Natural History and Genotype-Phenotype Correlation in Female X-Linked Alport Syndrome

Yamamura, Tomohiko ; Nozu, Kandai ; Fu, Xue Jun ; Nozu, Yoshimi ; Ye, Ming Juan ; Shono, Akemi ; Yamanouchi, Satoko ; Minamikawa, Shogo ;…

(Citation)
Kidney International Reports,2(5):850-855

(Issue Date)
2017-09

(Resource Type)
journal article

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/90004457

KI REPORTS
KIReports.org
CLINICAL RESEARCH

Natural History and Genotype–Phenotype Correlation in Female X-Linked Alport Syndrome

Tomohiko Yamamura[1], Kandai Nozu[1], Xue Jun Fu[1], Yoshimi Nozu[1], Ming Juan Ye[1], Akemi Shono[1], Satoko Yamanouchi[1], Shogo Minamikawa[1], Naoya Morisada[1], Koichi Nakanishi[2], Yuko Shima[2], Norishige Yoshikawa[2], Takeshi Ninchoji[1], Ichiro Morioka[1], Hiroshi Kaito[1] and Kazumoto Iijima[1]

[1]Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; and [2]Department of Pediatrics, Wakayama Medical University, Wakayama, Japan

Introduction: X-linked Alport syndrome (XLAS) is a hereditary disease characterized by progressive nephritis, hearing loss, and ocular abnormalities. Affected male patients usually progress to end-stage renal disease in early or middle adulthood, and disease severity is strongly correlated with genotype. However, the clinical course in female patients has rarely been reported.

Methods: We conducted a retrospective analysis of females with genetically proven XLAS (n = 275) and their affected female family members (n = 61) from 179 Japanese families. Patients suspected to have Alport syndrome from pathologic findings or a family history who were referred from anywhere in Japan for genetic diagnosis between 2006–2015 were included in this study. Clinical and laboratory data were collected from medical records at the time of registration for genetic analysis.

Results: Proteinuria was detected in 175 genetically proven patients (72.6%), and the median age for developing proteinuria was 7.0 years. Fifty-two of 336 patients developed end-stage renal disease with a median renal survival age of 65.0 years. No obvious genotype–phenotype correlation was observed. Additionally, targeted sequencing for podocyte-related genes in patients with severe phenotypes revealed no obvious variants considered to be modifier genes except for 1 patient with a *COL4A3* gene variant.

Discussion: This study revealed that phenotypes in female XLAS patients may be severe, but genotype does not help to predict the disease severity. Clinicians must therefore pay careful attention to the clinical course and appropriate treatment in females with XLAS.

Kidney Int Rep (2017) **2,** 850–855; http://dx.doi.org/10.1016/j.ekir.2017.04.011
KEYWORDS: *COL4A5*; genotype–phenotype correlation; modifier gene

X-linked Alport syndrome (XLAS) is a hereditary disease caused by mutations of the *COL4A5* gene encoding the type IV collagen α5 chain. It is characterized by progressive nephritis, hearing loss, and ocular abnormalities. Affected male patients generally develop end-stage renal disease (ESRD) in early or middle adulthood, and a strong genotype-phenotype correlation has been reported.[1–3] In contrast, female patients have rarely been studied, and only 1 large-scale study has previously been published.[4] This previous study reported that female patients showed various degrees of disease severity, ranging from asymptomatic genetic carriers to early-onset ESRD, with no genotype–phenotype correlation. We conducted the largest retrospective analysis of the natural history of female XLAS and also conducted next-generation sequencing to pick up modifier genes in women with severe XLAS.

MATERIALS AND METHODS

All procedures were reviewed and approved by the Institutional Review Board of Kobe University School of Medicine, and informed consent for this study was obtained from all the patients or their parents.

