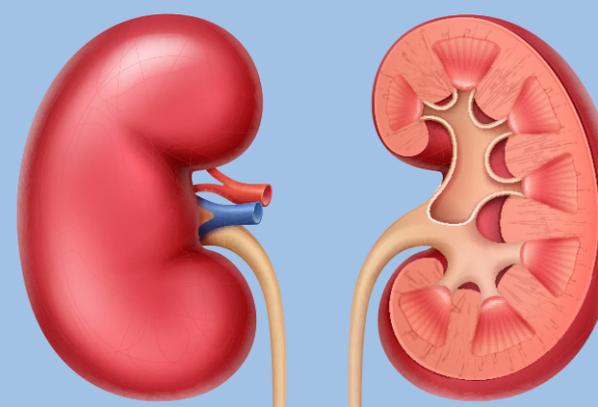


# Childhood Kidney Diseases

Vol. 26 No. 1, June 2022



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Childhood Kidney Diseases

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### Aims & Scope

*Childhood Kidney Diseases* (*Child Kidney Dis*; formerly Journal of the Korean Society of Pediatric Nephrology; ISSN 1226-5292, launched in 1997), the official journal of the Korean Society of Pediatric Nephrology, is a local peer-reviewed journal. It aims to improve kidney health in children and adolescent by covering clinical, and research works relevant to all aspects of pediatric nephrology. Its expected readers are clinicians and researchers around the world, although it has a particular focus on pediatric patients in Asia. Its publication types include reviews, original articles, case reports, editorials, and letters to the editor. The journal aims to serve pediatricians through the prompt publication of significant advances in pediatric nephrology and to rapidly disseminate recently updated knowledge to the public. Additionally, it will initiate dynamic, international, academic discussions concerning the major topics related to pediatric nephrology.

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# Management strategies for congenital isolated hydronephrosis and the natural course of the disease

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Congenital isolated hydronephrosis encompasses a spectrum of physiologic states that spontaneously resolve and pathologic obstruction that necessitates surgical intervention. Distinguishing patients whose condition will resolve, those who will require stringent follow-up, and those who will eventually need surgical intervention present a challenge to clinicians, particularly because no unified guidelines for assessment and follow-up have been established. The recognition of the natural course and prognosis of hydronephrosis and a comprehensive understanding of the currently proposed consensus guidelines may aid in multidisciplinary treatment and in providing proper counseling to caregivers. In this review, we aimed to summarize the literature on the grading systems and management strategies for congenital isolated hydronephrosis.

**Keywords:** Congenital; Hydronephrosis; Ureteral obstruction

## Introduction

Hydronephrosis refers to dilatation of the renal collecting system due to a build-up of urine resulting from drainage problems. Congenital hydronephrosis occurs in up to 1%–5% of all pregnancies [1,2]. More than half of the cases are transient and physiologic, whereas other cases are caused by disorders of the ureteropelvic junction (UPJ) including intrinsic stenosis (10%–30%), vesicoureteral reflux (VUR; 10%–30%), and congenital anomalies leading to secondary dilatation of the urinary tract [1,3,4]. To date, studies have shown that low-grade isolated hydronephrosis usually resolves during the first few years of life [5,6], whereas high-grade hydronephrosis requires interven-

tion to prevent the progression of obstruction or deterioration of renal function [7].

Distinguishing children who require follow-up or intervention, determining the possibility of resolution and the time to resolution, deciding about performing pyeloplasty to relieve the obstruction and determining the timing of the procedure, and preserving the patient's renal function are crucial issues for both clinicians and family members. To stratify the risk of early surgical intervention or the possibility of resolution, attempts have been made to create a unified grading system for urinary tract dilatation that can be used during the prenatal or postnatal period; however, no definitive consensus guidelines have been established to date [8].

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In this review, we aimed to summarize the literature (to date) on the proposed grading systems and management strategies for congenital isolated hydronephrosis, as well as to describe the natural history.

**Detection and classification–grading systems**

Currently, no standardized protocol exists for defining, classifying, and grading congenital hydronephrosis. Different terminologies with overlapping meanings are used to describe the status of dilatation (e.g., pelviectasia, pelviectasis, hydronephrosis, and urinary tract dilatation), and different clinicians from different subspecialties (e.g., pediatric urology, pediatric radiology, pediatric nephrology, and obstetrics) may use the terms to refer to different conditions [9,10]. Consequently, several grading systems have been developed, leading to the use of various nomenclatures and causing a misunderstanding between the radiologist and the clinician [11]. In this context, we will review the most widely used and the recently proposed grading systems aimed at providing a unified classification during the perinatal period, including their validation in the literature.

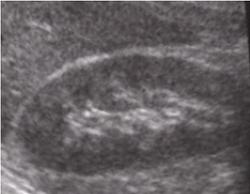
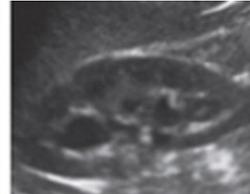
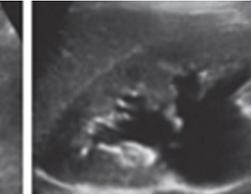
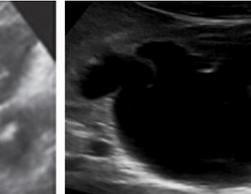
**Society for Fetal Urology classification**

In 1993, the Society for Fetal Urology (SFU) proposed a 5-point numeric grading system (0–IV) based on the postnatal appearance of the renal pelvis, calyces, and renal parenchyma on ultrasonography (USG) images (Fig. 1) [1,6,12]. The SFU classification remains the most widely used grading system owing to its intuitiveness and ease of use, especially in cases of isolated hydronephrosis. However, interobserver and intraobserver

variabilities exist owing to the nature of morphologic classification. Furthermore, since it was not originally developed for use in antenatal evaluation, it has not been widely adopted in subspecialties other than pediatric urology radiology (e.g., obstetrics and neonatology).

**Grading based on the anteroposterior pelvic diameter**

Anteroposterior pelvic diameter (APD) measurement, obtained from a transverse section of the renal hilum, is also widely used by itself or in conjunction with SFU grading. APD is an objective quantitative parameter widely used as a predictor of pathology and outcome, as well as of the resolution of the condition or the need for an intervention [1,8,13]. However, it also has limitations. Because APD measurement does not provide descriptive details of the renal parenchyma, calyces, ureter, and lower urinary tract, it does not accurately reflect the degree of hydronephrosis according to different renal pelvic configurations. Some studies argue that there is no threshold separating nonobstructive from obstructive dilatation of the kidney because renal dilatation is affected by many factors (e.g., hydration status, bladder filling, position, and respiration of the patient) giving its dynamic character [11,14]. Because of the advantages and disadvantages of both grading systems, SFU grading and APD measurement are commonly used together complementarily in clinical practice. As the authors have previously demonstrated, in cases showing a discrepancy between morphologic classification and APD measurement (i.e., higher grade in the SFU classification than that based on APD measurement), the resolution time should be predicted using the APD measurement rather than the SFU grade [15]. This is because normalization of morphology occurs before improvement in the APD measurements.

SFU grade 0	SFU grade I	SFU grade II	SFU grade III	SFU grade IV
Normal, no splitting	Urine in the pelvis barely splits the sinus	Urine fills the pelvis, major calyces dilated	Uniformly dilated minor calyces, parenchyma preserved	Parenchymal compromise with thinning
				

**Fig. 1.** Society for Fetal Urology (SFU) hydronephrosis grading system. The SFU grading system is based on the degree of renal-pelvic and calyceal dilatation seen on renal ultrasonography images and the integrity of the renal parenchyma [12].

**Urinary tract dilation classification system**

The urinary tract dilation (UTD) classification system was developed in 2014 as a collaborative effort among eight different medical and surgical societies (American College of Radiology, American Institute of Ultrasound in Medicine, American Society of Pediatric Nephrology, SFU, Society for Maternal-Fetal Medicine, Society for Pediatric Urology, Society for Pediatric Radiology, and Society of Radiologists in Ultrasound) in an attempt to establish a standardized and simplified description of hydronephrosis that can be consistently applied across specialties for prenatal and postnatal evaluation and management [8,16]. This classification includes parameters such as the APD of the renal pelvis (normal, <10 mm), presence of central and peripheral calyceal dilatation, renal parenchymal abnormalities, ureteral abnormalities, and bladder abnormalities in two antenatal (UTD-A1 and UTD-A2) and three postnatal (UTD-P1, UTD-P2, and UTD-P3) categories (Fig. 2). This system is intended to stratify the risk of postnatal uropathies and the clinical outcomes and to conduct a cost-effective evaluation in high-risk patients rather than being a mere descriptive grading system [8,17]. However, the system may also cause confusion because the classification suggests the general term “urinary tract dilatation” to indicate all types of ureteral and kidney dilatation, including UPJ obstruction (UPJO), ureterovesical junction-type

hydroureteronephrosis, VUR, bladder pathologies, and posterior urethral valve formation. Its additional limitations include interrater discrepancy in the assessment of calyceal dilatation, the wide range encompassed by the UTD-P3 grade, and the complexity and time-consuming nature of its application in real clinical practice [11]. Nonetheless, some studies have validated the usefulness of the UTD grading system in predicting the need for surgical intervention or predicting urinary tract infection by showing a relationship between UTD grade and clinical outcomes [17-24].

**Onen grading system**

The Onen system was developed in 2007 for the assessment of prenatal and postnatal hydronephrosis with UPJ pathology, with emphasis on the quality of the renal parenchyma, and was updated in 2016 [11,25]. It is based on nonsubjective parameters (presence of dilatation of the pelvicalyceal system and quality of the renal parenchyma based on exact criteria) (Fig. 3). Although the system is not widely used because of low recognition by practitioners, a few groups have recently reported its low subjectivity with a decreased interobserver agreement in Onen grades 2 to 3 [26,27].

Among the aforementioned grading systems, the SFU classification of congenital hydronephrosis seems to remain the

	<b>UTD-P1 (low risk)</b>	<b>UTD-P2 (intermediate risk)</b>	<b>UTD-P3 (high risk)</b>
APD	10 to <15 mm	≥15 mm	≥10 mm
	<b>OR</b>	<b>OR</b>	<b>OR</b>
Calyces	Central dilatation (pelviectasis, pelvicaliectasis)	Peripheral dilatation	Any dilatation
		<b>OR</b>	
Ureter		≥4 mm (with APD ≥10 mm or calyceal dilatation)	
			<b>AND</b>
Parenchymal abnormality, bladder abnormality or prenatal oligohydramnios			Yes

**Fig. 2.** Urinary tract dilation (UTD) classification system (postnatal categories). Although the UTD classification system has two antenatal categories (UTD-A1 and UTD-A2-3) and three postnatal categories (UTD-P1, UTD-P2, and UTD-P3) [8,16], only the postnatal categories (>48 hours) are presented here. APD, anteroposterior pelvic diameter. Adapted from Nguyen et al. [16] with permission from Springer.

Onen-1	Onen-2	Onen-3	Onen-4
Only renal pelvic dilatation (APD not important)	Pelvic+calyceal dilatation (APD not important, renal parenchyma normal)	Medulla short and thin, postnatal 3–7 mm (APD not important, corticomedullary differentiation normal)	Severe parenchymal loss with thin cortex, postnatal <3 mm no medulla (APD not important, no corticomedullary differentiation)
			

**Fig. 3.** Onen grading system. The diagram summarizes the Onen grading system, reflecting the 2016 update on grades 3 and 4 [26]. APD, anteroposterior pelvic diameter.

most widely used classification system by clinicians owing to familiarity and established practice patterns, followed by APD measurement and the UTD system [17,28,29].

### Risk-based management and follow-up plan after birth

As previously mentioned, no universal guideline exists on the frequency and timing of USG examination and the required duration of follow-up. In addition, heterogeneity in clinical management exists among pediatric radiologists, pediatric urologists, and maternal-fetal obstetricians, partly because of the lack of prospective studies and different practices across different centers [10]. This section will cover the postnatal management of congenital hydronephrosis according to the current literature in the context of clinical decision-making (Fig. 4).

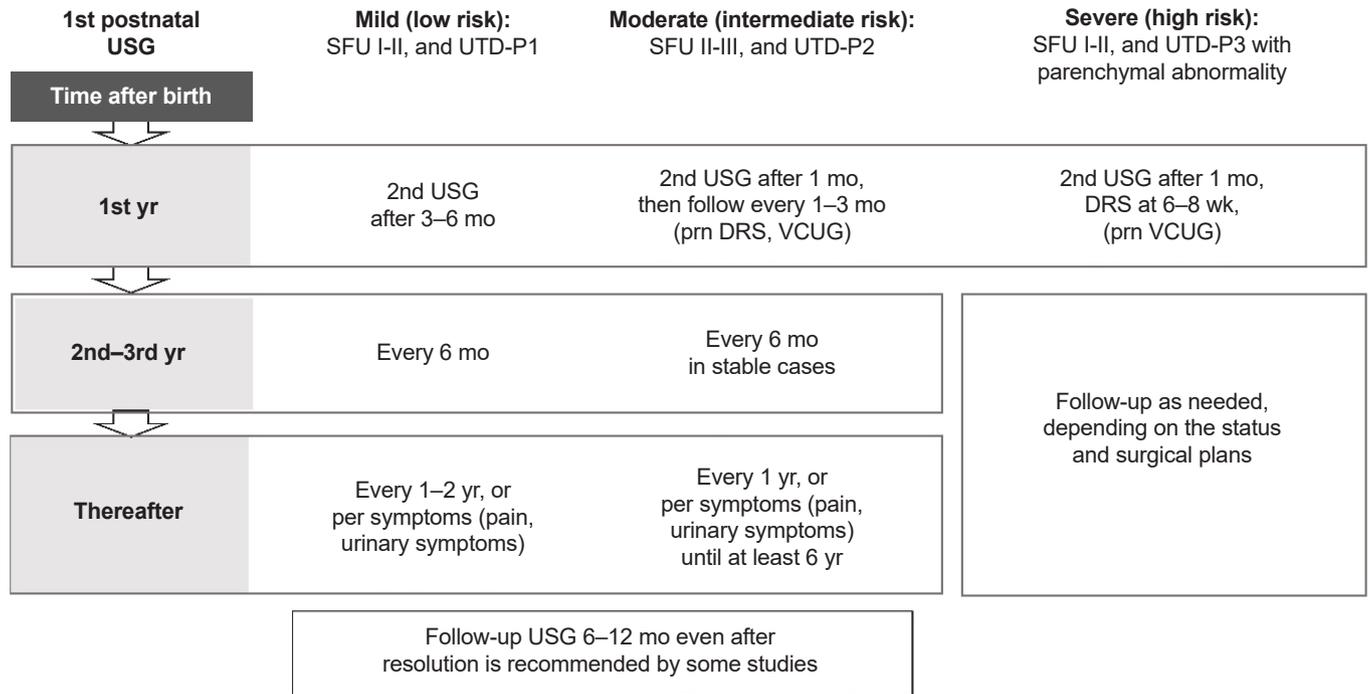
For unilateral hydronephrosis, postnatal evaluation should begin within the first week (after the second day, usually from the fifth to seventh days, to ensure adequate hydration) of life using renal USG. For bilateral hydronephrosis, early postnatal imaging is recommended. After the initial evaluation, follow-up and management are stratified according to severity, as assessed using the aforementioned grading systems.

In cases of known prenatal hydronephrosis that show normalization on the first postnatal USG, the follow-up may be terminated. However, 15% to 45% of patients with normalized initial USG results show abnormal USG results on follow-up, suggesting the need for a second USG examination at 1 to 6 months of age (varying among studies) despite normal findings on the first USG [1,14,30,31].

In the case of mild hydronephrosis (generally grade I and

unilateral grade II in SFU grading and UTD-P1), observational studies anecdotally recommend less aggressive imaging follow-up [14,31], or no further follow-up [32], owing to the nature of spontaneous resolution during the first 2 to 3 years [15,19,33]. Irrespective of the suggestions, an APD of approximately 10 to 20 mm (cutoff value may vary among studies) can be managed conservatively [14,15,34]. Follow-up evaluations using USG after 3 to 6 months for the first year, every 6 months until 3 years, and every 1 to 2 years thereafter (or according to the symptoms [flank pain, dysuria] articulated by the patient) are usually recommended [8,14,31]. Although extremely rare, late worsening after spontaneous resolution can occur in some patients (1%–5%) in a few months (up to 5–6 years) [35], even in patients with mild congenital hydronephrosis [1,33,36]. Clinicians should be aware of this possibility and educate the patients and caregivers about the possible need for follow-up imaging in intervals (varying from 1 to 6–12 months among studies) after resolution or when symptoms such as abdominal pain and urinary symptoms appear [1,8,14,36–38].

For moderate hydronephrosis with an intermediate risk of progression (bilateral SFU grade II, SFU grade III, and UTD-P2), a second USG examination is recommended in the first month and every 1 to 3 months thereafter, during the first year, depending on the stability of the patient's condition. For the next 2 years, follow-up every 6 months is recommended. Annual follow-up or follow-up according to the symptoms (flank pain and dysuria) articulated by the toddler until 6 years is also recommended [8,14,31]. Diuretic renal scan (DRS), which can be performed from 6 to 8 weeks of age, is the most commonly used modality for assessing the presence of upper urinary tract obstruction in infants. It is usually recommended when two



**Fig. 4.** Suggested follow-up and management strategies for congenital isolated hydronephrosis. The diagram summarizes the proposed guidelines from the literature. USG, ultrasonography; SFU, Society for Fetal Urology; UTD, urinary tract dilatation; DRS, diuretic renal scan; VCUG, voiding cystourethrography; prn, pro re nata.

renal USG examinations, during at least 3 months, show no improvement or suggest the aggravation of moderate hydronephrosis. The indications for voiding cystourethrography (VCUG) include bilateral hydronephrosis, ureteral dilatation, abnormal renal echogenicity, and abnormal appearance of the bladder, which are suggestive of lower urinary tract disease (e.g., posterior urethral valve or VUR) [5]. However, the decision to recommend DRS or VCUG is dependent on the clinician's discretion because less invasiveness and cost-effectiveness in evaluation are recently being emphasized, supported by the fact that most patients remain asymptomatic without severe pathology [8,31]. Duong et al. [39] suggested that DRS should only be performed in patients with APD  $\geq 30$  mm, major calyceal dilatation ( $>10$  mm), or renal parenchymal thinning and emphasized the need for more conservative management among patients with mild-to-moderate hydronephrosis.

For severe, high-risk hydronephrosis (SFU grade IV, UTD-P3), USG examination should be repeated at 1 month, followed by DRS at age 6 to 8 weeks [8,31]. The possibility of a later follow-up using USG examination and DRS/VCUG depends on the results of second USG with DRS, and the plans for surgery. The index of obstructive uropathy (UPJO) and the indications for surgical

intervention will be addressed later.

### Natural course of isolated hydronephrosis

According to existing studies,  $>50\%$  to  $70\%$  of all cases of isolated hydronephrosis resolve regardless of the grade [5,6,15,40]. The resolution rate differs according to the baseline severity of hydronephrosis. Prior studies on low-grade hydronephrosis (SFU grades I–II and APD  $<10$ – $20$  mm) showed resolution or improvement in  $56.0\%$  to  $97.4\%$  of the cases, implying a benign condition [6,41,42]. Elmaci and Donmez [13] evaluated the congenital hydronephrosis's time to resolution in patients with APD  $<20$  mm; those with APD  $<10$  mm showed complete resolution in a median of 5 months, whereas those with an APD of 10 to 20 mm showed complete resolution in a median of 11 months. In addition, cumulative resolution rates were reported by several prospective and retrospective studies. In a prospective study, Braga et al. [19] reported the cumulative resolution rate at 3 years for each grade in the SFU and UTD systems (98% for SFU I, 87% for SFU II, 76% for SFU III, and 56% for SFU IV; 90% for UTD-P1, 81% for UTD-P2, and 71% for UTD-P3). In addition, the authors previously reported the cumulative resolution rates

of isolated hydronephrosis at 2 years in a retrospective study (81.7%, 65.6%, 37.6%, and 5.2% for SFU grades I, II, III, and IV, respectively) [15]. Among cases of high-grade hydronephrosis associated with UPJ stenosis, approximately 27% show resolution, >50% remain stable, and the rest progress with possible renal function deterioration [3]. Surgical intervention has been required in approximately 25% of all cases, ranging from 5% to 50% depending on the study [1,15,43-46]. Therefore, after at least 2 to 3 years of watchful observation and evaluation, termination of follow-up may be possible since the outcome would be determined within these years.

## Prediction and decision of intervention

Disagreements about the definition of obstruction and the indications and timing of surgery in hydronephrosis due to UPJO remain. The appearance of symptoms of UPJO, such as pain and urinary tract infection, is indicative of the need for surgery. A differential renal function of <40% with impaired drainage ( $T_{1/2}$  >20 minutes) on DRS or a >10% deterioration of renal function on a serial renal scan is also generally considered a surgical indication [6,31,38,47].

Additional studies have presented the predictors of surgery and their corresponding cutoff values, including initial postnatal APD, cortical tissue transit time on DRS, renal pyramidal thickness, and renal parenchyma-to-hydronephrosis area ratio (PHAR) [34,47-51].

### Initial postnatal APD

Postnatal APD has been widely used as an index for evaluating and anticipating the presence of obstruction, with advantages of wide availability and absence of radiation exposure. In clinical practice, sequential changes in APD are mainly used to determine management plans. Although no absolute cutoff value of APD for performing pyeloplasty has been defined, several studies suggested different APD values, ranging from 15 to 30 mm, as significant predictors of surgical intervention [8,34,37,50,52]. Arora et al. [47] performed a prospective multivariate analysis and showed that an APD of up to 24 mm in the first week after birth can predict the need for surgical intervention (sensitivity, 73.1%; specificity, 88.0%). The prospective cohort studies of Coelho et al. [37] and Dias et al. [34] suggested an APD of >15 and 18 mm as the cutoff value, respectively.

### Delayed tissue transit time in $^{99m}\text{Tc}$ -mercaptoacetyltriglycine DRS

Some recent studies have shown that delayed tissue transit time, which is defined as an absence of activity in the subcortical structures or in the pelvis on a  $^{99m}\text{Tc}$ -mercaptoacetyltriglycine (MAG3) DRS within 3 or 8 minutes of tracer injection, can predict deterioration of UPJO in pediatric populations [51,53-55]. Song et al. [56] proposed that delayed tissue transit time on  $^{99m}\text{Tc}$ -MAG3 DRS is a significant predictor of renal function improvement after pyeloplasty in patients with UPJO. Therefore, they suggested that delayed tissue transit time should be considered a candidate predictor of immediate pyeloplasty and decreased differential renal function.

