A review of multi-dimensional diagnosis of Alport syndrome in 22 children in Northeast China

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Abstract

**Background** Alport syndrome (AS) is progressive hereditary nephritis due to different gene mutations. Affected individuals usually develop hematuria during childhood with gradual deterioration of renal functions. We adopted multi-dimensional methods to diagnose Alport syndrome in order to decrease the misdiagnosis.

**Methods** Twenty-two children were diagnosed and managed by the Department of Pediatric Nephrology of Jilin University First Hospital between January 2017 and January 2020 through multi-dimensional methods. Information collected included age of onset, age at diagnosis, clinical manifestations, family history (FH), renal pathology and their genotype.

**Results** All patients presented with hematuria with various degrees of proteinuria in some patients. While three children suffered from hearing loss, none of the children in the cohort had any visual problem or renal failure. Besides five patients estimated as Stage 2, the remain seventeen cases were at Stage 0. Renal biopsy were obtained in eighteen patients and fourteen of them showed glomerular basement membranes (GBM)-specific abnormalities. Thirteen children had mutations of the collagen IV genes.

**Conclusion** Combined with the importance of early diagnosis and economic factors, we adopted multi-dimensional methods to improve the diagnosis of Alport syndrome and estimate the risk of progression. We also reviewed the therapy progress.

**Background**

Alport syndrome (AS) is a hereditary type IV collagen disease, which always results in progressive renal fibrosis and end-stage renal disease (ESRD).\(^1\) AS arises from mutations in the COL4A3, COL4A4, and COL4A5 genes. X-linked AS (XL-AS), which is due to mutations in the gene encoding the α5 chain of COL4 (COL4A5) present on chromosome X, is the most common. The frequencies of XL-AS, autosomal recessive AS (ARAS), and autosomal dominant AS (ADAS) were previously estimated to be 80-85%, 15%, and 1-5%, respectively.\(^2\) Recent reports from literature suggests that around 60% of patients with AS belongs to the X-linked type, while ARAS accounts for about 15% and ADAS accounts for the rest 25%.\(^3-4\) However, considering many patients due to heterozygous mutations in
the **COL4A3** or **COL4A4** genes remain undiagnosed because of the subclinical course of the disease and incomplete penetrance, it is difficult to determine the accurate prevalence.\[^5\]

Besides hematuria and progressive renal failure, affected patients also frequently suffer extra-renal illnesses that involve ears (sensorineural deafness) and eyes (peri-macular flecks and lenticous).\[^6\]

With the advancement in medical technology, in addition to electron microscopy,\[^7\] other modes of investigations such as collagen IV analysis and genetic testing\[^8\] have broadened the clinical and research repertoire that we can use to detect the changes in AS. The diagnostic criteria includes family history (FH) of hematuria, sensorineural hearing loss, characteristic eye signs, diffuse esophageal leiomyomatosis, ultrastructural changes and abnormal distributions of the α(IV) collagen chains by immunohistochemical staining of GBM, and genetic mutations of **COL4A3**, **COL4A4** or **COL4A5**.\[^9\]

However, most of the time, not every patient can afford all of the examinations and meet all of the criteria, which made many patients be underdiagnosed. Meanwhile, clinicians may not be aware of this disease, either because of an incomplete evaluation or an atypical presentation. That urged us to define the working hypothesis using new methods to improve our diagnosis. In this study, we adopted multi-dimensional methods to diagnose AS, especially to cover those with atypical manifestations. In addition, we estimated the risk of progressions and reviewed the therapy progress.

**Methods**

### 2.1 Diagnostic criteria

In order to unify standardized diagnosis, we adopted the criteria established by the Working Group for Alport Syndrome in the Japanese Society of Pediatric Nephrology (JSPN) in 2015 (Table1).\[^10\]

1. **Diagnostic criteria:** In addition to the primary feature, patients should satisfy one or more secondary features or satisfy two or more of the accessory features. 2. If patients only have the primary feature and a family member diagnosed with Alport syndrome, the case is set as a “suspected case”. 3. If patients have any one feature of type IV collagen (II-1 or II-2) among the secondary features, the case is set as “asymptomatic carriers”. 4. Features caused by other diseases should be excluded, for example, a family history of kidney failure due to diabetes.
2.2 Risk evaluation criteria

We adopted three criteria (Table 2-4)\(^6,11-12\) to estimate risk of renal progression, including clinical estimate and genotype-phenotype correlation in X-linked Alport syndrome (XL-AS).

