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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.

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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 asymptomatic (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 urinary 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 standards 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 having 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 analyzed data from other individuals possessing the heterozygous 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 applicable), 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 introduced or kidney transplantation was performed. The eGFR (milliliters per minute per 1.73 m^2) 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. Genomic DNA was isolated from patient peripheral blood leukocytes with the QuickGene-Mini80 system (Kurabo Industries, 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 November 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 heterozygous mutations were not identified by direct sequencing, mRNA or multiplex ligation–dependent probe amplification 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 uniformity in the urinalysis procedure, we used variant information from the HGMD variant database (https://portal.biobase-international.com/hgmd/pro/start.php) to determine which variants could cause urinary abnormalities (autosomal 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[a]	Urinary Findings Allele[b]
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	+	−		*COL4A3*	2	0
155–1	Women	33	21	ESKD	+	Band keratopathy		*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	−	−	Negative	*COL4A3*	1	1
169	Men	19	(107.8)	6.3	+	−	Negative	*COL4A3*	1	1
170	Women	7	(119.5)	0.80	−	−	Negative	*COL4A3*	1	1
245	Men	41	(91.5)	7.4	+	−	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				*COL4A3*	1	1
412	Men	19	(80.7)	1.7	−	−		*COL4A3*	N/A	1
94	Women	17	(64.1)	0.24	−	−	Positive	*COL4A3*	0	1
114	Men	20	(34.0)	1.6	+	−	Negative	*COL4A3*	0	0
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	−	−		*COL4A3*	0	1
130	Women	16	15	ESKD	−	Perimacular fleck		*COL4A3*	0	2
130–1	Women	18	11	ESKD				*COL4A3*	0	2
137	Men	20	13	ESKD	+			*COL4A3*	0	1
137–1	Women	27	26	ESKD	−			*COL4A3*	0	1
167	Women	21	(158.7)	2.1	+	−	Negative	*COL4A3*	0	1
168	Men	19	19	ESKD	−	−	Negative	*COL4A3*	0	2
171	Men	16	9	ESKD	+	−		*COL4A3*	0	2
171–1	Women	11	11	ESKD	+	−		*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	−	−		*COL4A3*	0	2
595	Women	19	18	ESKD	−	−		*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	−	−		*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	−	−		*COL4A4*	0	1
270	Women	47	(45.5)	1.9	−	−	Positive	*COL4A4*	0	1
468	Women	4	(153.9)	0.22	−	−		*COL4A4*	0	1

Table 1. (Continued)

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[a]	Urinary Findings Allele[b]
476	Women	21	(125.0)	0.37	–	–	Positive	*COL4A4*	0	2
601	Women	22	(82.1)	4.1	–	–	Positive	*COL4A4*	0	1
741	Women	7	(132.3)	8.1	–	–		*COL4A4*	0	2
738	Men	37	N/A	N/A	+	–		*COL4A4*	N/A	1
738–1	Women	33	27	ESKD	+	–		*COL4A4*	N/A	1

–, no indication at the time of genetic testing; +, it was pointed out at the time of genetic testing; N/A, not available.
[a]The number of alleles with truncating mutation.
[b]The number of alleles that can cause urinary findings with heterozygous 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.

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.

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 significantot significant.

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

Genotype-Phenotype Correlation Analyses of Truncating Mutations

Missense mutations, insertions, and deletions of bases in multiples of three and skipping of bases in exons in multiples of three were defined as nontruncating mutations, whereas nonsense mutations, insertions, and deletions of bases in multiples of other than three, exon skipping of bases in multiples other than three, and large deletions were defined as truncating mutations. Three patients (patient identifications 412, 738, and 738–1) had a mutation in the splicing consensus sequence that was expected to cause exon skipping; however, the transcript was not analyzed and could not be included in this analysis. Twenty-six patients possessed no alleles with a truncating mutation, whereas 14 patients possessed one such allele, and six patients possessed two such alleles. The median age of ESKD onset for these different groups was as follows: no allele, 26 years; one allele, 31 years; two alleles, 21 years; and overall, 31 years (Figure 2, Table 2). There were no statistically significant differences between the three groups. The eGFR at the timing of genetic testing of these patients who had not developed ESKD is shown in Table 1. There was no tendency of the decrease of the eGFR between the three groups ($P=0.53$).

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 ($n=34$).

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.

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

Table 3. Distribution of patients on the basis of the number of alleles containing variants that could cause urinary abnormalities in the heterozygous state

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.

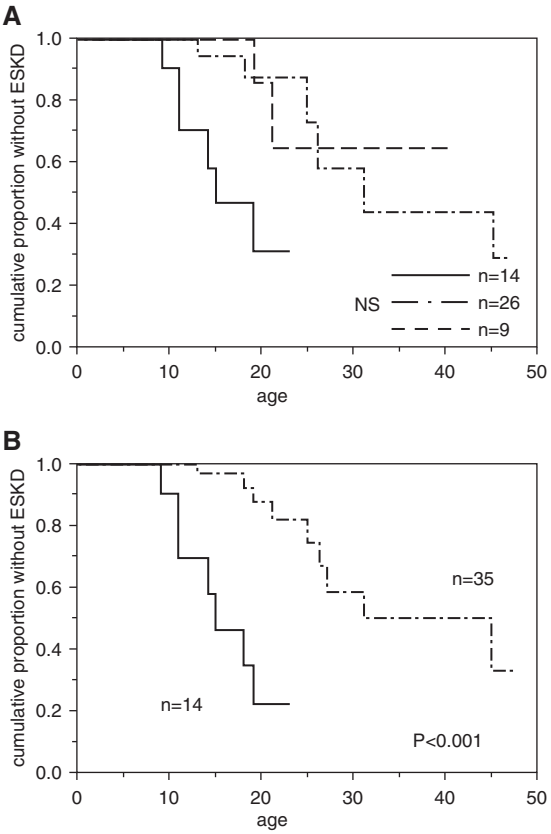

Figure 3. | Probability of developing ESKD was associated with heterozygous urinary abnormality–causing mutations. (A) Probability of patients with autosomal recessive Alport syndrome developing ESKD on the basis of the number of alleles containing heterozygous mutations associated with urinary abnormalities. 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 significantot 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).

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. Nagano, K. Nozu, R. Rossanti, N. Sakakibara, and T. Yamamura were responsible for data curation; T. Horinouchi, C. Nagano, 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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Title Page

Original Article

Heterozygous urinary abnormality-causing variants of *COL4A3* and *COL4A4* affect severity of autosomal recessive Alport syndrome

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Table S1. Patient clinical and genetic information

Patient ID	Gender	Age	ESRD age (Cr-eGFR)	Hearing loss	Ocular lesion	Gene	Mutation1			Mutation2		
							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	Exon3(90bp)skipping	-
108	male	16	(107.0)	-	-	COL4A3	c.4028-27 A>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)

143	female	2	(122.1)			COL4A3	c.4793T>G	p.Leu1598Arg	ID 143's M : OB	c.2125G>T	p.Gly709Term	-
155	male	36	19	+	-	COL4A3	c.4463-523 C>G	Cryptic exon(139bp)	-	c.4463-523 C>G	Cryptic exon(139bp)	-
155-1	female	33	21	+	Band-keratopathy	COL4A3	c.4463-523 C>G	Cryptic exon(139bp)	-	c.4463-523 C>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	Exon43-44del (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

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.Trp1651Term	-	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 ID 741's F: OB	c.1795G>C	p.Gly599Arg	ID 741's M: OB 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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