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# Genetic, Clinical, and Pathologic Backgrounds of Children With X-Linked Alport Syndrome in China: A Monocenter Study

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**Abstract**

*Background*. Characteristics of X-linked Alport syndrome (XLAS) in a cohort of Chinese children. *Methods*. This work is a retrospective study covering the clinical information, pathological data, and gene sequencing results of 32 cases with XLAS from 2011 to 2022. *Results*. Among these 32 patients, the youngest age of onset was 3 months. Renal biopsy was performed on 29 children. The lamellated glomerular basement membrane was observed in 19 children using electron microscopy (65.5%). Of the 26 samples tested, 73.1% were found to be negative for collagen-a5 under immunohistochemical staining, showing clinical significance. Next-generation sequencing (NGS) detected 27 pathogenic gene mutations. A total of 15.4% of patients carried de novo mutations. *Conclusions*. The boys with XLAS showed more typical pathological performance than the girls. Patients with severe mutation were more likely to have proteinuria and hearing impairment. Renal pathology combined with NSG is an important means of diagnosis of AS.

**Keywords**

nephritis, hereditary, next-generation sequencing, diagnosis

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## Introduction

Alport syndrome (AS) is a hereditary disorder in which chronic kidney disease (CKD) progresses to end-stage renal disease (ESRD), sensorineural hearing loss, and ocular abnormalities.[1] Alport syndrome has 3 modes of inheritance: X-linked Alport syndrome (XLAS), autosomal recessive Alport syndrome (ARAS), and autosomal dominant Alport syndrome (ADAS).The more severe XLAS affects about one in 2000,[2] because of a mutation in the *COL4A5* gene, which encodes the type 4 collagen a5 chain.[3]

In this study, we provide the clinical information, pathological data, and gene sequencing results of 32 cases with childhood XLAS.

## *Materials and Methods*

*Diagnostic criteria for XLAS.* All cases were diagnosed in accordance with the following guideline[3–5]:

Alport syndrome is suspected when there is persistent glomerular hematuria. The likelihood is higher if the patient has a family history of Alport syndrome or renal failure with no other obvious cause and if any one of the characteristic clinical features (hearing loss, lenticonus, or retinopathy) is present or the glomerular basement membrane (GBM) lacks the collagen IV a3, a4, and a5 chains. The diagnosis can be confirmed if there is a lamellated GBM or a pathogenic mutation in the *COL4A5* gene.

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*Patients.* The inclusion criteria for our study participants are as follows: (1) clinical manifestations such as hematuria; (2) confirmation of XLAS diagnosis through pathological or genetic testing. Exclusion criteria encompass: (1) other hereditary kidney diseases not indicative of XLAS diagnosis; (2) non-cooperative children and their families.

We retrospectively reviewed data on all pediatric patients with XLAS identified from January 2011 to December 2022. When we found records for 2 or more patients from the same family, only the first child presented to the pediatric nephrology department was included in the study as a proband.

*Genetic analyses*

1. Specimen collection: The EDTA-treated specimens were collected with informed consent of the patients tested.
2. DNA extraction: The peripheral blood genomic DNA was extracted using the Blood Genome Column Medium Extraction Kit (Kangweishiji, China) according to the kit instructions. The extracted DNA samples were subjected to quality controlling using Qubit 2.0 fluorimeter and electrophoresis with 0.8% agarose gel for further protocol.
3. Whole exome library construction: Liquid hybridization of the genomic DNA was performed using Roche Nimble Gen Seq EZ Exome Enrichment Kit V2.0 and Seq EZ Exome Enrichment Kit V2.0 capture probes (Roche, USA), and the target DNA fragments were enriched to construct exome library covering 19 119 genes with whole exons and partial introns. Each enriched region shared 40 hypokalemiaMb of targeted sequences.
4. Sequencing: High-throughput sequencing was performed by Illumina NovaSeq 6000 series sequencer (PE150), and not less than 99% of target sequence were sequenced. The sequencing process was performed by the Zhiyin Oriental Translational Medicine Research Center.
5. bioinformatics analysis:
   a. Quality control: Raw data were cleaned after adapters removing, low-quality reads filtering and other quality control protocol.
   b. Variants calling: The clean data were aligned to the NCBI human reference genome (hg18) using BWA and variants were called using GATK. Samtools and Pindel were used to call SNPs (Single Nucleotide Polymorphisms) and indels, respectively. The clean data were than filtered, according to the quality of the sequencing, for further protocol.
   c. Variants annotation and prediction: Nonsynonymous substitutions and SNPs with MAF (Minor Allele Frequency) lower than 5% were filtered using SIFT. The function of mutated genes and their pathogenicity were then analyzed referencing to dbSNP, 1000 Genomes Project, ExAC, ESP, OMIM, Swiss-var, HGMD, ClinVar, and other disease databases.
   d. The variants with unknown pathogenicity of single bases were screened by Provean, SIFT, Polyphen2-HVAR, Polyphen2-HDIV, Mutationtster and other protein structure prediction software. MaxEntScan were used to screen potential splice sites.

