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

Tomohiko Yamamura¹, Kandai Nozu¹, Xue Jun Fu¹, Yoshimi Nozu¹, Ming Juan Ye¹, Akemi Shono¹, Satoko Yamanouchi¹, Shogo Minamikawa¹, Naoya Morisada¹, Koichi Nakanishi², Yuko Shima², Norishige Yoshikawa², Takeshi Ninchoji¹, Ichiro Morioka¹, Hiroshi Kaito¹ and Kazumoto Iijima¹

¹Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; and ²Department 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.

KEYWORDS: COL4A5; genotype–phenotype correlation; modifier gene
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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 and 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 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.

### Table 1. Clinical features of 275 female XLAS patients

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age of diagnosis (yr [range])</td>
<td>24 (0–92)</td>
</tr>
<tr>
<td>Hematuria</td>
<td>232 (97.9)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>175 (72.6)</td>
</tr>
<tr>
<td>ESRD*</td>
<td>33 (12)</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>15 (5.5)</td>
</tr>
<tr>
<td>Specific ocular changes</td>
<td>4 (1.5)</td>
</tr>
</tbody>
</table>

ESRD, end-stage renal disease; XLAS, X-linked Alport syndrome.

*Definition of ESRD: age at starting any renal replacement therapy.

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

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>Number of cases</th>
<th>Number of families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missense mutation</td>
<td>137</td>
<td>88</td>
</tr>
<tr>
<td>Splicing mutation</td>
<td>49</td>
<td>31</td>
</tr>
<tr>
<td>Small deletion</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>Nonsense mutation</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Large rearrangement</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Insertion + duplication</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

XLAS, X-linked Alport syndrome.
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 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.

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. 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. 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,1–5,9 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, 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, 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. 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. reported that digenic mutations in COL4A5 and MYH9 affected the severity of XLAS symptoms. Mencarelli et al. 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.

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.


