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Early Clinical Manifestations and Long-term Renal Outcomes by Genotype in Children with X-Linked Alport Syndrome

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Research Article

Keywords: XLAS, Children, Genotype, phenotype, prognosis

Posted Date: March 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8992013/v1

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Version of Record: A version of this preprint was published at Pediatric Nephrology on October 28th, 2026. See the published version at https://doi.org/10.1007/s00467-026-07585-z.

Abstract

Background X-linked Alport syndrome (XLAS) is the most common pediatric genetic kidney disease. The correlation between genotype, phenotype, and prognosis in children remains poorly defined. This study aims to elucidate these relationships.

Methods We analyzed associations between genotype and clinical features at onset, including hematuria, proteinuria, and serum creatinine(Scr). Longitudinal data on creatinine and 24-hour urinary protein (24h UP) were assessed to evaluate genotype-prognosis correlations.

Results All children presented with hematuria (33.8% microscopic, 13.2% gross, 52.9% with proteinuria). Extrarenal manifestations occurred in 5.9%. Genetic testing revealed missense (67.7%), splicing (13.2%), frameshift (14.7%), and deletion mutations (4.4%). At onset, males had significantly higher proteinuria than females. Among males, frameshift mutations and mutations beyond exon 40 (> EX40) were associated with more severe phenotypes. During follow-up (10.3% lost), gender and mutation type independently predicted proteinuria progression, with faster progression in males and patients with splicing or deletion mutations. Intronic mutations predicted better outcomes. For renal function decline, gender, mutation type, and site were independent risk factors, with faster decline in males, deletion mutations, and > EX40 mutations.

Conclusions In XLAS, gender and genotype closely correlate with disease severity at onset and prognosis. Males, frameshift mutations, and > EX40 sites predict severe initial manifestations. Male gender, splicing/deletion mutations, and > EX40 sites are independent risk factors for faster progression, while intronic mutations predict favorable outcomes.

Introduction

Alport syndrome (AS) is a hereditary glomerular disorder resulting from a single gene mutation that causes a deficiency in type IV collagen. This condition is characterized by structural damage and functional impairment of the glomerular basement membrane (GBM)[1]. Clinically, AS presents with persistent hematuria and proteinuria, a progressive decline in renal function, and eventual progression to end-stage kidney disease (ESKD). Additionally, some patients may experience extrarenal manifestations, such as sensorineural hearing loss and ocular abnormalities affecting the lens and fundus. AS is responsible for 0.5% [2]of new-onset ESKD cases in adults and 12.9% [3]in children.The prevalence of Alport syndrome (AS) varies according to its genetic inheritance pattern, with an overall incidence estimated at approximately 1 in 5,000 to 10,000 individuals. XLAS is the most prevalent form, constituting approximately 80% to 85% of cases [4]. XLAS results from mutations in the COL4A5 gene, which encodes the α5 chain of type IV collagen. This gene is situated on the long arm of the X chromosome, specifically at Xq21-q22 [5], and over 1,000 mutations have been identified to date [6].

The severity and progression of XLAS are influenced by factors such as the patient's sex, age, and specific mutation type [7]. Jais et al. [8–9] have documented that the severity of XLAS in patients from

European and American populations is associated with sex. Hemizygous male patients typically present with more severe phenotypes and have a higher likelihood of developing ESKD or hearing loss compared to heterozygous female patients. In contrast, heterozygous females generally exhibit milder symptoms, and even within the same family, a range of phenotypic expressions may be observed.Gong et al.[10] conducted a comprehensive analysis involving 141 patients with XLAS from 88 Chinese families. Consistent with findings from international studies, the male XLAS patients in China exhibited more severe phenotypic manifestations compared to their female counterparts, particularly in relation to proteinuria and renal function impairment. Notably, over 50% of the male patients experienced renal function impairment, a prevalence significantly higher than that observed in female patients. Patients harboring deletion mutations demonstrated a higher propensity for progression to ESKD. Approximately 50% of the male patients with deletion mutations also presented with hearing loss, and around 25% exhibited ocular lesions. In contrast, one-third of the male patients with missense mutations experienced hearing loss, while fewer than one-sixth had ocular lesions.

The clinical manifestations and prognosis of XLAS are intricately linked to the specific type of genetic mutation present. Therefore, early identification, diagnosis, and intervention in AS are essential for mitigating the progression of renal dysfunction towards ESKD.Previous research on XLAS has predominantly concentrated on adult populations in regions such as Europe, America, and Japan.The sample size for children was relatively small. Due to the young age of these children, many had not yet progressed to ESKD, leading to a scarcity of specific prognostic data for this demographic. The correlation between genotype and clinical phenotype, as well as the prognosis of pediatric AS, remains inadequately understood. Notably, there is a significant gap in research concerning the relationship between specific mutation sites and their clinical manifestations and prognostic outcomes. This study aims to address these gaps by conducting a retrospective analysis of the genotypes—including mutation types and sites—and clinical phenotypes of 68 pediatric AS patients treated at our center. The objective is to elucidate the factors influencing the clinical presentations and prognoses of children with XLAS at disease onset and to provide a theoretical foundation for predicting the prognosis of pediatric patients with varying AS genotypes.

Patients and Methods

Patients

The subjects of this study comprised children diagnosed with AS at our center, all of whom underwent whole-genome sequencing. Inclusion criteria adhered to the "Expert Recommendations for Diagnosis and Treatment of Alport Syndrome," as published in the Chinese Journal of Nephrology in 2018 [11]. These criteria required that patients with persistent glomerular hematuria, or hematuria accompanied by proteinuria, meet at least one of the following conditions: (1) Abnormal immunofluorescence staining of type IV collagen α3, α4, and α5 chains in the glomerular basement membrane, or abnormal staining of the type IV collagen α5 chain in the skin basement membrane; (2) Electron microscopy of renal tissue revealing tearing and layering of the dense layer of the glomerular basement membrane; (3)

Identification of a pathogenic mutation in the COL4A5 gene, or two pathogenic mutations in either the COL4A3 or COL4A4 genes. Exclusion criteria included: (1) Children in whom no suspicious mutations were detected through genetic testing; (2) Children with mutations in genes other than COL4A5; (3)Children with incomplete clinical data at the onset of the disease.

Methods

1. Clinical Data Collection: Patient data were retrospectively collected, including gender, height/length, weight, age, age of onset, mode of onset, levels of hematuria and proteinuria, and renal function status. Renal function was primarily assessed through Scr levels, and the glomerular filtration rate (GFR) was calculated using the Schwartz formula. The Chronic Kidney Disease (CKD) stage was determined based on the following formula: eGFR = K × height / serum creatinine, where the K value is 40 for ages 0–18 months, 49 for girls aged 2–16 and boys aged 2–13, and 62 for boys aged 13–16. Additional data collected included family history, presence or absence of high-frequency sensorineural deafness, retinal lesions, and other extrarenal manifestations, as well as genetic test results. Renal biopsy findings were analyzed through light microscopy, immunofluorescence staining for type IV collagen α3 and α5 chains, and electron microscopy.

2. Follow-up Data Collection: A cohort of 68 children was monitored longitudinally, with 7 cases (10.3%) lost to follow-up. The remaining 61 children underwent biannual evaluations at our hospital or local hospitals, which included assessments of Scr levels and 24-h UP, among other parameters. Verification of test results was conducted via electronic medical records or telephone follow-ups. The initiation of follow-up was marked by the onset of symptoms in each child. Given that children with XLAS are predominantly in the early stages of the disease and the incidence of progression to ESKD is relatively low, this study defined the follow-up endpoints as follows: (1) a doubling of the 24-h UP from baseline, and (2) a 25% reduction in estimated glomerular filtration rate (eGFR) from baseline.