*Statistical analyses.* Continuous Results were expressed asmeanand SD, and results compared using independent t test. Categorical results were expressed as frequents and percent and tested by x2 test or Fisher's exact test (DNA Stata). A *P* value of less than .05 was considered significant, and a *P* values less than 0.10, a trend.

## Results

*Clinical features.* A total of 32 children were included in the study, 24 boys and 8 girls. The age of onset ranged from 3 months to 12 years. Of the included children, 37.2%, saw disease onset when they were 3 years old or younger. A total of 24 patients had a family history of kidney disease. The parents of 2 children (lost to follow-up) declined to provide a family history. Six patients had no family history of kidney disease, 12 patients had microscopic hematuria, and 20 patients had hematuria and proteinuria. Only 1 patient (the patient No.22), a 5years and 9 months old boy, had an eGFR of less than 90 ml/min per 1.73 $m^2$, and the eGFR of the other patients was normal. Of the 32 children, 14 children (12 boys and 2 girls) were able to cooperate for the examination of ocular lesions, which was performed by an ophthalmologist, and underwent pure tone audiometry tests, while the remaining 18 children were too young for examination. None of these 14 children had ocular lesions, but 6 of the 12 boys had hearing impairment.

*Pathological findings.* Renal biopsy was performed on 29 patients. Samples were examined using light microscopy, and 21 biopsies showed mesangial proliferation, 5 showed minor glomerular abnormalities, and 3 showed focal segmental glomerulosclerosis (FSGS). Due to the lack of testing reagents, we were able to perform immunohistochemical staining for collagen-a1/a5 on only 26 renal samples. The glomerular distribution of the collagen-a1 chains served as an internal control. Glomerular distribution of collagen chains was defined as diffuse,

segmental, or absent. Normal glomerular distribution of the collagen-a1 chains was observed in all cases. A total of 73.1% of samples tested using immunohistochemical staining were negative for collagen-a5 (19/26, 16 boys vs 3 girls). Lamellated GBM (the gold standard for diagnosing AS using EM) was detected in 19 patients (65.5%, 17 boys *vs* 2 girls), and the other 10 patients (5 boys vs 5 girls) showed isolated areas of thinning glomerular basement membrane. Boys were more typical in pathological performance than girls.

*Mutation detection.* NGS detected 27 pathogenic gene mutations in 26 patients: 20 patients inherited the gene from their mothers, 2 patients inherited it from their fathers, and 4 patients (15.4%) carried *de novo* mutations. The Table 1 shows the clinical phenotypes and detected mutations.

These patients include a 9-year-old boy (the patient No.20). He presented with edema, gross hematuria, and nephrotic range proteinuria combined with hypocomplementemia. His family history showed his mother had microscopic hematuria with normal renal function. He underwent a kidney puncture and NGS test. The final diagnosis was X-linked Alport syndrome combined with C3 glomerulonephritis. The relevant articles have been published in *Pediatric Nephrology.*[6,7]

We divided the patients into 2 groups according to the type of gene mutation. Group A had 16 patients (missense mutations). Group B had 12 patients, all of whose genes had severe mutations (large deletions, nonsense changes, mutations at the donor or acceptor site, frameshift mutations).