2.3 Clinical investigation

Clinical data collected included age of onset, diagnosis age, duration from onset of symptoms to diagnosis, hematuria, proteinuria, estimated glomerular filtration rate (eGFR), extrarenal symptoms and family history. Renal pathohistological findings of light microscopy (LM) and electron microscopy (EM), immunofluorescence staining and immunohistochemical staining of type IV collagen were also collected for analysis. Gene data was obtained form some children when AS is highly suspected.

2.4 Statistical analysis

SPSS 20.0 statistical software was used to process the data. The non-normal distribution data were expressed as median (range) and the counting data were expressed as percentage (%).

Results

Twenty-two children were diagnosed as AS from January 2017 to January 2020 in Department of Pediatric Nephrology of Jilin University First Hospital. All of them met the diagnostic criteria including the primary feature and at least one of the secondary features (Table 5). The clinical characteristics, renal pathology characteristics and gene mutations were elaborated as follows.

3.1 Clinical characteristics

The diagnosis age of the 22 patients (12 boys and 10 girls) ranged from 34 to 170 months (median: 84 months). The interval time between onset and diagnosis ranged from 1 to 97 months (median: 5.5 months). All children (100%) had hematuria with dysmorphic red cells; eighteen of them (81.8%) had paroxysmal macroscopic hematuria during upper respiratory infection. Non-nephrotic range proteinuria was presented in 10 children (45.5%), in which all of their proteinuria level were less than 30 mg albumin per g creatinine or per day (Stage 0). Five other children (22.7%) had nephrotic range proteinuria (P2, P9, P15, P19 and P20) who were at Stage 2. At diagnosis, while the eGFR and vision of all children was within the normal range, 3 children (13.6%) were confirmed to have mild to moderate sensorineural hearing loss (P2, P9 and P19). Positive FH was identified in 16 patients (72.7%) .
depicts the full details of the clinical findings.

### 3.2 Renal pathology characteristics

Renal biopsies were performed in 18 children. Sixteen (88.9%) of the examined biopsies showed minor glomerular abnormalities (MGA) in LM. In additional, one case of focal segmental glomerulosclerosis (FSGS) and one MsPGN (mesangial proliferative glomerulonephritis) was showed respectively. Immunoflourescence staining were negative in 6 (33.3%) while the others presenting with non-specific deposition of immune complex. GBM-specific abnormalities were observed in 14 cases (77.8%) in EM. Two cases presented atypical, with extensive thinning of the GBM in P8 and irregular thinning of the GBM in P13, respectively. Type IV collagenα2 and α 5 chain expression were tested for in 11 children. All patients showed normal positive staining of GBM and tubular basement membranes for Type IV collagenα2 chain. Three (27.3%) patients showed Type IV collagen abnormal expression, in which P18 and P19 (males) showed negative staining of α5(IV) while P21 (female) showed discontinuous α5 chain, and the remaining 8 (72.7%) had intact staining for α 5 chain. Seeing in Table 5.

Twenty-two children were diagnosed as AS from January 2017 to January 2020 in Department of Pediatric Nephrology of Jilin University First Hospital. All of them met the diagnostic criteria including the primary feature and at least one of the secondary features (Table 5). The clinical characteristics, renal pathology characteristics and gene mutations were elaborated as follows.

### 3.3 Gene detection

Thirteen children and their parents were tested using high throughput-targeted next generation sequencing (NGS) technologies (performed by Beijing Zhiyin Oriental Transforming Medical Research Center Co., Ltd, Beijing Jinzhun Gene Science or Centre of Genetic Diagnosis of Jilin University First Hospital). The pathogenicity was predicted by online Polyphen2 and SIFT software.

Table 6 depicted the details of these mutations of the collagen IV. Ten of the mutations (76.9%) were inherited in X linked manner (8 from maternal and 1 from paternal sides, 1 indeterminacy). There are five boys with COL4A5 missense mutation, in which P2 got premature stop (Type S), three boys (P1, P9 and P14) got glycine-X-Y substitutions involving exons 21-47 (Type MS), and one boys (P15) got
glycine-XY substitutions involving exons 1-20 (Type M). There are five girls with COL4A5 mutation including: P4 got non-glycine-X-Y missense mutation (Type MS); P6 and P11 got glycine-X-Y substitutions involving exons 21-47 (Type MS); P10 got glycine-XY substitutions involving exons 1-20 (Type M); P12 got compound heterozygous mutations of COL4A5, in which one sequence variant led to premature stop (Type S). P3 was identified with compound heterozygous mutations of COL4A4, while P8 was identified with autosomal dominant mutation of COL4A3. P13 got both sequence variants of COL4A3 and COL4A5.