Statistical analysis

Statistical analyses were performed using SPSS software. For measurement data not conforming to a normal distribution, the median and interquartile range were utilized for representation. The Mann-Whitney U test was applied for comparing two independent samples, while the Kruskal-Wallis test was used for three or more independent samples. Statistical significance was determined at a $P<0.05$. For normally distributed measurement data, the mean ± standard deviation was employed. The t-test was utilized for two independent samples, and one-way analysis of variance was applied when more than two groups were involved, with statistical significance set at a $P<0.05$. For categorical data, the Fisher's exact test was used, with significance also determined at a $P<0.05$. Cox regression analysis was conducted to identify risk factors influencing disease progression, with statistical significance considered at a $P< 0.05$.

Results

Genetic test results and Clinical phenotypes at the onset of XLAS children

1.General Demographics: In this cohort of 68 patients, 43 were male (63.2%) and 25 were female (36.8%), resulting in a male-to-female ratio of 1.72:1. The median age of onset for male patients was 2 (2-4)years , while for female patients it was 3 (2-5) years .

2.Hematuria and Proteinuria at Onset: All patients (n=68) presented with hematuria. Of these, 23 patients (33.8%) exhibited isolated microscopic hematuria, 9 patients (13.2%) had isolated gross hematuria, and 36 patients (52.9%) presented with proteinuria. Additionally, 14 patients (20.6%) had concurrent microscopic hematuria and proteinuria, whereas 22 patients (32.4%) had concurrent gross hematuria and proteinuria.

3.Renal Function at Onset: At the time of presentation, 2 patients had progressed to chronic kidney disease stages 2-5, both of whom were male. One patient, aged 3 years, had an eGFR of 69.0 ml/min/1.73m², while the other, aged 14 years, had an eGFR of 42.1 ml/min/1.73m².

4.Extrarenal Manifestations: Among the cohort, four patients exhibited extra-renal manifestations at the onset, all of whom were male. One male patient presented with both fundus lesions and high-frequency hearing impairment, with the onset occurring at 14 years of age. The remaining three male patients exhibited only high-frequency hearing impairment, with onset ages of 1 year, 1 year, and 2 years, respectively.

5.Renal Pathology: Renal tissue biopsy was performed on 51 patients, accounting for 75.0% of the cohort. Light microscopy findings revealed that 28 cases exhibited mild lesions, 14 cases were diagnosed with mesangial proliferative glomerulonephritis, 1 case with focal segmental glomerulosclerosis, and 8 cases demonstrated typical Alport changes. Immunofluorescence staining was conducted on all patients, revealing continuous positive α3 and α5 in 13 cases, negative α3 and α5 in 28 cases, intermittent positive α3 and α5 in 6 cases, intermittent positive α3 and positive α5 in 2 cases, and intermittent positive α3 and negative α5 in 2 cases. Electron microscopy data were available for 26 patients, with 4 cases (15.4%) exhibiting typical Alport changes, 7 cases (26.9%) showing mesangial proliferative glomerulonephritis, 9 cases (34.6%) displaying mild lesions, and 1 case (3.9%) presenting with focal segmental glomerulosclerosis,2 cases (7.7%) exhibited an absence of glomeruli, while 3 cases (11.5%) showed no abnormalities.

6.Genetic test results:Genetic testing was conducted on 68 patients, representing 65 families, which included 3 pairs of siblings, revealing 63 distinct mutation sites. Among these patients, 46 cases (67.7%) presented with missense mutations, 9 cases (13.2%) with splicing mutations, 10 cases (14.7%) with frameshift mutations, and 3 cases (4.4%) with deletion mutations. Notably, 35 of the 46 missense mutations were of the Gly-X-Y type. Sanger sequencing was employed to verify familial mutations in 64 families (excluding 1 adopted patient without available family verification), revealing that 34 mutations were maternally inherited, 8 were paternally inherited, and 25 were de novo mutations.

Table 1. Clinical phenotypes and genetic testing results of XLAS patients at onset

	Total	Male	Female
Number of cases	68	43（63.2%）	25（36.8%）
Age of onset (y), median(IQR)		2（2-4）	3（2-5）
Onset manifestations			
Only microscopic hematuria	23（33.8%）	11	12
Only gross hematuria	9（13.2%）	7	2
Microscopic hematuria and proteinuria	14（20.6%）	10	4
Gross hematuria and proteinuria	22（32.4%）	15	7
CKD stage 2-5	4（5.9%）	4	0
Extrarenal manifestations			
Ocular manifestations	1（1.5%）	1	0
Hearing impairment	4（5.9%）	4	0
Renal Pathology			
Mild lesions	28（41.1%）	18	10
Mesangial proliferative glomerulonephritis	14（20.6%）	9	5
Focal segmental glomerulosclerosis	1（1.5%）	1	0
Typical Alport changes	8（11.8%）	7	1
No-Biopsy	17（25.0%）	8	9
Immunofluorescence staining			
Negative α3 and α5	28（41.1%）	22	6
Intermittent positive α3 and/or α5	10（14.7%）	6	4
Continuous positive α3 and α5	13（19.1%）	7	6
Types of mutation			
Missense mutations	46（67.7%）	30	16
Splicing mutations	9（13.2%）	5	4
Frameshift mutations	10（14.7%）	6	4
Deletion mutations	3（4.4%）	2	1

Correlation between Genotypes and Clinical Phenotypes at Onset in XLAS Patients

Previous research has indicated that the clinical manifestations of adult patients with XLAS are influenced by both gender and mutation type. However, there is a paucity of studies examining the factors affecting the clinical manifestations of pediatric patients with XLAS. This study, therefore, investigated the relationship between gender, mutation type, and the clinical manifestations of pediatric patients with XLAS at disease onset. The findings revealed that the age of onset for male patients was 2(2-4) years , while for female patients it was 3(2-5) years . No statistically significant difference was observed in the age of onset between genders (*P* = 0.248, Figure 1a). At disease onset, the 24-h UP for male patients was 0.20(0.11 - 0.52) g , compared to 0.11 (0.055 - 0.195) g for female patients, with this difference being statistically significant (*P* = 0.028, Figure 1b), indicating more severe proteinuria in male patients. The number of red blood cells in the urine sediment at disease onset was 881.3(366.0 - 3000.0) /μL for male patients and 752 (302.68 - 2050.5) /μL for female patients, with no significant statistical difference between genders(*P* = 0.434, Figure 1c).At the onset of the disease, the eGFR for male patients was 159.7(117.9 -188.5) ml/(min·1.73 m²) , whereas for female patients, it was 184.5(135.7 -204.7) ml/(min·1.73 m²) . Statistical analysis revealed no significant difference in eGFR between genders (*P* = 0.067, Figure 1d).

The mutation type is a critical factor influencing the clinical manifestations in patients with XLAS. However, the impact of mutation type on the clinical manifestations of children with XLAS at disease onset remains unclear. Consequently, this study examined the mutation patterns and clinical manifestations in a cohort of 68 children with XLAS.