Group B patients were more likely to have proteinuria and hearing impairment, and they were more likely to have no detectable collagen-a5 in their glomerular basement membrane. The differences between the 2 groups were statistically significant (The data are shown in Table 2).

## Discussion

AS is a form of familial nephritis caused by defects in type IV collagen, which is composed of a heterotrimer of α chains 3, 4, and 5. Type IV collagen makes up most of the network of basement membranes in the glomerular basement membrane, cochlea, cornea, lens capsule, and retina, which explains much of the phenotype.[8–10]

AS may present clinical symptoms in infancy. In our data, children under 3 years old were often misdiagnosed with urinary tract infections. Doctors in primary hospitals did not screen patients by family history, which suggests that the primary doctors in China lack knowledge of AS, consistent with Ding Jie's report.[11] We only diagnosed 4 cases of XLAS before 2016. With the development of renal biopsy and genetic testing, the number of XLAS diagnosis is increasing annually.

None of our ophthalmic examinations had any positive results. The specific ocular manifestations include anterior lenticonus, posterior subcapsular cataract, posterior polymorphous dystrophy, and retinal flecks.[12,13] These abnormalities occur in up to 70% of all patients with AS.[13] Due to the progressive nature of the disease, the ocular findings of AS are more prevalent in adult patients.[14] However, ophthalmologists' inadequate skill may have kept the detection rate of ocular lesions artificially low.[15] Seda Karaca Adıyeke found that the optical coherence tomography (OCT) examination provides valuable information suitable for identifying the structural changes associated with AS and evaluating relevant ocular findings.[16] We should pay attention to the OCT examination during follow-up. We found the rate at which immunohistochemical staining did not detect collagen-a5 and the lamellated GBM to be relevant. However, the earliest change in AS is diffuse thinning of the GBM. Women and children with XLAS may show thinning of the GBM as their only symptom.[17] In our data, 3 girls showed undetectable levels of a5 (COLIV) chain (3/7, 42.86%), and only 2 girls (2/7, 28.57%) had typical changes in GBM. Attempting to diagnose AS using pathological manifestations alone may lead to missed diagnosis, particularly in girls. If AS is suspected in female children, especially when the renal pathology is atypical, genetic testing should be performed for further confirmation. Here, 15.4% of patients were found to carry *de novo* mutations, which indicates that the renal pathology combined with NSG is an important means of diagnosing AS.

The kidney function of patient No. 22 has declined. His mother had microscopic hematuria, and his uncle passed away at the age of 20 due to end-stage renal disease. This patient's genetic variation site is c.2215(exon28) C>G, a mutation previously reported. According to ACMG guidelines,[4] this mutation is classified as a probable pathogenic variant. Notably, Patients No. 10 and No. 11 share the same genetic variation, indicating genetic heterogeneity in this cohort.

Some scholars have reported that kidney prognosis is significantly closely related to mutation type.[18–20] We found that patients still in childhood who had severe genetic mutations were more likely to have proteinuria and hearing impairment than children with missense mutations. The rate at which collagen-a5 was not detected in kidney tissues under immunofluorescence staining was higher than in children with missense mutations.

Bekheirnia also found deduced premature termination of the collagen-5(IV) chain to be associated with