Patients

Patients enrolled in this study were referred to our hospital for clinical evaluation or genetic analysis from

Correspondence: Kandai Nozu, Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo, Kobe, Hyogo 6500017, Japan. E-mail: nozu@med.kobe-u.ac.jp
Received 14 October 2016; revised 11 April 2017; accepted 22 April 2017; published online 4 May 2017

Table 1. Clinical features of 275 female XLAS patients

Clinical features	Age
Median age at diagnosis (yr [range])	24 (0–92)
Hematuria	232 (97.9)
Proteinuria	175 (72.6)
ESRD[a]	33 (12)
Hearing loss	15 (5.5)
Specific ocular changes	4 (1.5)

ESRD, end-stage renal disease; XLAS, X-linked Alport syndrome.
Values are n (%) unless otherwise indicated.
[a]Definition of ESRD: age at starting any renal replacement therapy.

2006 to 2015. Most of them were followed in various local hospitals in Japan. They were suspected of having Alport syndrome from their pathological findings or family histories. All clinical and laboratory findings were obtained from the patients' medical records at the point when genetic analysis was performed. Annual urinary screening is available for all students and most adults in Japan. When hematuria or proteinuria is detected during screening, patients are referred to their family doctor for further urinalysis that is performed at least 3 times. If their abnormal findings persist, they are referred to specialists for further evaluation. Therefore, most cases are diagnosed with Alport syndrome during the very early stages of disease.

In this study, proteinuria was defined by a protein-creatinine ratio >0.2 g/gCre in the early morning first urine that persisted for >3 months. All patients were required to be evaluated for ocular lesions by an ophthalmologist before the application for genetic analysis. Audiometry screening is also available for all students 6, 7, 8, 10, 13, and 15 years of age in Japan and hearing loss can be detected by this screening system.

A total of 314 families were genetically diagnosed with Alport syndrome from January 1, 2006 to December 31, 2015. Sixty-four families were excluded

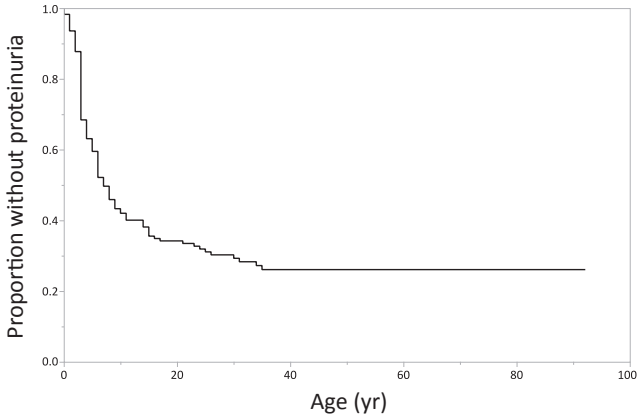

Figure 1. Probability of developing proteinuria. The median age for developing proteinuria was 7.0 years (n = 172).

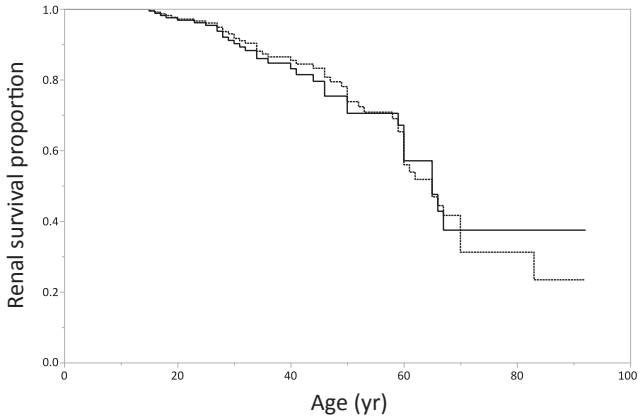

Figure 2. Probabilities of developing end-stage renal disease (ESRD). Solid line indicates genetically proven cases (n = 250). The median age for developing ESRD was 65 years. Dots indicate all female cases of X-linked Alport syndrome, including genetically unconfirmed affected family members (n = 312). The median age for developing ESRD was 65 years.

because they were genetically diagnosed with autosomal dominant or autosomal recessive Alport syndrome with *COL4A3*/*COL4A4* mutations. Among the remaining 250 families with an identified *COL4A5* mutation, 275 female patients and 61 affected female family members from 179 families were selected for this study. All the patients and family members in this study are Japanese.