### Renal pyramidal thickness

The renal pyramid is the first portion of renal parenchyma that becomes affected in high-grade hydronephrosis. The parenchymal thickness changes with age, making its clinical application difficult in a growing child. In contrast, the renal pyramid is a part of the parenchyma that grows slowly and shows only small changes in the first 9 years of life; thus, it is a feasible parameter for evaluation and comparison between serial USG images [57]. Pyramidal thickness measurement was not previously performed in patients with hydronephrosis until Hodhod et al. [48] recently measured pyramidal thickness in the supine position in the middle third of the sagittal plane. In their study, multivariate analysis showed that a renal pyramidal thickness of  $\leq 3$  mm (sensitivity, 98.1%; specificity, 89.7%) predicted the need for surgical intervention.

### Renal PHAR

Some studies have attempted to simultaneously measure the renal parenchymal volume and the grade of hydronephrosis using USG (without a renal scan) as a surrogate of renal function in patients with hydronephrosis [49,58]. In this regard, Rickard et al. [49] showed that the renal PHAR predicted the need for surgery (cutoff value, <0.5) in high-grade hydronephrosis (area under the receiver operating characteristic curve, 0.816;  $P < 0.001$ ) more efficiently than the APD measurement, SFU grade, and UTD classification.

## Risk of urinary tract infection

The existing studies commonly suggest that the risk of urinary tract infection increases with the degree of hydronephrosis.

Observational studies have shown that patients with moderate or severe hydronephrosis show an increased incidence (13.8%–40.0% for moderate-to-severe hydronephrosis vs. 4.1%–14.0% for mild hydronephrosis) of urinary tract infection [37,59–61]. Patients with hydronephrosis with obstructive drainage patterns on renal scans, without VUR, have a higher risk than those without obstructive patterns [61–63]. Furthermore, in terms of the benefits of antibiotic prophylaxis, different outcomes have been reported. Braga et al. [64,65] demonstrated a protective effect of antibiotic prophylaxis, especially in patients with high-grade hydronephrosis, in their systematic review and meta-analysis, whereas Estrada et al. [66] showed significant improvement in infection after prophylaxis even in patients with mild hydronephrosis. In clinical practice, the use of prophylactic antibiotics remains nonuniform owing to the absence of recommendations or guidelines from randomized control studies [67,68]. An ongoing randomized control trial on hydronephrosis with UPJO-like and non-refluxing megaureter by Braga et al. (Clinical Trials Registry no. NCT01140516) might aid in elucidating the effect of chemoprophylaxis. Therefore, clinicians are currently advised to decide whether they want to make use of antibiotics on a case-by-case basis while keeping in mind that high-grade hydronephrosis may confer an increased risk of urinary tract infection.

## Conclusions

Predicting the natural course of prenatally detected hydronephrosis has become possible with increasing knowledge and accumulated outcomes from cases treated with surgical intervention. Since no definite consensus exists about using a certain grading system in clinical practice, a practical, user-friendly system, combined with the use of an objective imaging modality that is generally accepted by multidisciplinary specialists, is needed. Furthermore, the establishment of the timing of the initial evaluation and follow-up intervals according to disease severity can aid in efficient management and help inform the caregivers and patients about the prognosis and follow-up plans.

## Conflicts of interest

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 All authors read and approved the final manuscript.

## References

1. Nguyen HT, Herndon CD, Cooper C, Gatti J, Kirsch A, Kokorowski P, et al. The Society for Fetal Urology consensus statement on the evaluation and management of antenatal hydronephrosis. *J Pediatr Urol* 2010;6:212-31.
2. Choi HA, Lee DJ, Shin SM, Lee YK, Ko SY, Park SW. The prenatal and postnatal incidence of congenital anomalies of the kidneys and urinary tract (CAKUT) detected by ultrasound. *Child Kidney Dis* 2016;20:29-32.
3. Corbett HJ, McCarthy L. Hydronephrosis in children: pelviureteric junction dysfunction. *Surgery (Oxford)* 2013;31:135-9.
4. Kim MY, Im YJ, Hyun HS, Kang HG, Ha IS, Cheong HI, et al. A case of an ureteropelvic junction obstruction caused by a crossing vessel. *Child Kidney Dis* 2018;22:28-31.
5. Davenport MT, Merguerian PA, Koyle M. Antenatally diagnosed hydronephrosis: current postnatal management. *Pediatr Surg Int* 2013;29:207-14.
6. Sidhu G, Beyene J, Rosenblum ND. Outcome of isolated antenatal hydronephrosis: a systematic review and meta-analysis. *Pediatr Nephrol* 2006;21:218-24.
7. Sarhan OM, El Helaly A, Al Otay AH, Al Ghanbar M, Nakshabandi Z. Prenatally detected, unilateral, high-grade hydronephrosis: can we predict the natural history? *Can Urol Assoc J* 2018;12:E137-41.
8. Nguyen HT, Benson CB, Bromley B, Campbell JB, Chow J, Coleman B, et al. Multidisciplinary consensus on the classification of prenatal and postnatal urinary tract dilation (UTD classification system). *J*

- Pediatr Urol* 2014;10:982-98.
9. Chalmers DJ, Meyers ML, Brodie KE, Palmer C, Campbell JB. Inter-rater reliability of the APD, SFU and UTD grading systems in fetal sonography and MRI. *J Pediatr Urol* 2016;12:305.
  10. Zanetta VC, Rosman BM, Bromley B, Shipp TD, Chow JS, Campbell JB, et al. Variations in management of mild prenatal hydronephrosis among maternal-fetal medicine obstetricians, and pediatric urologists and radiologists. *J Urol* 2012;188:1935-9.
  11. Onen A. Grading of hydronephrosis: an ongoing challenge. *Front Pediatr* 2020;8:458.
  12. Fernbach SK, Maizels M, Conway JJ. Ultrasound grading of hydronephrosis: introduction to the system used by the Society for Fetal Urology. *Pediatr Radiol* 1993;23:478-80.
  13. Elmaci AM, Donmez MI. Time to resolution of isolated antenatal hydronephrosis with anteroposterior diameter  $\leq$  20 mm. *Eur J Pediatr* 2019;178:823-8.
  14. Timberlake MD, Herndon CD. Mild to moderate postnatal hydronephrosis: grading systems and management. *Nat Rev Urol* 2013;10:649-56.
  15. Jung J, Lee JH, Kim KS, Park YS. Utility of Society for Fetal Urology and anteroposterior pelvic diameter grading systems for estimating time to resolution of isolated hydronephrosis: a single center study. *J Urol* 2020;204:1048-53.
  16. Nguyen HT, Phelps A, Coley B, Darge K, Rhee A, Chow JS. 2021 update on the urinary tract dilation (UTD) classification system: clarifications, review of the literature, and practical suggestions. *Pediatr Radiol* 2022;52:740-51.
  17. Gray MC, Zillioux JM, Varda B, Herndon CD, Kurtz MP, Chow JS, et al. Assessment of urinary tract dilation grading amongst pediatric urologists. *J Pediatr Urol* 2020;16:457.
  18. Braga LH, McGrath M, Farrokhyar F, Jegatheeswaran K, Lorenzo AJ. Associations of initial Society for Fetal Urology grades and urinary tract dilatation risk groups with clinical outcomes in patients with isolated prenatal hydronephrosis. *J Urol* 2017;197(3 Pt 2):831-7.
  19. Braga LH, McGrath M, Farrokhyar F, Jegatheeswaran K, Lorenzo AJ. Society for Fetal Urology classification vs urinary tract dilation grading system for prognostication in prenatal hydronephrosis: a time to resolution analysis. *J Urol* 2018;199:1615-21.
  20. Cakici EK, Aydog O, Eroglu FK, Yazilitas F, Ozlu SG, Uner C, et al. Value of renal pelvic diameter and urinary tract dilation classification in the prediction of urinary tract anomaly. *Pediatr Int* 2019;61:271-7.
  21. Hodhod A, Capolicchio JP, Jednak R, El-Sherif E, El-Doray Ael-A, El-Sherbiny M. Evaluation of urinary tract dilation classification system for grading postnatal hydronephrosis. *J Urol* 2016;195:725-30.
  22. Han M, Kim HG, Lee JD, Park SY, Sur YK. Conversion and reliability of two urological grading systems in infants: the Society for Fetal Urology and the urinary tract dilatation classifications system. *Pediatr Radiol* 2017;47:65-73.
  23. Rickard M, Easterbrook B, Kim S, Farrokhyar F, Stein N, Arora S, et al. Six of one, half a dozen of the other: a measure of multidisciplinary inter/intra-rater reliability of the Society for Fetal Urology and urinary tract dilation grading systems for hydronephrosis. *J Pediatr Urol* 2017;13:80.
  24. Kaspar CD, Lo M, Bunchman TE, Xiao N. The antenatal urinary tract dilation classification system accurately predicts severity of kidney and urinary tract abnormalities. *J Pediatr Urol* 2017;13:485.
  25. Onen A. An alternative grading system to refine the criteria for severity of hydronephrosis and optimal treatment guidelines in neonates with primary UPJ-type hydronephrosis. *J Pediatr Urol* 2007;3:200-5.
  26. Cho HY, Jung I, Kim YH, Kwon JY. Reliability of Society of Fetal Urology and Onen grading system in fetal hydronephrosis. *Obstet Gynecol Sci* 2019;62:87-92.
  27. Kim SY, Kim MJ, Yoon CS, Lee MS, Han KH, Lee MJ. Comparison of the reliability of two hydronephrosis grading systems: the Society for Foetal Urology grading system vs. the Onen grading system. *Clin Radiol* 2013;68:e484-90.
  28. Swenson DW, Darge K, Ziniel SI, Chow JS. Characterizing upper urinary tract dilation on ultrasound: a survey of North American pediatric radiologists' practices. *Pediatr Radiol* 2015;45:686-94.
  29. Suson KD, Preece J. Do current scientific reports of hydronephrosis make the grade? *J Pediatr Urol* 2020;16:597.
  30. Aksu N, Yavascan O, Kangin M, Kara OD, Aydin Y, Erdogan H, et al. Postnatal management of infants with antenatally detected hydronephrosis. *Pediatr Nephrol* 2005;20:1253-9.
  31. Yalcinkaya F, Ozcakar ZB. Management of antenatal hydronephrosis. *Pediatr Nephrol* 2020;35:2231-9.
  32. Djahangirian O, Young I, Dorgalli C, Bissaillon A, Tran NA, Walia A, et al. Safe discharge parameters for patients with isolated antenatal hydronephrosis. *J Pediatr Urol* 2018;14:321.
  33. Kohata E, Kimata T, Onuma C, Yamanouchi S, Tsuji S, Ohashi A, et al. Natural course of isolated mild congenital hydronephrosis: a 2-year prospective study at a single center in Japan. *Int J Urol* 2019;26:643-7.
  34. Dias CS, Silva JM, Pereira AK, Marino VS, Silva LA, Coelho AM, et al. Diagnostic accuracy of renal pelvic dilatation for detecting surgically managed ureteropelvic junction obstruction. *J Urol* 2013;190:661-6.
  35. Gatti JM, Broecker BH, Scherz HC, Perez-Brayfield MR, Kirsch AJ. Antenatal hydronephrosis with postnatal resolution: how long are

- postnatal studies warranted? *Urology* 2001;57:1178.
36. Matsui F, Shimada K, Matsumoto F, Takano S. Late recurrence of symptomatic hydronephrosis in patients with prenatally detected hydronephrosis and spontaneous improvement. *J Urol* 2008;180:322-5.
  37. Coelho GM, Bouzada MC, Pereira AK, Figueiredo BF, Leite MR, Oliveira DS, et al. Outcome of isolated antenatal hydronephrosis: a prospective cohort study. *Pediatr Nephrol* 2007;22:1727-34.
  38. Riccabona M, Avni FE, Blickman JG, Dacher JN, Darge K, Lobo ML, et al. Imaging recommendations in paediatric urology: minutes of the ESPR workgroup session on urinary tract infection, fetal hydronephrosis, urinary tract ultrasonography and voiding cystourethrography, Barcelona, Spain, June 2007. *Pediatr Radiol* 2008;38:138-45.
  39. Duong HP, Piepsz A, Collier F, Khelif K, Christophe C, Cassart M, et al. Predicting the clinical outcome of antenatally detected unilateral pelviureteric junction stenosis. *Urology* 2013;82:691-6.
  40. Thomas DF. Prenatal diagnosis: what do we know of long-term outcomes? *J Pediatr Urol* 2010;6:204-11.
  41. Madden-Fuentes RJ, McNamara ER, Nseyo U, Wiener JS, Routh JC, Ross SS. Resolution rate of isolated low-grade hydronephrosis diagnosed within the first year of life. *J Pediatr Urol* 2014;10:639-44.
  42. Sarhan OM, Helaly AE, Al Otay A, Ghanbar MA, Nakshabandi Z. Isolated low grade prenatally detected unilateral hydronephrosis: do we need long term follow-up? *Int Braz J Urol* 2018;44:812-8.
  43. Thornburg LL, Pressman EK, Chelamkuri S, Hulbert W, Rabinowitz R, Mevorach R. Third trimester ultrasound of fetal pyelectasis: predictor for postnatal surgery. *J Pediatr Urol* 2008;4:51-4.
  44. Mesrobian HG, Mirza SP. Hydronephrosis: a view from the inside. *Pediatr Clin North Am* 2012;59:839-51.
  45. Chertin B, Pollack A, Koulikov D, Rabinowitz R, Hain D, Hadas-Halpren I, et al. Conservative treatment of ureteropelvic junction obstruction in children with antenatal diagnosis of hydronephrosis: lessons learned after 16 years of follow-up. *Eur Urol* 2006;49:734-9.
  46. Ismaili K, Hall M, Piepsz A, Alexander M, Schulman C, Avni FE. Insights into the pathogenesis and natural history of fetuses with renal pelvis dilatation. *Eur Urol* 2005;48:207-14.
  47. Arora S, Yadav P, Kumar M, Singh SK, Sureka SK, Mittal V, et al. Predictors for the need of surgery in antenatally detected hydronephrosis due to UPJ obstruction: a prospective multivariate analysis. *J Pediatr Urol* 2015;11:248.
  48. Hodhod A, Capolicchio JP, Jednak R, Eid H, El-Doray AE, El-Sherbiny M. Is the renal pyramidal thickness a good predictor for pyeloplasty in postnatal hydronephrosis? *J Pediatr Urol* 2018;14:277.
  49. Rickard M, Lorenzo AJ, Braga LH. Renal parenchyma to hydronephrosis area ratio (PHAR) as a predictor of future surgical intervention for infants with high-grade prenatal hydronephrosis. *Urology* 2017;101:85-9.
  50. Killi I, Avlan D, Taskinlar H, Kara PP, Apaydin FD, Delibas A, et al. Effective predictors for surgical decision in antenatal hydronephrosis: a prospective multiparameter analysis. *Turk J Urol* 2017;43:361-5.
  51. Lee JN, Kang JK, Jeong SY, Lee SM, Cho MH, Ha YS, et al. Predictive value of cortical transit time on MAG3 for surgery in antenatally detected unilateral hydronephrosis caused by ureteropelvic junction stenosis. *J Pediatr Urol* 2018;14:55.
  52. Longpre M, Nguan A, Macneily AE, Afshar K. Prediction of the outcome of antenatally diagnosed hydronephrosis: a multivariable analysis. *J Pediatr Urol* 2012;8:135-9.
  53. Harper L, Bourquard D, Grosos C, Abbo O, Ferdynus C, Michel JL, et al. Cortical transit time as a predictive marker of the need for surgery in children with pelvi-ureteric junction stenosis: preliminary study. *J Pediatr Urol* 2013;9(6 Pt B):1054-8.
  54. Piepsz A, Tondeur M, Nogarede C, Collier F, Ismaili K, Hall M, et al. Can severely impaired cortical transit predict which children with pelvi-ureteric junction stenosis detected antenatally might benefit from pyeloplasty? *Nucl Med Commun* 2011;32:199-205.
  55. Schlotmann A, Clorius JH, Clorius SN. Diuretic renography in hydronephrosis: renal tissue tracer transit predicts functional course and thereby need for surgery. *Eur J Nucl Med Mol Imaging* 2009;36:1665-73.
  56. Song SH, Park S, Chae SY, Moon DH, Park S, Kim KS. Predictors of renal functional improvement after pyeloplasty in ureteropelvic junction obstruction: clinical value of visually assessed renal tissue tracer transit in 99mTc-mercaptoacetyltriglycine renography. *Urology* 2017;108:149-54.
  57. Kadioglu A. Renal measurements, including length, parenchymal thickness, and medullary pyramid thickness, in healthy children: what are the normative ultrasound values? *AJR Am J Roentgenol* 2010;194:509-15.
  58. Rodriguez LV, Lock J, Kennedy WA, Shortliffe LM. Evaluation of sonographic renal parenchymal area in the management of hydronephrosis. *J Urol* 2001;165:548-51.
  59. Szymanski KM, Al-Said AN, Pippi Salle JL, Capolicchio JP. Do infants with mild prenatal hydronephrosis benefit from screening for vesicoureteral reflux? *J Urol* 2012;188:576-81.
  60. Tombesi MM, Alconcher LF. Short-term outcome of mild isolated antenatal hydronephrosis conservatively managed. *J Pediatr Urol* 2012;8:129-33.
  61. Lee JH, Choi HS, Kim JK, Won HS, Kim KS, Moon DH, et al. Nonrefluxing neonatal hydronephrosis and the risk of urinary tract infection.

- J Urol 2008;179:1524-8.
62. Yavascan O, Aksu N, Anil M, Kara OD, Aydin Y, Kangin M, et al. Postnatal assessment of growth, nutrition, and urinary tract infections of infants with antenatally detected hydronephrosis. *Int Urol Nephrol* 2010;42:781-8.
  63. Lidfelt KJ, Herthelius M. Antenatal hydronephrosis: infants with minor postnatal dilatation do not need prophylaxis. *Pediatr Nephrol* 2008;23:2021-4.
  64. Braga LH, Mijovic H, Farrokhyar F, Pemberton J, DeMaria J, Lorenzo AJ. Antibiotic prophylaxis for urinary tract infections in antenatal hydronephrosis. *Pediatrics* 2013;131:e251-61.
  65. Braga LH, Easterbrook B, Jegatheeswaran K, Lorenzo AJ. From research question to conducting a randomized controlled trial on continuous antibiotic prophylaxis in prenatal hydronephrosis: a rational stepwise process. *Front Pediatr* 2016;4:27.
  66. Estrada CR, Peters CA, Retik AB, Nguyen HT. Vesicoureteral reflux and urinary tract infection in children with a history of prenatal hydronephrosis: should voiding cystourethrography be performed in cases of postnatally persistent grade II hydronephrosis? *J Urol* 2009;181:801-7.
  67. Merguerian PA, Herz D, McQuiston L, Van Bibber M. Variation among pediatric urologists and across 2 continents in antibiotic prophylaxis and evaluation for prenatally detected hydronephrosis: a survey of American and European pediatric urologists. *J Urol* 2010;184(4 Suppl):1710-5.
  68. Silay MS, Undre S, Nambiar AK, Dogan HS, Kocvara R, Nijman RJ, et al. Role of antibiotic prophylaxis in antenatal hydronephrosis: a systematic review from the European Association of Urology/European Society for Paediatric Urology Guidelines Panel. *J Pediatr Urol* 2017;13:306-15.

# BK polyomavirus-associated nephropathy

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BK polyomavirus (BKPyV) is a ubiquitous virus residing in the kidney tubules and is clinically significant only in immunocompromised patients. In clinical practice, BKPyV is a causative pathogen of BKPyV-associated nephropathy (BKVAN) in kidney allograft recipients or hemorrhagic cystitis of hematopoietic stem cell transplant recipients. Currently, there is no effective treatment for BKVAN; therefore, careful monitoring and prudent modification of immunosuppression are necessary to prevent BKVAN. In this article, the epidemiology, pathophysiology, and current management strategies for BKVAN are reviewed.

**Keywords:** BK virus; Immunosuppression therapy; Kidney transplantation

## Introduction

BK polyomavirus (BKPyV) is a non-enveloped double-stranded DNA virus that was first isolated from a kidney allograft recipient and described in 1971 [1]. More than 90% of the general population is infected with this virus [2]. Primary infection of BKPyV usually occurs subclinically during childhood, and the virus remains in a latent state in the uroepithelium and renal tubular epithelial cells. Upon immunosuppression, BKPyV is reactivated, leading to tubular cell lysis and viruria. One-third to one-half of those who show viruria ( $>10^8$  copies/mL) develop BKPyV-DNAemia after 2 to 6 weeks along with tubulointerstitial lesions; half of these patients develop BKPyV-associated nephropathy (BKVAN) after another 2 to 6 weeks, especially if plasma BKPyV loads are  $>10,000$  copies/mL.

BKVAN occurs more commonly with more potent immunosuppression, and it is currently one of the most important

causes of kidney allograft failure [3-5]. In addition, this virus is associated with ureteral stenosis and hemorrhagic cystitis [6]. Moreover, sporadic cases of pneumonitis, retinitis, colitis, capillary-leak syndrome, liver disease, meningoencephalitis, encephalitis, hemophagocytic syndrome, and urothelial cancer caused by BKPyV have been described [7]. BKVAN has a poor prognosis, and it has currently no treatment.

## Epidemiology

BKVAN usually occurs within the first 2 years after kidney transplantation (KT). Viruria is first noted in 30% to 40% of KT recipients, with decoy cells positive in 20% to 30%, detectable BKPyV-DNA in 10% to 20%, BKVAN in approximately 10%, and graft loss from BKVAN in approximately 5% [8-11]. Interestingly, BKPyV viruria is identified in only 10% of immune-competent hosts; however, its prevalence is 30% to 60% in immuno-

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compromised hosts. In addition, BKPyV-DNA clears within 2 to 12 hours after allograft nephrectomy for BKVAN, implying the presence of replication foci in the kidney allograft. BKPyV-DNAemia is associated with worse outcomes after KT. The 36-month graft survival rate if BKPyV is detected within 6 months post-KT is 79%, compared with 90% in controls [12]. Risk factors for BKVAN include tacrolimus use, potent immunosuppression, acute graft rejection, male gender, old age, younger age for children, delayed graft function, use of cadaveric graft, previous transplantation, human leukocyte antigen mismatches, ABO incompatibility, highly sensitized status, history of hemodialysis (vs. peritoneal dialysis), and a ureteral stent [13]. In other solid organ transplantations, BKPyV-related complications are not common, although cases have been reported following heart and lung transplantations [14,15].

In hematopoietic stem cell transplant (HCT) recipients, hemorrhagic cystitis occurs in up to one-fourth of patients [16,17], 1-month post-HCT [18] and usually lasts more than a month. BKPyV-DNAemia or viruria, which was associated with acute kidney injury, long-term poor kidney function, and mortality, were noted in 18% and 45% of HCT recipients, respectively, in the first 3 months post-HCT [19].