Discussion
Mutations in the collagen IV genes leading to AS is well documented. Novel mutations and genotype and phenotype correlation have been under study in China.\[^{13-14}\] However, as a developing country, there are still many economic less-developed regions in China, especially in Northeast China. Whole exome sequencing (WES) can not be accepted in all families due to economic reason, while renal biopsy not be accepted for conservative ideas. Combined with the above reasons, we put forward the hypothesis and adopted the Japanese diagnostic criteria\[^{10}\] for multi-dimensional diagnosis of this disease. We hope to reduce misdiagnosis and improper treatment, estimate the risk of the progressive renal disease, provide timely intervention, and minimize economic costs.

Through the diagnostic criteria above, 22 children were diagnosed as AS. In addition to the primary feature, patients should satisfy one or more secondary features or satisfy two or more of the accessory features. Patients (100%) in our cohort generally presented with persistent hematuria which is the primary feature in the criteria, partially with proteinuria. That is similar to published articles.\[^{15}\] As we have said, not all of the patients have done WES or renal biopsy. Only 5 patients (P3, P6, P10, P14, P15) did both WES and also showed the GBM-specific abnormalities. Six patients with positive FH, 4 patients (P1, P9, P11 and P12) who did not done the renal biopsy, 2 patients (P2, P4) who refused to do the renal histopathology in EM, were confirmed with COL4A5 variants by WES. P8 showed diffuse thinning of the GBM while P13 showed irregular thinning of the GBM. Although they were not typical in EM, they were confirmed by WES. Meanwhile, they also had positive FH. The remaining 9 patients were confirmed AS due to the typical GBM abnormalities. According to the
criteria, they satisfied the primary feature, at least one secondary feature, accompanied with or without one or more accessory features. Although some of them didn't have a renal biopsy and some didn't have a genetic test, the diagnosis was credible. In contrast to previous general diagnostic criteria, the Japanese criteria improved our diagnosis and covered the patients who are easy to be ignored or ambiguous.

Type IV collagen, which is a component of the GBM, is a triple helix composed of three α chains. The α3(IV), α4(IV) and α5(IV) chains are present in GBM, Bowman's capsule and the basement membranes of distal and collecting tubules. In our cohort, P18 and P19 (males) showed negative staining of α5(IV) while P21 (female) showed discontinuous α5 chain, which satisfied type IV collagen abnormal expression. For P19, besides presenting proteinuria of nephrotic range, he had hearing loss when he was diagnosed. Similarly, Samar et al.\(^{[16]}\) stated that negative staining for α5 chain correlates with worse prognosis and more severe ultrastructural alterations in males with Alport Syndrome. There were still 8 patients who had intact staining for α5 chain. The hypothesized cause might include the type and location of sequence variants. Hashimura et al.\(^{[17]}\) hypothesized that some missense and inframe mutations of XL-AS might affect the structure of this triple helix, but its rate of degradation is low. They also suggest that mutations located between exons 1 and 25 may lead to a less critical disruption of triple helix-forming process. That may explain the positive staining in male patients of XL-AS who had milder clinical manifestation. The mechanism in autosomal AS hasn't been studied clearly yet.

Though five of them have manifested with nephrotic-range proteinuria which made them be estimated as Stage 2 of AS, three of them have displayed hearing loss, fourteen cases presented with GBM-specific abnormalities in electron microscope, none of them showed renal failure. That may due to they were diagnosed within their first or second decades of lifetime. It gives us time and opportunity to estimate the risk of progression and provide appropriate treatment.