Mutational Analysis

Mutational analysis of *COL4A5* was performed by several methods: (i) targeted next-generation sequencing using a custom disease panel; (ii) conventional direct sequencing using the Sanger method for all exons and exon–intron boundaries; (iii) multiplex ligation-dependent probe amplification to detect copy-number variations; and (iv) reverse transcription–polymerase chain reaction of mRNA and direct sequencing to detect abnormal splicing. We initially performed methods (i) or (ii), and if no mutations were detected, we then performed methods (iii) and (iv).

Additionally, 24 female patients with clinically severe phenotypes underwent targeted sequencing for

Table 2. Type of mutations in all 275 female XLAS patients

Type of mutation	Number of cases	Number of families
Missense mutation	137	88
Splicing mutation	49	31
Small deletion	31	21
Nonsense mutation	25	17
Large rearrangement	21	14
Insertion + duplication	12	8

XLAS, X-linked Alport syndrome.

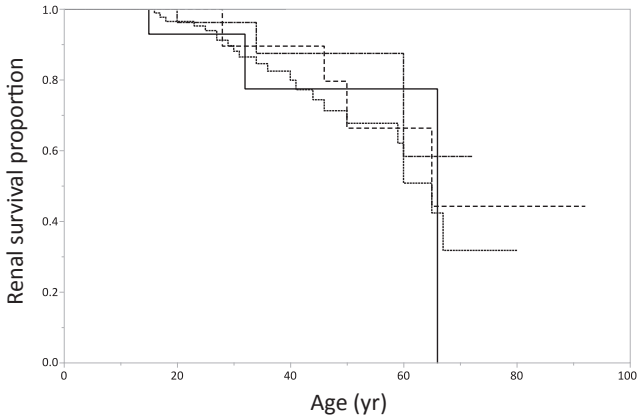

Figure 3. Probability of developing end-stage renal disease (ESRD) according to mutation types. Solid line indicates patients with nonsense mutations (n = 23). The median age for developing ESRD was 66 years. Dots indicate patients with missense mutations (n = 129). The median age for developing ESRD was 65 years. Dashes indicate splice site mutations (n = 43). The median age for developing ESRD was 65 years. Dash-dots indicate small mutation (small deletion, insertion, duplication) (n = 36). The median age for developing ESRD could not be calculated because the probability of having ESRD does not reach 50%. A curve for patients with large rearrangements is not shown because none of them had developed ESRD because of their relatively young age.

45 podocyte-related genes that are known to be causative of inherited focal segmental glomerulosclerosis or Alport syndrome (Supplementary Table S1). Clinically severe cases were defined by (i) developing ESRD before the age of 60 years (n = 14) or (ii) starting to show heavy proteinuria (urine protein-creatinine ratio >1.0 g/gCre) in early morning first urine analysis with the detection of proteinuria before the age of 3 years.

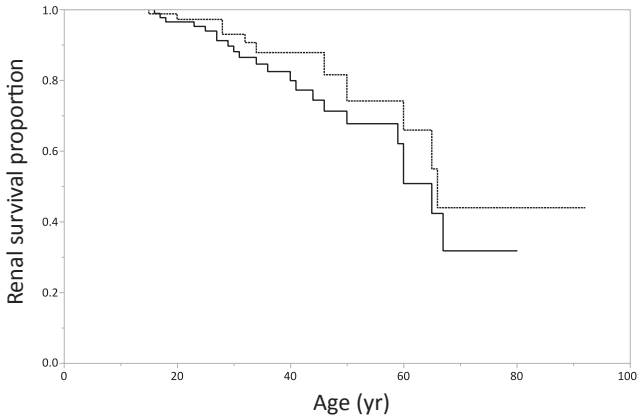

Figure 4. Probability of developing end-stage renal disease (ESRD) according to mutation with or without missense mutation. Solid line indicates patients with missense mutations (n = 129). The median age for developing ESRD was 65 years. Dots indicate patients with other mutations (n = 121). The median age for developing ESRD was 66 years.