## Pathophysiology

The BKPyV dsDNA is enclosed in a viral capsid comprised of an outer layer of VP1 pentamer and an inner layer of VP2 and VP3 proteins [20]. Its genome is composed of circular dsDNA of approximately 5 kb that contains the early viral gene region, which codes the regulatory large and small tumor antigens promoting cell cycle entry/progression and viral replication, the late viral gene region, which codes the viral capsid proteins VP1, VP2, VP3 for entry and assembly of progeny virions, and the non-coding control region [8]. Once infection occurs, BKPyV hijacks the host cell's DNA replication machinery for its own reproduction [20]. Therefore, antiviral agents targeting viral DNA replication are ineffective against this virus. After replication, lysis of the host cells along with inflammation and transition to the latent phase follow. Upon immunosuppression, viral replication resumes, causing acute tubular injury, interstitial nephritis, and severe interstitial fibrosis.

## Screening of BKVAN

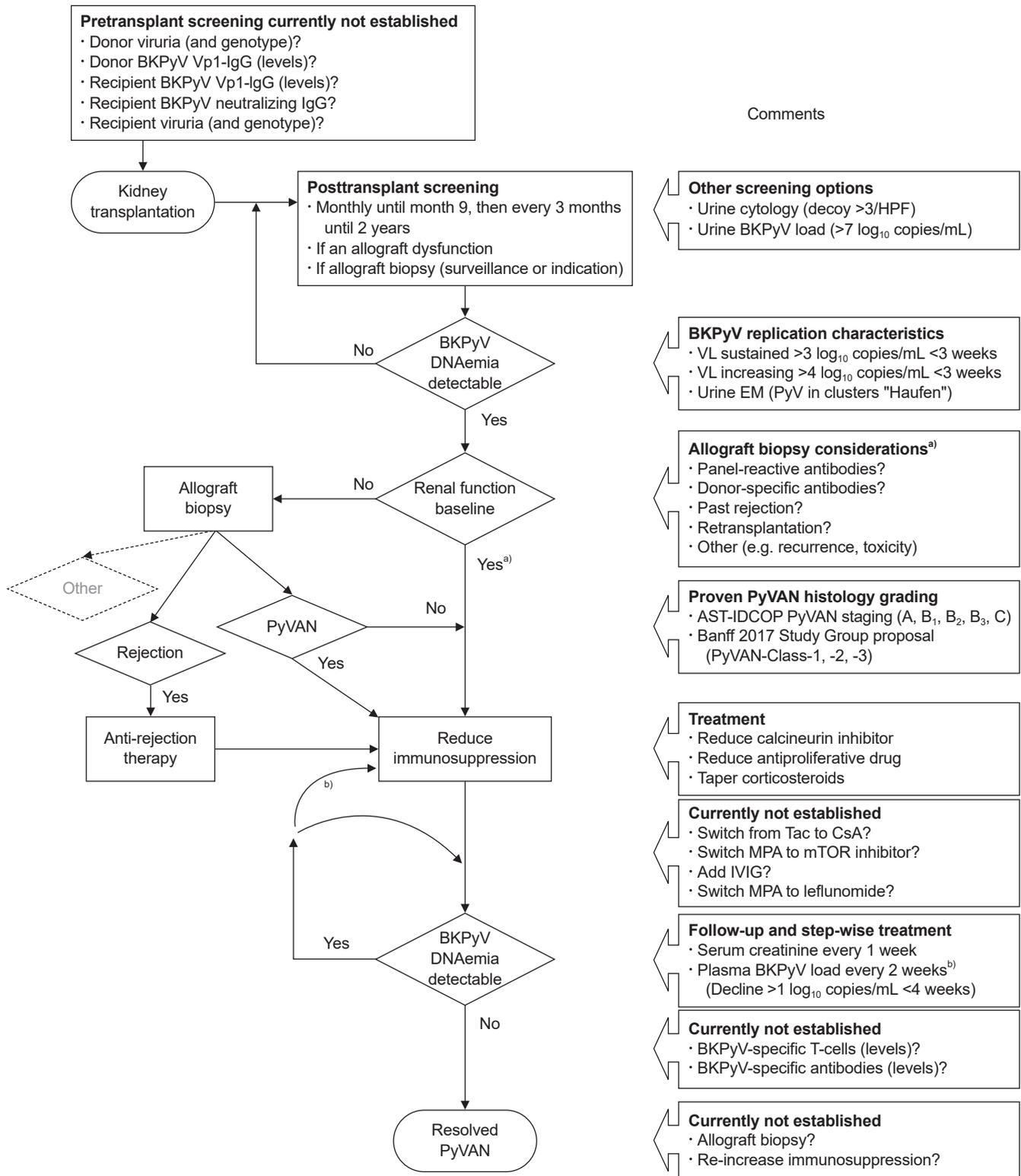
Since there is no effective treatment for BKVAN, screening

for BKPyV is the most important strategy to prevent BKVAN. Recently, the American Society of Transplantation Infectious Disease Community of Practice (AST-IDCOP) recommended a monitoring and management strategy for BKVAN (Fig. 1) [7]. Prospective screening of the plasma or urine can identify early viral replication, permitting early intervention and preventing progression to nephropathy or allograft loss. For screening, plasma DNA load is measured monthly for 9 months, and then every 3 months thereafter for 2 years after KT [5,7,21] or when allograft biopsy is performed for surveillance or as indicated and when unexplained allograft dysfunction develops (Fig. 1). BKVAN is suspected when the BKPyV viral load is >10,000 copies/mL with or without serum creatinine level elevation. Histological findings of tubular atrophy, fibrosis, and inflammatory lymphocytic infiltrates need to be differentiated from those of acute cellular rejection. Intranuclear BKPyV inclusion bodies suggest BKVAN, which can be identified with special staining of large T antigen [22].

## Diagnosis of BKVAN

Diagnosis of BKVAN is confirmed only by allograft kidney biopsy, with features of interstitial nephritis and large T antigen positivity with immunohistochemistry. If the plasma viral load either increases to >10,000 copies/mL in one of two measurements within 3 weeks or is sustained at >1,000 copies/mL in two measurements within 3 weeks, these are considered presumptive or probable BKVAN, respectively, which requires modification of immunosuppression and kidney biopsy if there is a risk of acute rejection and/or impaired kidney function (Fig. 1). Additionally, urine BKPyV viruria >10,000,000 copies/mL or presence of decoy cells indicates possible BKVAN, warranting plasma BKPyV viral load monitoring. If BKVAN is established, immunosuppression needs to be reduced, which can be accomplished even without biopsy confirmation. In 10% to 30% of cases, false-negative results were obtained as biopsies were taken early after BKPyV-DNAemia onset, and medullary tissue was not sampled [21].

The pathology of BKVAN is described using the histologic patterns of BKVAN proposed by the 2013 AST-IDCOP. In addition to viral cytopathic changes, acute tubular injury, interstitial nephritis, and severe interstitial fibrosis are denoted as patterns A, B, C, respectively, along with the degree of interstitial nephritis (Table 1) [23]. Meanwhile, the Banff 2017 Working Group Classification takes into account the intrarenal PyV load



**Fig. 1.** Monitoring and management strategy for BK polyomavirus (BKPyV)-associated nephropathy (BKVAN). HPF, high-power field; VL, viral load; EM, electron microscopy; PyV, polyomavirus; PyVAN, polyomavirus-associate nephropathy; AST-IDCOP, American Society of Transplantation Infectious Diseases Community of Practice; Tac, tacrolimus; CsA, cyclosporine-A; MPA, mycophenolic acid or equivalent; mTOR, mammalian target of rapamycin; IVIG, intravenous immunoglobulin. <sup>a)</sup> Allograft biopsy should be considered in patients with baseline allograft function, if concurrent (subclinical) acute rejection is likely; <sup>b)</sup> If decline in plasma BKPyV-DNAemia is <1 log<sub>10</sub> copies/mL in <4 weeks, further immunosuppression is required. Reuse from Hirsch et al. Clin Transplant [7] with permission from John Wiley and Sons.

**Table 1.** Histological patterns for BKVAN according to American Society of Transplantation 2013

Pattern	Biopsy findings	Risk of graft loss
<b>A</b>		
Viral cytopathic changes	Mild ( $\leq 25\%$ )	<10%
Interstitial inflammation	Minimal ( $\leq 10\%$ )	
Tubular atrophy	Minimal ( $\leq 10\%$ )	
Interstitial fibrosis	Minimal ( $\leq 10\%$ )	
<b>B</b>		
Viral cytopathic changes	Variable (11% to >50%)	50%
Interstitial inflammation	Significant (11% to >50%)	
Tubular atrophy	Moderate ( $< 50\%$ )	
Interstitial fibrosis	Moderate ( $< 50\%$ )	
B1: Interstitial inflammation	Moderate (11% to 25%)	25%
B2: Interstitial inflammation	Significant (26% to 50%)	50%
B3: Interstitial inflammation	Extensive ( $> 50\%$ )	75%
<b>C</b>		
Viral cytopathic changes	Variable (variable)	>80%
Interstitial inflammation	Variable (variable)	
Tubular atrophy	Extensive ( $> 50\%$ )	
Interstitial fibrosis	Extensive ( $> 50\%$ )	

BKVAN, BK polyomavirus-associated nephropathy. Adapted from Hirsch et al. Am J Transplant [23].

**Table 2.** Banff histologic classification system of BKVAN

Class 1		Class 2		Class 3	
pvl <sup>a)</sup>	Banff ci score <sup>b)</sup>	pvl <sup>a)</sup>	Banff ci score <sup>b)</sup>	pvl <sup>a)</sup>	Banff ci score <sup>b)</sup>
1	0–1	1	2–3	NA	NA
NA	NA	2	0–3	NA	NA
NA	NA	3	0–1	3	2–3

BKVAN, BK polyomavirus-associated nephropathy; ci, interstitial fibrosis in cortex; NA, not available.

<sup>a)</sup>The pvl scoring is on the basis of the extent of virally induced tubular changes. The overall percentage of positive tubular cross-sections is estimated in the entire biopsy sample (all available cores, cortex, and medulla): pvl 1,  $\leq 1\%$  of all tubules/ducts with viral replication; pvl 2,  $> 1\%$  to  $\leq 10\%$  of all tubules/ducts with viral replication; and pvl 3,  $> 10\%$  of all tubules/ducts with viral replication. <sup>b)</sup>The Banff ci score evaluates the extent of interstitial fibrosis in cortex: ci 0,  $\leq 5\%$  of cortical area; ci 1, 6%–25% of cortical area (mild); ci 2, 26%–50% of cortical area (moderate); and ci 3,  $> 50\%$  of cortical area (severe).

Adapted from Nickleleit et al. J Am Soc Nephrol [22].

(extent of virally induced tubular changes with intranuclear viral inclusion bodies and/or a positive immunohistochemistry reaction for SV40 T antigen) and Banff ci scores (Table 2) [22]. These histological patterns/classifications indicate the risks of allograft kidney loss, which range from <10% to >80%. In cases wherein there is evidence of rejection or intimal arteritis, or a positive C4d stain is observed, intensifying immunosuppression to treat rejection should first be considered before treating BKVAN.

## Treatment

### Reduction in immunosuppression

The first-line management for BKVAN is the reduction in im-

munosuppressive agents (Fig. 1). Usually, a stepwise approach to reduce immunosuppression is adapted; calcineurin inhibitors (CNI) are initially reduced by 25% to 50%, followed by mycophenolate mofetil (MMF) by 50%, and finally MMF discontinuation if there is no improvement [24]. Another approach is initial MMF reduction by 50%, then CNI reduction by 25% to 50%, and finally MMF discontinuation. Steroids are often limited to prednisolone 10 mg or less, and targets of CNI trough levels are <6 ng/mL with tacrolimus and <150 ng/mL with cyclosporine. Additionally, mammalian target of rapamycin (mTOR) inhibitors were shown to decrease BKPyV-DNAemia and/or BKVAN [25]. Since cyclosporine and sirolimus, an mTOR inhibitor, inhibit BKPyV replication *in vitro*, switching immunosuppressants from tacrolimus to cyclosporine, CNI to sirolimus, MMF to si-

rolimus, or MMF to leflunomide can be considered, albeit with weak evidence [8,21]. However, reducing or modifying immunosuppression may be inadequate to prevent rejection, whereas excessive immunosuppression will worsen BKVAN and cause allograft dysfunction, tubulointerstitial nephritis, and fibrosis [7]. Therefore, prior to modifying immunosuppression, patient's immunological risk, viral load, and kidney dysfunction must be considered [26].

### Other management

No randomized clinical study has proven the efficacy of other adjunctive managements aside from modification of immunosuppression.

#### *Intravenous immunoglobulins*

Intravenous immunoglobulins, which have indirect immunomodulatory effects, contain high titers of potent BKPyV neutralizing antibodies that can directly neutralize BKPyV activity [7]. For BKVAN, 0.1–2.0 g/kg/dose is used.

#### *Cidofovir*

Cidofovir is a nucleoside analog licensed by the U.S. Food and Drug Administration for the treatment of cytomegalovirus retinitis [23]. Its efficacy in BKVAN is controversial; however, cidofovir concentration in renal tissues and urine is high. Therefore, cidofovir can theoretically be effective against viral infection in the kidneys. Coincidentally, drug-induced anterior uveitis has been reported in 12% to 35% of cases. Cidofovir is given as a low-dose regimen at 0.25–1.0 mg/kg/dose every 2 to 4 weeks, and serum creatinine, white blood cell count, ocular and visual symptoms should be monitored every 2 weeks [24].

#### *Leflunomide*

Leflunomide is a disease-modifying antirheumatic drug that inhibits dihydroorotate dehydrogenase, which is necessary for pyrimidine synthesis [27]. Its anti-proliferative activity and anti-inflammatory effects are a result of the selective inhibition of mTOR signaling. It has been shown to inhibit BKPyV viral DNA synthesis *in vitro* [28]. According to a systematic review, clearance of BKPyV-DNAemia was reported in 33.3% to 92.3% of cases in different studies and 27 (10.1%) graft losses were reported in 267 patients [27,29]. Considering its immune-modulating effects, leflunomide is often used in place of MMF in cases of BKVAN [7]. Adverse events of leflunomide include hepatitis, hemolysis, thrombotic microangiopathy, myelosuppres-

sion, and fungal pneumonia. Thus, monitoring the complete blood count and performing liver function tests every 4 weeks is mandated.

#### *Fluoroquinolones*

Fluoroquinolones, including ciprofloxacin, inhibit BKPyV replication by affecting the helicase activity of the virus-encoded large T antigen [8]. However, in a randomized controlled trial to determine the effectivity of a 3-month course of ciprofloxacin as BKPyV prophylaxis in KT, ciprofloxacin not only failed to improve the allograft outcome but also increased levels of BKPyV-DNA and incidence of fluoroquinolone-resistant Gram-negative infections [30].

## Special consideration for children

Similar to other common infections, children often require immunosuppression before primary infection with BKPyV. Therefore, they are more likely to be BKPyV-seronegative, which increases both the risk, severity, and duration of viral replication [31–34]. Thus, children may benefit more from intravenous immunoglobulins administration [35]. If they are BKPyV-seropositive, this means exposure to BKPyV was a recent event, which is why younger children harbor higher levels of immune effectors. Children with end-stage kidney disease often have urinary tract anomalies, which carry a risk of viral reactivation similar to a ureteric stent [31]. In addition, there may be hyperfiltration due to donor-recipient size mismatch in pediatric KT, which may delay the diagnosis of BKVAN [7]. Therefore, screening in children must be extended to a longer period [7].

## Conclusions

BKVAN, although uncommon, threatens allograft survival in KT. Currently, there is no approved and effective treatment for BKVAN. To prevent BKVAN, meticulous screening up to the third year post-KT and appropriate modification of immunosuppression is necessary to improve outcomes.

## Conflicts of interest

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## References

- Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* 1971;1:1253-7.
- Hurdiss DL, Frank M, Snowden JS, Macdonald A, Ranson NA. The structure of an infectious human polyomavirus and its interactions with cellular receptors. *Structure* 2018;26:839-47.
- Mengel M, Marwedel M, Radermacher J, Eden G, Schwarz A, Haller H, et al. Incidence of polyomavirus-nephropathy in renal allografts: influence of modern immunosuppressive drugs. *Nephrol Dial Transplant* 2003;18:1190-6.
- Patel H, Rodig N, Agrawal N, Cardarelli F. Incidence and risk factors of kidney allograft loss due to BK nephropathy in the pediatric population: a retrospective analysis of the UNOS/OPTN database. *Pediatr Transplant* 2021;25:e13927.
- Cho MH. Monitoring BK virus infection in pediatric kidney transplant recipients. *Korean J Pediatr* 2019;62:414-5.
- Myint TM, Chong C, Wyld M, Nankivell B, Kable K, Wong G. Polyoma BK virus in kidney transplant recipients: screening, monitoring, and management. *Transplantation* 2022;106:e76-89.
- Hirsch HH, Randhawa PS; AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant* 2019;33:e13528.
- Bohl DL, Brennan DC. BK virus nephropathy and kidney transplantation. *Clin J Am Soc Nephrol* 2007;2 Suppl 1:S36-46.
- Vanichanan J, Udomkarnjananun S, Avihingsanon Y, Jutivorakool K. Common viral infections in kidney transplant recipients. *Kidney Res Clin Pract* 2018;37:323-37.
- Kwon Y, Kim JY, Lee Y, Cho H. Clinical manifestations of BK virus infection in pediatric kidney transplant patients. *Korean J Pediatr* 2019;62:422-7.
- Lee YH, Kang HG. Pediatric kidney transplantation. *Child Kidney Dis* 2021;25:8-13.
- Schold JD, Rehman S, Kayle LK, Magliocca J, Srinivas TR, Meier-Kriesche HU. Treatment for BK virus: incidence, risk factors and outcomes for kidney transplant recipients in the United States. *Transpl Int* 2009;22:626-34.
- Demey B, Tinez C, Francois C, Helle F, Choukroun G, Duverlie G, et al. Risk factors for BK virus viremia and nephropathy after kidney transplantation: a systematic review. *J Clin Virol* 2018;109:6-12.
- Ducharme-Smith A, Katz BZ, Bobrowski AE, Backer CL, Pahl E. BK polyomavirus infection in pediatric heart transplant recipients: a prospective study. *Pediatr Transplant* 2017;21:e12830.
- Dufek S, Haitel A, Muller-Sacherer T, Aufrecht C. Duct Bellini carcinoma in association with BK virus nephropathy after lung transplantation. *J Heart Lung Transplant* 2013;32:378-9.
- Bedi A, Miller CB, Hanson JL, Goodman S, Ambinder RF, Charache P, et al. Association of BK virus with failure of prophylaxis against hemorrhagic cystitis following bone marrow transplantation. *J Clin Oncol* 1995;13:1103-9.
- Cesaro S, Dalianis T, Hanssen Rinaldo C, Koskenvuo M, Pegoraro A, Einsele H, et al. ECIL guidelines for the prevention, diagnosis and treatment of BK polyomavirus-associated haemorrhagic cystitis in haematopoietic stem cell transplant recipients. *J Antimicrob Chemother* 2018;73:12-21.
- Ruderfer D, Wu M, Wang T, Srivaths PR, Krance RA, Naik S, et al. BK virus epidemiology, risk factors, and clinical outcomes: an analysis of hematopoietic stem cell transplant patients at Texas Children's Hospital. *J Pediatric Infect Dis Soc* 2021;10:492-501.
- Laskin BL, Denburg MR, Furth SL, Moatz T, Altrich M, Kleiboeker S, et al. The natural history of BK polyomavirus and the host immune response after stem cell transplantation. *Clin Infect Dis* 2020;71:3044-54.
- Helle F, Brochot E, Handala L, Martin E, Castelain S, Francois C, et al. Biology of the BKPyV: an update. *Viruses* 2017;9:327.
- Sawinski D, Trofe-Clark J. BK virus nephropathy. *Clin J Am Soc Nephrol* 2018;13:1893-6.
- Nickeleit V, Singh HK, Randhawa P, Drachenberg CB, Bhatnagar R, Bracamonte E, et al. The Banff Working Group classification of definitive polyomavirus nephropathy: morphologic definitions and clinical correlations. *J Am Soc Nephrol* 2018;29:680-93.
- Hirsch HH, Randhawa P; AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation. *Am J Transplant* 2013;13 Suppl 4:179-88.
- Ryu TH, Kim Hy, Oh JS, Kim JK. Combination therapy of low-dose

- cidofovir and leflunomide in a kidney transplant recipient with BK virus nephropathy: a case report. *Korean J Transplant* 2021;35(Supple 1):S61.
25. Mallat SG, Tanios BY, Itani HS, Lotfi T, McMullan C, Gabardi S, et al. CMV and BKPyV infections in renal transplant recipients receiving an mTOR inhibitor-based regimen versus a CNI-based regimen: a systematic review and meta-analysis of randomized, controlled trials. *Clin J Am Soc Nephrol* 2017;12:1321-36.
  26. Ahlenstiel-Grunow T, Pape L. Immunosuppression, BK polyomavirus infections, and BK polyomavirus-specific T cells after pediatric kidney transplantation. *Pediatr Nephrol* 2020;35:625-31.
  27. Schneidewind L, Neumann T, Drager DL, Kranz J, Hakenberg OW. Leflunomide in the treatment of BK polyomavirus associated nephropathy in kidney transplanted patients: a systematic review. *Transplant Rev (Orlando)* 2020;34:100565.
  28. Bernhoff E, Tylden GD, Kjerpeseth LJ, Gutteberg TJ, Hirsch HH, Rinaldo CH. Leflunomide inhibition of BK virus replication in renal tubular epithelial cells. *J Virol* 2010;84:2150-6.
  29. Jung YH, Moon KC, Ha JW, Kim SJ, Ha IS, Cheong HI, et al. Leflunomide therapy for BK virus allograft nephropathy after pediatric kidney transplantation. *Pediatr Transplant* 2013;17:E50-4.
  30. Patel SJ, Knight RJ, Kuten SA, Graviss EA, Nguyen DT, Moore LW, et al. Ciprofloxacin for BK viremia prophylaxis in kidney transplant recipients: results of a prospective, double-blind, randomized, placebo-controlled trial. *Am J Transplant* 2019;19:1831-7.
  31. Hocker B, Schneble L, Murer L, Carraro A, Pape L, Kranz B, et al. Epidemiology of and risk factors for BK polyomavirus replication and nephropathy in pediatric renal transplant recipients: an international CERTAIN registry study. *Transplantation* 2019;103:1224-33.
  32. Schmidt T, Adam C, Hirsch HH, Janssen MW, Wolf M, Dirks J, et al. BK polyomavirus-specific cellular immune responses are age-dependent and strongly correlate with phases of virus replication. *Am J Transplant* 2014;14:1334-45.
  33. Smith JM, McDonald RA, Finn LS, Healey PJ, Davis CL, Limaye AP. Polyomavirus nephropathy in pediatric kidney transplant recipients. *Am J Transplant* 2004;4:2109-17.
  34. Ginevri F, De Santis R, Comoli P, Pastorino N, Rossi C, Botti G, et al. Polyomavirus BK infection in pediatric kidney-allograft recipients: a single-center analysis of incidence, risk factors, and novel therapeutic approaches. *Transplantation* 2003;75:1266-70.
  35. Miettinen J, Lautenschlager I, Weissbach F, Wernli M, Auvinen E, Mannonen L, et al. BK polyomavirus viremia and antibody responses of pediatric kidney transplant recipients in Finland. *Pediatr Transplant* 2019;23:e13324.

