagnosis in carriers | Nucleotide change | Amino acid change | Urinary/Historical findings in carriers |
|-----------|--------|-----|-------------------|--------------|--------------|------|-----------------|-----------------|------------------------------------------------|-----------------|-----------------|----------------------------------|
| 94        | female | 17  | (64.1)           | -            | -            | COL4A3 | c.4793T>G       | p.Leu1598Arg     | ID 143’s M: OB                                      | c.145-2A>G      |                  |                                  |
| 108       | male   | 16  | (107.0)          | -            | -            | COL4A3 | c.4028-27A>G    | Exon46(126bp)skipping | ID 108’s F: OB                                         | c.2698_2714del  | p.Ile900Profs*34 |                                    |
| 114       | male   | 20  | (34.0)           | +            | -            | COL4A3 | c.3266G>A       | p.Gly1089Asp    | -                                                        | c.3266G>A       | p.Gly1089Asp     |                                    |
| 115       | female | 19  | (57.8)           | +            | -            | COL4A3 | c.1844dup       | p.Pro616Thrfs*30 | -                                                        | c.3687del       | p.Gly1231Valfs*33 |                                    |
| 125       | female | 22  | (138.1)          | - Retinal regeneration | COL4A3 | c.2330G>A       | p.Gly777Asp    | ID 130’s M: Pro/OB                                     | c.4354A>T       | p.Ser1452Cys     |                                    |
| 125-1     | male   | 21  | (8.9)            | -            | -            | COL4A3 | c.2330G>A       | p.Gly777Asp    | ID 130’s M: Pro/OB                                     | c.4354A>T       | p.Ser1452Cys     |                                    |
| 125-2     | male   | 11  | (126.2)          | -            | -            | COL4A3 | c.2330G>A       | p.Gly777Asp    | ID 130’s M: Pro/OB                                     | c.4354A>T       | p.Ser1452Cys     |                                    |
| 130       | female | 16  | 15               | - Perimacular fleck | COL4A3 | c.2330G>A       | p.Gly777Asp    | ID 130’s M: Pro/OB                                     | c.4793T>G       | p.Leu1598Arg     | ID 143’s M:OB                                  |
| 130-1     | female | 18  | 11               |               |              | COL4A3 | c.2330G>A       | p.Gly777Asp    | ID 130’s M: Pro/OB                                     | c.4793T>G       | p.Leu1598Arg     | ID 143’s M:OB                                  |
| 137       | male   | 20  | 13               | +             |              | COL4A3 | c.4928G>A       | p.Arg1643Lys    | -                                                        | c.40_63del      | p.Leu14_Leu21del | Longo et al. (ADAS)               |
| 137-1     | female | 27  | 26               | -             |              | COL4A3 | c.4928G>A       | p.Arg1643Lys    | -                                                        | c.40_63del      | p.Leu14_Leu21del | Longo et al. (ADAS)               |
| ID  | Gender | Age  | Height | Diagnosis       | COL4A3 Mutation 1  | ID 143’s M  | COL4A3 Mutation 2 | ID 143’s M  | Other Observations |