**Table1.** Clinical Phenotypes and Analysis of Gene Mutations in This Study.

| Patient number | Sex | Age, years | Kidney disease (age, years) | Sensorineural hearing loss | GBM α1 | GBM α5 | Light microscope | Electron microscope | Transcripts | The position in chromosomal | Variants | Effect on protein | Mution | From | HGMD | Previously report |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Male | 6 years 5 months | HU and PU (1) | Positive | D (++) | A | MsPGN | S | NM_033380 | chrX:107838814 | c.1498 (exon22) delG | p.G500Vfs*57 | Frameshift | Mother | LP | _____ |
| 2 | Male | 1 year 7 months | HU (3M) | Negtive | D (++) | A | MsPGN | S | NM_033380 | chrX:107863584 | c.2605G>A (exon31) | p.G869R | Missense | De novo | LP | Yes |
| 3 | Male | 4 years 8 years | HU and PU (4) | Negtive | D (++) | A | MsPGN | TM | NM_033380 | chrX:107910395 | c.3586G-A (exon40) | p.G1196R | Missense | Mother | LP | Yes |
| 4 | Male | 3 years 2 months | HU (3) | None | D (++) | A | MsPGN | TM | NM_033380 | — | Exon 37-53 is missing (large deletion) | — | Large deletion | Mother | LP | Yes |
| 5 | Male | 4 years 11 months | HU (4) | Negtive | D (++) | A | MsPGN | S | NM_033380 | chrX:107827724 | c.1001G>A (exon18) | p.G334D | Missense | Mother | P | Yes |
| 6 | Male | 2 years | HU (1) | None | D (++) | A | MsPGN | S | NM_033380 | chrX:107898633 | c.3319G>A (exon37) | p.G1107R (NM_033380) | Missense | Mother | LP | Yes |
| 7 | Female | 3 years 1 month | HU (3) | Negtive | D (++) | D (++) | GML | S | NM_033380 | chrX:107844631 | c.1957G>T (exon26) | p.G653W (NM_033380) | Missense | Mother | LP | Yes |
| 8 | Male | 12 years 3 months | HU and PU (12) | Positive | D (++) | A | MsPGN | TM | NM_033380 | chrX:107840286 | c.1573 (exon23) delC | p.P525Pfs*32 (NM_033380) | Frameshift | Mother | LP | _____ |
| 9 | Female | 2 years 10 months | HU and PU (1) | Negtive | D (++) | D (++) | GML | S | NM_033380 | chrX:107821521 | c.688 (exon13) G>A | p.G230S (NM_033380) | Missense | Mother | P | Yes |
| 10 | Female | 4 years 1 month | HU and PU (2) | Negtive | D (++) | D (+) | GML | S | NM_033380 | chrX:107846262 | c.2215C>G (exon28) | p.P739A (NM_033380) | Missense | Father | LP | Yes |
| 11 | Male | 8 years | HU (8) | Negtive | D (++) | D (+) | MsPGN | TM | NM_033380 | chrX:107846262 | c.2215 (exon28 ) C>G | p.P739A (NM _000495) | Missense | Mother | LP | Yes |
| 12 | Male | 3 years 6 months | HU and PU (3) | Negtive | D (+) | A | MsPGN | TM | NM_033380 | chrX:107821560 | c.727 (exon13 C>T) | p.Q243X,1449 (NM _033380) | Nonsense(LOF) | Mother | P | _____ |
| 13 | Male | 2 years 1 month | HU and PU (2) | Negtive | D (++) | A | MsPGN | TM | NM_033380 | chrX:107863621 | c.2642 (exon31 ) G>A | p .G881E (NM _000495) | Missense | Mother | LP | Yes |
| 14 | Female | 6 years | HU and PU (5) | Negtive | D (++) | S (+) | MsPGN | S | NM_033380 | chrX:107908790 | c.3427 (exon38 ) G>T | p .G1143C (NM _033380 ) | Missense | De novo | P | _____ |
| 15 | Male | 2 years 5 months | HU and PU (2) | Negtive | D (++) | A | MsPGN | TM | NM_033380 | chrX:107846193-107939608 | Deletion (exon: 28–53) | — | Large deletion | De novo | P | _____ |
| 16 | Male | 3 years 11 months | HU and PU (4) | Negtive | None | None | MsPGN | TM | | | c.3958 (exon45) C>T | p.P1320S (p. Pro1320Ser) (NM_033380) | Missense | Mother | P | _____ |
| 17 | Male | 4 years 8 months | HU (2) | Negtive | None | None | MsPGN | TM | NM_033380 | chrX:107936155 | c.4688 (exon48 ) G>A | p .R1563Q (p .Arg1563Gl n) (NM _000495) | Missense | Mother | P | Yes |
| 18 | Male | 9 years 3 months | HU and PU (8) | Positive | None | None | MsPGN | TM | NM_033380 | chrX:107683437 | c.81 + 1 (IVS1) G>C | "-(NM_0004 95) | Splicing and Frameshift | Mother | P | Yes |
| 19 | Male | 1 year 2 months | HU and PU (1) | None | None | None | None | None | NM_033380 | chrX:107869487 | c.3154 (exon 36) C>T | p.Q1052X, 63 4 (p. Gln105 2Stop, 634) (NM_000495) | Nonsense | Mother | P | Yes |