# Renal involvement in pediatric rheumatologic diseases

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Pediatric rheumatologic diseases are rare systemic diseases that can involve various organs, including the kidneys. Each rheumatologic disease can exhibit characteristic renal involvement, which requires proper treatment and diagnosis. In this review, we discuss renal involvement in classic rheumatologic diseases, including juvenile idiopathic arthritis, Sjogren's syndrome, systemic sclerosis, and juvenile dermatomyositis. Reviews addressing lupus nephritis and antineutrophil cytoplasmic antibody-associated renal disease are complex and tend to cover a wide array of topics, and thus were excluded from this review.

**Keywords:** Arthritis, juvenile; Kidney diseases; Rheumatic diseases; Scleroderma, systemic; Sjogren's syndrome

## Introduction

The kidneys are important target organs involved with systemic disease, which includes rheumatologic disease. The field of pediatric rheumatology originated in the first half of the 20th century and has a relatively short history compared to other medical fields. It started principally with interest in juvenile chronic inflammatory arthritis, the most common childhood rheumatologic disease [1]. Currently, its scope is expanding to address rare disease groups that have recently been elucidated, including autoinflammatory syndrome. Pediatric rheumatologic disease mainly involves various acute and chronic diseases targeting the musculoskeletal system, blood vessels, and other tissues, and is still a significant cause of chronic illness in children worldwide; although it remains among one of the smallest pediatric subspecialties [2]. Pediatric rheumatologic diseases are frequently associated with renal disease as a part of systemic autoimmune disease, and in some diseases such

as systemic lupus erythematosus and antineutrophil cytoplasmic antibody-associated vasculitis, the kidney is the main target organ that can indicate the long-term prognosis. Renal manifestations in childhood rheumatologic disease vary from asymptomatic to end-stage kidney disease (Fig. 1). It is important to recognize that renal abnormalities can be a symptom of rheumatologic disease because they can provide important signals towards establishing a personalized treatment plan. In addition, kidney abnormalities may be a presenting symptom of rheumatologic disease; in this case, clinicians should attempt to identify the underlying disease. In this paper, we review kidney problems that can be accompanied by representative pediatric rheumatologic diseases, including juvenile idiopathic arthritis (JIA), Sjogren's syndrome (SS), systemic sclerosis/scleroderma, and juvenile dermatomyositis (JDM). Lupus nephritis and antineutrophil cytoplasmic antibody-associated renal disease tend to expand over a wide range of topics, and thus were excluded from this review.

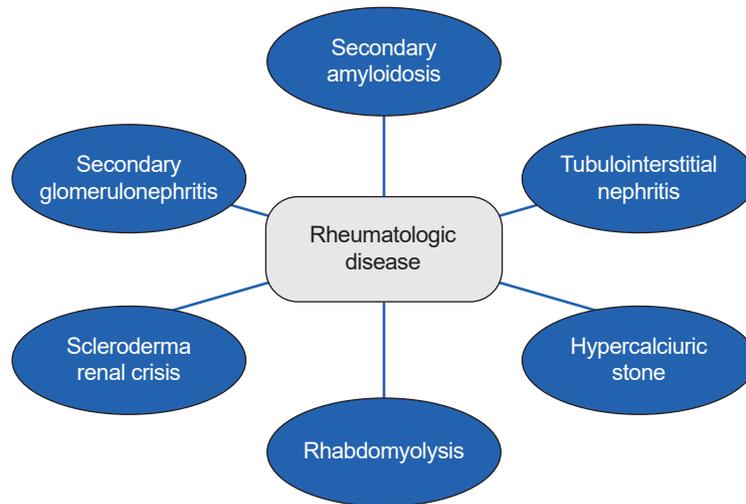
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**Fig. 1.** The spectrum of renal involvement in pediatric rheumatologic diseases.

## Juvenile idiopathic arthritis

JIA is characterized by chronic noninfectious inflammation of the joints and encompasses a complex group of diseases. It is the most famous and frequent rheumatoid disease in children and is classified into several groups, according to clinical and laboratory characteristics. In the early history of JIA, gold nephropathy was an interesting kidney disease associated with the use of intramuscular gold salts, although there is currently no gold treatment. The pathological picture of gold nephropathy is drug-induced membranous glomerulonephritis that usually resolves over time if gold treatment is stopped [3,4].

Although it is difficult to determine the cause of renal abnormalities in JIA, according to one prospective study in adults, proteinuria and decreased renal function were mainly due to drug side effects, while hematuria was associated with the disease itself [5]. The renal diseases associated with JIA have rarely been reported, yet include renal amyloidosis, glomerulonephritis, and drug-induced tubulointerstitial nephritis (TIN). Amyloidosis is the most characteristic lesion associated with chronic systemic inflammation in JIA [6].

### Renal amyloidosis

Amyloidosis is characterized by the deposition of amyloid fibrils in organs and there are a number of subtypes. Amyloid A (AA) amyloidosis is caused by the overproduction of the precursor of AA protein, which is produced in response to systemic inflammation, while amyloid light-chain amyloidosis is caused by the

overproduction of monoclonal immunoglobulin light chains. Only AA amyloidosis (secondary amyloidosis) can occur in children with JIA [7,8]. In the past, amyloidosis was the main cause of death in JIA; however, recently, the prognosis has improved [8-10]. Renal amyloidosis occurs most commonly in systemic onset JIA (sJIA), followed by polyarticular JIA [8,9]. Renal amyloidosis insidiously progresses, causing massive proteinuria from an asymptomatic state, and consequently leading to end-stage renal disease. Hematuria is rarely accompanied [8,11]. Regular urinalysis is required in patients with sJIA or polyarticular JIA as asymptomatic proteinuria is the most common initial symptom [7]. It can be confirmed by renal biopsy which demonstrates amyloid fibrils, although the correlation between the degree of amyloid deposition and clinical symptoms are not clear. Treatments that control the inflammatory cascade caused by the underlying diseases are critical. Several disease-modifying antirheumatic drugs and biologics have been used to control these diseases. Since renal amyloidosis occurs in a situation where JIA is not well controlled by standard drugs such as methotrexate, sulfasalazine, and hydroxychloroquine, it is usually treated by adding disease-modifying antirheumatic drugs or biologics or switching biologics after the diagnosis of amyloidosis [12]. Interleukin-6 inhibitor, tocilizumab has become the standard treatment for sJIA, and it can play an important role in treating secondary amyloidosis by suppressing serum AA levels [13,14].

According to one large study conducted in 2008, of the 3,500 patients with JIA, 24 patients with biopsy-proven amyloidosis

were detected. Ten patients died, but the cause of death was associated with JIA itself rather than with amyloidosis. Of the 14 survivors, three patients underwent kidney transplants, and 11 patients maintained normal renal function at last follow-up. Proteinuria improved completely in four patients who initially had proteinuria [8]. Renal disease can be improved by early intensive treatment. Therefore, it is essential to monitor regularly the occurrence of amyloidosis in JIA patients.

### Glomerulonephritis and drug-induced TIN

Several studies on adult rheumatoid arthritis (RA) suggest that there is an association between RA and different types of glomerulonephritis, although studies on glomerulonephritis in JIA are extremely rare. Mesangial proliferative glomerulonephritis is the most commonly reported type of glomerulonephritis in adults with RA [15]. In JIA, membranous nephropathy, mesangial glomerulonephritis, focal segmental glomerulosclerosis, and crescentic glomerulonephritis have also reported [15-21]. Nephrotic syndrome in JIA is extremely rare and is usually caused by amyloidosis rather than glomerulonephritis [20,22]. The pathogenesis of renal involvement in JIA remains unclear. Immunologic abnormalities related to the occurrence of JIA, including hypergammaglobulinemia, abnormal B and T cell mitogen responsiveness, decreased T suppressor activity, and uncontrolled cytokine production, are presumed to lead to renal involvement [23,24]. In JIA, the treatment for glomerulonephritis is generally conservative with angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers. However, severe cases with rapidly progressive glomerulonephritis or massive proteinuria require intensive treatments with immunosuppressants [16-22]. One should be aware that drugs used as treatments can also cause renal abnormalities. Drugs commonly used in JIA include nonsteroidal anti-inflammatory drugs, proton pump inhibitor, methotrexate, sulfasalazine, leflunomide, etc. Since these drugs can often cause renal abnormalities such as acute tubular necrosis and TIN. Clinicians should be careful to identify the cause of renal abnormalities in patients taking these drugs.

### Sjogren's syndrome

Primary SS is a chronic autoimmune disease characterized by inflammation of exocrine glands including salivary and lacrimal glands [25]. In addition, it can demonstrate various exocrinopathy involving respiratory, urogenital tract and skin.

Furthermore, extraglandular and systemic symptoms can also be accompanied [26]. SS is typically classified as primary or secondary. Primary SS has no association with other autoimmune diseases, while secondary SS has another underlying or combined autoimmune disease such as systemic lupus erythematosus, RA, and mixed connective tissue disease [27]. The suggested pathogenesis is that genetically susceptible individuals are exposed to environmental factors such as infection, leading to inflammatory processes in target tissues [28]. Definite diagnosis of SS is difficult, because cardinal symptoms in SS can be commonly seen and there is no diagnostic gold standard test. Clinicians generally use classification criteria for diagnosis, such as rheumatologic diseases. Autoantibodies to nuclear antigens Ro/SSA and La/SSB are the hallmarks of SS, but typically are not present in 30% to 50% of SS.

Renal involvement is uncommon in patients with SS; 5% to 33% of adult SS patients and approximately 10% of pediatric SS patients having renal involvement [29,30]. TIN is most frequently reported. TIN usually results from activated lymphocytic infiltration (primarily CD4+ T lymphocyte) to tubular epithelium and interstitium around the renal tubules, and this pathophysiology is similar to the process that occurs in exocrine glands [31]. TIN presents tubular dysfunction including renal tubular acidosis (mostly distal type but, proximal or mixed type is also possible), renal Fanconi syndrome, Bartter and Gitelman syndromes and nephrogenic diabetes insipidus [30,32-36]. Hypokalemia is a general symptom in SS-related renal disease, which is observed in about 40% of patients [32]. Actually, rheumatologic diseases are the second common cause of TIN accounting for 10% to 20% of all TIN cases [37]. Several rheumatologic diseases associated with TIN are described in Table 1.

Glomerular disease is less prevalent than tubular disease, and its pathophysiological process is mediated by the immune complex, while TIN is mediated by direct lymphocytic infiltration. Membranoproliferative glomerulonephritis due to cryoglobulinemia is the most commonly reported condition. In a study of 22 children with SS, 13 children showed renal involvement, only three of whom had glomerulonephritis, including mesangial proliferative glomerulonephritis, immunoglobulin A (IgA) deposits, membranous glomerulonephritis, and pauci-immune crescentic glomerulonephritis [29,38-40].

Immunosuppressants are not universally required in patients with SS; however, immunosuppressants such as steroid may be helpful in cases of severe systemic symptoms, includ-

**Table 1.** Rheumatologic diseases associated with TIN

Rheumatologic diseases	Diagnostic clues	
	Laboratory	Clinical
Sjogren's syndrome	Anti-Ro, anti-La, RF, ANA, cryoglobulin, (very) high ESR, high serum IgG	Recurrent parotitis, sicca syndrome, arthritis, neurological involvement (peripheral neuropathy and demyelinating disease), interstitial lung disease
Sarcoidosis	Hypercalcemia with/without hypercalciuria Increased 1,25(OH) <sub>2</sub> vitamin D High inflammatory markers Increased serum ACE level (not consistent, unclear in children) Autoantibodies usually (-)	Constitutional symptoms (weight loss, fatigue, fever), pulmonary symptoms (hilar adenopathy, pulmonary infiltration), pericarditis, subcutaneous nodules, polyarticular arthritis, ocular diseases (iridocyclitis, uveitis) myositis, hepatosplenomegaly
IgG4-related disease	(Very) high serum IgG High serum IgG4 (>140 mg/dL), IgG4 (+) plasma cell (pathology) Hypocomplementemia (hypocomplementemic TIN with extensive deposits) Autoantibodies usually (-)	Enlargement of the lacrimal and salivary gland (similar to Sjogren's syndrome), submandibular lymphadenopathy, ocular inflammation pancreatitis, hepatobiliary disease, retroperitoneal fibrosis, arthritis, thyroiditis, periaortitis
Scleroderma (mostly systemic sclerosis)	Scl-70, ANA, centromere	Raynaud phenomenon, digital ulcers, skin thickness, sclerodactyly, pulmonary hypertension, joint contractures, arthritis, interstitial lung disease
TINU syndrome	Increased ESR, mild anemia	Eye pain and redness, photophobia, vision change, constitutional symptoms (fever, fatigue), abdominal pain, arthralgia

TIN, tubulointerstitial nephritis; RF, rheumatoid factor; ANA, antinuclear antibody; ESR, erythrocyte sedimentation rate; IgG, immunoglobulin G; ACE, angiotensin-converting enzyme; TINU, tubulointerstitial nephritis and uveitis.

ing arthritis, fatigue, and recurrent parotitis [28]. Patients with symptomatic TIN also require treatment. Patients with TIN associated with SS generally respond well to steroid alone. In cases of steroid dependency, other immunosuppressants may be effective in reducing steroids. Steroid with rituximab and plasmapheresis are effective [25].

It is important to monitor for renal involvement in known SS patients, and it is also important to consider SS as the cause in patients with renal disease, primarily TIN, especially for nephrologists. However, it is challenging because symptoms such as sicca are insidious and commonly unclear [30]. In this case, high serum immunoglobulin G (IgG) level, positive rheumatoid factor, positive anti-Ro, anti-La, very high erythrocyte sedimentation rate, and cryoglobulins are clues suggesting SS [28,41]. In a previous adult study, long-term renal prognosis appeared to be good, however, the risk of chronic kidney disease is significantly elevated relative to the general population [42].

### IgG4-related disease

IgG4-related disease (IgG4-RD) is a new disease entity that has emerged in the last decade and shares several common symptoms with SS, such as glandular enlargement, sicca, arthralgia, and high levels of IgG [43]. It is characterized by highly

increased levels of IgG4 (>140 mg/dL) and striking IgG-producing plasma cell infiltration in the affected organ. The clinical manifestations varied widely. Diagnosis is usually made by histological examination demonstrating a classic fibrotic lesion with IgG4+ plasma cells. IgG4-RD is rare, occurring mainly in middle-aged men. TIN is the most common renal disease in IgG4-RD. Obstructive acute kidney injury (AKI) caused by retroperitoneal fibrosis can occur [44]. The renal IgG4-RD in children has been reported very limitedly. In most cases, it occurred concomitantly with other organ manifestations, except for one isolated renal IgG4 pseudotumor [45,46]. The use of steroid tends to be effective in most cases. IgG4-RD needs to be considered in cases with TIN accompanied by involvement of several organs, although it is rare.

### Systemic sclerosis and localized scleroderma

Scleroderma disorders include both systemic sclerosis (SSc) and localized scleroderma (LS). LS is mainly restricted to the skin, whereas SSc affects the skin, vessels, and internal organs, including the gastrointestinal, pulmonary, and musculoskeletal organs [47]. It is an autoimmune disease characterized by vasculopathy and fibrosis. Renal involvement is uncommon in both SSc and LS, although mild renal dysfunction commonly

occurs due to vasculopathy in SSc with a close frequency in children and adults with SSc. The most specific renal involvement is scleroderma renal crisis (SRC), which is more common in SSc. It is characterized by progressive AKI with severe hypertension, microangiopathic hemolytic anemia, and thrombocytopenia and renal involvement may continue asymptomatic until the late stages. In the past, it was a major cause of death, but recently, with appropriate treatment, the mortality rate has decreasing [22,48,49]. SRC has rarely been reported in children with SSc [47]. The pathogenesis of SRC still remains elusive, but the essential process is suspected to be injury of endothelial cells, leading to intimal thickening and proliferation of branched renal arteries.

The common histologic finding is an onion skin lesion of the renal interlobular artery. Additionally, episodic vasospasm in cortical arteries contributes to renal ischemia or hypoperfusion and activation of the renin-angiotensin system [48,50]. This mechanism is often called the renal Raynaud phenomenon, in connection with the fact that Raynaud phenomenon in fingers and toes is a typical and essential symptom in SSc. Early recognition of SRC is essential for its management. In adult guidelines, ACE inhibitors are recommended as the first-line treatment. If treatment is delayed, there is a possibility of irreversible kidney damage and death [51]. Special attention should be drawn to the development of SRC in patients taking steroid since studies have demonstrated an association between SRC and steroid in adult. In severe cases, treatment with eculizumab, a C5 blocker, may be needed, similar to refractory systemic thrombotic microangiopathy [48]. The effect of prophylactic ACE inhibitor is not evident. Although, compared to other rheumatologic diseases, secondary amyloidosis is very rare in SSc, it should be considered in cases of long-standing and progressive SSc with proteinuria [52].

## Juvenile dermatomyositis

JDM is a rare systemic autoimmune disease mainly affects skin and muscles and accounts for 80% to 85% of all inflammatory myopathies in children. It is characterized by proximal muscle weakness and typical skin rashes, such as heliotrope rashes and Gottron papules. Other organs can be involved including the lungs, heart, gastrointestinal tract, and kidneys. Constitutional symptoms such as fever, fatigue, anorexia, and weight loss are common, and the onset is usually insidious. Histological findings are characterized by vascular and perivascular inflamma-

tion, and vasculopathy is considered the key to the pathogenesis of myositis and cutaneous symptoms. It is also associated with other manifestations including intestinal perforation, ulcerations, pulmonary disease, and cutaneous calcinosis [53]. The spectrum of renal disease in inflammatory myopathies in adults includes AKI, chronic kidney disease, glomerulonephritis, myoglobinuria, and hypertension, while data on children with JDM are lacking [54]. Membranous nephropathy was the most common chronic renal sequela in adults with inflammatory myopathy [55]. Few case reports in JDM demonstrated IgA nephropathy and nephrotic syndrome with AKI.

The children responded well to treatment with steroids and methotrexate for the primary disease, JDM, and usually do not require additional drugs for renal involvement except ACE inhibitors or angiotensin receptor blockers. However, in cases of IgA nephropathy with nephrotic-range proteinuria or progressive AKI, additional immunosuppressants such as cyclosporin, mycophenolate mofetil, azathioprine, and cyclophosphamide should be considered [56,57].

Rhabdomyolysis with AKI is extremely rare in JDM, but can occur especially in fulminant JDM with multiorgan involvement [58]. Macrophage activation syndrome may occur as a result of excessive systemic inflammation in JDM such as sJIA, and one case of thrombotic microangiopathy secondary to macrophage activation syndrome in JDM has been reported [59].

## Conclusions

Rheumatologic diseases in children can affect various organs, including the musculoskeletal, cutaneous, pulmonary, heart, gastrointestinal, central nervous system, and kidneys. It is necessary to understand the type of renal disease associated with each rheumatologic disease to properly monitor and treat renal involvement in patients with rheumatologic diseases. In certain cases where patients are initially expressing symptoms of renal disease alone, we should try to find the underlying diseases, and then renal disease can be a diagnostic clue for underlying rheumatologic diseases.