Among male XLAS patients with missense mutations, the age of onset was 3 (2-4) years, for frameshift mutations it was 2 (0.9-5)years , for splice mutations it was 2 (2-3) years, and for deletion mutations, both cases had an onset age of 2 years. There was no statistically significant difference in the age of onset among children with different mutation types (*P* = 0.375, refer to Figure 2a). In male patients with XLAS, the 24-h UP at the onset of missense mutations was measured at 0.14(0.0775 - 0.34) g . For frameshift mutations, the protein level was 1.025(0.5375-2.5125) g , and for splicing mutations, it was 0.15(0.045 - 0.8) g . In the case of deletion mutations, two male XLAS patients exhibited 24-h UP levels of 0.21 g and 0.12 g, respectively. A statistically significant difference was observed in the 24-h UP levels among the different mutation types in male XLAS patients (*P* = 0.011, Figure 2b). Notably, the 24-h UP level in children with frameshift mutations at onset was significantly higher than those with missense and splicing mutations. Regarding the number of red blood cells in urine sediment, male XLAS patients with missense mutations had a count of 709.25(281.825 - 2837.3) /μL , those with frameshift mutations had a count of 2484.5(540.275 - 4299.6) /μL , and those with splicing mutations had a count of 614.00 (306.5 - 2659.0) /μL. For the two male XLAS patients with deletion mutations, the red blood cell counts in urine sediment at onset were 1490.00 /μL and 4305.70 /μL, respectively. No significant difference was found in the number of red blood cells in urine among the different mutation types(*P*=0.36,Figure 2c).In male patients with XLAS, the eGFR at the onset of missense mutations was 150.9 (116.725 - 187.75)ml/(min·1.73 m²) , for frameshift mutations it was 194.5 (96.25 - 205.425)ml/(min·1.73 m²), and for splicing mutations it was 174.2(116.3 - 205.5) ml/(min·1.73 m²) . In the two male patients with deletion mutations, the eGFR at onset was 167.6 ml/(min·1.73 m²)and 148.8 ml/(min·1.73 m²),

respectively. Statistical analysis revealed no significant differences in eGFR among the various mutation types in male patients at onset (*P* = 0.847, Figure 2d).

The age of onset for female XLAS patients with missense mutations was 3.5 (2 - 6.5) years , for frameshift mutations it was 3 (1.25 - 4) years , for splice mutations it was 3 (2.25 - 3) years . For the only one female XLAS patient with deletion mutations, the age of onset was 7 years old. There was no significant difference in the age of onset among different mutation types for female XLAS patients (*P* = 0.773, Figure 3a). The 24-h UP at the time of onset for female XLAS patients with missense mutations was 0.12 (0.08 - 0.275) g; for frameshift mutations it was 0.115 (0.0575 - 0.1725) g; for splice mutations it was 0.12 (0.08 - 0.4225) g; for the only one female XLAS patient with deletion mutation, the 24-h UP was 0.23 g. There was no significant difference in the 24-h UP levels among different mutation types for female XLAS patients (*P*=0.999, Figure 3b). In female patients with XLAS, the initial number of red blood cells in urine sediment was observed to be 677.7 (280.52 - 1835.25) /μl for those with missense mutations, 1155.45 (353.75 - 2369.8) /μl for frameshift mutations, and 1876.85 (254.92 - 6551.5) /μl for splice mutations. There was a single case of deletion mutation in this cohort, with a red blood cell count of 582.9 /μl in urine sediment at disease onset. Statistical analysis revealed no significant differences in the red blood cell counts among the different mutation types (*P*=0.706, Figure 3c). Additionally, the eGFR at onset was recorded as 194.7 (147.4 - 212.775) ml/(min·1.73 m²) for missense mutations, 149.9 (131.15 - 174.05) ml/(min·1.73 m²) for frameshift mutations, and 187.3 (136.5 - 199.475) ml/(min·1.73 m²) for splice mutations. No significant differences were found in eGFR values among the different mutation types in female XLAS patients (*P* = 0.247, Figure 3d).

Gross et al. [12] observed that patients harboring mutations in exons 21-47 (EX21-47) exhibited more severe phenotypes and progressed to ESKD earlier than those with mutations in exons 1-20 (EX1-20). Consequently, this study categorized mutation sites into three groups: exons 1-20 (EX1-20), exons 21-47 (EX21-47), and exons beyond 47 (>EX47). The age of onset for male patients with XLAS was 2 (2-4)years for those with EX1-20 mutations, 2.5 (1.25-4) years for those with EX21-47 mutations, and 2(2-2.25) years for those with >EX47 mutations. Statistical analysis revealed no significant differences in the age of onset among the different mutation groups (*P*=0.62, Figure 4a). However, the 24-h UP at disease onset was 0.12 (0.09-0.285)g for EX1-20 mutations, 0.18 (0.075-0.535) g for EX21-47 mutations, and 1.0 (0.3375-2.5125) g for >EX47 mutations. There was a statistically significant difference in the 24-h UP levels at disease onset among the mutation groups (*P* = 0.024, Figure 4b), with male XLAS patients harboring mutations in >EX47 exhibiting significantly higher proteinuria levels compared to those with mutations in EX1-20 and EX21-47. At the onset of the disease, the number of red blood cells in the urine sediment for male XLAS patients in the EX1-20 group was 1490 (643.7-2854.5) cells/μL, in the EX21-47 group was 490 (242-2440.5) cells/μL, and in the >EX47 group was 2954.6 (1585.25-5801.5) cells/μL. A statistically significant difference was observed in the number of red blood cells in the urine sediment among patients with different mutation sites (*P*=0.037, Figure 4c). Specifically, the >EX47 group exhibited a significantly higher number of red blood cells compared to the EX21-47 group. Regarding the estimated eGFR at disease onset, the values for the EX1-20 group were 167.5 (128- 202.25) ml/(min·1.73 m²), for the EX21-47 group were 138.4 (109.2 - 170.55) ml/(min·1.73 m²), and for the >EX47 group were

187.65 (178.075 - 215.15ml/(min·1.73 m²). There was a statistically significant difference in eGFR among patients with different mutation sites (*P*= 0.028, Figure 4d), with the EX21-47 group showing a significantly lower eGFR compared to the >EX47 group.

The age of onset for female patients with XLAS in the EX1-20 mutation group was 3.5 (1.25 - 6.5)years , in the EX21-47 group it was 3(1.75 - 5.625) years , and in the >EX47 group it was 3 (2) years. Statistical analysis revealed no significant differences in the age of onset among patients with varying mutation sites (*P* = 0.972, Figure 5a). The 24-h UP at disease onset for the EX1-20 group was 0.095(0.065 - 0.215) g, for the EX21-47 group it was 0.12 (0.0875 - 0.1675)g , and for the >EX47 group it was 0.11(0.02) g. No statistically significant differences were observed in the 24-h UP levels at onset across different mutation sites (*P*= 0.628, Figure 5b). The number of red blood cells in urine sediment at disease onset was 837.5(229.74 - 1835.25) /μL in the EX1-20 group, 701.7(372.5 - 1697.2) /μL in the EX21-47 group, and 2656.1(205)/μL in the >EX47 group. There were no statistically significant differences in the number of red blood cells in urine sediment at onset among the different mutation groups (*P* = 0.582, Figure 5c). At the onset of the eGFR in the EX1-20 cohort of female patients with XLAS was 183.65(123.9 - 195.35) g , in the EX21-47 cohort it was 205.4(174.05 - 214.825) g , and in the >EX47 cohort it was 135.8(129.6) g . Statistical analysis revealed no significant differences in eGFR at disease onset among patients with varying mutation sites (*P* = 0.081, Figure 5d).

Association between genotype and prognosis

Using the doubling of 24-h UP as the follow-up endpoint, both the recording time (in months) and status (not doubled/doubled) were documented. A COX multivariate regression analysis was performed, considering variables such as gender, mutation type, and mutation site. The analysis revealed that gender is an independent risk factor influencing the progression of 24-h UP (HR=0.22, 95%CI:0.08-0.63, *P*=0.005), with males experiencing greater impact than females(Figure 6a). Additionally, mutation type emerged as an independent risk factor, with splice mutations (HR=9.84, 95%CI:1.86-51.96, *P*=0.007) and deletion mutations (HR=8.93, 95%CI:1.92-41.41.96, *P*=0.005) showing more rapid progression in 24-h UP compared to missense mutations(Figure 6b). Furthermore, within the mutation site group comparison, it was observed that mutations located in intron(IN) were associated with a longer duration for 24-h UP to double compared to those in EX1-20 (HR=0.11, 95%CI:0.01-0.99, *P*=0.04) (Figure 6c).