A total of ten \(\text{COL4A5}\) mutation, one compound heterozygous mutations in \(\text{COL4A4}\), one autosomal dominant (AD) mutation of \(\text{COL4A3}\), and a Digenic AS with mutation of \(\text{COL4A3}\) and \(\text{COL4A5}\) were
identified in this study. In XL-AS, hemizygous male patients have a 100% risk of progression to ESRD, although rate of progression and timing of extrarenal manifestations are related to \textit{COL4A5} genotype.\textsuperscript{11} Heterozygous female patients have a lifetime risk of progression to ESRD of approximately 25%. But that depends on many risk factors including a history of gross hematuria in childhood, sensorineural deafness, proteinuria, and extensive GBM thickening and lamellation.\textsuperscript{18} Few Gly substitutions are non-pathogenic. Substitutions of Gly with a charged residue, such as Arg, Glu or Asp, often result in early-onset renal failure and more extrarenal features.\textsuperscript{19} However, it is much more difficult to distinguish between pathogenic and benign variants for non-Gly substitutions.\textsuperscript{20} Since our patients with \textit{COL4A5} mutation were estimated from Type MS to Type S, with risk factors in majority, renal function should be monitored closely within the next decade.

Autosomal Alport syndrome associated with biallelic mutations (homozygous or compound heterozygous) in \textit{COL4A3} or \textit{COL4A4} exhibits a recessive inheritance pattern and is associated with a 100% risk of ESRD, with rate of progression and timing of extrarenal manifestations influenced by genotype.\textsuperscript{11} P3 in our study got the compound heterozygous mutations of \textit{COL4A4} with GBM-specific renal pathology. According to this, he is estimated with a 100% risk of ESRD. Patients with heterozygous mutations in \textit{COL4A3} or \textit{COL4A4} are considered affected if they exhibit hematuria or proteinuria and include patients who would have previously been diagnosed with thin basement membrane nephropathy (TBMN). In these individuals the risk of ESRD is up to 20% among those with risk factors for progression, which include proteinuria, sensorineural deafness, family history of progression to ESRD, and renal biopsy findings of focal segmental glomerulosclerosis, or GBM thickening and lamellation, or all of these. Recent systematic review states there is a striking difference in the percentage of patients reaching ESRD.\textsuperscript{21} Like in a large cohort, many patients were misdiagnosed since heterozygous \textit{COL4A3/COL4A4} mutations as a cause of TBMN associated with FSGS.\textsuperscript{22} These figures of risk of ESRD are not always solid which depend on different patients and diverse age range. P8 who has been diagnosed with TBMN was identified to have the \textit{COL4A3} dominant mutation. Since her mother presented isolated hematuria without ESRD, it is hopeful to look
forward to the benign progression. Also, the risk of digenic inheritance need further study.

Literature\cite{23-24} reports that \textit{COL4A3/A4} mutations in \textit{cis}, resembles an AD inheritance with a more severe phenotype, \textit{COL4A3/A4} mutations in \textit{trans}, mimicks an autosomal recessive inheritance with a less severe phenotype and \textit{COL4A5} combined with \textit{COL4A3} triggered a more severe phenotype. In our cohort, P13 was a bit different. He got glycine-XY substitutions involving exons 1-20 in \textit{COL4A5} and irregular thinning of the GBM, but he also got a \textit{COL4A3} mutation, which contributed him high risk of renal progression. We are actively following these children and closely monitoring their renal progression.

Not all of our patients were correctly diagnosed to have AS at presentation. The median of interval time between onset and diagnosis was 5.5months, ranging from 1 to 97 months. That means it took nearly half of a year to get diagnosed since onset. For those patients who were willing to accept necessary examminations, we just spent one month to identify the etiology. However, more patients may need much longer time, even 4 to 8 years. In that case, many patients accepted some improper treatments under a ambiguous diagnosis.

P2 was initially diagnosed to have glomerulonephritis at the age of 3 years at local clinic due to hematuria and nephrotic range of proteinuria. Subsequent renal biopsy, which was done at his 9-year-old, was compatible with minimal change disease (MCD). Despite further history did reveal his mother had persistent hematuria and proteinuria of unknown etiology and his grandfather died of uremia, his parents refused to undergo further investigation (Seeing in Fig.1A). The child was initially managed by using corticosteroids, followed by cyclophosphamid and mycophenolate mofetil, due to steroid resistance. After accepted the treatment of Tacrolimus, he obtained partial remission with urine protein being controlled below 1 gram per day. He was later confirmed to have XL-AS (a missense mutation of \textit{COL4A5} inherited from his mother) at the age of 11 by genetic testing (Seeing in Fig.1B). He is currently treated with tacrolimus and ACE inhibitors. According to the Japanese criteria, the boy should be set as a “suspected case” long before being diagnosed. Therefore, it is important to choose the right time for both renal biopsy or re-biopsy or genetic testing for those “suspected cases”.