## Conflicts of interest

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## References

- Schaller JG. The history of pediatric rheumatology. *Pediatr Res* 2005;58:997-1007.
- Srinivasalu H, Riebschleger M. Medical education in pediatric rheumatology: unique challenges and opportunities. *Clin Rheumatol* 2020;39:643-50.
- Husserl FE, Shuler SE. Gold nephropathy in juvenile rheumatoid arthritis. *Am J Dis Child* 1979;133:50-2.
- Rovenska E, Kapeller K, Rossmann P. Gold nephropathy in rheumatoid arthritis and in juvenile chronic arthritis. *Czech Med* 1979;2:125-33.
- Koseki Y, Terai C, Moriguchi M, Uesato M, Kamatani N. A prospective study of renal disease in patients with early rheumatoid arthritis. *Ann Rheum Dis* 2001;60:327-31.
- Deschenes G, Prieur AM, Hayem F, Broyer M, Gubler MC. Renal amyloidosis in juvenile chronic arthritis: evolution after chlorambucil treatment. *Pediatr Nephrol* 1990;4:463-9.
- Bilginer Y, Akpolat T, Ozen S. Renal amyloidosis in children. *Pediatr Nephrol* 2011;26:1215-27.
- Immonen K, Savolainen A, Kautiainen H, Hakala M. Longterm outcome of amyloidosis associated with juvenile idiopathic arthritis. *J Rheumatol* 2008;35:907-12.
- David J, Vouyiouka O, Ansell BM, Hall A, Woo P. Amyloidosis in juvenile chronic arthritis: a morbidity and mortality study. *Clin Exp Rheumatol* 1993;11:85-90.
- Schneider R, Passo MH. Juvenile rheumatoid arthritis. *Rheum Dis Clin North Am* 2002;28:503-30.
- Dhillon V, Woo P, Isenberg D. Amyloidosis in the rheumatic diseases. *Ann Rheum Dis* 1989;48:696-701.
- Chantarogh S, Vilaiyuk S, Tim-Aroon T, Worawichawong S. Clinical improvement of renal amyloidosis in a patient with systemic-onset juvenile idiopathic arthritis who received tocilizumab treatment: a case report and literature review. *BMC Nephrol* 2017;18:159.
- Gupta A, Bagri NK, Tripathy SK, Barwad A, Phulwara RH, Hari P. Successful use of tocilizumab in amyloidosis secondary to systemic juvenile idiopathic arthritis. *Rheumatol Int* 2020;40:153-9.
- Okuda Y, Takasugi K. Successful use of a humanized anti-interleukin-6 receptor antibody, tocilizumab, to treat amyloid A amyloidosis complicating juvenile idiopathic arthritis. *Arthritis Rheum* 2006;54:2997-3000.
- Mielants H, Van den Bosch F. Extra-articular manifestations. *Clin Exp Rheumatol* 2009;27(4 Suppl 55):S56-61.
- Adu D, Berisa F, Howie AJ, Emery P, Bacon PA, McConkey B, et al. Glomerulonephritis in rheumatoid arthritis. *Br J Rheumatol* 1993;32:1008-11.
- Bandin F, Merhenberger M, Modesto A, Brochard K, Decramer S. Steroid-responsive nephrotic syndrome in a child with juvenile idiopathic arthritis. *Pediatr Nephrol* 2008;23:651-4.
- Breedveld FC, Valentijn RM, Westedt ML, Weening JJ. Rapidly progressive glomerulonephritis with glomerular crescent formation in rheumatoid arthritis. *Clin Rheumatol* 1985;4:353-9.
- Helin HJ, Korpela MM, Mustonen JT, Pasternack AI. Renal biopsy findings and clinicopathologic correlations in rheumatoid arthritis. *Arthritis Rheum* 1995;38:242-7.
- Gedalia A, Mendez EA, Craver R, Vehaskari M, Espinoza LR. Renal involvement in juvenile rheumatoid arthritis: report of two cases. *Clin Rheumatol* 2001;20:153-6.
- Varma S. Juvenile rheumatoid arthritis with focal segmental glomerulosclerosis: a rare association. *Pediatr Nephrol* 2010;25:2189-90.
- Kari JA, Bamashmous H, Mahan JD. Steroid-sensitive nephrotic syndrome and juvenile idiopathic arthritis. *Pediatr Nephrol* 2002;17:975-6.
- Lipnick RN, Tsokos GC, Magilavy DB. Immune abnormalities in the pathogenesis of juvenile rheumatoid arthritis. *Rheum Dis Clin North Am* 1991;17:843-57.
- Foster BJ, Duffy CM, Sharma AK. Systemic juvenile rheumatoid arthritis complicated by two different renal lesions. *Pediatr Nephrol* 1998;12:113-6.
- Francois H, Mariette X. Renal involvement in primary Sjogren syndrome. *Nat Rev Nephrol* 2016;12:82-93.
- Pessler F, Emery H, Dai L, Wu YM, Monash B, Cron RQ, et al. The spectrum of renal tubular acidosis in paediatric Sjogren syndrome. *Rheumatology (Oxford)* 2006;45:85-91.
- Brito-Zeron P, Baldini C, Bootsma H, Bowman SJ, Jonsson R, Mariette X, et al. Sjogren syndrome. *Nat Rev Dis Primers* 2016;2:16047.
- Tucker L, Ciurtin C. Sjogren syndrome and immunoglobulin-G4 disease. In: Petty RE, Laxer RM, Lindsley CB, Wedderburn LR, Mellins ED, Fuhlbrigge RC, editors. *Textbook of pediatric rheumatology*. 8th ed. Philadelphia: Elsevier; 2021. p. 417-26.
- Zhao J, Chen Q, Zhu Y, Zhao M, Liu J, Zhang Z, et al. Nephrological disorders and neurological involvement in pediatric primary Sjogren

- syndrome: a case report and review of literature. *Pediatr Rheumatol Online J* 2020;18:39.
30. Aiyegbusi O, McGregor L, McGeoch L, Kipgen D, Geddes CC, Stevens KI. Renal disease in primary Sjogren's syndrome. *Rheumatol Ther* 2021;8:63-80.
  31. Katsifis GE, Rekka S, Moutsopoulos NM, Pillemer S, Wahl SM. Systemic and local interleukin-17 and linked cytokines associated with Sjogren's syndrome immunopathogenesis. *Am J Pathol* 2009;175:1167-77.
  32. Goules A, Geetha D, Arend LJ, Baer AN. Renal involvement in primary Sjogren's syndrome: natural history and treatment outcome. *Clin Exp Rheumatol* 2019;37 Suppl 118:123-32.
  33. Gu X, Su Z, Chen M, Xu Y, Wang Y. Acquired Gitelman syndrome in a primary Sjogren syndrome patient with a SLC12A3 heterozygous mutation: a case report and literature review. *Nephrology (Carlton)* 2017;22:652-5.
  34. Ren H, Wang WM, Chen XN, Zhang W, Pan XX, Wang XL, et al. Renal involvement and followup of 130 patients with primary Sjogren's syndrome. *J Rheumatol* 2008;35:278-84.
  35. Cohen EP, Bastani B, Cohen MR, Kolner S, Hemken P, Gluck SL. Absence of H(+)-ATPase in cortical collecting tubules of a patient with Sjogren's syndrome and distal renal tubular acidosis. *J Am Soc Nephrol* 1992;3:264-71.
  36. Pedro-Botet J, Tomas S, Soriano JC, Coll J. Primary Sjogren's syndrome associated with Bartter's syndrome. *Clin Exp Rheumatol* 1991;9:210-2.
  37. Oliva-Damaso N, Oliva-Damaso E, Payan J. Acute and chronic tubulointerstitial nephritis of rheumatic causes. *Rheum Dis Clin North Am* 2018;44:619-33.
  38. Jung SK, Park KH, Yim HE, Yoo KH, Hong YS, Lee JW, et al. Primary Sjogren's syndrome with mesangial proliferative glomerulonephritis and IgA deposits in a child. *Pediatr Nephrol* 2010;25:567-8.
  39. Yoshida K, Suzuki J, Kume K, Suzuki S, Isome M, Kato K, et al. Sjogren's syndrome with membranous glomerulonephritis detected by urine screening of schoolchildren. *Acta Paediatr Jpn* 1996;38:533-6.
  40. Kagan M, Bervina N, Vorobyeva O, Wouters C, Levtchenko E. Pauci-immune crescentic glomerulonephritis complicating Sjogren's syndrome in a 12-year-old girl. *Pediatr Nephrol* 2011;26:991-2.
  41. Dorfman RF, Berry GJ. Kikuchi's histiocytic necrotizing lymphadenitis: an analysis of 108 cases with emphasis on differential diagnosis. *Semin Diagn Pathol* 1988;5:329-45.
  42. Lin CY, Wu CH, Chen HA, Hsu CY, Wang LH, Su YJ. Long-term renal prognosis among patients with primary Sjogren's syndrome and renal involvement: a nationwide matched cohort study. *J Autoimmun* 2020;113:102483.
  43. Fragoulis GE, Zampeli E, Moutsopoulos HM. IgG4-related sialadenitis and Sjogren's syndrome. *Oral Dis* 2017;23:152-6.
  44. Boffa JJ, Esteve E, Buob D. Renal involvement in IgG4-related disease. *Presse Med* 2020;49:104017.
  45. Johnson JS, Saltzman AF, Treece AL, Cost NG. A case of IgG4-related renal pseudotumor in a child with history of Wilms tumor. *Urol Case Rep* 2018;21:107-9.
  46. Karim F, Loeffen J, Bramer W, Westenberg L, Verdijk R, van Hagen M, et al. IgG4-related disease: a systematic review of this unrecognized disease in pediatrics. *Pediatr Rheumatol Online J* 2016;14:18.
  47. Torok KS, Stevens AM. Juvenile systemic sclerosis. In: Petty RE, Laxer RM, Lindsley CB, Wedderburn LR, Mellins ED, Fuhlbrigge RC, editors. *Textbook of pediatric rheumatology*. 8th ed. Philadelphia: Elsevier; 2021. p. 377-401.
  48. Chrabaszcz M, Malyszko J, Sikora M, Alda-Malicka R, Stochmal A, Matuszkiewicz-Rowinska J, et al. Renal involvement in systemic sclerosis: an update. *Kidney Blood Press Res* 2020;45:532-48.
  49. Denton CP, Khanna D. Systemic sclerosis. *Lancet* 2017;390:1685-99.
  50. Herrick AL. Vascular function in systemic sclerosis. *Curr Opin Rheumatol* 2000;12:527-33.
  51. Steen VD. Scleroderma renal crisis. *Rheum Dis Clin North Am* 2003;29:315-33.
  52. Pamuk GE, Pamuk ON, Altiparmak MR, Tanverdi M, Uygun N, Yurdakul S. Secondary amyloidosis in progressive systemic sclerosis. *Clin Rheumatol* 2001;20:285-7.
  53. Papadopoulou C, McCann LJ. The vasculopathy of juvenile dermatomyositis. *Front Pediatr* 2018;6:284.
  54. Sangeetha G, Dhanabal D, Mouttou Prebagarane S, Janarthanan M. Juvenile dermatomyositis: a case of delayed recognition with unusual complication of nephrocalcinosis. *BMJ Case Rep* 2021;14:e241152.
  55. Couvrat-Desvergnès G, Masseau A, Benveniste O, Bruel A, Hervier B, Mussini JM, et al. The spectrum of renal involvement in patients with inflammatory myopathies. *Medicine (Baltimore)* 2014;93:33-41.
  56. Mantoo MR, Tripathy SK, Phulware RH, Bagri NK, Hari P, Barwad A. Juvenile dermatomyositis with IgA nephropathy: case-based review. *Rheumatol Int* 2019;39:577-81.
  57. Wyatt RJ, Julian BA. IgA nephropathy. *N Engl J Med* 2013;368:2402-14.
  58. Stahl JL, Rutledge JC, Gordillo R. The unusual suspects: a curious case of acute kidney injury: answers. *Pediatr Nephrol* 2019;34:1213-5.
  59. Minoia F, Tibaldi J, Muratore V, Gallizzi R, Bracaglia C, Arduini A, et al. Thrombotic microangiopathy associated with macrophage activation syndrome: a multinational study of 23 patients. *J Pediatr* 2021;235:196-202.

# Mechanism, clinical consequences, and management of dyslipidemia in children with nephrotic syndrome

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Dyslipidemia in nephrotic syndrome (NS) is often characterized by marked increases in the levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol, and other lipoproteins, such as very low-density lipoprotein, intermediate-density lipoprotein, and lipoprotein(a). It has been suggested that impaired catabolism of lipoproteins and cholesterol is mainly due to decreased lipoprotein lipase and hepatic lipase activity, and increased biosynthesis of lipoproteins in the liver. The management strategies for dyslipidemia in patients with NS consist of lifestyle modification, lipid-lowering agents represented by statins, second-line agents such as fibrates and bile acid sequestrants, and lipid apheresis. Compared with dyslipidemia in adult NS patients, whose risks of atherosclerotic disease and progressive renal injury are considered high, clinical data on dyslipidemia in pediatric NS patients are limited. Therefore, it is necessary to pay more attention to the evaluation and management of dyslipidemia in pediatric patients with NS in clinical practice.

**Keywords:** Child; Dyslipidemias; Nephrotic syndrome

## Introduction

Nephrotic syndrome (NS) is a clinical syndrome characterized by severe proteinuria, hypoalbuminemia, generalized edema, and dyslipidemia. It is mainly caused by the leakage of large amounts of protein from the blood into the urine due to malfunction of the glomerular filtration barrier. Primary (idiopathic) NS comprises >90% of cases of non-hereditary NS in children and adolescents, with pathological findings including minimal changes disease and focal segmental glomerulosclerosis. Although the incidence rate varies according to race and region, it is known to be approximately 2 per 100,000 children under the age of 15 to 18 years. In South Korea, the annual inci-

dence of NS in pediatric patients is reported to be approximately 2 to 7 cases per 100,000 people, and the prevalence of NS is approximately 12 to 16 per 100,000 people [1,2].

It is well known that dyslipidemia in adult patients with NS significantly increases the risk of myocardial infarction and coronary artery disease, as well as progression of chronic kidney disease (CKD). However, studies on the risk of dyslipidemia in pediatric patients with NS are limited. Therefore, the importance of dyslipidemia management in pediatric patients with NS is often overlooked [3].

In this review, we describe the pathogenic mechanism, clinical consequences, and management of dyslipidemia in pediatric and adult patients with NS.

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## Pathogenesis of lipid abnormality

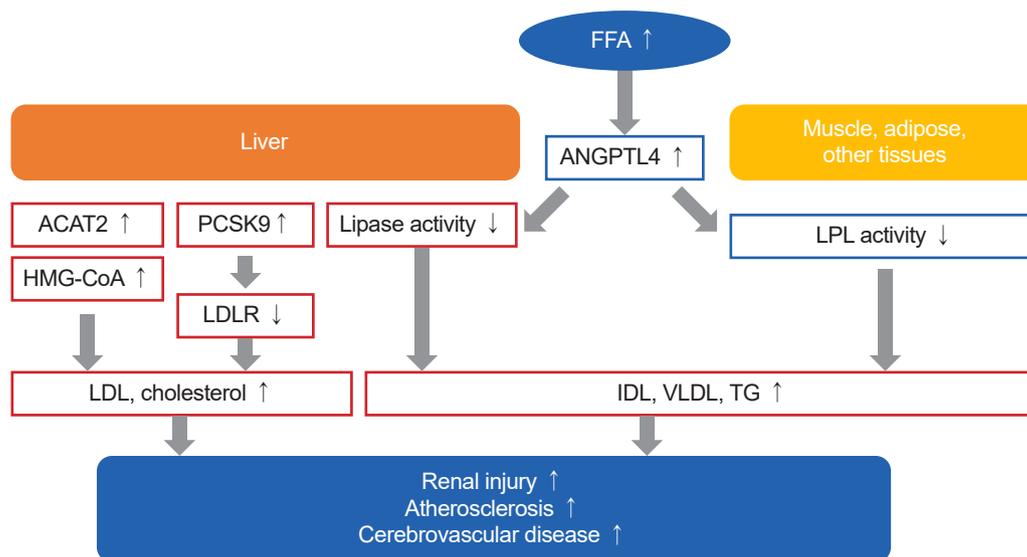
Lipids, mainly triglycerides (TG) and cholesterol, circulate in the body in the form of lipoproteins. Lipoproteins are formed from lipids packaged in apolipoproteins and phospholipids. The main forms of lipoproteins are chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL), which differ in composition and function.

It is known that there are three main pathways responsible for the production and transport of lipids within the body. First, in the exogenous pathway, dietary lipids ingested as food are packaged into chylomicrons in the intestinal mucosal cells and enter blood circulation through the lymphatic system. In the blood, TG, the main component of dietary lipids, is released as free fatty acids by lipoprotein lipase (LPL) in the capillary endothelium and transported to muscle, adipose tissue, and other peripheral tissues for absorption, and the remaining chylomicrons are transported to the liver for clearance. Second, in the endogenous pathway, VLDL generated in the liver is converted into IDL by LPL in the circulation and then absorbed into the

liver by the LDL receptor (LDLR), releasing TG and free fatty acids in this process. In addition, IDL is transformed into LDL by hepatic lipase and LDL is removed from the blood by binding to LDLR in the liver and extrahepatic tissues. Third, the lipid metabolism pathway, known as reverse cholesterol transport, occurs via HDL. HDL is the so-called anti-atherogenic lipoprotein or good cholesterol because it captures cholesterol from peripheral tissues and other lipoproteins and transports them back to the liver [3].

Dyslipidemia in NS is characterized by an increase in total cholesterol (TC), TG, LDL cholesterol, and other lipoproteins, such as VLDL, IDL, and lipoprotein(a). In contrast, HDL remains almost normal, but the ratio of HDL cholesterol to TC is decreased [4]. This lipid abnormality is mainly caused by impaired catabolism of lipoproteins and cholesterol and, to a lesser extent, increased biosynthesis of lipoproteins in the liver.

It has been suggested that impaired lipoprotein clearance is due to decreased hepatic lipase and LPL activity in the endothelium and peripheral tissues such as muscle and adipose tissues [3,5,6]. In detail, as shown in Fig. 1, proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates the expression of



**Fig. 1.** The pathophysiology and clinical consequences of dyslipidemia in nephrotic syndrome. In nephrotic syndrome, decreased hepatic lipase and lipoprotein lipase (LPL) activity in the extrahepatic tissues such as endothelium, muscle, and adipose tissue leads to impaired lipoprotein clearance, and thus increasing plasma levels of intermediate-density lipoproteins (IDL), very low-density lipoproteins (VLDL), triglycerides (TG). In addition, due to proteinuria and reduced free fatty acids (FFA) catabolism, as the ratio of FFA to albumin increases, levels of angiopoietin-like 4 (ANGPTL4) which inhibits lipase activity increase. Meanwhile, as the intrahepatic expression of proprotein convertase subtilisin/kexin type 9 (PCSK9) increases, low-density lipoproteins receptor (LDLR) degradation increases, resulting in reduced uptake of low-density lipoproteins (LDL) into hepatocytes. Furthermore, increased expression and activity of acetyl CoA acetyltransferase 2 (ACAT2) and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase lead to elevation of plasma levels of LDL and cholesterol via increased synthesis and esterification of free cholesterol within the liver. Consequently, accumulation of oxidized LDL and IDL may cause glomerulosclerosis and other adverse effects on kidney by mesangial cell proliferation, podocyte injury, and tubular cell injury. Also, increased lipoproteins and cholesterol promote atherosclerosis represented by cerebrovascular disease.

hepatic LDLR. In patients with NS, LDLR degradation increases as the intrahepatic expression of PCSK9 increases. Therefore, the uptake of LDL into hepatocytes is reduced and the clearance of LDL is disturbed [7-9]. In addition, when the ratio of free fatty acids to albumin in the blood increases due to proteinuria, circulating angiopoietin-like 4 levels increase, which leads to downregulation of hepatic lipase and a decrease in IDL clearance. Furthermore, as the permeability of the glomerular basement membrane increases and LPL activators decrease, LPL activity decreases and levels of IDL and VLDL increase [10,11]. Meanwhile, the level of immature HDL in the blood increases due to the reduction of cholesterol efflux through ATP-binding cassette subfamily A member 1, which is present in peripheral organs [3]. In patients with NS, the expression and activity of acetyl CoA acetyltransferase 2 (ACAT2) in the liver increases, which promotes cholesterol esterification and reduces the concentration of free cholesterol in cells. In an animal experiment using rats, it was reported that pharmacological inhibition of ACAT improved dyslipidemia and alleviated proteinuria [12,13]. Increase in 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity and cholesterol production have been observed in the liver of patients with NS [14,15].

## Clinical consequences of dyslipidemia in NS

### Atherosclerosis and cerebrovascular disease

In general, it is well known that dyslipidemia promotes atherosclerosis and is a risk factor for myocardial infarction or cerebrovascular disease (CVD). Dyslipidemia exacerbates platelet hyperreactivity, which increases the risk of thrombosis, and is often accompanied by atherosclerosis. Although clinical studies to confirm the increased risk of CVD in patients with NS are scarce, a case-control study reported that non-diabetic adults with NS have a 5.5-fold significantly higher risk of MI (95% confidence interval, 1.6–18.3) compared to those without NS [16]. Recently, a case-control study comprising 66 children with NS and 128 age- and sex-matched controls showed that carotid-intima media thickness as a surrogate marker of CVD is significantly higher in children with NS aged over 4 years [17]. Therefore, in patients with persistent NS and accompanying dyslipidemia, especially in the presence of other cardiovascular risk factors, lowering cholesterol levels is important for preventing the progression of atherosclerotic lesions [18].

### Renal injury

It has been suggested in several experiments and observations that dyslipidemia, accompanied with proteinuria and hypoalbuminemia, may increase renal organ damage and cause glomerulosclerosis by directly affecting mesangial cells, podocytes, and tubular cells, which is referred to as “nephrotoxicity hypothesis” [3,19].

Under normal conditions, LDL is metabolized and used by mesangial cells; however, when excess LDL is stored in the extracellular matrix in dyslipidemia, it is oxidized and causes an increase in cytotoxic agents such as prostaglandin E2 and tumor necrosis factor. These cytotoxic agents have the potential to damage the glomerular epithelial and endothelial cells, resulting in sclerosis.

In addition, it is hypothesized that increased free fatty acids bind to albumin and promote micropinocytosis of podocytes through lipid-binding G-protein coupled receptors, resulting in podocyte injury and loss, leading to end-stage renal disease [20,21]. Furthermore, it is reported that albumin-binding fatty acid may induce infiltration of macrophages and T lymphocytes into the tubulointerstitial space, leading to renal tubular cell injury and acute interstitial nephritis [22,23].

Several clinical studies, including prospective cohorts, reported that dyslipidemia could be a risk factor for renal injury represented as CKD progression. However, the findings were inconsistent among studies; some studies have reported that a high TC, TG, and LDL and a low HDL were associated with CKD progression, yet others did not [24].

## Management of dyslipidemia in NS

The main approach for managing dyslipidemia is to treat the underlying renal disease that causes NS. Dyslipidemia usually improves when the underlying disease is treated with steroids, immunosuppressants, or angiotensin converting enzyme inhibitors/angiotensin-receptor blockers.

### Lifestyle modification

A heart-healthy diet, physical activity, and weight reduction are recommended as the first-line treatments for pediatric patients with NS. In early studies, 20 NS patients with persistent proteinuria were administered a low-fat, low-protein, vegetarian soy diet rich in unsaturated fatty acids and fiber for 8 weeks instead of their usual diet. It was reported that lipid profiles (TC, LDL, HDL, apolipoprotein A, and apolipoprotein B), except for

TG and proteinuria, were significantly improved due to the diet. However, lipid profiles and proteinuria returned to baseline levels after resumption of their original diet [25,26]. It was also suggested that omega-3 fatty acids decreased serum TG and postprandial chylomicron in patients with NS [27,28].

#### **Pharmacological treatment: statins**

Patients with NS should decide whether to initiate lipid-lowering medication based on CVD risk and renal function. Currently, there are no agreed upon guidelines for initiating lipid-lowering medications in patients with NS. Studies that provide clear evidence for the use of lipid-lowering medications are lacking. Therefore, it is necessary to consider the potential benefits and risks of such medication for each patient with NS.

Statins are the most commonly used drugs for the treatment of dyslipidemia in patients with NS. Statins inhibit HMG-CoA reductase competitively, reduce hepatic cholesterol production, and promote LDL absorption in blood. However, studies on statin administration in patients with NS are scarce. It was reported that TC and LDL were effectively reduced by 20% to 45% in adult NS patients treated with statins, but there was a lesser reduction in TG and apolipoprotein levels [29,32].

Meanwhile, in a meta-analysis including four randomized controlled trials (RCTs) using statins as lipid-lowering agents, only one RCT had a significant HDL improvement, and the others did not show a clear blood lipid improvement [33]. It was reported that statins also reduce lipoprotein(a) levels in NS patients in cases with high baseline values [34].

Studies on the treatment of dyslipidemia in pediatric patients with NS using lipid-lowering agents are very limited. Because long-term safety data are lacking and the U.S. Food and Drug Administration has approved its limited use in pediatric patients with familial hypercholesterolemia, the use of lipid-lowering agents is relatively low in pediatric patients compared to that in adults [3,35]. Most studies on pediatric patients with NS used statins, and it was shown that there is 30% to 40% lipid-lowering effect than before statin treatment [36,37]. Hepatotoxicity and muscle-related effects, including myopathy and rhabdomyolysis, have been reported as major side effects of statins, with common effects comprising gastrointestinal symptoms, such as diarrhea and musculoskeletal symptoms, as well as joint pain [35].

#### **Pharmacological treatment: second-line agents**

Fibrates, such as gemfibrozil, fenofibrate, and clofibrate, increase LPL activity, decrease TG production, and decrease plasma concentrations of TG and LDL. There have been small-scale RCT studies in NS patients that reported that gemfibrozil treatment reduced plasma TG concentrations by approximately 50% and the LDL concentration by 13% to 30% compared to placebo [38,39]. However, it is known that myopathy risk increases when fibrates are used in combination with statins [40].