The follow-up endpoint was defined as a 25% reduction in eGFR. Both the duration (in months) and the occurrence of a 25% decrease were documented. A Cox multivariate regression analysis was performed, considering variables such as gender, mutation type, and mutation site. The analysis revealed that gender is an independent risk factor influencing the progression of renal function (HR=0.397, 95%CI:0.13-1.216, *P*=0.016), with male children exhibiting a higher likelihood of experiencing a 25% reduction in eGFR compared to female children(Figure 6d). Additionally, mutation type emerged as an independent risk factor , with deletion mutations (HR=13.703, 95%CI:3.333-56.331, *P*=0.001) leading to a more rapid decline in eGFR relative to missense mutations(Figure 6e). Furthermore, the mutation site was identified as an independent risk factor, with accelerated disease progression observed in children

with mutations in EX□40 compared to those in EX1-20 (HR=4.101, 95%CI:1.079-15.591,*P* = 0.038,Figure 6f).

Discussion

AS is the most prevalent hereditary glomerular disease in pediatric populations. Its characteristic clinical manifestations include hematuria, proteinuria, sensorineural hearing loss, ocular abnormalities, and progressive decline in renal function. AS typically presents insidiously, with early lesions being mild and often manifesting solely as microscopic hematuria, which may or may not be accompanied by proteinuria. Abnormalities in renal function generally emerge after school age or during adolescence. The initial severity of the disease and the rate of progression in patients with XLAS are influenced by factors such as the patient's sex, age, and mutation type [7]. Data compiled by Jais et al. [8–9] from multiple families indicate that disease severity is associated with sex; hemizygous male patients typically exhibit a more severe phenotype compared to heterozygous female patients, with a higher likelihood of developing ESKD or hearing loss, while the onset symptoms in heterozygous females tend to be relatively mild. The study encompassed a cohort of 68 children, comprising 43 males (63.2%) and 25 females (36.8%), yielding a male-to-female ratio of 1.72:1. The median age of onset was 2 years for male children and 3 years for female children, consistent with previous findings [13]. Statistical analysis revealed no significant difference in the age of onset between genders. All participants exhibited varying degrees of hematuria, with 23 cases (33.8%) presenting with isolated microscopic hematuria, 9 cases (13.2%) with isolated macroscopic hematuria, 36 cases (52.9%) with proteinuria, 14 cases (20.6%) with concurrent microscopic hematuria and proteinuria, and 22 cases (32.4%) with concurrent macroscopic hematuria and proteinuria. In a prior study by Gross et al. [12], microscopic hematuria was documented in all male subjects, and approximately 98% of female subjects exhibited hematuria; however, the study did not ascertain whether hematuria severity was gender-dependent. The present study determined that there was no significant difference in the severity of hematuria at onset between male and female children. Proteinuria levels serve as a significant indicator of the severity of AS and the progression of renal function deterioration. Presently, the primary objective in the management of AS is to decrease proteinuria levels. Numerous studies have demonstrated that reducing proteinuria can delay the progression of renal impairment [14–16]. In this study, out of 68 pediatric patients, 36 (52.9%) presented with proteinuria at disease onset. Among these, 22 cases (61.1%) exhibited mild proteinuria, which contrasts with previous reports indicating that 30%-40% of patients reach nephrotic syndrome levels [17]. The 24-h UP in male children at disease onset was 0.20 (0.11–0.52) g, compared to 0.11 (0.055–0.195) g in female children, indicating a statistically significant difference. Proteinuria was more severe in male children at disease onset. Nonetheless, the overall 24-h UP levels at disease onset in this cohort were not elevated, potentially due to the young age of the patients and their early stage of disease progression. In this study, only two cases (2.9%) of the pediatric participants exhibited renal function deterioration to CKD stages 2–5 at disease onset. One case involved a 3-year-old child with an eGFR of 69.0 ml/(min·1.73m^2), while the other involved a 14-year-old with an eGFR of 42.1 ml/(min·1.73m^2). The majority of the children maintained normal renal function at the onset of the disease. Furthermore, only

four cases demonstrated extrarenal manifestations at disease onset, all of whom were male. Among these, one male child presented with both fundus lesions and high-frequency hearing impairment, whereas the remaining three exhibited only high-frequency hearing impairment. The predominance of children without extrarenal manifestations at disease onset aligns with findings from previous studies.

Jais et al. [8, 9] reported that male patients with specific gene mutation types, including large segment deletions, nonsense mutations, or frameshift mutations, exhibited more severe clinical manifestations. The likelihood of progressing to ESKD before the age of 30 was 90%, with a 20-year kidney survival rate of 50%. Patients with splicing mutations demonstrated moderate to severe kidney involvement, with a 70% probability of developing ESKD before the age of 30 and a 25-year kidney survival rate of 50%. For those with missense mutations, the probability of developing ESKD before the age of 30 was 50%, and the 32-year kidney survival rate was 50%. Gong et al. [10] conducted an analysis of 141 patients with X-linked Alport syndrome (XLAS) from 88 Chinese families. Their findings revealed that among 24 Chinese families, 24 deletion mutations were identified, affecting 43 patients. The average age of symptom onset was 9.1 ± 6.0 years. All patients exhibited microscopic hematuria, 84.6% presented with proteinuria, and only 36.9% of the male patients had visible hematuria.In the remaining 64 Chinese families, 63 missense mutations were identified, affecting a total of 98 patients. The mean age of symptomatic individuals was 20.6 ± 13.8 years, which is significantly higher than that observed in cases with deletion mutations. Nearly all patients exhibited microscopic hematuria, while 81.1% presented with proteinuria, and only 22.6% experienced visible hematuria. Consistent with findings from international studies, male patients with X-linked Alport syndrome (XLAS) in China exhibit more severe phenotypes compared to female patients, particularly regarding proteinuria and renal function impairment. More than half of the male patients demonstrate impaired renal function, a proportion significantly higher than that observed in female patients. Patients with deletion mutations are more predisposed to progressing to ESKD. Approximately 50% of male patients with deletion mutations experience hearing loss, and about 25% present with ocular lesions. In contrast, approximately one-third of male patients with missense mutations suffer from hearing loss, and fewer than one-sixth exhibit ocular lesions.This study identified that male children with XLAS exhibiting frame-shift mutations demonstrated significantly elevated 24-h UP at disease onset compared to those with other mutation types. The type of mutation did not significantly influence the age of onset, severity of hematuria, or eGFR. Similarly, mutation type did not significantly affect the initial phenotype in female XLAS patients. In male children, proteinuria was more severe in those with frame-shift mutations, whereas it was milder in those with deletion mutations. This finding may be attributed to the limited number of deletion mutation cases (three cases) and the shorter disease onset duration observed in this study. No significant differences were observed in the clinical manifestations among female patients with different mutation types at onset. Furthermore, previous research comparing renal survival curves of 250 female patients with known renal conditions found no significant differences in the time to progression to end-stage kidney disease (ESKD) across mutation types [18].