Likewise, P9, P15, P19 and P20 presented with heavy proteinuria who would have been managed as
nephrotic syndrome if further examinations were not performed. Interestingly, P9 and P15 were finally treated with tacrolimus after genetic confirmation of AS, and their proteinuria reduced to below 1 gram per day, this phenomenon corroborates with the previous studies that showed therapeutic benefits of calcineurin inhibitors in AS patients.[25]

The presentation of AS can occasionally mimic other clinical entities. P4 of our cohort, who had a missense mutation of COL4A5 inherited from her mother (Seeing in Fig.2B), presented with macroscopic hematuria during infection, and she did not suffer from any hearing loss or visual problem. Her renal biopsy revealed minor glomerular abnormalities with mild IgA deposition that was compatible with IgA nephropathy. If not for the presence of family history (Seeing in Fig.2A) which makes her as “suspected case”, she would have been managed as IgA nephropathy and genetic testing would not be offered. Interestingly, a child with similar clinical presentations to our patient was misdiagnosed to have IgA nephropathy.[26] His renal biopsy did not show features of AS until he had his second renal biopsy 4 years later. Similarly, in another recent Chinese report,[27] the proband who presented with hematuria and proteinuria was initially diagnosed as IgAN by renal biopsy and Immunofluorescence detection. Because of the poor treatment outcome, he was identified with a novel mutation of COL4A5 under the gene detection. By the time he was diagnosed, he has been treated with prednisolone accompanied with mycophenolate mofetil and tacrolimus successively.

Different from the typical manifestations, P3 displayed as isolated hematuria with negative family history. He accidentally found microscopic hematuria during a health check. During the eight-month follow-up, the urine red blood cell count fluctuated from 10/HPF to 30/HPF. GBM-specific abnormalities were observed in renal biopsy. Gene detection revealed compound heterozygous mutaitons of COL4A4. The renal pathology, gene mutation and family pedigree of P3 are showed in Fig.3(A-G).

In addition, whether the characteristic changes in GBM can be present depend on multiple factors, such as the age of biopsy and different mutation. In our cohort, 14 of the 18 renal biopsies showed GBM-specific abnormalities, while two cases presented as extensive thinning of the GBM (P8) and irregular thinning of the GBM (P13) respectively. TBMN is a relatively common disease that has been
reported in 1% of the general population.[28] According to the newest classification,[11] TBMN is now considered to be a lesion description rather than a diagnosis, and it’s likely that some of our patients previously diagnosed to have TBMN were actually patients suffered from AS. P8 had extensive thinning of GBM and would have been diagnosed to have TBMN if genetic testing was not performed. Different from other cases, she got an autosomal dominant mutation of COL4A3. The renal pathology, gene mutation and the family pedigree are showed in Fig.4(A-F). P13 had irregular thinning of the GBM and digenic mutation of COL4A3 and COL4A5. We showed the detailed information in Fig.5(A-G).

There is no radical cure for the disease and attempts to use various stem cell therapies in animal models have been met with ambiguous success. It is reported that with the exception of cyclosporine, a calcineurin inhibitor, the use of which remains controversial due to its possible long term nephrotoxic effects,[25] Renin-Angiotensin-Aldosterone System (RAAS) inhibitors are efficient and well tolerated to retard chronic kidney disease (CKD) progression in AS.[29] Gross et al.[30] reported a double-blind, randomized, placebo-controlled, multicentre phase III trial in order to clarify the safety and efficacy of ramipril in pediatric patients with AS, in which they discussed about the efficacy of ramipril when they are presenting only with microhaematuria. So far, The Alport Syndrome Classification Working Group recommended to use ACEI when presenting hematuria and overt proteinuria.[11] In addition, future therapies including stem cells, chaperon therapy, collagen receptor blockade and anti-microRNA therapy will expand our perspective in protecting the kidneys of Alport patients from further damage.[31] Through different mechanism, therapies such as Bardoxolone, anti-miRNA-21, paricalcitol, lipid-lowering agents and epidermal growth factor receptor inhibition play role in decreasing renal fibrosis.[32] Meanwhile, Chaperone and Stem-cell based therapies are expected to be therapeutic at collagen chains and GBM level respectively. However, when kidney failure is inevitable, patients with Alport syndrome who undergo renal transplantation would have generally excellent outcomes.[33] Although genotype-phenotype correlation is prominent, severe mutations do not impact on patient and graft survival after transplantation.[34]