Bile acid sequestrants such as cholestyramine and colestipol inhibit intestinal reabsorption of bile and block enterohepatic circulation of bile. Consequently, the expression of various liver enzymes involved in bile production increases, which in turn increases hepatic cholesterol breakdown and LDL absorption from the blood. In patients with NS, it has been reported that when cholestyramine was used, LDL was reduced by 19%, and when colestipol was used, it was reduced by approximately 30% [29,41]. However, the gastrointestinal side effects of bile acid sequestrants have been reported to be high; therefore, their use was often limited [3].

Nicotinic acid and ezetimibe can also be used to treat dyslipidemia; however, there are no available clinical data on patients with NS until now.

Monoclonal antibodies against PCSK9 (e.g., evolocumab and alirocumab) bind to and inactivate it, eventually increasing LDLR on hepatocyte surfaces to promote LDL uptake [3]. Recent studies reported that remission in NS patients was associated with a decrease in cholesterol and PCSK9 blood levels, and the LDL reduction effect in the group treated with a PCSK9 inhibitor was significant [42-44]. In addition, ACAT inhibitors were reported to improve proteinuria and dyslipidemia in NS animal models [13].

#### **Lipid apheresis**

Lipid apheresis has been used to treat patients with homozygous familial hypercholesterolemia and has recently been applied to the treatment of dyslipidemia in NS patients [3]. It has been reported that adult and pediatric patients with steroid-resistant NS treated with lipid apheresis with or without steroid showed reduced proteinuria and improved lipid profiles. This effect might be explained by the improvement in dyslipidemia, removal of autoantibodies, reduced potential vascular permeability factors and inflammatory cytokines, and improved responsiveness to immunosuppressants [20,45-47].

## Conclusions

In this article, the authors reviewed recently described mechanisms, clinical impacts, and several treatment methods for dyslipidemia in patients with NS. Compared with adult patients with NS whose risks of atherosclerotic cardiovascular disease, such as myocardial infarction or coronary arterial disease, are high, studies on dyslipidemia in pediatric patients with NS are still lacking. However, there are also possible risks of atherosclerotic cardiovascular disease and progressive renal injury due to severe dyslipidemia, even in pediatric NS patients. In conclusion, more attention should be paid to the screening and treatment of dyslipidemia in pediatric patients with NS in clinical practice.

## Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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## Author contributions

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## References

1. Larkins NG, Liu ID, Willis NS, Craig JC, Hodson EM. Non-corticosteroid immunosuppressive medications for steroid-sensitive nephrotic syndrome in children. *Cochrane Database Syst Rev* 2020;4:CD002290.
2. Kang HG, Cheong HI. Nephrotic syndrome: what's new, what's hot? *Korean J Pediatr* 2015;58:275-82.
3. Agrawal S, Zaritsky JJ, Fornoni A, Smoyer WE. Dyslipidaemia in nephrotic syndrome: mechanisms and treatment. *Nat Rev Nephrol* 2018;14:57-70.
4. Vaziri ND. Disorders of lipid metabolism in nephrotic syndrome: mechanisms and consequences. *Kidney Int* 2016;90:41-52.
5. Vaziri ND, Yuan J, Ni Z, Nicholas SB, Norris KC. Lipoprotein lipase deficiency in chronic kidney disease is accompanied by down-regulation of endothelial GPIIb/IIIa expression. *Clin Exp Nephrol* 2012;16:238-43.
6. Sato T, Liang K, Vaziri ND. Down-regulation of lipoprotein lipase and VLDL receptor in rats with focal glomerulosclerosis. *Kidney Int* 2002;61:157-62.
7. Liu S, Vaziri ND. Role of PCSK9 and IDOL in the pathogenesis of acquired LDL receptor deficiency and hypercholesterolemia in nephrotic syndrome. *Nephrol Dial Transplant* 2014;29:538-43.
8. Morris AW. Nephrotic syndrome: PCSK9: a target for hypercholesterolaemia in nephrotic syndrome. *Nat Rev Nephrol* 2016;12:510.
9. Jin K, Park BS, Kim YW, Vaziri ND. Plasma PCSK9 in nephrotic syndrome and in peritoneal dialysis: a cross-sectional study. *Am J Kidney Dis* 2014;63:584-9.
10. Moorhead JF, Chan MK, El-Nahas M, Varghese Z. Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. *Lancet* 1982;2:1309-11.
11. Clement LC, Mace C, Avila-Casado C, Joles JA, Kersten S, Chugh SS. Circulating angiotensin-like 4 links proteinuria with hypertriglyceridemia in nephrotic syndrome. *Nat Med* 2014;20:37-46.
12. Vaziri ND, Liang K. Up-regulation of acyl-coenzyme A:cholesterol acyltransferase (ACAT) in nephrotic syndrome. *Kidney Int* 2002;61:1769-75.
13. Vaziri ND, Liang KH. Acyl-coenzyme A:cholesterol acyltransferase inhibition ameliorates proteinuria, hyperlipidemia, lecithin-cholesterol acyltransferase, SRB-1, and low-density lipoprotein receptor deficiencies in nephrotic syndrome. *Circulation* 2004;110:419-25.
14. Vaziri ND, Liang KH. Hepatic HMG-CoA reductase gene expression during the course of puromycin-induced nephrosis. *Kidney Int* 1995;48:1979-85.
15. Vaziri ND, Sato T, Liang K. Molecular mechanisms of altered cholesterol metabolism in rats with spontaneous focal glomerulosclerosis. *Kidney Int* 2003;63:1756-63.
16. Ordonez JD, Hiatt RA, Killebrew EJ, Fireman BH. The increased risk of coronary heart disease associated with nephrotic syndrome. *Kidney Int* 1993;44:638-42.
17. Mehta A, Mishra S, Ahmad K, Tiwari HC, Singh V, Singh A. Carotid intima media thickness in children with nephrotic syndrome: an observational case control study. *Sudan J Paediatr* 2019;19:110-6.
18. Radhakrishnan J, Appel AS, Valeri A, Appel GB. The nephrotic syndrome, lipids, and risk factors for cardiovascular disease. *Am J Kidney Dis* 1993;22:135-42.
19. Gyebi L, Soltani Z, Reisin E. Lipid nephrotoxicity: new concept for an old disease. *Curr Hypertens Rep* 2012;14:177-81.
20. Chung JJ, Huber TB, Godel M, Jarad G, Hartleben B, Kwok C, et al. Albumin-associated free fatty acids induce macropinocytosis in podocytes. *J Clin Invest* 2015;125:2307-16.
21. Allison SJ. Podocyte biology: free fatty acid-induced macropinocytosis.

- sis in podocytes. *Nat Rev Nephrol* 2015;11:386.
22. Eddy AA, Michael AF. Acute tubulointerstitial nephritis associated with aminonucleoside nephrosis. *Kidney Int* 1988;33:14-23.
  23. Eddy AA, McCulloch L, Liu E, Adams J. A relationship between proteinuria and acute tubulointerstitial disease in rats with experimental nephrotic syndrome. *Am J Pathol* 1991;138:1111-23.
  24. Cases A, Coll E. Dyslipidemia and the progression of renal disease in chronic renal failure patients. *Kidney Int Suppl* 2005;(99):S87-93.
  25. D'Amico G, Gentile MG, Manna G, Fellin G, Ciceri R, Cofano F, et al. Effect of vegetarian soy diet on hyperlipidaemia in nephrotic syndrome. *Lancet* 1992;339:1131-4.
  26. Gentile MG, Fellin G, Cofano F, Delle Fave A, Manna G, Ciceri R, et al. Treatment of proteinuric patients with a vegetarian soy diet and fish oil. *Clin Nephrol* 1993;40:315-20.
  27. Hall AV, Parbtani A, Clark WF, Spanner E, Huff MW, Philbrick DJ, et al. Omega-3 fatty acid supplementation in primary nephrotic syndrome: effects on plasma lipids and coagulopathy. *J Am Soc Nephrol* 1992;3:1321-9.
  28. Bell S, Cooney J, Packard CJ, Caslake MJ, Deighan CJ. The effect of omega-3 fatty acids on the atherogenic lipoprotein phenotype in patients with nephrotic range proteinuria. *Clin Nephrol* 2012;77:445-53.
  29. Rabelink AJ, Hene RJ, Erkelens DW, Joles JA, Koomans HA. Effects of simvastatin and cholestyramine on lipoprotein profile in hyperlipidaemia of nephrotic syndrome. *Lancet* 1988;2:1335-8.
  30. Thomas ME, Harris KP, Ramaswamy C, Hattersley JM, Wheeler DC, Varghese Z, et al. Simvastatin therapy for hypercholesterolemic patients with nephrotic syndrome or significant proteinuria. *Kidney Int* 1993;44:1124-9.
  31. Wheeler DC, Bernard DB. Lipid abnormalities in the nephrotic syndrome: causes, consequences, and treatment. *Am J Kidney Dis* 1994;23:331-46.
  32. Massy ZA, Ma JZ, Louis TA, Kasiske BL. Lipid-lowering therapy in patients with renal disease. *Kidney Int* 1995;48:188-98.
  33. Kong X, Yuan H, Fan J, Li Z, Wu T, Jiang L, et al. Lipid-lowering agents for nephrotic syndrome. *Cochrane Database Syst Rev* 2013;(12):CD005425.
  34. Brown CD, Azrolan N, Thomas L, Roberts KG, Bostom A, Zhao ZH, et al. Reduction of lipoprotein(a) following treatment with lovastatin in patients with unremitting nephrotic syndrome. *Am J Kidney Dis* 1995;26:170-7.
  35. Khurana M, Silverstein DM. Etiology and management of dyslipidemia in children with chronic kidney disease and end-stage renal disease. *Pediatr Nephrol* 2015;30:2073-84.
  36. Coleman JE, Watson AR. Hyperlipidaemia, diet and simvastatin therapy in steroid-resistant nephrotic syndrome of childhood. *Pediatr Nephrol* 1996;10:171-4.
  37. Sanjad SA, al-Abbad A, al-Shorafa S. Management of hyperlipidemia in children with refractory nephrotic syndrome: the effect of statin therapy. *J Pediatr* 1997;130:470-4.
  38. Groggel GC, Cheung AK, Ellis-Benigni K, Wilson DE. Treatment of nephrotic hyperlipoproteinemia with gemfibrozil. *Kidney Int* 1989;36:266-71.
  39. Buyukcelik M, Anarat A, Bayazit AK, Noyan A, Ozel A, Anarat R, et al. The effects of gemfibrozil on hyperlipidemia in children with persistent nephrotic syndrome. *Turk J Pediatr* 2002;44:40-4.
  40. Jacobson TA. Myopathy with statin-fibrate combination therapy: clinical considerations. *Nat Rev Endocrinol* 2009;5:507-18.
  41. Valeri A, Gelfand J, Blum C, Appel GB. Treatment of the hyperlipidemia of the nephrotic syndrome: a controlled trial. *Am J Kidney Dis* 1986;8:388-96.
  42. Di Bartolo B, Scherer DJ, Brown A, Psaltis PJ, Nicholls SJ. PCSK9 inhibitors in hyperlipidemia: current status and clinical outlook. *BioDrugs* 2017;31:167-74.
  43. Haas ME, Levenson AE, Sun X, Liao WH, Rutkowski JM, de Ferranti SD, et al. The role of proprotein convertase subtilisin/kexin type 9 in nephrotic syndrome-associated hypercholesterolemia. *Circulation* 2016;134:61-72.
  44. Jatem E, Lima J, Montoro B, Torres-Bondia F, Segarra A. Efficacy and safety of PCSK9 inhibitors in hypercholesterolemia associated with refractory nephrotic syndrome. *Kidney Int Rep* 2020;6:101-9.
  45. Hattori M, Chikamoto H, Akioka Y, Nakakura H, Ogino D, Matsunaga A, et al. A combined low-density lipoprotein apheresis and prednisone therapy for steroid-resistant primary focal segmental glomerulosclerosis in children. *Am J Kidney Dis* 2003;42:1121-30.
  46. Muso E. Beneficial effect of LDL-apheresis in refractory nephrotic syndrome. *Clin Exp Nephrol* 2014;18:286-90.
  47. Raina R, Krishnappa V. An update on LDL apheresis for nephrotic syndrome. *Pediatr Nephrol* 2019;34:1655-69.

# Alport syndrome: new advances in the last decade

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Alport syndrome (AS) is a progressive hereditary nephritis that is often accompanied by sensorineural hearing loss and ocular abnormalities. It is inherited in three modes of X-linked AS (XLAS), autosomal recessive AS (ARAS), and autosomal dominant AS (ADAS). XLAS is caused by pathogenic variants in *COL4A5*, while ARAS and ADAS are caused by those in *COL4A3* or *COL4A4*. There is currently no curative treatment for AS; however, angiotensin-converting enzyme inhibitors (ACEi) can improve the outcome of AS. In the past decade, multiple studies have shown that early intervention with ACEi upon isolated microscopic hematuria or microalbuminuria could delay disease progression, and early diagnosis is crucial for early treatment. Therefore, a new classification of AS based on molecular diagnoses has been proposed, including the paradigm shift of re-classifying female “carriers” to “patients” and “thin basement membrane nephropathy” to “ADAS.” In addition, with the detection of *COL4A* mutations in some patients with biopsy-confirmed IgA nephropathy, focal segmental glomerulosclerosis, and chronic kidney disease of unknown origin, it is suggested that the phenotype of AS should be expanded. In this review, we highlight the landmark studies and guidelines published over the past decade and introduce strategies for early diagnosis and treatment to improve the outcomes of AS.

**Keywords:** Angiotensin-converting enzyme inhibitor; Early diagnosis; Early medical intervention; Hematuria, benign familial; Nephritis, hereditary

## Introduction

Alport syndrome (AS) is a progressive inherited kidney disease often accompanied by sensorineural hearing loss (SNHL) and ocular abnormalities such as lenticonus or retinal flecks [1]. Kidney symptoms of AS start with isolated microscopic hematuria, followed by the appearance of microalbuminuria (urine microalbumin-to-creatinine ratio, 30–300 mg/g), which progresses to overt proteinuria and a decline in kidney function [2]. AS, which affects one in approximately 5,000 people, is the second most common cause of inherited kidney failure after autosomal dominant polycystic kidney disease [3]. It accounts for

0.5% of new-onset chronic kidney disease (CKD) stage G5 (glomerular filtration rate [GFR] <15 mL/min/1.73 m<sup>2</sup> or treatment by dialysis [4]) cases in adults and 12.9% in children [5]. AS is caused by pathogenic variants in the *COL4A3*, *COL4A4*, and *COL4A5* genes that encode type IV collagen  $\alpha 3$ ,  $\alpha 4$ , and  $\alpha 5$  chains, respectively. Type IV collagen has six different  $\alpha$  chains,  $\alpha 1$  to  $\alpha 6$ , which construct triple-helix structures, the major components of the basement membrane. In the embryonic glomerulus, classical chains of the  $\alpha 1$ - $\alpha 1$ - $\alpha 2$  triple helix form the glomerular basement membrane (GBM), and with development, these are gradually replaced by novel chains  $\alpha 3$ - $\alpha 4$ - $\alpha 5$  [6]. Basement membranes of the cochlea and ocular lens have similar type

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IV collagen compositions as the GBM. Since the novel chains of type IV collagen are defective in AS, basement membranes of the glomerulus, cochlea, and ocular lens become progressively defective [5]. Inheritance patterns of AS are X-linked (XLAS; a defect of *COL4A5* on chromosome X), autosomal recessive (ARAS), and autosomal dominant (ADAS; defect of *COL4A3* or *COL4A4* in chromosome 2), comprising 80%, 15%, and 5%, respectively [1,5].

## Inheritance of AS

### X-linked AS

Approximately 85% of XLAS patients have a family history of hematuria (with or without proteinuria), kidney failure, or extrarenal manifestations (SNHL or ocular abnormalities). All male patients present with hematuria and early-onset proteinuria, eventually progressing to kidney failure. Ninety percent of patients reached CKD stage G5 by the age of 40 years (median, 25 years) [7]. In males, truncating mutations (rearrangement, nonsense, and frameshift) show a severe phenotype of earlier kidney failure and hearing loss (HL), and non-truncating mutations (in-frame, missense) exhibit a milder phenotype with later kidney failure and HL [7-9]. Hematuria is present in most (>95%) female patients, and proteinuria appears at the median age of 7 years in 75% of females. One-fourth of female patients progress to CKD stage G5 during their lifetime (median age, 65 years), with this progression occurring by the age of 40 years in 15% of them. The phenotype is highly variable in females, ranging from isolated microscopic hematuria with normal kidney function throughout life to kidney failure at a young age [10,11]. SNHL occurs in 90% of males and 12% of females by the age of 40 years [7,10].

### Autosomal recessive AS

There are no gender differences in the clinical symptoms, incidence, and prognosis of ARAS. Extrarenal symptoms are common. Both males and females exhibit poor prognoses similar to those with XLAS male patients (the median age of onset is 2.5 years for hematuria, 21 years for developing CKD stage G5, and 13-20 years for SNHL) [12,13].

### Autosomal dominant AS

In general, both males and females have a good prognosis, and the median ages for developing proteinuria and kidney failure are 17 and 70 years, respectively [5,14]. However, the phenotype

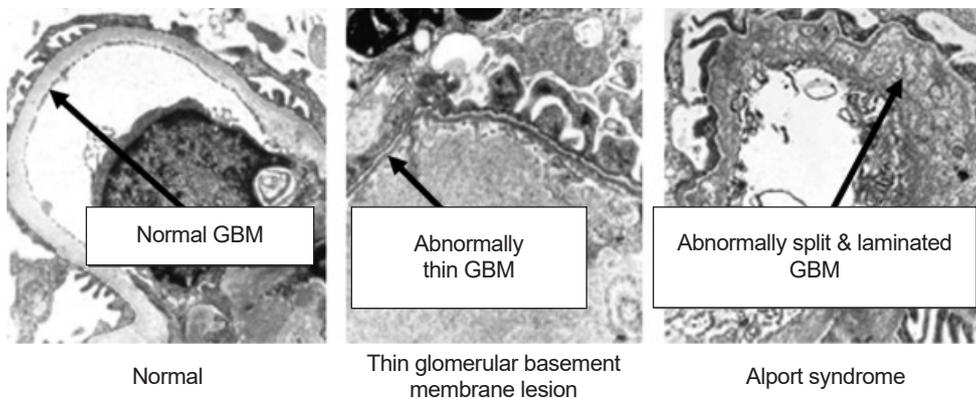
is very diverse even within a family, from isolated microscopic hematuria to CKD stage G5. Extrarenal symptoms are uncommon. Their pathologic findings can be either thin basement membrane nephropathy (TBMN) or focal segmental glomerulosclerosis (FSGS). Not rarely, patients with ADAS are misdiagnosed as familial FSGS or IgA nephropathy (IgAN) [14].

## Clinical suspicion of AS

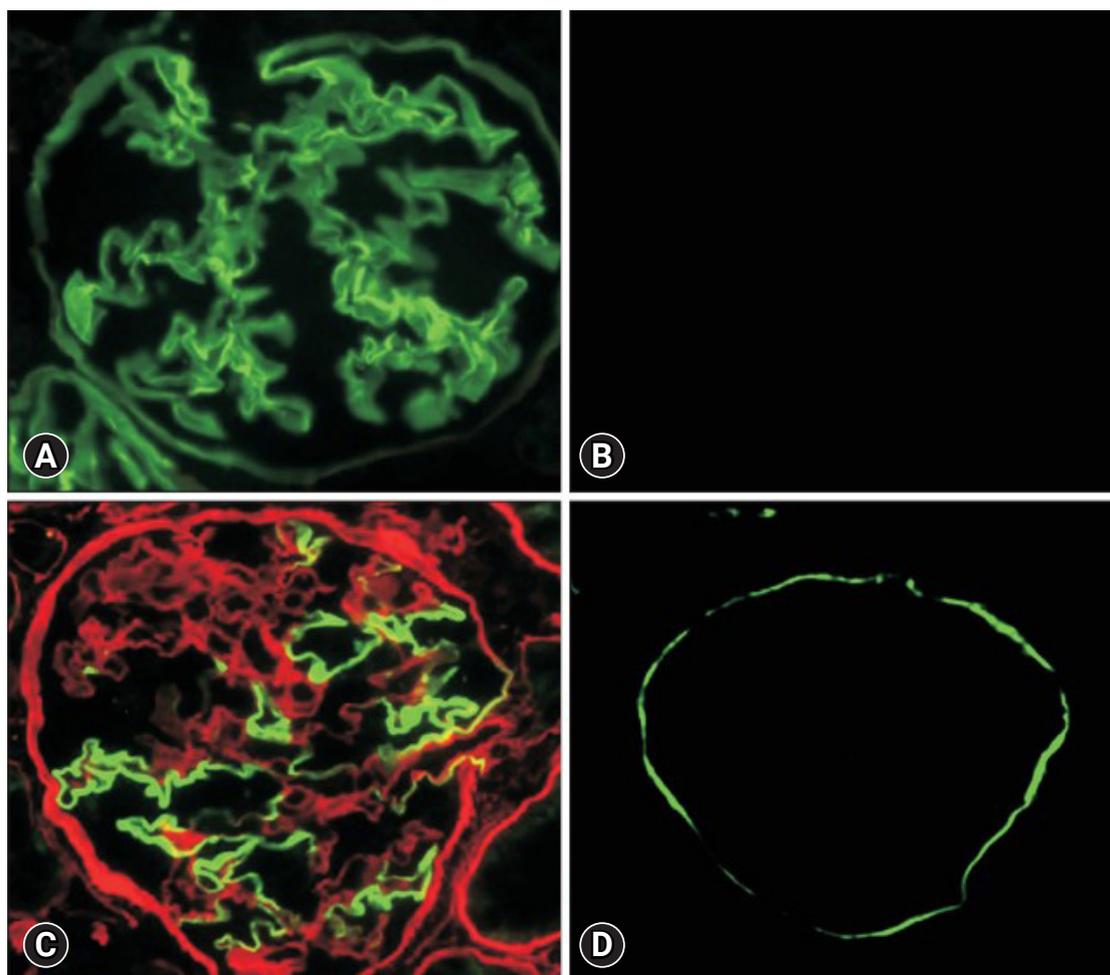
The most common symptom of AS is hematuria, which is persistent in 100% of XLAS male and ARAS patients, and may appear intermittently in approximately 95% of XLAS females and 50% of ADAS patients (GeneReviews). Recurrent gross hematuria (especially following upper respiratory infection) occurs in infancy or early childhood in 40% to 60% of cases, with an average age of 3.5 years in males and 9 years in females. Males without hematuria by the age of 10 years are unlikely to have AS [15]. Importantly, proteinuria does not appear without hematuria [16]. Ocular abnormalities of AS are less sensitive than the associated HL; however, they are more specific, which means that they could be diagnostic. Lenticonus and central fleck retinopathy occur only in AS and are associated with kidney failure before 30 years in male patients with XLAS [17].