Research on the progression of XLAS patients to ESKD consistently indicates distinct clinical phenotypes between genders. Male patients typically exhibit more severe clinical manifestations and are

more likely to progress to ESKD, with a reported risk of 100% [19]. In contrast, female patients with X-linked inheritance have a 25% risk of developing ESKD, often at a later age. Proteinuria is detectable in male patients during early childhood and may escalate to nephropathy. Furthermore, 90% of male patients reach ESKD before the age of 40, with a median onset age of 25 years. This study corroborates that gender significantly influences the progression of proteinuria and the decline in the eGFR, with male XLAS children experiencing a more rapid disease progression. In examining mutation types, the mutation type present in affected children serves as an independent risk factor influencing the progression of 24-h UP. Splice and deletion mutations are associated with a more rapid progression of 24-h UP compared to missense mutations. Although children with frameshift mutations exhibit higher levels of 24-h UP at disease onset than those with other mutation types, the progression rate does not significantly differ from that of missense mutations. This may be attributed to the inherently high level of proteinuria associated with frameshift mutations, necessitating a longer follow-up period to observe progression to doubling. Furthermore, mutation type independently affects renal function progression, with deletion mutations leading to a more accelerated decline in the eGFR compared to missense mutations. In a pioneering study, Jais et al. [20] demonstrated that the type of mutation in male patients with XLAS significantly influences renal prognosis. Specifically, male patients harboring mutations such as large segment deletions, nonsense mutations, or frameshift mutations exhibited more severe clinical manifestations, with a 90% likelihood of progressing to ESKD before the age of 30. This was followed by those with splicing mutations, who had a 70% probability of developing ESKD before 30. Conversely, patients with missense mutations experienced the mildest outcomes, with a 50% probability of ESKD onset before the age of 30. The study further integrated gender and mutation type into a Cox regression analysis, revealing that both 24-h UP and the progression of the eGFR were associated with mutation type. Although current studies have not established a definitive correlation for female children, this research identified that deletion mutations were associated with a more severe phenotype in both male and female children. However, the study's findings are limited by the small sample size, as only three children with deletion mutations were included, one of whom was a female with a large segment deletion mutation. Consequently, larger-scale, multi-center studies are necessary to validate these findings.

Currently, there is a limited body of research examining the correlation between mutation sites and clinical phenotypes, as well as prognosis. Existing literature suggests that patients with mutations located in EX1-20 exhibit a later median age of progression to the ESKD and have a more favorable prognosis compared to those with mutations in EX21-47 [12]. Our analysis reveals that male children with XLAS exhibit distinct clinical manifestations at disease onset depending on their mutation sites. Specifically, male XLAS children with mutations >EX47 demonstrate significantly higher levels of proteinuria and a greater number of urinary red blood cells at disease onset compared to those in the EX1-20 and EX21-47 groups. Additionally, the clinical manifestations at disease onset in male XLAS children with mutations > EX47 are more severe. This study identified that the eGFR at disease onset was significantly lower in the EX21-47 mutation site group compared to the >EX47 group; however, the eGFR in both groups remained within the normal range. Furthermore, the study determined that the mutation site serves as an independent risk factor influencing the progression of renal function.

Specifically, the eGFR in children with mutations >EX40 declined more rapidly than in those with mutations in EX1-20. Currently, there is a lack of mechanism-based research to elucidate the underlying cause of this phenomenon. It is hypothesized that this may be attributed to the presence of numerous Gly-X-Y repeat sequences in type IV collagen, with missense mutations being the most prevalent type in X-linked Alport syndrome (XLAS). These glycine substitutions may alter the triple helix folding structure of type IV collagen, which initiates from the NC1 domain and progresses towards the N-terminal in a zipper-like fashion. Mutations in EX1-20 may result in less severe disruption of this process. Comparable findings have been observed in osteogenesis imperfecta involving type I collagen folding [21].

In conclusion, this study identified a significant correlation between gender and genotype in XLAS and the clinical phenotype observed at disease onset. In males, frameshift mutations and mutations occurring beyond exon 40 are associated with more severe initial clinical presentations. Gender, mutation type, and mutation location were found to be independent risk factors influencing the prognosis of XLAS. Specifically, males with splice or deletion mutations exhibit rapid disease progression, whereas those with intronic mutations tend to have a comparatively better prognosis. Conversely, males with deletion mutations and mutations beyond exon 40 generally have a poor prognosis. These findings may aid in predicting the clinical manifestations and prognosis of children with XLAS across different genotypes.

Declarations

Funding source

The author(s) declared that financial support was received for this work and/or its publication. This study was supported by the National Natural Science Foundation of China (82170683, Wen- Yan Huang) and Shanghai Science and Technology Commission Natural Foundation Project (23JC1401202, Wen-Yan Huang).

Ethical appproval

The study was approved by the Research Ethics Committee of Shanghai Children’s Hospital (NO.2022R102-E02) and all methods were performed in accordance with the relevant guidelines and regulations (Declaration of Helsinki). Written informed consent was obtained from all participants or their guardians

Conflict of interest for all authors

No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

Author contributions

Yu-Jie Hu,Chang Yuan: formal analysis, methodology, and writing–original draft.

Dan Feng: data curation, investigation.

Bing-Xue Huang,Lei Sun: data curation, investigation, and methodology.

Sheng Hao, Wen-Yan Huang: conceptualization, funding acquisition, project administration, supervision, validation, and writing–review and editing

Data availability The research data associated with this paper is available.

Competing interests The authors declare any competing interests.