Conclusion
In conclusion, considering the importance of early diagnosis and economic factors, we adopted multi-dimensional methods to diagnose AS and estimate the risk of progression. In condition-limited settings, it is important to follow a pragmatic approach. The Japanese criteria do improve our diagnosis. RAAS inhibitors are testified to have safety and efficacy in delaying renal progression. Patients after renal transplantation and the graft survival rates are excellent. Future therapies are on the way to change the “inevitable” outcome of disease.

Abbreviations

AS: Alport syndrome; FH: family history; GBM: glomerular basement membrane; ESRD: end-stage renal disease; ARAS: autosomal recessive Alport syndrome; ADAS: autosomal dominant Alport syndrome; XL-AS: X-linked Alport syndrome; eGFR: estimated glomerular filtration rate; LM: light microscopy; EM: electron microscopy; MGA, minor glomerular abnormalities; FSGS, focal segmental glomerulosclerosis; MsPGN: mesangial proliferative glomerulonephritis; NGS, next generation sequencing; WES, whole exome sequencing; TBMN: thin basement membrane nephropathy; MCD: minimal change disease; RAAS: Renin-Angiotensin-Aldosterone System; CKD: chronic kidney disease.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Hospital of Jilin University. Permission was obtained from the hospital to access the mentioned data.

Consent for publication

Written informed consents were obtained from the parents of patients for publication of case history and clinical results. A copy of the written consent is available for review by the Editor of this journal.

Availability of data and materials

The datasets used analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

ZL collected the clinical and the gene information and wrote the manuscript. SBC supported the data collection, interpretation of the data. ZBG carried out genetic studies and evaluated the mutant using expression and functional studies. MQS was involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Features

I. Primary feature:
   I-1. Persistent hematuria

II. Secondary features:
   II-1. Mutations in type IV collagen genes
   II-2. Type IV collagen abnormal expression
   II-3. Glomerular basement membrane (GBM)-specific abnormalities

III. Accessory features
   III-1. Family history of kidney diseases
   III-2. Bilateral sensorineural deafness
   III-3. Ocular abnormalities
   III-4. Diffuse leiomyomatosis

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Tables

Table 1 Diagnostic features of Alport syndrome\textsuperscript{[10]}

| Features                                         |
|--------------------------------------------------|
| I. Primary feature:                              |
| I-1. Persistent hematuria                         |
| II. Secondary features:                          |
| II-1. Mutations in type IV collagen genes         |
| II-2. Type IV collagen abnormal expression        |
| II-3. Glomerular basement membrane (GBM)-specific abnormalities |
| III. Accessory features                           |
| III-1. Family history of kidney diseases          |
| III-2. Bilateral sensorineural deafness           |
| III-3. Ocular abnormalities                       |
| III-4. Diffuse leiomyomatosis                     |
Stage 0  microscopic hematuria (<30 mg albumin per g creatinine or per day)

Stage 1  microalbuminuria (30–300 mg albumin per g creatinine or per day)

Stage 2  gross proteinuria (>300 mg albumin per g creatinine or per day)

Stage 3  impaired renal function (GFR <60 ml/min/1.73m²)

Stage 4  end-stage renal disease

GFR: glomerular filtration rate

Table 3 New classification system for Alport syndrome and related disorders[11]
| Inheritance | Affected gene(s) | Genetic state | Rate of progression to ESRD and timing of extrarenal manifestations |
|-------------|-----------------|---------------|---------------------------------------------------------------|
| X-linked    | COL4A5          | Hemizygous    | Risk factors for progression: gross hematuria, SNHL, proteinuria, GBM thickening and lamellation, SNHL, or evidence of progression in patient or family, genetic modifiers |
|             |                 | (male subjects) |                                                               |
|             |                 | Heterozygous  | Inheritance pattern does not simulate any Mendelian transmission |
|             |                 | (female subjects) |                                                               |
| Autosomal   | COL4A3 or COL4A4| Recessive (homozygous or compound heterozygous) | Rate of progression to ESRD and timing of extrarenal manifestations |
|             |                 | Dominant | Hematuria includes patients previc proteinuria, FSGS, GBM thickening patient |
|             | COL4A3, COL4A4, | COL4A3 and COL4A4 mutations in trans | Clinical findings and pedigree |
| Digenic     | and COL4A5      | COL4A3 and COL4A4 mutations in cis | Clinical findings and pedigree |
|             |                 | Mutations in COL4A5 and in COL4A3 or COL4A4 | Inheritance pattern does not simulate any Mendelian transmission |

BFH, benign familial hematuria; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; SNHL, sensorineural hearing loss; TBMN, thin basement membrane nephropathy.