## Histologic findings

Conventionally, the diagnosis of AS was made pathologically; however, this can be challenging. There are no specific light microscopic findings in AS; mesangial proliferation, FSGS, and interstitial infiltration containing lipid-laden foam cells may be observed [18,19]. Electron microscopy findings typically show irregular thickening and thinning of the GBM, lamellation, and splitting in the lamina densa of a "basket-weave" appearance (Fig. 1) [20]. While these findings are very characteristic of AS, they appear later in the course of the condition; therefore, electron microscopy may show only diffuse GBM thinning in young male patients with XLAS or XLAS females, or ARAS/ADAS patients [5,21]. Immunofluorescence staining of type IV collagen  $\alpha 5$  can be diagnostic, irrespective of the patient's age. Typically, collagen type IV  $\alpha 5$  chain expression is completely absent in XLAS male patients (both at GBM and Bowman capsule) and ARAS (GBM) patients; however, more than 20% of XLAS males and 20% of ARAS patients exhibit normal expressions of  $\alpha 5$  if their pathologic variants are non-truncating [13,22], as well as ADAS patients. XLAS female patients exhibit a mosaic pattern



**Fig. 1.** Electron microscopy findings: the glomerular basement membrane (GBM) abnormalities caused by *COL4A* mutations. The GBM in Alport syndrome (AS) patients demonstrates irregular thickening with abnormal splitting and lamination. In patients with a thin basement membrane lesion, which is currently proposed to be regarded as AS, the GBM shows abnormally diffuse thinning. Reused from Warady et al. [20]. Images were used with permission from J. Charles Jennette. www.unckidneycenter.org. Accessed March 9, 2022.



**Fig. 2.** Immunofluorescence staining of type IV collagen in glomerulus. (A) Normal control exhibits full expression in both the glomerular basement membrane (GBM) and Bowman capsule (BC). (B) An X-linked Alport syndrome (XLAS) male patient shows entirely negative expression in both GBM and BC. (C) An XLAS female patient displays a mosaic pattern of expression in both GBM and BC. Green and red colors indicate  $\alpha 5$  and  $\alpha 2$  chains of type IV collagen, respectively. (D) An autosomal recessive AS patient presents negative expression only on GBM, but positivity on BC. Reused from Nozu et al. [1].

of  $\alpha 5$  staining (Fig. 2) [1]. The collagen type IV  $\alpha 5$  stain in skin biopsy is valid only for XLAS since the epidermal basement membrane is mainly comprised of the triple helix of  $\alpha 5$ - $\alpha 5$ - $\alpha 6$  as the Bowman capsule [23]. Therefore, the normal expression of collagen type IV  $\alpha 5$  AS cannot exclude AS. Furthermore, non-specific focal GBM thinning may also be seen in IgAN [24]. Thus, kidney biopsy plays a supportive, not confirmatory role in the diagnosis of AS [25].

## Advances in the last decade

Due to these limitations of histopathology and the remarkable development of molecular-based techniques in the past decade, genetic testing has become the first-line diagnostic technique for AS. Because *COL4A* genes are large, targeted next-generation sequencing, including all three novel chain genes, has become the primary genetic screening method instead of Sanger sequencing [3,26]. Although there is currently no curative therapy for AS, nephroprotective drugs such as angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers can delay the progression to kidney failure for years or even decades. In a large cohort study of XLAS males in Japan [27], kidney replacement therapy (KRT) was delayed by 17 years and 12 years in the non-truncating and truncating mutant groups, respectively, in the group of patients treated with either ACEi or angiotensin receptor blockers compared to those in the untreated group. In addition, multiple prospective and retrospective studies have reported the beneficial effects of early interventions with ACEi. The 2020 guideline emphasizes early molecular diagnosis to enable early treatment and avoid immunosuppression [2].

### The rationale for early ACEi treatment

In a study using the mouse model of ARAS, those treated with ramipril at 4 weeks survived twice as long as mice not treated with ramipril, whereas the group that started treatment at 7 weeks did not differ in survival compared to the untreated group [28]. A retrospective cohort study in Europe showed that among those who began treatment when they had only isolated hematuria or microalbuminuria, no one needed KRT until the age of at least 40 years [29]. Treatment initiation when patients had proteinuria ( $>0.3$  g/day) with normal kidney function delayed KRT for 18 years (median age of KRT, 40 years). Later initiation at CKD stage G3 (GFR, 30–59 mL/min/1.73 m<sup>2</sup>) or G4 (GFR, 15–29 mL/min/1.73 m<sup>2</sup>) had the effect of postponing KRT by only

3 years (median age, 25 years) compared to the untreated group with a median KRT age of 22 years. Based on these studies, the Early Prospective Therapy European Community Trial in Alport syndrome (EARLY PRO-TECT Alport) was conducted in Germany to determine whether starting ramipril treatment on noticing isolated hematuria or microalbuminuria could delay the progression of the disease to its next phase (microalbuminuria or proteinuria, respectively). Early treated group (n=11, open-label [n=42]) developed disease progression in 27%, 41%, for each, whereas the placebo group (n=9) in 56%. Although not statistically significant, the authors concluded that the early initiation of ramipril might be beneficial in delaying progression by more than 40% [30].

## Expanded phenotypes of AS

With the broad implementation of next-generation sequencing, several cases are rediagnosed with AS even when their symptoms are not always compatible with AS. In a large-scale analysis of whole-exome sequencing in 3,315 patients with CKD of all causes, 10% were found to have genetic causes, of which 30% were diagnosed as AS with *COL4A* mutations. The majority of these patients (62%) had been misdiagnosed with hypertensive nephropathy, other kinds of glomerulonephropathy such as steroid-resistant nephrotic syndrome, IgAN, or FSGS [20,31]. *COL4A* defects were the most common causes in adult-onset FSGS, accounting for up to 20% of familial FSGS [32]. Therefore, the *COL4A3-COL4A5* mutations must be considered in CKD of unknown etiology, steroid-resistant nephrotic syndrome, FSGS, and IgAN, even if the clinical manifestations and biopsy findings are not fully compatible with AS, especially if there is a contributive family history [26]. Recent guidelines emphasize that FSGS or IgAN with pathogenic variants of *COL4A* should be considered “AS” and not as “coincidental disease” [2].

## The new classification scheme for AS

### From “carrier” to “patient” in XLAS females

Traditionally, females with *COL4A5* heterozygous mutations have been considered “carriers.” While previously regarded as benign, 25% of them develop CKD stage G5 during their lifetime. Risk factors for progression include a history of gross hematuria in childhood, proteinuria, nephrotic syndrome, SNHL, and the presence of diffuse thickening and lamellation of the GBM. Therefore, experts stressed that females with *COL4A5*

heterozygous mutations should be considered “patients” rather than benign “carriers.” Female AS patients need to be monitored regularly for proteinuria and kidney function [33,34].

#### From “TBMN” and “ADAS”

TBMN, traditionally known as “benign familial hematuria,” is characterized by hematuria with or without mild proteinuria, with diffuse GBM thinning. This condition was considered a benign one that does not progress to CKD stage G5. However, TBMN, in many cases [35], is caused by heterozygous mutation of *COL4A3* or *COL4A4*, same as “ADAS,” and there had been no definite distinction [5]. Moreover, the progression to CKD stage G5 in TBMN has been reported, especially in patients with risk factors of proteinuria, FSGS, or GBM thickening and lamellation on kidney biopsy, SNHL, and family history of progressive kidney disease [33,36]. So, is TBMN equal to ADAS? A guideline published in 2019 did not agree, as the likelihood of kidney failure is minimal [3]. However, the latest guidelines emphasize that all diseases caused by heterozygous mutations of *COL4A3* or *COL4A4* should be classified as “ADAS” for an earlier and more aggressive intervention since earlier ACEi treatment delays kidney failure for longer [2,33]. In a recent study, Yamamura et al. [37] reported that ADAS accounted for 17% of all AS cases, which is much higher than the 5% found in previous reports, suggesting that the diagnosis of ADAS is underestimated [37]. Therefore, even when diagnosed with TBMN, especially if associated with *COL4A3* or *COL4A4* variants, regular follow-up is strongly recommended for the risk of kidney failure.

## Early diagnostic tactics

#### Early genetic test necessity

The latest guideline proposed in 2020 by Kashtan and Gross [2] recommends that genetic testing should be performed if AS is suspected in patients with persistent glomerular hematuria. Kidney biopsy is recommended if genetic testing has uncertain pathogenicity and AS is not suspected based on clinical data or family history. Kidney biopsy should include transmission electron microscopy, and if transmission electron microscopy is not available, immunofluorescence of the type-IV collagen stain is required.

## Treatment guidelines

#### When should we start treatment and how do we monitor it?

The clinical practice recommendations from the Alport Syndrome Research Collaborative emphasize initiating early interventions. In XLAS male and ARAS patients, who progress to CKD stage G5 in 100% of cases, treatment should be started as soon as possible when the diagnosis is obtained in patients older than 1 year regardless of proteinuria (strong recommendation). For XLAS female and ADAS patients, treatment initiation is recommended if microalbuminuria develops during annual monitoring [2]. This differs significantly from their previous guideline in 2013 that did not recommend treatment with isolated hematuria and recommended optional treatment with microalbuminuria in XLAS males [38].

#### Which drugs should we use?

The drug recommended by the above-mentioned guideline is either ramipril or lisinopril. Ramipril is a well-established drug with evidence of efficacy and safety, which was adopted from protocols in the ESCAPE (Effect of Strict blood pressure Control and ACE Inhibition on the Progression of CKD in Pediatric Patients) trials [39] and the EARLY PRO-TECT trial for children with AS [30]. Lisinopril is another ACEi with a comparable duration of action to that of ramipril, and it has obtained evidence from some pediatric studies [40,41]. In the ARAS mouse model study, ramipril prolonged the lifespan significantly more than candesartan did (111% vs. 38%, respectively) [42]. However, no AS studies in humans have compared ramipril with other drugs. One small study compared the antiproteinuric effects of enalapril to those of losartan in children with AS and found no significant difference between the two [43].

#### How to increase the dose of the ACEi?

It is emphasized that ACEi should be up titrated more rapidly and aggressively than previously recommended. The previous guideline in 2013 recommended increasing ramipril dose “every” 3 months until the target urine protein-to-creatinine ratio (UPCR; less than half of the baseline value) is reached [38]. However, the recent guideline in 2020 recommends increasing the dose “over” the first 3 to 4 months starting 1 mg/m<sup>2</sup>/day to a maximum of 6 mg/m<sup>2</sup>/day of ramipril irrespective of the degree of proteinuria if the patient can tolerate such an increase. The dosage needs to be increased as the child grows to maintain the maximum dose [2].

## Dual renin-angiotensin-aldosterone system blockade

Data from the ESCAPE trial in pediatric CKD reported that a UPCR <1.0 mg/mg was associated with better kidney outcomes [44]. Therefore, the latest guideline suggested that considering the use of dual renin-angiotensin-aldosterone system (RAAS) blockade may be reasonable if the UPCR exceeds 1.0 mg/mg despite the maximum tolerated dose of ramipril or lisinopril. Losartan can be added in small amounts, at an initial dose of 0.8 mg/kg/day [2]. However, there is limited evidence of the efficacy and safety of dual RAAS blockade, and the Kidney Disease: Improving Global Outcomes (KDIGO) guideline recommends not using dual blockade in another glomerulopathy. When using dual RAAS blockade, adverse effects such as hyperkalemia, kidney insufficiency, and hypotension should be periodically monitored [2,45].

## Cyclosporine

Cyclosporine (CsA) is not recommended in patients with AS [2]. CsA diminishes proteinuria by directly stabilizing the podocyte cytoskeleton [46]; however, the long-term use of this drug may stimulate profibrotic mediators such as transforming growth factor-beta, leading to interstitial fibrosis and tubular atrophy [47]. In small, uncontrolled studies conducted in Spain [48,49], France [50], and Italy [51], proteinuria significantly decreased throughout CsA treatment (5 mg/kg/day). However, the effect was temporary: proteinuria nearly returned to the baseline value after discontinuation in most patients, and kidney outcomes were conflicting with stable GFRs over 8 years [48,49] versus kidney function decline over 6 months, and some patients developed interstitial fibrosis 20 to 27 months after CsA initiation [50].

## Hearing and ophthalmologic evaluation and follow-up

HL in children, even in mild cases, affects speech-language, social-behavior, cognitive development, and academic performances [52]. Several papers have shown that HL in AS is sensorineural, progressive, and bilateral, often affecting the middle and particularly high frequencies [53]. Therefore, it can only be detected by formal hearing tests, especially during early childhood. Approximately 30% of XLAS males and 20% of ARAS

patients exhibit detectable HL by age 10. In males with XLAS, HL increases to approximately 60% by age 20 and shows a genotype-phenotype correlation. ARAS patients show a high rate of HL when they have one or more truncating mutations [8,13,54]. Therefore, a hearing evaluation is recommended annually for XLAS males and ARAS, starting at the age of 5 to 6 years, and earlier if overt proteinuria occurs or symptoms suggestive of HL, such as speech delay, develop. For XLAS females, the probability of HL is less than 10% by the age of 40 years; however, HL eventually occurs in 30% of them [10]. It is recommended that females with XLAS perform a formal hearing test when overt proteinuria is present. All AS patients should avoid loud sounds. If HL develops, it usually responds well to hearing aids [2].

Ocular abnormalities in AS, lenticonus, and central fleck retinopathy have high diagnostic and prognostic values [17]. For XLAS males with truncating mutations and ARAS patients, it is strongly recommended that ocular investigations begin at age 15 (earlier if they have an abnormal vision) and annual check-ups should be performed. For females with XLAS and ADAS patients, ophthalmologic assessments are recommended if clinically indicated [2].

## Other recommendations

Hypertension can accelerate the deterioration of kidney function. Therefore, it should be strictly controlled, and the target blood pressure should be in the 50th percentile [25]. As lifestyle modifications, maintaining a body mass index of <25 kg/m<sup>2</sup>, moderating one's dietary intake of meat protein and salt, and avoiding smoking are also recommended [2].

## Research horizons

### Future therapeutic options

In a clinical trial on AS-induced CKD [55], bardoxolone, an activator of nuclear factor erythroid-related factor 2 (Nrf2), improved the GFR. However, another study of this drug in patients with type 2 diabetes mellitus and CKD stage G4 (BEACON trial) was prematurely terminated due to fatal cardiovascular complications (relative risk, 1.83;  $P<0.001$ ) [56]. For safety and efficacy reasons, the Food and Drug Administration recently denied the approval of the drug in AS.

Other investigational approaches in AS include microRNA-21 antagonists as anti-fibrotic agents and exon-skipping gene therapy. Both were effective in animal studies and are now un-

der clinical trials [2,57].

## Conclusions

Advances in molecular genetics and clinical studies over the past decade have made early diagnosis and intervention possible in patients with AS. Also, histopathologic diagnoses other than AS cannot exclude AS, since some of such patients harbor disease-causing variants of AS. Therefore, even when the clinical phenotype and biopsy results are not compatible with AS, the *COL4A3-COL4A5* mutations need to be considered, especially if there is a contributive family history. Female subjects affected by XLAS are no longer considered “carriers” but “patients,” and individuals with heterozygous mutations in *COL4A3* and *COL4A4* (even diagnosed with “TBMN”) should be monitored regularly due to the risk of kidney failure. XLAS males and ARAS patients older than 12 months need to undergo ACEi regardless of proteinuria, while other types can be monitored for microalbuminuria appearance, which indicates treatment.

## Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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## Author contributions

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## References

- Nozu K, Takaoka Y, Kai H, Takasato M, Yabuuchi K, Yamamura T, et al. Genetic background, recent advances in molecular biology, and development of novel therapy in Alport syndrome. *Kidney Res Clin Pract* 2020;39:402-13.
- Kashtan CE, Gross O. Clinical practice recommendations for the diagnosis and management of Alport syndrome in children, adolescents, and young adults-an update for 2020. *Pediatr Nephrol* 2021;36:711-9.
- Savige J, Ariani F, Mari F, Bruttini M, Renieri A, Gross O, et al. Expert consensus guidelines for the genetic diagnosis of Alport syndrome. *Pediatr Nephrol* 2019;34:1175-89.
- Levey AS, Eckardt KU, Dorman NM, Christiansen SL, Hoorn EJ, Inzeggfingher JR, et al. Nomenclature for kidney function and disease: report of a Kidney Disease: Improving Global Outcomes (KDIGO) consensus conference. *Kidney Int* 2020;97:1117-29.
- Nozu K, Nakanishi K, Abe Y, Udagawa T, Okada S, Okamoto T, et al. A review of clinical characteristics and genetic backgrounds in Alport syndrome. *Clin Exp Nephrol* 2019;23:158-68.
- Boutaud A, Borza DB, Bondar O, Gunwar S, Netzer KO, Singh N, et al. Type IV collagen of the glomerular basement membrane: evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains. *J Biol Chem* 2000;275:30716-24.
- Bekheirnia MR, Reed B, Gregory MC, McFann K, Shamshirsaz AA, Masoumi A, et al. Genotype-phenotype correlation in X-linked Alport syndrome. *J Am Soc Nephrol* 2010;21:876-83.
- Jais JP, Knebelmann B, Giatras I, Marchi M, Rizzoni G, Renieri A, et al. X-linked Alport syndrome: natural history in 195 families and genotype-phenotype correlations in males. *J Am Soc Nephrol* 2000;11:649-57.
- Gross O, Netzer KO, Lambrecht R, Seibold S, Weber M. Meta-analysis of genotype-phenotype correlation in X-linked Alport syndrome: impact on clinical counselling. *Nephrol Dial Transplant* 2002;17:1218-27.
- Jais JP, Knebelmann B, Giatras I, De Marchi M, Rizzoni G, Renieri A, et al. X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a “European Community Alport Syndrome Concerted Action” study. *J Am Soc Nephrol* 2003;14:2603-10.
- Yamamura T, Nozu K, Fu XJ, Nozu Y, Ye MJ, Shono A, et al. Natural history and genotype-phenotype correlation in female X-linked Alport syndrome. *Kidney Int Rep* 2017;2:850-5.
- Lee JM, Nozu K, Choi DE, Kang HG, Ha IS, Cheong HI. Features of autosomal recessive Alport syndrome: a systematic review. *J Clin Med* 2019;8:178.
- Oka M, Nozu K, Kaito H, Fu XJ, Nakanishi K, Hashimura Y, et al. Natural history of genetically proven autosomal recessive Alport syndrome. *Pediatr Nephrol* 2014;29:1535-44.
- Kamiyoshi N, Nozu K, Fu XJ, Morisada N, Nozu Y, Ye MJ, et al. Genetic, clinical, and pathologic backgrounds of patients with autosomal dominant Alport syndrome. *Clin J Am Soc Nephrol* 2016;11:1441-9.
- Kashtan CE. Alport syndrome: an inherited disorder of renal, ocular, and cochlear basement membranes. *Medicine (Baltimore)* 1999;78:338-60.
- Sessa A, Meroni M. Alport's syndrome: Orphanet encyclopedia [Internet]. Paris: Orphanet; 2001 [cited 2022 Mar 2]. Available from: <https://doi.org/10.33399/ckd.22.022>

- <https://www.orpha.net>.
17. Savige J, Sheth S, Leys A, Nicholson A, Mack HG, Colville D. Ocular features in Alport syndrome: pathogenesis and clinical significance. *Clin J Am Soc Nephrol* 2015;10:703-9.
  18. Adam J, Connor TM, Wood K, Lewis D, Naik R, Gale DP, et al. Genetic testing can resolve diagnostic confusion in Alport syndrome. *Clin Kidney J* 2014;7:197-200.
  19. Yao XD, Chen X, Huang GY, Yu YT, Xu ST, Hu YL, et al. Challenge in pathologic diagnosis of Alport syndrome: evidence from correction of previous misdiagnosis. *Orphanet J Rare Dis* 2012;7:100.
  20. Warady BA, Agarwal R, Bangalore S, Chapman A, Levin A, Stenvinkel P, et al. Alport syndrome classification and management. *Kidney Med* 2020;2:639-49.
  21. Heidet L, Gubler MC. The renal lesions of Alport syndrome. *J Am Soc Nephrol* 2009;20:1210-5.
  22. Hashimura Y, Nozu K, Kaito H, Nakanishi K, Fu XJ, Ohtsubo H, et al. Milder clinical aspects of X-linked Alport syndrome in men positive for the collagen IV  $\alpha 5$  chain. *Kidney Int* 2014;85:1208-13.
  23. Nakanishi K, Yoshikawa N, Iijima K, Kitagawa K, Nakamura H, Ito H, et al. Immunohistochemical study of alpha 1-5 chains of type IV collagen in hereditary nephritis. *Kidney Int* 1994;46:1413-21.
  24. IgA nephropathy combined with thin basement membrane nephropathy in children. *Kidney Res Clin Pract* 2013;32:194-5.
  25. Lennon R, Fornoni A. Could this be Alport syndrome? *Clin J Am Soc Nephrol* 2021;16:1743-5.
  26. Savige J, Storey H, Watson E, Hertz JM, Deltas C, Renieri A, et al. Consensus statement on standards and guidelines for the molecular diagnostics of Alport syndrome: refining the ACMG criteria. *Eur J Hum Genet* 2021;29:1186-97.
  27. Yamamura T, Horinouchi T, Nagano C, Omori T, Sakakibara N, Aoto Y, et al. Genotype-phenotype correlations influence the response to angiotensin-targeting drugs in Japanese patients with male X-linked Alport syndrome. *Kidney Int* 2020;98:1605-14.
  28. Gross O, Beirowski B, Koepke ML, Kuck J, Reiner M, Addicks K, et al. Preemptive ramipril therapy delays renal failure and reduces renal fibrosis in COL4A3-knockout mice with Alport syndrome. *Kidney Int* 2003;63:438-46.
  29. Gross O, Licht C, Anders HJ, Hoppe B, Beck B, Tonshoff B, et al. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. *Kidney Int* 2012;81:494-501.
  30. Gross O, Tonshoff B, Weber LT, Pape L, Latta K, Fehrenbach H, et al. A multicenter, randomized, placebo-controlled, double-blind phase 3 trial with open-arm comparison indicates safety and efficacy of nephroprotective therapy with ramipril in children with Alport's syndrome. *Kidney Int* 2020;97:1275-86.
  31. Groopman EE, Marasa M, Cameron-Christie S, Petrovski S, Aggarwal VS, Milo-Rasouly H, et al. Diagnostic utility of exome sequencing for kidney disease. *N Engl J Med* 2019;380:142-51.
  32. Gast C, Pengelly RJ, Lyon M, Bunyan DJ, Seaby EG, Graham N, et al. Collagen (COL4A) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 2016;31:961-70.
  33. Kashtan CE, Ding J, Garosi G, Heidet L, Massella L, Nakanishi K, et al. Alport syndrome: a unified classification of genetic disorders of collagen IV  $\alpha 345$ . A position paper of the Alport Syndrome Classification Working Group. *Kidney Int* 2018;93:1045-51.
  34. Grunfeld JP, Noel LH, Hafez S, Droz D. Renal prognosis in women with hereditary nephritis. *Clin Nephrol* 1985;23:267-71.
  35. Rana K, Wang YY, Powell H, Jones C, McCredie D, Buzza M, et al. Persistent familial hematuria in children and the locus for thin basement membrane nephropathy. *Pediatr Nephrol* 2005;20:1729-37.
  36. Imafuku A, Nozu K, Sawa N, Nakanishi K, Ubara Y. How to resolve confusion in the clinical setting for the diagnosis of heterozygous COL4A3 or COL4A4 gene variants? Discussion and suggestions from nephrologists. *Clin Exp Nephrol* 2020;24:651-6.
  37. Yamamura T, Nozu K, Minamikawa S, Horinouchi T, Sakakibara N, Nagano C, et al. Comparison between conventional and comprehensive sequencing approaches for genetic diagnosis of Alport syndrome. *Mol Genet Genomic Med* 2019;7:e883.
  38. Kashtan CE, Ding J, Gregory M, Gross O, Heidet L, Knebelmann B, et al. Clinical practice recommendations for the treatment of Alport syndrome: a statement of the Alport Syndrome Research Collaborative. *Pediatr Nephrol* 2013;28:5-11.
  39. ESCAPE Trial Group, Wuhl E, Trivelli A, Picca S, Litwin M, Peco-Antic A, et al. Strict blood-pressure control and progression of renal failure in children. *N Engl J Med* 2009;361:1639-50.
  40. Nakanishi K, Iijima K, Ishikura K, Hataya H, Awazu M, Sako M, et al. Efficacy and safety of lisinopril for mild childhood IgA nephropathy: a pilot study. *Pediatr Nephrol* 2009;24:845-9.
  41. Gipson DS, Trachtman H, Kaskel FJ, Greene TH, Radeva MK, Gassman JJ, et al. Clinical trial of focal segmental glomerulosclerosis in children and young adults. *Kidney Int* 2011;80:868-78.
  42. Gross O, Schulze-Lohoff E, Koepke ML, Beirowski B, Addicks K, Bloch W, et al. Antifibrotic, nephroprotective potential of ACE inhibitor vs AT1 antagonist in a murine model of renal fibrosis. *Nephrol Dial Transplant* 2004;19:1716-23.
  43. Webb NJ, Shahinfar S, Wells TG, Massaad R, Gleim GW, McCrary Sisk C, et al. Losartan and enalapril are comparable in reducing proteinuria in children with Alport syndrome. *Pediatr Nephrol* 2013;28:737-