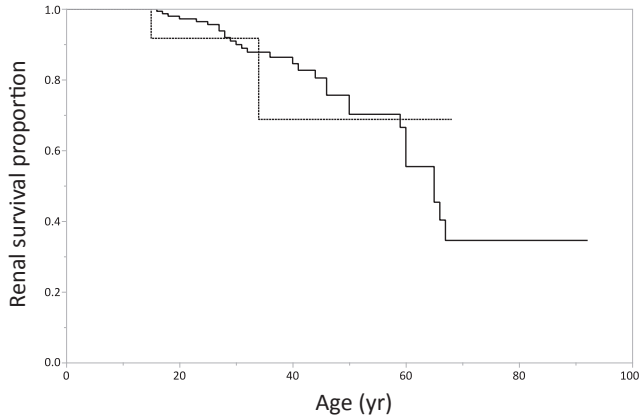

Figure 5. Probability of developing end-stage renal disease (ESRD) according to presence or absence of hearing loss. Solid line indicates patients without hearing loss (n = 236). The median age for developing ESRD was 65 years. Dots indicate patients with hearing loss (n = 14). The median age for developing ESRD could not be calculated because the probability of having ESRD does not reach 50%.

Statistical Analysis

All calculations were performed using standard statistical software (JMP version 10 Package for Windows; SAS Institute, Cary, NC). The occurrence of events (age of developing proteinuria, renal survival period) was analyzed according to the Kaplan-Meier method.

RESULTS

Clinical Features

The clinical features of the cohort are shown in Table 1. *COL4A5* mutations in all 179 families are shown in Supplementary Table S2. Their median age at genetic testing was 24 years (range, 0–92 years). Proteinuria was detected in 175 patients (72.6%) and the median age for developing proteinuria was 7.0 years (Figure 1). Thirty-three patients developed ESRD, with a median renal survival period of 65.0 years, and 15% of patients reaching ESRD by the age of 40 (Figure 2, Supplementary Table S3). Specific ocular changes were only detected in 4 patients (1.5%) and hearing loss was detected in 15 (5.5%). Regarding treatment, only patients of a relatively young age who started to show proteinuria had started treatment with angiotensin-covering enzyme inhibitor or angiotensin receptor blocker, and none of the older patients including those who reached ESRD were treated with these drugs.

We also constructed a renal-survival curve including genetically unconfirmed affected family members who had abnormal urinalysis results or ESRD or both, excluding 25 cases whose age was unknown (n = 312). Fifty-two patients reached ESRD, with a

median age of developing ESRD of 65.0 years (Figure 2). No one with hematuria alone without proteinuria developed ESRD.

Genotype–Phenotype Correlation

The detected mutation types are shown in Table 2. We compared renal-survival curves for 250 patients with known renal status according to the type of mutation (Figure 3). There was no difference between these groups in terms of the age of reaching ESRD in each mutation type. We also compared renal-survival curves for patients with or without missense mutations (Figure 4) and found no significant difference.

Hearing Loss and Kidney Prognosis

We also compared renal prognoses according to the presence or absence of hearing loss, but found no significant difference between patients with and without hearing loss (Figure 5).

Next-Generation Sequencing Analysis

We conducted targeted sequencing analyses of 24 clinically severe cases who reached ESRD before the age of 60 (n = 14) or who showed heavy proteinuria from a young age (n = 10) to identify genetic factors enhancing disease severity in female cases. Identical pathogenic variants in *COL4A5* were detected by both Sanger sequencing and targeted sequencing in all patients. In addition, only 1 patient was revealed to have the heterozygous nonsense mutation in *COL4A3* (c.1216C>T, p.Arg406Term), which was already reported as a causative mutation of autosomal Alport syndrome.[5] She is a 10-year-old girl with a large deletion of *COL4A5* exon 1 who showed heavy proteinuria (urine protein-creatinine ratio, 1.35 g/gCre) under treatment of angiotensin-covering enzyme inhibitor and angiotensin receptor blocker. In this case, the digenic variants in *COL4A3* and *COL4A5* might have affected the severity of Alport syndrome. None of the other 23 cases possessed modifier gene variants in 45 podocyte-related genes.

DISCUSSION

In this study, we reported the genetic and clinical characteristics of female XLAS in patients with proven *COL4A5* variants. Only 1 large-scale observational study of female cases of XLAS has previously been reported.[4] The current study involved a similar sample size, with the added advantage of ethnic homogeneity, given that all the patients in the current study were Japanese.