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Figures

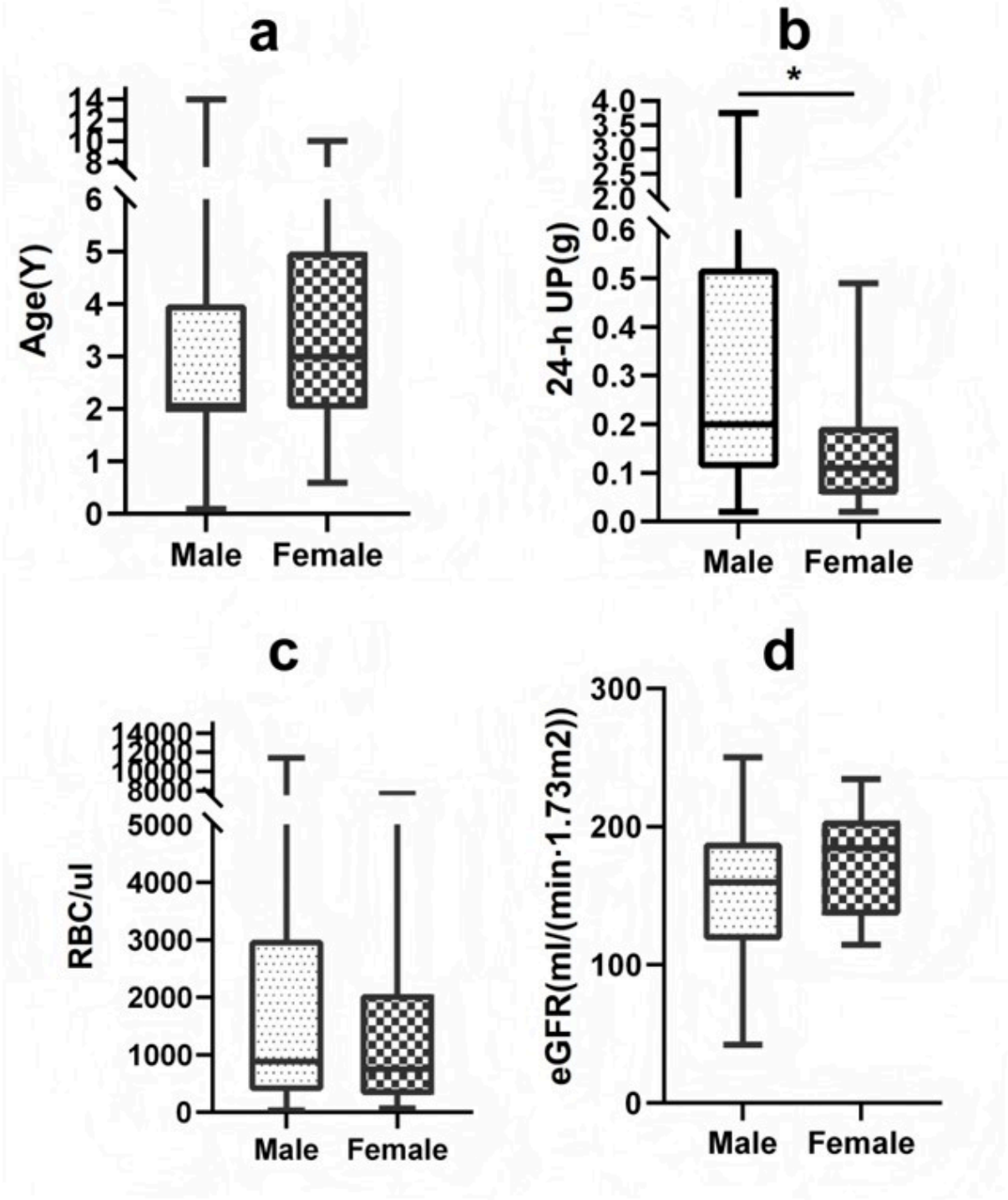


Figure 1

Clinical manifestations of XLAS in pediatric patients across different genders at disease onset. **a** Age of onset **b** 24-hour urine total protein **c** Red blood cells in urine sediment **d** The estimated glomerular filtration rate. The 24-h UP in male patients was significantly elevated compared to female patients(*P<0.05).No statistically significant difference was observed in the age,RBC inurine sediment and the eGFR of onset between genders.

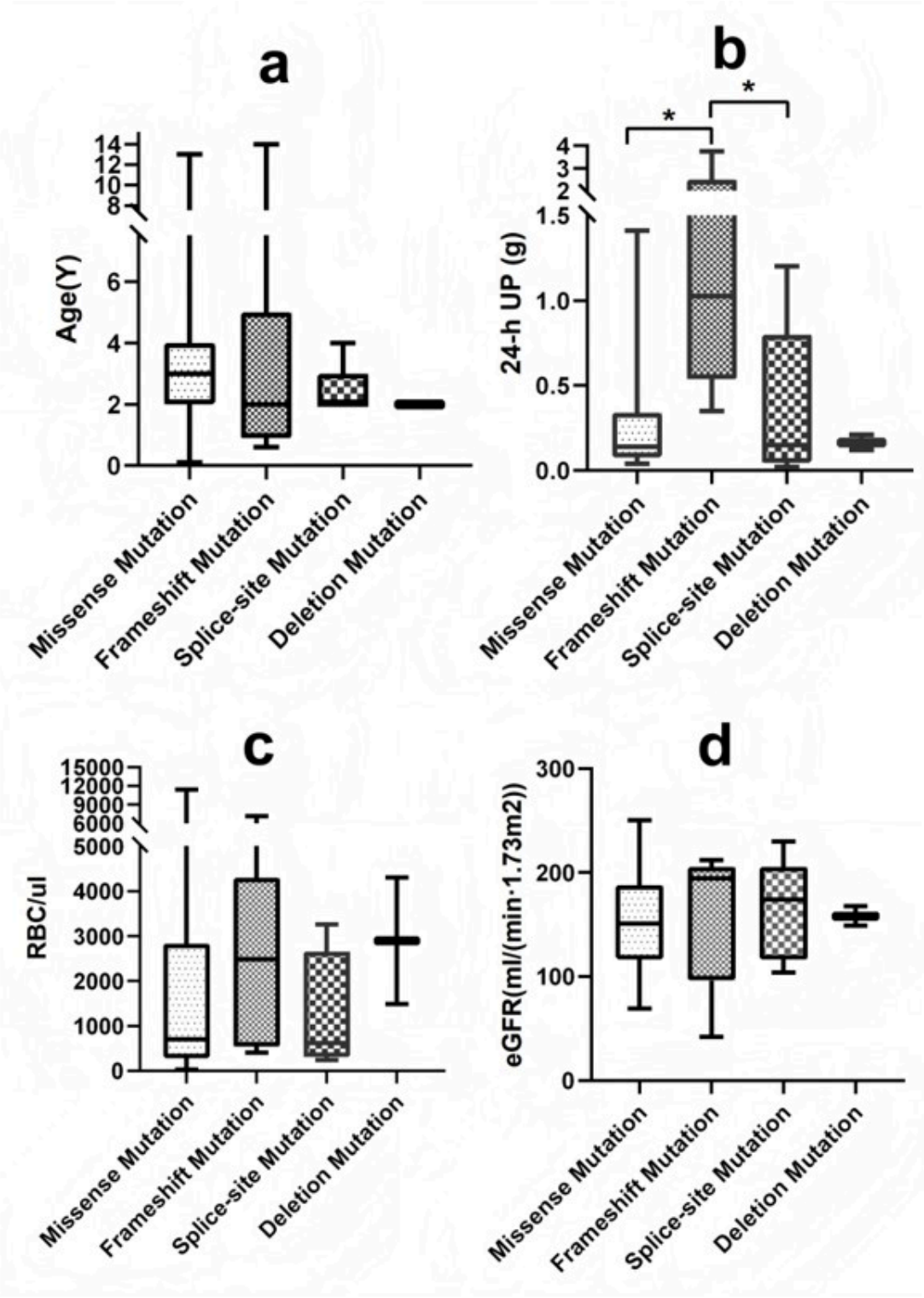


Figure 2

Clinical manifestations of XLAS in male pediatric patients across different mutation types at disease onset.**a** Age of onset **b** 24-hour urine total protein **c** Red blood cells in urine sediment **d** The estimated glomerular filtration rate. The 24-h UP in children with frameshift mutations at onset was significantly higher than those with missense and splicing mutations(*P<0.05).No statistically significant difference was observed in the age,RBC in urine sediment and the eGFR of onset between different mutation types.