|-----|--------|------|--------|-----------------|---------------------|--------------|-------------------|--------------|-------------------|
| 143 | female | 2    | 122.1  |                 | COL4A3 c.4793T>G   | p.Leu1598Arg | c.2125G>T        | p.Gly709Term | -                 |
| 155 | male   | 36   | 19     | +               | COL4A3 c.4463-523C>G | Cryptic exon(139bp) | -              | c.4463-523C>G | Cryptic exon(139bp) | - |
| 155-1 | female | 33   | 21     | +               | COL4A3 c.4463-523C>G | Cryptic exon(139bp) | -              | c.4463-523C>G | Cryptic exon(139bp) | - |
| 165 | male   | 6    | 167.8  | -               | COL4A3 c.689G>A    | p.Gly230Asp  | ID 165’s M or F: OB | c.1576-20_1576-6del | Exon25(183bp)skipping | ID 165’s M or F: OB |
| 166 | female | 18   | 18     | -               | COL4A3 c.1855G>A   | p.Gly619Arg  | ID 166’s M: OB ID 473’s M: OB | c.1060G>T | p.Gly354Term | - |
| 167 | female | 21   | 158.7  | +               | COL4A3 c.4708T>C   | p.Cys1570Arg | -               | c.40_63del | p.Leu14_Leu21del | Longo et al. (ADAS) |
| 168 | male   | 19   | 19     | -               | COL4A3 c.1918G>A   | p.Gly640Arg  | ID 168’s M: OB, ID 168’s F: OB•TBM | c.1918G>A | p.Gly640Arg | ID 168’s M: OB, ID 168’s F: OB•TBM |
| 169 | male   | 19   | 107.84 | +               | COL4A3 c.4793T>G   | p.Leu1598Arg | ID 143’s M: OB | c.3752-511_3955+576del (131+73bp) | - | - |
| 170 | female | 7    | 119.52 | -               | COL4A3 c.1354G>A   | p.Gly452Arg  | ID 170’s F: OB | c.3821dup | p.His1275Profs*34 | - |
| 171 | male   | 16   | 9      | +               | COL4A3 c.40_63del  | p.Leu14_Leu21del | Longo et al. (ADAS) | c.40_63del | p.Leu14_Leu21del | Longo et al. (ADAS) |
| 171-1 | female | 11   | 11     | +               | COL4A3 c.40_63del  | p.Leu14_Leu21del | Longo et al. (ADAS) | c.40_63del | p.Leu14_Leu21del | Longo et al. (ADAS) |
| 173 | male   | 25   | 25     | +               | COL4A3 c.3464G>A   | p.Gly1155Asp | -               | c.4793T>G | p.Leu1598Arg | ID 143’s M: OB |
| 179 | female | 45   | 45     | +               | COL4A3 c.2863G>A   | p.Gly955Arg  | -               | c.4793T>G | p.Leu1598Arg | ID 143’s M: OB |
| 245 | male   | 41   | 91.54  | +               | COL4A3 c.933+1G>A  | Exon16(45bp)skipping | -               | c.3650_3657del | p.Pro1217Hisfs*89 | - |
|   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 412 | male | 19 | (80.70) | - | - | COL4A3 | c.1576G>T | p.Gly526Cys | ID 412’s M: OB | c.3883-1G>C | N/A | - |
| 415 | female | 17 | (107.70) | + | - | COL4A3 | c.4708T>C | p.Gly1507Arg | - | c.4441C>T | p.Arg1481Term | - |
| 473 | female | 8 | (73.93) | - | - | COL4A3 | c.1855G>A | p.Gly619Arg | ID 166’s M: OB ID 473’s M: OB | c.4793T>G | p.Leu1598Arg | ID 143’s M: OB |
| 525 | female | 11 | (129.84) | + | - | COL4A3 | c.1994G>A | p.Gly665Asp | ID 525’s M: OB | c.1216C>T | p.Arg406Term | - |
| 570 | male | 45 | (53.79) | - | - | COL4A3 | c.3427G>A | p.Gly1143Arg | ID 570’s M: OB (Any) | c.4085del | p.Pro1362Hisfs*23 | ID 570’s M: OB (Any) |
| 570-1 | male | 47 | 31 | - | - | COL4A3 | c.3427G>A | p.Gly1143Arg | ID 570’s M: OB (Any) | c.4085del | p.Pro1362Hisfs*23 | ID 570’s M: OB (Any) |