Table 4 Genotype-phenotype correlation in XL-AS[12]

| Genotype | Genotype |
|----------|----------|
| Type S (Severe) | large rearrangements, premature stop, frameshift, donor splice site mutations, and i domain, 15% de novo mutations |
| Type MS (Moderate-severe) | non-glycine-X-Y missense, glycine-X-Y involving exons 21-47, in-frame and acceptor splice mutations (5% de novo glycine-X-Y mutations) |
| Type M (Moderate) | glycine-XY mutations involving exons 1-20, 5% de novo mutations |

Table 5 Diagnostic features of 22 Patients
| Patient ID | Gender | Age at onset/diagnosis age (months) | Persistent Hematuria (I-1) | Macroscopic hematuria | Proteinuria Of nephrotic range | Stage | ESRD | Mutations in tvr collagen genes (II-1) |
|-----------|--------|------------------------------------|--------------------------|----------------------|-------------------------------|-------|------|-----------------------------------|
| P1        | M      | 38/45                              | +                        | +                    | -                             | 0     | -    | COL4A5                             |
| P2        | M      | 36/132                             | +                        | +                    | +                             | 2     | -    | COL4A5                             |
| P3        | M      | 108/120                            | +                        | -                    | -                             | 0     | -    | COL4A4/COL4                        |
| P4        | F      | 108/109                            | +                        | +                    | -                             | 0     | -    | COL4A5                             |
| P5        | M      | 96/97                              | +                        | +                    | -                             | 0     | -    | NA                                 |
| P6        | F      | 96/97                              | +                        | +                    | -                             | 0     | -    | COL4A5                             |
| P7        | F      | 60/61                              | +                        | +                    | -                             | 0     | -    | NA                                 |
| P8        | F      | 48/49                              | +                        | -                    | -                             | 0     | -    | COL4A3                             |
| P9        | M      | 48/84                              | +                        | +                    | +                             | 2     | -    | COL4A5                             |
| P10       | F      | 60/84                              | +                        | -                    | -                             | 0     | -    | COL4A5                             |
| P11       | F      | 24/60                              | +                        | -                    | -                             | 0     | -    | COL4A5                             |
| P12       | F      | 33/34                              | +                        | +                    | -                             | 0     | -    | COL4A5/COL4                        |
| P13       | M      | 60/61                              | +                        | +                    | -                             | 0     | -    | COL4A3/COL4                        |
| P14       | M      | 72/73                              | +                        | +                    | -                             | 0     | -    | COL4A5                             |
| P15       | M      | 143/170                            | +                        | +                    | +                             | 2     | -    | COL4A5                             |
| P16       | F      | 107/108                            | +                        | +                    | -                             | 0     | -    | NA                                 |
| P17       | F      | 24/73                              | +                        | +                    | -                             | 0     | -    | NA                                 |
| P18       | M      | 36/85                              | +                        | +                    | -                             | 0     | -    | NA                                 |
| P19       | M      | 24/121                             | +                        | +                    | +                             | 2     | -    | NA                                 |
| P20       | M      | 59/61                              | +                        | +                    | +                             | 2     | -    | NA                                 |
| P21       | F      | 59/81                              | +                        | +                    | -                             | 0     | -    | NA                                 |
| P22       | M      | 118/122                            | +                        | +                    | -                             | 0     | -    | NA                                 |
| Total     |        | 22/122                             |                          |                      |                               | 13    |      |                                   |

M, male; F, female; ESRD, end-stage renal disease; LM, light microscope; EM, electron microscope; MGA, minor glomerular abnormalities;

FSGS, focal segmental glomerulosclerosis; MsPGN, mesangial proliferative glomerulonephritis;
GBM, glomerula basement membrane; FH, family history; NA, not available; A, atypical.