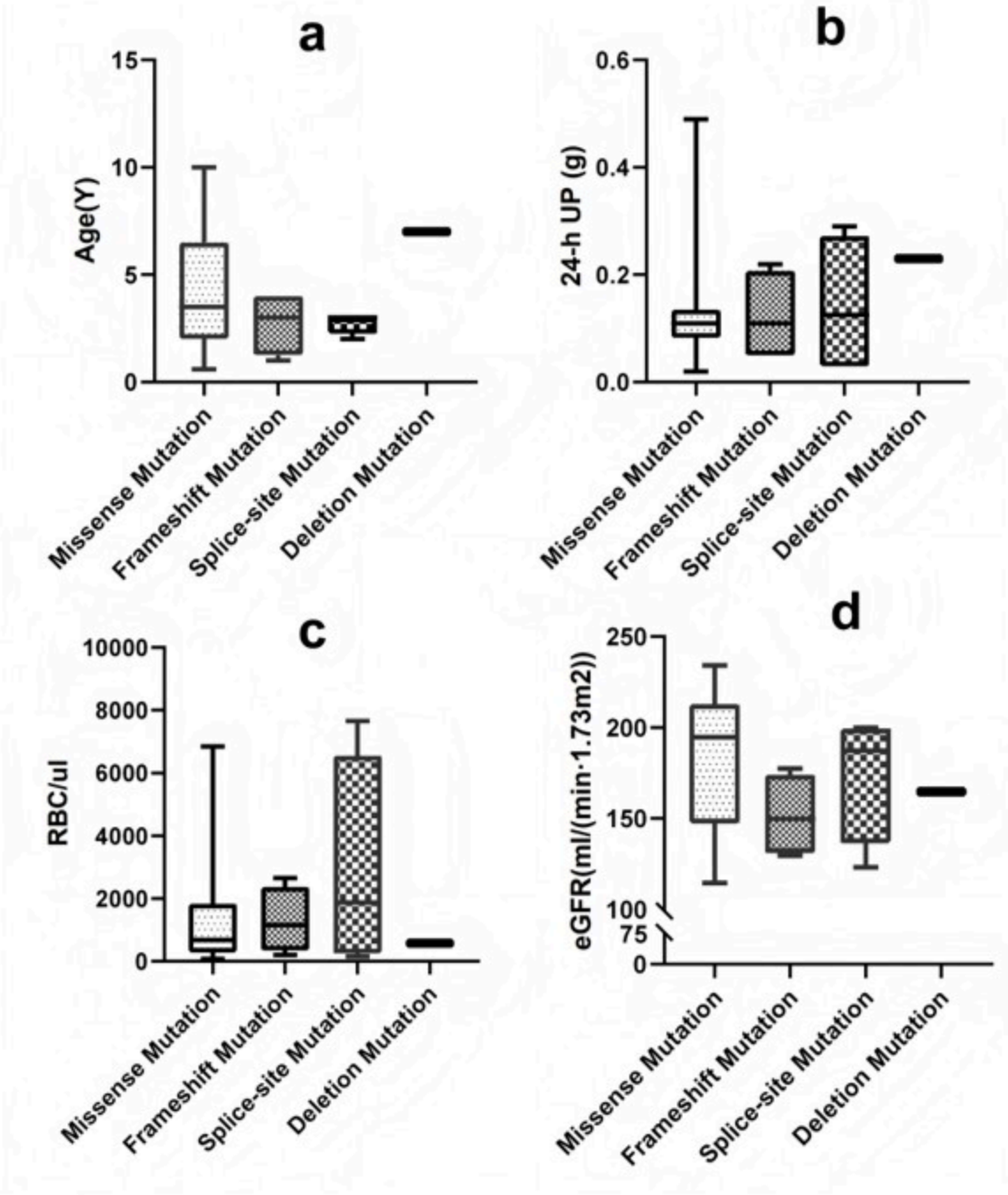


Figure 3

Clinical manifestations of XLAS in Female pediatric patients across different mutation types at disease onset.**a** Age of onset **b** 24-hour urine total protein **c** Red blood cells in urine sediment **d** The estimated glomerular filtration rate.The mutation types do not influence the initial clinical manifestations observed in female pediatric patients with XLAS.

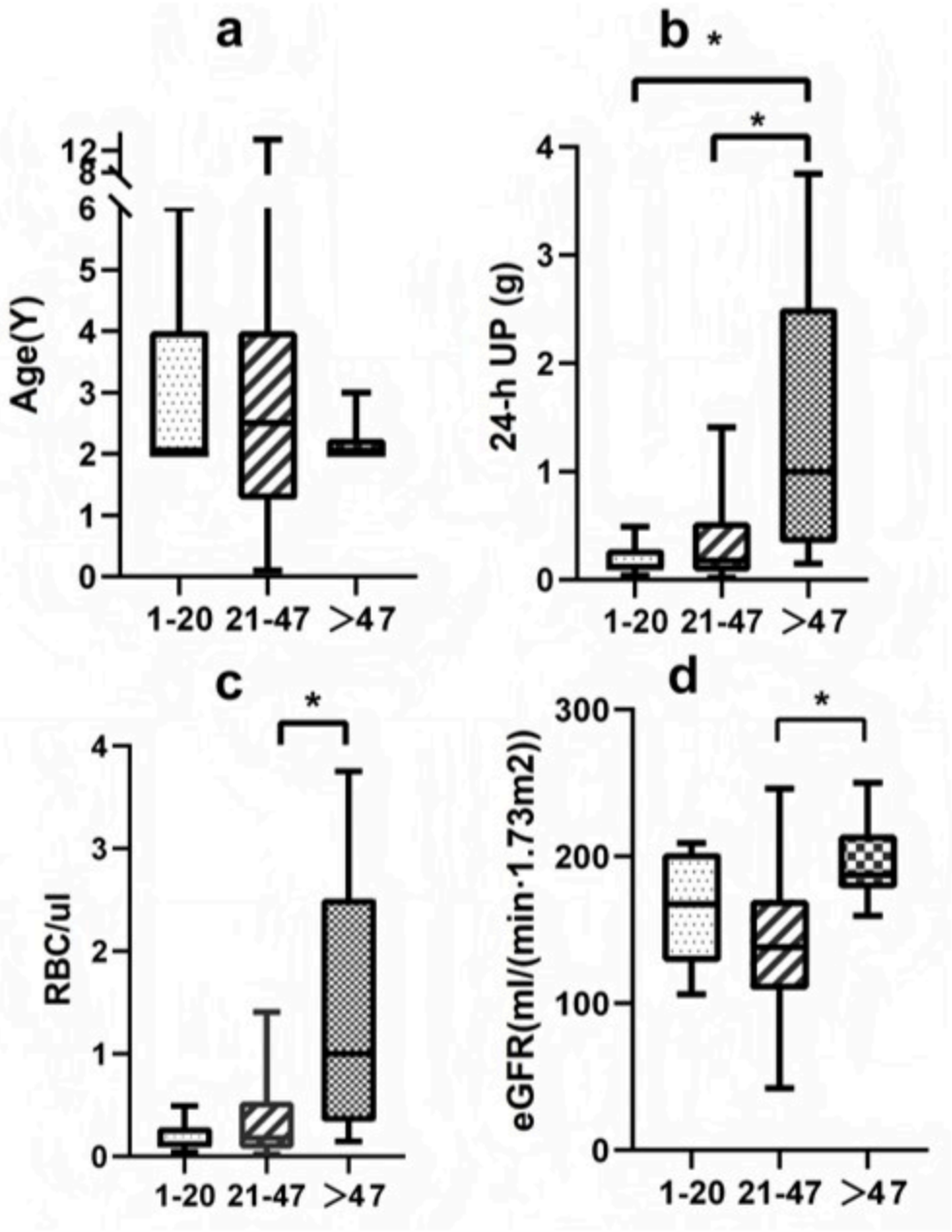


Figure 4

Clinical manifestations of XLAS in male pediatric patients across different mutation sites at disease onset.**a** Age of onset **b** 24-hour urine total protein **c** Red blood cells in urine sediment **d** The estimated glomerular filtration rate. Male XLAS patients harboring mutations in >EX47 exhibiting significantly higher proteinuria levels compared to those with mutations in EX1-20 and EX21-47(*P 0.05).The >EX47 group exhibited a significantly higher number of red blood cells and eGFR compared to the EX21-47 group(*P 0.05).