We detected a very high prevalence of hematuria (97.9%), consistent with the results of the previous report (95.5%). Although other studies found that all female carriers of XLAS had hematuria,[6,7] it is necessary to bear in mind that some heterozygous carriers of XLAS do not show obvious hematuria. The prevalence of proteinuria (72.6%) in the current study was also similar to that in the previous study (75.2%). Furthermore, the median age for developing proteinuria in female XLAS patients in our study was 7.0 years. Japan offers an annual school urinary screening system for all students, and these data are therefore relatively accurate. This study provides the first clarification of this information.

Regarding extrarenal symptoms, hearing loss and specific ocular abnormalities only developed in a few patients (5.5% and 1.5%, respectively). These prevalences were much lower than in the previous report (28% and 15%).[4] The low detection rate for ocular abnormalities may have been associated with insufficient ophthalmologic detection skills, while the low rate of hearing loss detection might reflect ethnic differences or the relatively young median age of our cohort, given that audiometry screening is also available for all students 6, 7, 8, 10, 13, and 15 years of age in Japan.

The renal prognosis of female XLAS has generally been considered to be favorable, and treatment for female XLAS is therefore minimal. Jais *et al.*[4] reported that 51 of 349 female XLAS patients developed ESRD, and 55% of them reached ESRD before the age of 40, the youngest at the age of 19. They revealed that the risk of developing ESRD before the age of 40 was 12%, while the median age for developing ESRD was not detected (>80 years). Flinter *et al.*[6] reported that among 113 female XLAS patients, 15% developed chronic renal failure at an average age of 40. In our cohort, 52 of 336 female XLAS patients developed ESRD, with the youngest developing it at the age of 15 years. We also constructed a renal-survival curve using the Kaplan-Meier method and showed a median renal survival period of 65.0 years. About 15% of patients reached ESRD before the age of 40. This suggests that the prognosis of female XLAS patients is not benign, and that suitable management, including early medication, should be considered. Recent expert guidelines suggested that women with XLAS should be monitored carefully and treated with renin-angiotensin blockade if they develop hypertension, microalbuminuria, or renal impairment.[8]

We investigated the genotype–phenotype correlation in female XLAS patients. Although a strong correlation has been reported in male XLAS cases,[1–3,9] this association has rarely been examined in female cases. We compared renal prognosis of female patients according to their mutation types, but we found no significant difference in terms of the age of reaching

ESRD. These results support previous data,[4] and we therefore concluded that disease severity was unrelated to genotype in female XLAS patients. Although renal prognosis of female XLAS patients can be influenced by continuous angiotensin-covering enzyme inhibitor or angiotensin receptor blocker treatment, or both, as previously reported,[10] its influence in our study result was limited because we accepted patients for genetic testing just after they were suspected to have Alport syndrome and before the initiation of treatment; moreover, our clinical data were obtained at the time of application for genetic testing. Therefore, only a few patients had started treatment with these nephroprotective drugs.

There are limitations to our study. First, we were unable to assess the impact of early onset proteinuria on ESRD development because the school urine screening system started about 40 years ago in Japan, so the onset age of proteinuria in the ESRD group was not clear. However, it is possible to state that no case with hematuria alone, without proteinuria, developed ESRD as is shown in the previous report.[4] Second, clinical information that might deteriorate renal function such as poorly controlled hypertension, nonsteroidal anti-inflammatory drug use, preeclampsia, or incidental glomerular disease was not collected, so we could not analyze the impact of these factors.

Strasser *et al.*[11] reported that digenic mutations in *COL4A5* and *MYH9* affected the severity of XLAS symptoms. Mencarelli *et al.*[12] showed that digenic variants in any 2 genes of *COL4A3*, *COL4A4*, or *COL4A5* caused more severe phenotypes compared with monogenic variants of 1 of these genes. We therefore conducted targeted sequencing to search for modifier genes among podocyte-related genes, reported as causative genes of familial focal segmental glomerular sclerosis or congenital nephrotic syndrome. However, we failed to identify any variants likely to act as modifier genes except for 1 case with a heterozygous nonsense mutation in *COL4A3*, suggesting that modifier genes might rarely contribute to the severity of female XLAS. We have also failed to detect *NPHS2* variants in our cohort; although these are very common in the European population, it is possible that they are quite rare in the Japanese population.[13]

In conclusion, the phenotype of female XLAS patients is not always mild, and clinicians should therefore pay close attention to its clinical course and treatment. There is no genotype–phenotype correlation in female XLAS, and no obvious modifier genes were detected in most of the clinically severe patients. It therefore seems likely that the mechanisms determining the severity of female XLAS are multifactorial.