Table 6. Mutation of gene in 13 children with AS

| ID | Gene  | Location of chr | Exon | Nucleotide Change | Amino acid Change | Conservation of the protein |
|----|-------|----------------|------|-------------------|-------------------|--------------------------|
| P1 | COL4A5| chrX:107858210  | exon30| c.[2465GA]       | p.(Gly822Glu)    | conserved               |
| P2 | COL4A5| chrX:107866028  | exon33| c.[2890GT]       | p.(Gly964X)      | conserved               |
| P3 | COL4A4| chr2:227875130  | exon46| c.[4421CT]       | p.(Thr1474Met)   | /                        |
|    | COL4A4| chr2:227896933-227896934 | exon39| c.[3636_3637del] | p.(Arg1212fs)   | /                        |
| P4 | COL4A5| chrX:107865935  | exon33| c.[2797CT]       | p.(Leu933Phe)    | conserved               |
| P6 | COL4A5| chrX:107867547  | exon34| c.[2999GT]       | p.(Gly1000Val)   | conserved               |
| P8 | COL4A3| chr2:228159760  | exon40| c.[3499GA]       | p.(Gly1167Arg)   | conserved               |
| P9 | COL4A5| chrX:107842091  | exon25| c.[1939GA]       | p.(Gly647Arg)    | conserved               |
| P10| COL4A5| chrX:107824232 | exon16| c.[911GA]       | p.(Gly304Glu)    | conserved               |
| P11| COL4A5| chrX:107842014  | exon25| c.[1862GA]      | p.(Gly621Ala)    | conserved               |
| P12| COL4A5| chrX:107829932  | exon19| c.[1120G>T]     | p.(Gly374X)      | conserved               |
|    | COL4A5| chrX:107829930  | exon19| c.[1118G>A]     | p.(Arg373Gln)    | conserved               |
| P13| COL4A3| chr2:228175529  | exon51| c.[4793T>G]     | p.(Leu1598Arg)   | /                        |
|    | COL4A5| chrX:107815050  | exon8 | c.[448G>C]      | p.(Gly150Arg)    | conserved               |
| P14| COL4A5| chrX:107909779  | exon39| c.[3508G>A]     | p.(Gly1170Ser)   | conserved               |
| P15| COL4A5| chrX:107815050  | exon8 | c.[448G>T]      | p.(Gly150Trp)    | conserved               |

Mo, mother; Fa, father; XL, X-linked; AR, autosomal recessive; AD, autosomal dominant; ACMG, The American College of Medical Genetics and Genomics; Het, heterozygous; hemi, hemizygote; VUS, uncertain significance
Figures
Reverse complementary chain

Proband

Father

Mother
Fig. 1A The family pedigree of P2; Fig. 1B The COL4A5 mutation inherited from his mother (c.2890G>T, p.G964X, exon33, chrX:107866028).
Figure 2

Fig.2A The family pedigree of P 4; Fig.2B The COL4A5 mutation inherited from her mother (c.2797C>T, p.L933F, exon33, chrX:107865935).
Fig.3A-G The renal pathology and gene mutation of P 3. Fig.3A LM: PAS; Fig.3B LM: PASM; Fig.3C-D EM; Fig.3E The COL4A4 mutation inherited from his father (c.3636_3637del, p.R1212fs, exon39, chr2:227896933-227896934, NM_000092). Fig.3F The COL4A4 mutation inherited from his mother (c.4421CT, p.T1474M, exon46, chr2:227875130, NM_000092). Fig.3G The family pedigree of P 3.
Fig. 4

Fig. 4A-F The renal pathology and gene mutation of P 8. Fig. 4A LM: PAS; Fig. 4B LM: PASM-MAS; Fig. 4C, D EM. Fig. 4E The COL4A3 mutation inherited from her mother (c.3499G>A, p.G1167R, exon 40, chr2:228159760, NM_000091). Fig. 4F The family pedigree of P8.
Fig. 5A-G The renal pathology and gene mutation of P 13. Fig. 5A LM: PAS; Fig. 5B LM: PASM; Fig. 5C-D EM; Fig. 5E The COL4A3 mutation inherited from his mother (c.4793T>G, p.L1598R,
exon51, chr2-228175529). Fig.5F The COL4A5 mutation inherited from his mother (c.448G>C, p.G150R, exon8, chrX:107815050). Fig.5G The family pedigree of P 13.