*(continued)*

**Table 1. (continued)**

| Patient number | Sex | Age, years | Kidney disease (age, years) | Sensorineural hearing loss | GBM α1 | GBM α5 | Light microscope | Electron microscope | Transcripts | The position in chromosomal | Variants | Effect on protein | Mution | From | HGMD | Previously report |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | Male | 9 years 4 months | HU and PU (9) | Negtive | A | A | MsPGN | TM | NM_033380 | chrX:107844690– 107844694 | c.2014 (exon26)_c.2015 (exon26) in sGCAGA | p.G672Gfs*8 (p.Gly672G lyfs*8) (NM_000495) | Frameshift | Mother | P | _____ |
| 21 | Male | 4 years 2 months | HU and PU (2) | Negtive | D (++) | A | MsPGN | TM | NM_033380 | chrX:107829929 | c.1117 (exon19) C>T | p.R373X, 13 13 (p.Arg37 3Stop, 1313) (NM_000495) | Nonsense | Mother | P | Yes |
| 22 | Male | 5 years 9 months | HU (1) | Negtive | None | None | None | None | NM_033380 | chrX:107846262 | c.2215 (exon28) C>G | p.P739A (p.Pro739Ala) (NM_000495) | Missense | Mother | U | Yes |
| 23 | Male | 11 years 11 months | HU and PU (11) | None | None | None | MsPGN | S | NM_033380 | chrX:107842049 | c.1897 (exon 25) G>A | p.E633K (p.Glu633Lys) (NM_000495 ) | Missense | Mother | LP | Yes |
| 24 | Male | 4 years | HU and PU (3) | Negtive | D (++) | S (+) | MsPGN | TM | NM_033380 | chrX:107939582 | c.5032 (exo n51) T>C | p.C1678 (p.Cys1678A rg) (NM_00049 5) | Missense | De novo | P | Yes |
| 25 | Female | 7 years 8 months | HU and PU (7) | Negtive | None | None | None | None | NM_033380 | chrX:107834847 | c.1396 (exon 21) G>A | p.G466R (p. Gly466Arg) (NM_000495) | Missense | Father | LP | Yes |
| 26 | Female | 5 years 8 months | HU (5) | Negtive | D (++) | A | MsPGN | S | NM_033380 | chrX:107823817 | c.834 + 1 (IV S14) G>T | -(NM_033380 ) | Splicing | Mother | P | Yes |
| 27 | Male | 6 years | HU and PU (6) | Negtive | D (+++) | A | MsPGN | TM | NT | | | | | | | _____ |
| 28 | Male | 7 years | HU (6) | Negtive | D (+++) | A | GML | TM | NT | | | | | | | _____ |
| 29 | Male | 6 years 6 months | HU and PU (6) | Positive | D (++) | A | MsPGN | TM | NT | | | | | | | _____ |
| 30 | Female | 3 years 11 months | HU (3) | Negtive | D (++) | A | FSGS | TM | NT | | | | | | | _____ |
| 31 | Male | 10 years 9 months | HU and PU (10) | Positive | D(++) | A | MsPGN | TM | NT | | | | | | | _____ |
| 32 | Male | 11 years | HU and PU (11) | Positive | D (++) | A | MsPGN | TM | NT | | | | | | | _____ |

The variant was classed according to American College of Medical Genetics and Genomics guidelines (4): P- pathogenic, LP- likely pathogenic, U-uncertain significance. SL-loss of canonical splice site.
Abbreviations: HU, Hematuria; PU, Proteinuria; None, Undone; D, diffuse; S, segmental; A, absent;
S, Most segments of glomerular basement membrane are thin; TM, Typical appearance of electron microscopy includes uneven thickness of basement membrane, tearing and delamination; MsPGN, mesangial proliferative glomerulonephritis; GML, Glomerular Minor Lesion; NT, No genetic testing.