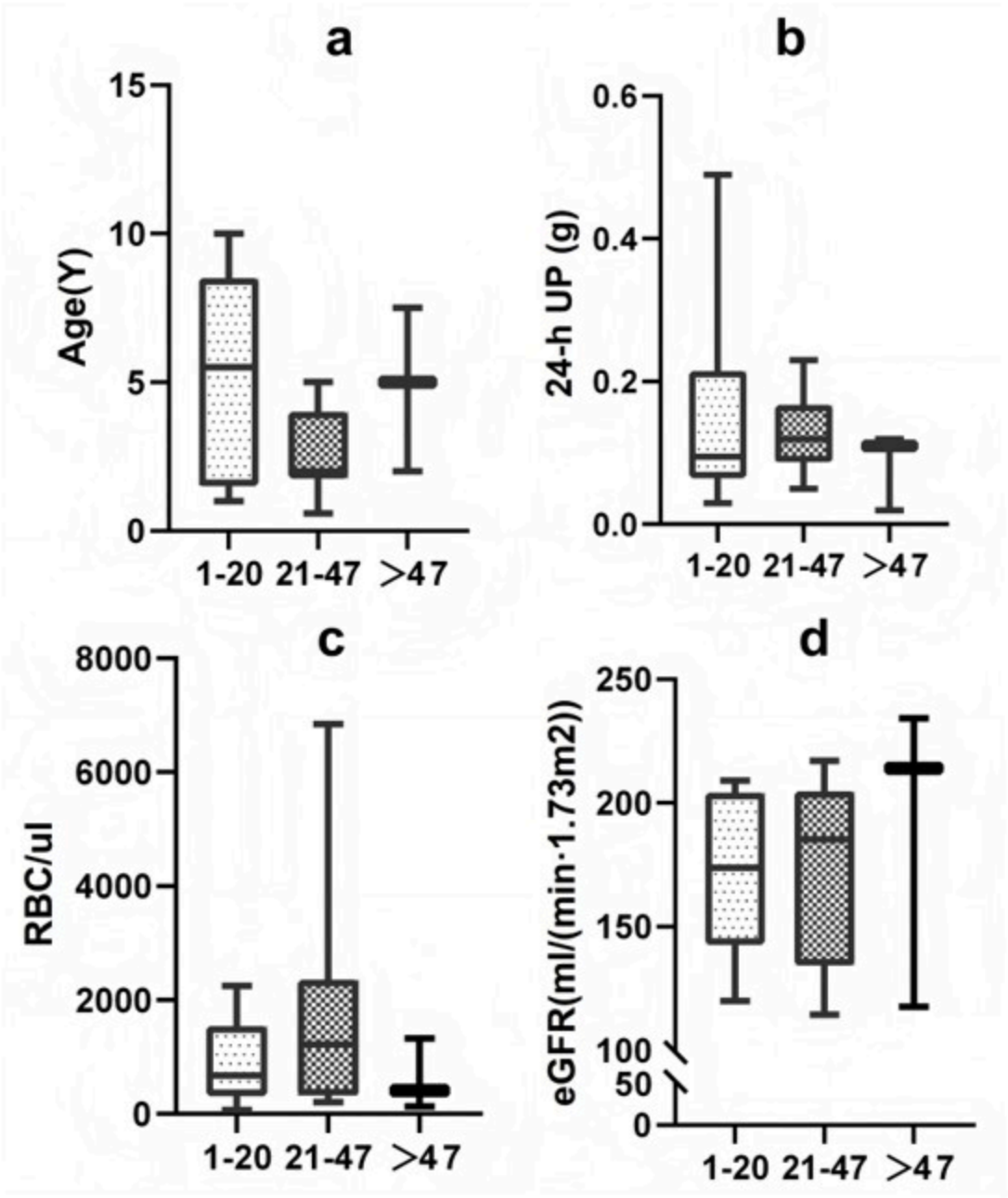


Figure 5

Clinical manifestations of XLAS in Female pediatric patients across different mutation sites at disease onset.**a** Age of onset **b** 24-hour urine total protein **c** Red blood cells in urine sediment **d** The estimated glomerular filtration rate.The mutation sites do not influence the initial clinical manifestations observed in female pediatric patients with XLAS.

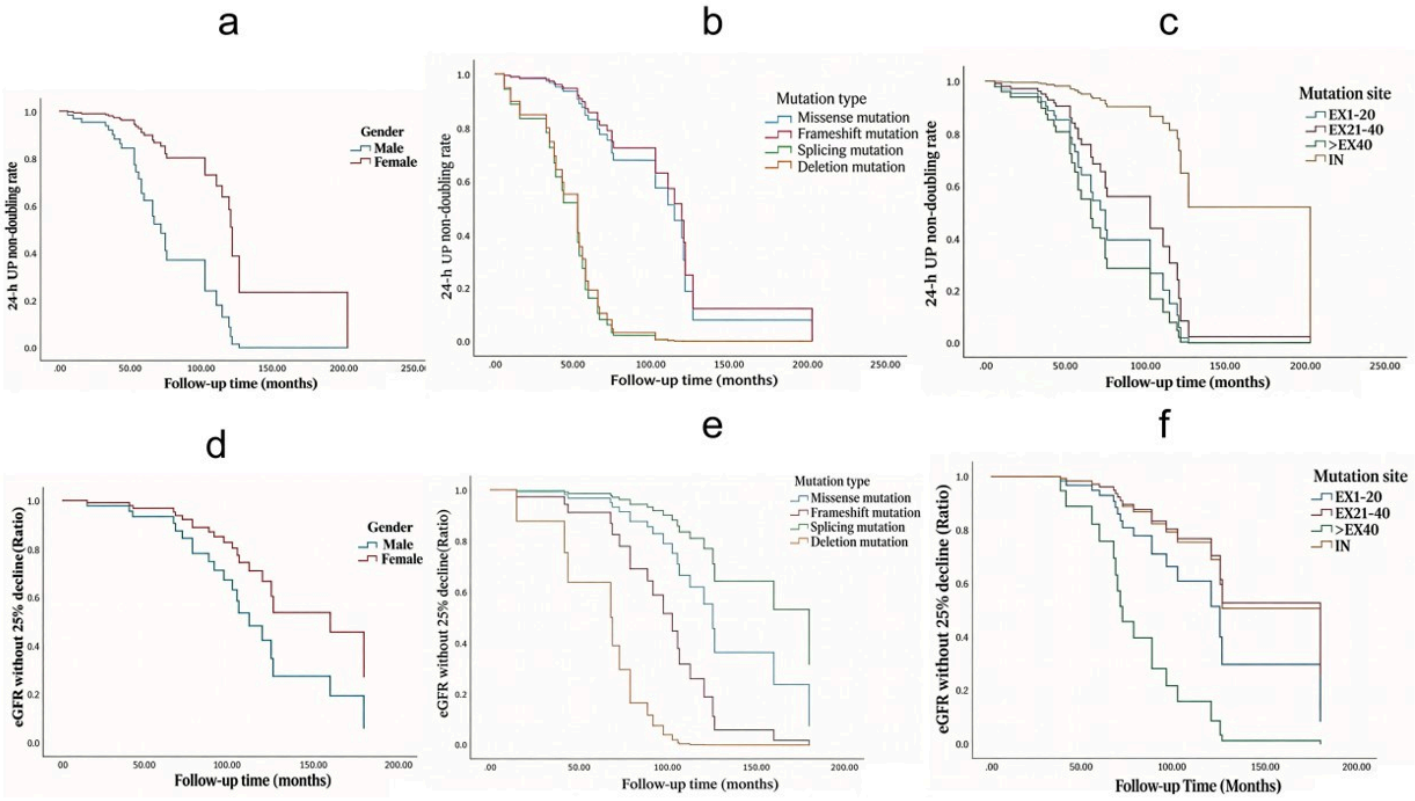


Figure 6

Kaplan-Meier analysis of disease progression by gender, mutation type, and mutation site. **a** Cumulative incidence of 24-h UP doubling by gender. Females had a significantly lower risk than males (HR = 0.22, 95% CI: 0.08–0.63, P = 0.005).

b Cumulative incidence of 24-h UP doubling by mutation type. Compared with missense mutations, splice mutations (HR = 9.84, 95% CI: 1.86–51.96, P = 0.007) and deletion mutations (HR = 8.93, 95% CI: 1.92–41.96, P = 0.005) were associated with higher risk. **c** Cumulative incidence of 24-h UP by mutation site. Intronic mutations conferred lower risk versus exons 1–20 (HR = 0.11, 95% CI: 0.01–0.99, P = 0.04).**d** Cumulative incidence of 25% eGFR decline by gender. Females had a lower risk than males (HR = 0.40, 95% CI: 0.13–1.22, P = 0.016). **e** Cumulative incidence of 25% eGFR decline by mutation type. Deletion mutations were associated with significantly higher risk compared to missense mutations (HR = 13.70, 95% CI: 3.33–56.33, P = 0.001). **f** Cumulative incidence of 25% eGFR decline by mutation site. Mutations beyond exon 40 (> EX40) had higher risk versus exons 1-20 (HR = 4.10, 95% CI: 1.08–15.59, P = 0.038).