DISCLOSURE

KN received lecture fees from Novartis Pharma K.K., Chugai Pharmaceutical Co., Ltd., Asahi Kasei Pharma Corp, and Taisho Pharm. Co. KI received grants from Daiichi Sankyo Co., Ltd, Japan. All other authors declared no competing interests.

ACKNOWLEDGMENTS

This study was supported by a grant from the Ministry of Health, Labour, and Welfare of Japan for Research on Rare Intractable Diseases in Kidney and Urinary Tract (H24-nanchitou (nan)-ippan-041 to KI) in the "Research on Measures for Intractable Diseases" Project; a Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Subject ID: 25893131 to KN and 26293203 to KI).

SUPPLEMENTARY MATERIAL

Table S1. Targeted genes screened for severe cases.

Table S2. Detected mutations of *COL4A5* gene in 179 families.

Table S3. List of 33 genetically proven patients who reached end-stage renal disease.

Supplementary material is linked to the online version of the paper at www.kireports.org.

REFERENCES

1. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history in 195 families and genotype-phenotype correlations in males. *J Am Soc Nephrol*. 2000;11:649–657.
2. Bekheirnia MR, Reed B, Gregory MC, et al. Genotype-phenotype correlation in X-linked Alport syndrome. *J Am Soc Nephrol*. 2010;21:876–883.
3. Gross O, Netzer KO, Lambrecht R, et al. Meta-analysis of genotype-phenotype correlation in X-linked Alport syndrome: impact on clinical counselling. *Nephrol Dial Transplant*. 2002;17:1218–1227.
4. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a "European Community Alport Syndrome Concerted Action" study. *J Am Soc Nephrol*. 2003;14:2603–2610.
5. Heidet L, Arrondel C, Forestier L, et al. Structure of the human type IV collagen gene *COL4A3* and mutations in autosomal Alport syndrome. *J Am Soc Nephrol*. 2001;12:97–106.
6. Flinter FA, Cameron JS, Chantler C, et al. Genetics of classic Alport's syndrome. *Lancet*. 1988;2:1005–1007.
7. Dagher H, Buzza M, Colville D, et al. A comparison of the clinical, histopathologic, and ultrastructural phenotypes in carriers of X-linked and autosomal recessive Alport's syndrome. *Am J Kidney Dis*. 2001;38:1217–1228.
8. Savige J, Gregory M, Gross O, et al. Expert guidelines for the management of Alport syndrome and thin basement

membrane nephropathy. *J Am Soc Nephrol*. 2013;24:364–375.

9. Hashimura Y, Nozu K, Kaito H, et al. Milder clinical aspects of X-linked Alport syndrome in men positive for the collagen IV alpha5 chain. *Kidney Int*. 2014;85:1208–1213.
10. Temme J, Peters F, Lange K, et al. Incidence of renal failure and nephroprotection by RAAS inhibition in heterozygous carriers of X-chromosomal and autosomal recessive Alport mutations. *Kidney Int*. 2012;81:779–783.
11. Strasser K, Hoefele J, Bergmann C, et al. *COL4A5*-associated X-linked Alport syndrome in a female patient with early inner ear deafness due to a mutation in *MYH9*. *Nephrol Dial Transplant*. 2012;27:4236–4240.
12. Mencarelli MA, Heidet L, Storey H, et al. Evidence of digenic inheritance in Alport syndrome. *J Med Genet*. 2015;52:163–174.
13. Maruyama K, Iijima K, Ikeda M, et al. *NPHS2* mutations in sporadic steroid-resistant nephrotic syndrome in Japanese children. *Pediatr Nephrol*. 2003;18:412–416.