**Table 2.** Clinical and Pathological Manifestations in Groups A and B.

| Clinical Manifestation | Group | | *P* |
|---|---|---|---|
| | A (N = 16) | B (N = 10) | |
| Age of onset (months), mean ± SD | 57.44 (32.27) | 68.70 (42.72) | .4519 |
| Sex | | | .1241 |
| Male | 10 (62.50) | 9 (90.00) | |
| Female | 6 (37.50) | 1 (10.00) | |
| Mode of onset | | | .0697 |
| Hematuria | 7 (43.75) | 1 (10.00) | |
| Hematuria + proteinuria | 9 (56.25) | 9 (90.00) | |
| Family history | | | .3451 |
| Negative | 4 (25.00) | 1 (10.00) | |
| Positive | 12 (75.00) | 9 (90.00) | |
| Pure tone audiometry test | | | .0381 |
| NA | 10 (62.50) | 6 (60.00) | |
| Abnormal | 0 (0.00) | 3 (30.00) | |
| Normal | 6 (37.50) | 1 (10.00) | |
| UALB, median (IQR) | 49.05 (24.45–113.0) | 411.50 (140–1280) | .0468 |
| a5(COLIV) in GBM | | | .0074 |
| Diffuse | 7 (58.33) | 0 (0.00) | |
| Absent | 5 (41.67) | 8 (100.00) | |
| GBM changes by EM | | | .0992 |
| Isolated thinner GBM | 8 (57.14) | 2 (22.22) | |
| Lamellated GBM | 6 (42.86) | 7 (77.78) | |

large and small deletions, and truncation and splice mutations cause the most severe clinical phenotype. Missense mutations result in a less severe phenotype.[21] Our data showed only one child with decreased renal function at the time of the study, but patients with severe genetic mutations were more likely to have proteinuria, and proteinuria is a high-risk factor for deterioration of renal function,[22,23] so long-term follow-up is needed.

Jais found the risk of developing hearing loss before 30 years of age was approximately 60% in patients with missense mutations, as opposed to 90% for the other types of mutations. The probability of developing hearing loss before the age of 30 years in male patients was affected by the type of *COL4A5* mutation.[18] Xiao Zhang reported that hearing loss in China usually started at school age and gradually increased over time. Xiao Zhang also found missense mutations less likely to lead to hearing loss than other mutations (rearrangement, deletion, insertion, and point mutations).[24] These conclusions are consistent with our observations.

Our study has some limitations. It is a single-center retrospective study with a small number of cases. Sample size calculation was not conducted.There were no significant differences in the mode of onset mode between Group A and Group B, which may be related to the number of cases. Additionally, we did not perform follow-up.

## Conclusions

In summary, our findings indicate that during childhood, boys with XLAS exhibited more typical pathological features compared to girls. Additionally, patients with severe mutations were more prone to developing proteinuria and hearing impairment. Combining renal pathology with NSG proves to be a crucial diagnostic approach for AS.

### Author Contributions

Ding Juan-Juan designed the study, drafted the manuscript, and managed the project. Wang Jia interpreted the results and drafted the manuscript. Wang Si and Zhao Pei-Wei analyzed the genetic data. Wang Xiao-Wen contributed to writing–review and editing. Luan Jiang-Wei and Sun Jie contributed to supervision. Liu Li-Li and Ke Li-Qin analyzed the pathological results. All authors contributed to manuscript revision, read, and approved the submitted manuscript.

### Data Availability Statement

Data are available from the corresponding author on reasonable request.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Ethical Approval/Patient consent

The study protocol followed the Declaration of Helsinki.All procedures were reviewed and approved by the Ethics committee of Wuhan Children 's Hospital(No2020R098-E01). Written informed consent was taken from the parents of all enrolled participants.

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