| 595 | female | 19 | 18 | - | - | COL4A3 | c.953G>A | p.Gly318Asp | ID 595’s F: OB/Pro CKD | c.4793T>G | p.Leu1598Arg | ID 143’s M: OB |
| 85 | female | 23 | (98.35) | - | - | COL4A4 | c.2510G>C | p.Gly837Ala | Kamiyoshi et al. (ADAS) | c.3151G>C | p.Gly1051Arg | ID 85’s F: OB |
| 145 | female | 26 | (106.13) | - | - | COL4A4 | c.3307G>A | p.Gly1103Arg | - | c.3307G>A | p.Gly1103Arg | - |
| 156 | female | 7 | (136.64) | - | - | COL4A4 | c.2608G>C | p.Gly870Arg | - | c.3687dup | p.Gly1230Argfs*23 | - |
| 172 | male | 16 | 14 | + | - | COL4A4 | c.2084G>A | p.Gly695Asp | ID 172’s M: OB | c.3612_3621del | p.Ile1205* | ID 172’s F: OB·TBM |
| 174 | male | 2 | (100.81) | - | - | COL4A4 | c.1733G>T | p.Gly578Val | - | c.4241_4254del | p.Asp1414Glyfs*14 | ID 174’s M: OB |
| 204 | male | 11 | (102.74) | - | - | COL4A4 | c.2084G>A | p.Gly695Asp | ID 172’s M: OB | c.4469G>C | p.Gly1490Ala | - |
| 218 | male | 12 | (65.77) | - | hyperopia | COL4A4 | c.2878G>A | p.Gly960Arg | ID 218’s F: OB | c.559-491_1460-808del insPolyT Exon8-Exon25 del | ID 218’s M: OB |
| 257 | female | 18 | (107.44) | - | - | COL4A4 | c.3160G>C | p.Gly1054Arg | ID 257’s F: OB | c.3307G>A | p.Gly1103Arg | - |
| 270 | female | 47 | (45.49) | - | - | COL4A4 | c.203G>A | p.Gly68Glu | - | c.2437G>C | p.Gly813Arg | ID 270’s F: OB |
| 309 | female | 2 | (126.66) | - | - | COL4A4 | c.2566C>T | p.Gln856Term | ID 309’s F: OB/Pro | c.3687del | p.Gly1230Valfs*58 | ID 309’s M: OB |
| ID   | Sex  | Age | BMI | Gender | COL4A4 Mutation | p.Gly527Val | p.Gly1054Cys | c.3160G>T | p.Gly837Ala | c.4817G>A | p.Gly1606Glu | p.Gly1103Arg | Kamiyoshi et al. (ADAS) | Baek et al. |
|------|------|-----|-----|--------|-----------------|-------------|--------------|-----------|------------|---------|--------------|-------------|-----------------------|----------|
| 468  | female | 4   | (153.85) | -      | COL4A4 c.1580G>T | p.Gly527Val | -            | c.3160G>T | p.Gly1054Cys | ID 468’s F:OB |
| 471  | female | 41  | (29.53) | +      | COL4A4 c.4953G>A | p.Trp1651Tern | -            | c.2930del | p.Pro977Leufs*61 | -   |
| 476  | female | 21  | (124.99) | -      | COL4A4 c.2510G>C | p.Gly837Ala | Kamiyoshi et al. (ADAS) | c.4817G>A | p.Gly1606Glu | Baek et al. |
| 601  | female | 22  | (82.08) | -      | COL4A4 c.3262G>C | p.Gly1088Arg | ID 601’s F: OB | c.3307G>A | p.Gly1103Arg | -   |
| 738  | male  | 37  | N/A   | +      | COL4A4 c.2617G>A | p.Gly873Arg | ID 738’s M: OB · 60yr ESRD | c.594+5G>A | N/A | -   |
| 738-1| female | 33  | 27    | +      | COL4A4 c.2617G>A | p.Gly873Arg | ID 738’s M: OB · 60yr ESRD | c.594+5G>A | N/A | -   |
| 741  | female | 7   | (132.27) | -      | COL4A4 c.1795G>C | p.Gly599Arg | ID 741’s M: OB · 60yr ESRD | c.1795G>C | p.Gly599Arg | ID 741’s M: OB · 60yr ESRD | ID 741’s F: OB |

ESRD: End-stage renal disease  
CKD: Chronic kidney disease  
OB: Occult blood  
Pro: Proteinuria  
TBM: Thin basement membrane  
ADAS: Autosomal dominant Alport syndrome  
M: Mother  
F: Father  
Kamiyoshi et al.: This mutation was reported by Kamiyoshi et al. (1)  
Longo et al.: This mutation was reported by Longo et al. (2)
Baek et al.: This mutation was reported by Baek et al. (3)
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