- 43.
44. van den Belt SM, Heerspink HJL, Gracchi V, de Zeeuw D, Wuhl E, Schaefer F, et al. Early proteinuria lowering by angiotensin-converting enzyme inhibition predicts renal survival in children with CKD. *J Am Soc Nephrol* 2018;29:2225-33.
45. Kaito H, Nozu K, Iijima K, Nakanishi K, Yoshiya K, Kanda K, et al. The effect of aldosterone blockade in patients with Alport syndrome. *Pediatr Nephrol* 2006;21:1824-9.
46. Faul C, Donnelly M, Merscher-Gomez S, Chang YH, Franz S, Delfgaauw J, et al. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat Med* 2008;14:931-8.
47. Naesens M, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol* 2009;4:481-508.
48. Callis L, Vila A, Carrera M, Nieto J. Long-term effects of cyclosporine A in Alport's syndrome. *Kidney Int* 1999;55:1051-6.
49. Callis L, Vila A, Nieto J, Fortuny G. Effect of cyclosporin A on proteinuria in patients with Alport's syndrome. *Pediatr Nephrol* 1992;6:140-4.
50. Charbit M, Gubler MC, Dechaux M, Gagnadoux MF, Grunfeld JP, Niaudet P. Cyclosporin therapy in patients with Alport syndrome. *Pediatr Nephrol* 2007;22:57-63.
51. Massella L, Muda AO, Legato A, Di Zazzo G, Giannakakis K, Emma F. Cyclosporine A treatment in patients with Alport syndrome: a single-center experience. *Pediatr Nephrol* 2010;25:1269-75.
52. Kim JH, Lee DH, Lee B, Lim SH, Ahn YH, Kang HG, et al. Renal syndromic hearing loss is common in childhood-onset chronic kidney disease. *J Korean Med Sci* 2020;35:e364.
53. Alves FR, de A Quintanilha Ribeiro F. Revision about hearing loss in the Alport's syndrome, analyzing the clinical, genetic and bio-molecular aspects. *Braz J Otorhinolaryngol* 2005;71:813-9.
54. Zhang X, Zhang Y, Zhang Y, Gu H, Chen Z, Ren L, et al. X-linked Alport syndrome: pathogenic variant features and further auditory genotype-phenotype correlations in males. *Orphanet J Rare Dis* 2018;13:229.
55. Reata Pharmaceuticals. Reata announces positive results from year 2 of the pivotal phase 3 CARDINAL study of bardoxolone methyl in patients with Alport syndrome [Internet]. Reata Plano: Pharmaceuticals; 2020 [cited 2022 Mar 5]. Available from: <https://www.reatapharma.com/investors/news/news-details/2020/Reata-Announces-Positive-Results-From-Year-2-of-the-Pivotal-Phase-3-CARDINAL-Study-of-Bardoxolone-Methyl-in-Patients-with-Alport-Syndrome/default.aspx>.
56. de Zeeuw D, Akizawa T, Audhya P, Bakris GL, Chin M, Christ-Schmidt H, et al. Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. *N Engl J Med* 2013;369:2492-503.
57. Gomez IG, MacKenna DA, Johnson BG, Kaimal V, Roach AM, Ren S, et al. Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. *J Clin Invest* 2015;125:141-56.

# Genetic analysis using whole-exome sequencing in pediatric chronic kidney disease: a single center's experience

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**Purpose:** Chronic kidney disease (CKD) has various underlying causes in children. Identification of the underlying causes of CKD is important. Genetic causes comprise a significant proportion of pediatric CKD cases.

**Methods:** In this study, we performed whole-exome sequencing (WES) to identify genetic causes of pediatric CKD. From January to June 2021, WES was performed using samples from pediatric patients with CKD of unclear etiology.

**Results:** Genetic causes were investigated using WES in 37 patients (17 males) with pediatric CKD stages 1 (n=5), 2 (n=7), 3 (n=2), 4 (n=2), and 5 (n=21). The underlying diseases were focal segmental glomerulosclerosis (n=9), congenital anomalies of the kidney and urinary tract including reflux nephropathy (n=8), other glomerulopathies (n=7), unknown etiology (n=6), and others (n=7). WES identified genetic causes of CKD in 12 of the 37 patients (32.4%). Genetic defects were discovered in the *COL4A4* (n=2), *WT1* (n=2), *ACTN4*, *CEP290*, *COL4A3*, *CUBN*, *GATA3*, *LAMA5*, *NUP107*, and *PAX2* genes. *WT1* defects were found in patients whose pathologic diagnosis was membranoproliferative glomerulonephritis, and identification of *CUBN* defects led to discontinuation of immunosuppressive agents. Genetic diagnosis confirmed the clinical diagnosis of hypoparathyroidism, sensorineural deafness, and renal disease; Alport syndrome; and Joubert syndrome in three of the patients with CKD of unknown etiology (*COL4A4* [n=2], *CUBN* [n=1]). Extrarenal symptoms were considered phenotypic presentations of *WT1*, *PAX2*, and *CEP290* defects.

**Conclusions:** WES provided a genetic diagnosis that confirmed the clinical diagnosis in a significant proportion (32.4%) of patients with pediatric CKD.

**Keywords:** Genetics; Pediatrics; Renal insufficiency, chronic; Whole exome sequencing

## Introduction

Chronic kidney disease (CKD) is a global health problem with increasing incidence and prevalence [1]. Children with CKD face mortality, lifelong morbidity, and a low quality of life [2-5]. Advancements in medical care have substantially improved the survival rate of children with CKD [1]. Identifying the underlying

cause of this disease is essential because the progression, treatment, and prognosis may differ according to etiology. However, traditional diagnostic approaches, such as kidney biopsy, could be unrevealing or contraindicated when the kidneys are already failing [6]. Therefore, new diagnostic methods are required to identify the etiology of CKD.

CKD is a complex genetically heterogeneous disease with

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both genomic and environmental causes. The heritability of CKD is relatively high (30%–75%) [7], and at least 15% to 20% of early onset CKD (before the age of 25 years) is caused by genetic variation. Nearly all children who progress to end-stage kidney disease (ESKD) have an inherited form of CKD [8,9]. In addition, approximately 17% of patients with ESKD do not have a primary renal disease diagnosis and are therefore labeled as patients with CKD of unknown etiology.

The diagnostic accuracy provided by genetic testing [6] could enable the establishment of treatment guidelines and aid in the accurate prediction of patient prognosis. Genetic diagnosis is crucial for identifying high-risk groups and for appropriate family planning. The prevalence of CKD caused by genetic defects is approximately 10% in unselected adults [9] and 20% to 30% in children with nephropathy [7,10]. These findings indicate that the clinical application of genetic testing could transform diagnostic pathways by providing a timely and accurate genetic diagnosis [11].

Sanger sequencing of the causative genes is typically performed to obtain a genetic diagnosis when CKD is suspected. However, the number of known causative genes for CKD is increasing, as is the number of CKD cases. Therefore, traditional Sanger sequencing is not cost-effective in most cases, making targeted exome sequencing or whole-exome sequencing (WES) the preferred approaches for genetic diagnosis [12–15]. WES can screen most genes associated with diseases and can therefore be applied across diverse categories of renal disorders. In addition, it can potentially identify novel etiological genes associated with nephropathy. Therefore, WES is emerging as the preferred diagnostic tool for hereditary disorders [12–17]. It has provided a genetic diagnosis in up to 11.5%, 26%, and 32.7% of patients with congenital kidney anomalies, steroid-resistant nephrotic syndrome, and ESKD, respectively [18,19].

In this study, we present the preliminary results of utilizing WES to identify the genetic causes of pediatric CKD in Republic of Korea.

## Methods

### 1. Study design and participants

We prospectively recruited 37 pediatric patients with CKD whose underlying etiology was uncertain or suspected to be monogenic. The male to female ratio of the patients was 20:17. All study participants were recruited from Seoul National University Children's Hospital, Seoul, Republic of Korea. Buccal

mucosal samples were collected and analyzed from January to June 2021. Details of DNA extraction and analysis of WES data have been previously described [20]. The identified variants were classified based on the American College of Medical Genetics and Genomics standards for the interpretation of sequence variants [21].

### 2. Statistical analysis

The diagnostic yield was calculated based on the variants classified as “pathogenic” or “likely pathogenic.” All statistical analyses were conducted using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and SPSS statistical software version 20 (IBM Corp, Armonk, NY, USA).

## Results

### 1. Cohort characteristics

A total of 37 patients with CKD were recruited for this study. The underlying causes of CKD among the study participants were focal segmental glomerulosclerosis, congenital anomalies of the kidney and urinary tract (CAKUT) including reflux nephropathy, ischemic disease, Fanconi syndrome, Bartter syndrome, and CKD of unknown etiology (Table 1). One patient had a family history of CKD. The median ages at the time of CKD diagnosis and recruitment were 6 and 13 years, respectively. The distributions of the CKD stages and clinical diagnoses are described in Tables 1 and 2.

### 2. Genetic findings and diagnostic yield

Diagnostic variants were detected in 12 of the 37 patients (32.4%), encompassing seven distinct monogenic disorders. The detected genes were *COL4A4* (n=2), *WT1* (n=2), *ACTN4* (n=1), *CEP290* (n=1), *COL4A3* (n=1), *CUBN* (n=1), *GATA3* (n=1), *LAMA5* (n=1), *NUP107* (n=1), and *PAX2* (n=1). An additional three patients had variants of uncertain significance. The diagnostic yield (Table 1) was the highest among patients with CKD of unknown etiology (n=6, 50%). In contrast, diagnostic variants were not detected in some (n=7) of the clinically diagnosed CKD cases, including Alport syndrome, CAKUT, and renal hypoplasia with diabetes mellitus.

### 3. Clinical implications of genetic diagnoses in the study

Table 3 summarizes the clinical characteristics and genetic results of the patients with diagnostic variants. In six cases including three patients with CKD of unknown etiology, WES

**Table 1.** Clinical category characteristic and diagnostic yield of genetic diagnose across clinical diagnostic categories

Clinical category	No. of patients (%)	Male sex	Sequencing performed	No. of diagnostic variant present (%)	Singleton genetic diagnoses
FSGS	9 (24.3)	3	9	3 (33.3)	0
CAKUT including reflux nephropathy	8 (21.6)	4	8	2 (25.0)	1
Other glomerulopathy	7 (18.9)	2	7	3 (42.8)	1
Unknown etiology	6 (16.2)	4	6	3 (50.0)	2
Others	7 (18.9)	4	7	1 (14.3)	1

FSGS, focal segmental glomerulosclerosis; CAKUT, congenital anomalies of kidney and urinary tract.

**Table 2.** Patient clinical characteristics of having whole-exome sequencing

Characteristics	Total (n=37)
Age at diagnosis of CKD (yr)	6.00 (3.00–10.25)
Sex, male:female	20:17
CKD stage	
Stage 1	5 (13.5)
Stage 2	7 (18.9)
Stage 3	2 (5.4)
Stage 4	2 (5.4)
Stage 5	21 (56.8)
Kidney transplantation state	18
On dialysis (HD or PD)	4

Values are presented as median (interquartile range) or number (%). CKD, chronic kidney disease; HD, hemodialysis; PD, peritoneal dialysis.

clarified the clinical diagnosis or reclassified the disease, which considerably affected clinical decision making. In five cases, WES confirmed the clinical diagnosis of hypoparathyroidism, sensorineural deafness, and renal disease; Alport syndrome; and Joubert syndrome.

Genetic diagnoses have direct consequences for medical management. The diagnosis of Alport syndrome enabled genetic counseling and screening for auditory and ophthalmological problems. Detection of *WT1* defects in patients with pathological diagnoses of membranoproliferative glomerulonephritis led to more careful monitoring of malignancy. In accordance with Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, identification of *CUBN* defects in a patient with persistent isolated proteinuria resulted in discontinuation of immunosuppressive agents [22]. Patients with extrarenal symptoms were examined to obtain a genetic diagnosis of *WT1* (sex reversal), *PAX2* (retinopathy), and *CEP290* (Joubert syndrome) defects.

## Discussion

In this study, we used WES to identify the genetic causes of

pediatric CKD in Republic of Korea. The diagnostic yield of this approach was 32.4%, encompassing 10 genes, one copy number variation, and one microdeletion. This is similar to previous studies reporting detection rates of causal mutations. Mutations were detected in approximately 20% and 30% of patients who presented with CKD [7] and ESKD [10], respectively. These causal mutations were detected in patients diagnosed before the age of 25 years. Furthermore, children with kidney failure had a detection rate of approximately 40% [6]. With rapid technological advances, we expect that regular re-analysis, re-interpretation, and reclassification of variants will increase these detection rates [23,24].

The primary diagnosis in patients with ESKD is often inaccurate [25], resulting in its characterization as a CKD of unknown origin. In adults, WES provided a genetic diagnosis in 22 out of 92 patients (24%) with CKD of unknown etiology [26], whereas our study established a diagnostic yield of 50%. This shows that WES is effective in determining the genetic causes of CKD of unknown etiology. However, it is important to note that the number of patients in our study was small.

Establishing a precise genetic diagnosis can allow for the preemptive screening of extrarenal manifestations. Patients clinically diagnosed with isolated CAKUT can have mutations in genes that cause syndromic diseases [27]. In other cases, extrarenal manifestations could be detected later in life. Moreover, subtle phenotypes may be initially overlooked and only identified through a genetic diagnosis. In these cases of reverse phenotyping, identification of a genetic mutation can lead to preemptive screening for extrarenal manifestations, leading to early provision of treatment where possible. In our study, extrarenal symptoms were identified to obtain a genetic diagnosis of *WT1*, *PAX2*, and *CEP290* defects.

Recent KDIGO guidelines recommend discontinuing immunosuppressive agents if a monogenic cause is discovered in a patient with focal segmental glomerulosclerosis. Furthermore, concerns regarding kidney donations by living donors may be

**Table 3.** Diagnostic variants identified in whole-exome sequencing

Patient no.	Age (yr)	Sex	CKD stage	Clinical presentation	Genetic diagnosis	Gene symbol	Nucleotide change	Amino acid change	Clinical implications of genetic information
1	14	F	KT	Infantile NS, Bx: FGSG		LAMA5	c.6883C>T; c.1447+4C>T	p.Gln2295Ter	
2	9	F	KT	Bx: membranoproliferative glomerulonephritis genital anomaly (-)		WT1			
3	18	M	1	Continuous microscopic HU, PU, Bx: GBM disease	Alport syndrome	2q36.3 deletion including COL4A4 (2:227773537-227890526x1)			Family counselling
4	14	F	1	Gross hematuria, proteinuria, brother: MELAS	Alport syndrome	COL4A3	c.1216C>T c.40_63del	p.Arg406Ter, p.Leu14_Leu21del	Family counselling
5	17	F	KT	ESKD, Bx: FGSG		ACTN4	c.718A>G;	p.Met240Val	
6	12	M	KT	ESKD, Bx: membranous glomerulonephritis genital anomaly (-)		WT1	c.1259A>G;	p.His420Arg	
7	18	F	KT	ESKD, Bx: FGSG		NUP107	c.2492A>C	p.Asp831Ala	
8	23	F	4	Azotemia, duplicated kidney, sensory neural hearing loss	HDR syndrome	CNV including GATA3			Family counselling
9	6	F	PD	Dandy-walker syndrome, developmental delay	Joubert syndrome	CEP290	c.6012-2T>A		
10	12	F	2	PU, azotemia, Bx: GBM disease	Alport syndrome	COL4A4	c.3161G>T	p.Gly1054Val	Family counselling
11	5	F	4	Azotemia, PU, hydronephrosis, CAKUT ocular anomaly (-)		PAX2	c.76dup	p.Val26GlyfsTer28	
12	1	M	1	PU		CUBN	c.4855+2C>G		Discontinuation of immunosuppressive agents

CKD, chronic kidney disease; F, female; M, male; KT, kidney transplantation; NS, nephrotic syndrome; Bx, biopsy; FGSG, focal segmental glomerulosclerosis; HU, hematuria; PU, proteinuria; GBM, glomerular basement membrane; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode syndrome; ESKD, end-stage kidney disease; FGSG, focal segmental glomerulosclerosis; HDR syndrome, hypoparathyroidism, sensorineural deafness and renal disease syndrome; CNV, copy number variant; PD, peritoneal dialysis; CAKUT, congenital anomalies of the kidney and urinary tract.

alleviated because of the reduced risk of recurrence [28]. In addition, genetic diagnosis aids in choosing potential donors among living relatives. For example, if Alport syndrome is diagnosed in a male patient, his mother needs to be tested for the presence of pathogenic variants before considering donating a kidney to her son. This is of clinical importance because we genetically identified Alport syndrome in patients with a clinical diagnosis of focal segmental glomerulosclerosis. This finding is an indication of variable phenotypic expressions caused by mutations in *Col4A* genes, such as hematuria or proteinuria, and is consistent with recent studies [29-31].

The limitations of our study include a relatively short study period and small sample size, in addition to its single-center design. Another limitation is that only one patient had a family history of CKD. In addition, some compound heterozygous variants were not investigated for phasing. The significance of variants of uncertain significance was not investigated further because it was beyond the scope of this study. The inherent shortcomings of WES include the possibility of missing mutations in introns, copy number variations, trinucleotide repeat expansions, methylation abnormalities, and mutations in exons with low coverage [23].

In conclusion, WES provided a genetic diagnosis in a considerable proportion of patients with pediatric CKD in Republic of Korea, which may confirm clinical diagnoses, provide guidelines for patient management, and aid in genetic counseling. Our study provides more evidence supporting WES as a new diagnostic method for identifying CKD etiology in children.

## Ethical statements

This study was approved by the Institutional Review Board of Seoul National University Hospital (No. 2011-048-1171). All patients were enrolled after informed consent was obtained from them and their caregivers.

## Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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## Author contributions

Conceptualization: YHA, HGK

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Formal analysis: HL

Funding acquisition: HGK

Investigation: YHA

Methodology: JM

Project administration: HGK

Visualization: YHA

Writing-original draft: HL

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## References

1. Becherucci F, Roperto RM, Materassi M, Romagnani P. Chronic kidney disease in children. *Clin Kidney J* 2016;9:583-91.
2. Kaspar CD, Bholah R, Bunchman TE. A review of pediatric chronic kidney disease. *Blood Purif* 2016;41:211-7.
3. Moreira JM, Bouissou Morais Soares CM, Teixeira AL, Simoes E Silva AC, Kummer AM. Anxiety, depression, resilience and quality of life in children and adolescents with pre-dialysis chronic kidney disease. *Pediatr Nephrol* 2015;30:2153-62.
4. Al-Uzri A, Matheson M, Gipson DS, Mendley SR, Hooper SR, Yadin O, et al. The impact of short stature on health-related quality of life in children with chronic kidney disease. *J Pediatr* 2013;163:736-41.
5. Gerson A, Hwang W, Fiorenza J, Barth K, Kaskel F, Weiss L, et al. Anemia and health-related quality of life in adolescents with chronic kidney disease. *Am J Kidney Dis* 2004;44:1017-23.
6. Chen J, Lin F, Zhai Y, Wang C, Wu B, Ma D, et al. Diagnostic and clinical utility of genetic testing in children with kidney failure. *Pediatr Nephrol* 2021;36:3653-62.
7. Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. *Nat Rev Nephrol* 2016;12:133-46.
8. Devuyst O, Knoers NV, Remuzzi G, Schaefer F; Board of the Working Group for Inherited Kidney Diseases of the European Renal Association and European Dialysis and Transplant Association. Rare inherited kidney diseases: challenges, opportunities, and perspectives. *Lancet* 2014;383:1844-59.
9. Groopman EE, Rasouly HM, Gharavi AG. Genomic medicine for kidney disease. *Nat Rev Nephrol* 2018;14:83-104.
10. Mann N, Braun DA, Amann K, Tan W, Shril S, Connaughton DM, et al.

- Whole-exome sequencing enables a precision medicine approach for kidney transplant recipients. *J Am Soc Nephrol* 2019;30:201-15.
11. Rabbani B, Mahdieh N, Hosomichi K, Nakaoka H, Inoue I. Next-generation sequencing: impact of exome sequencing in characterizing Mendelian disorders. *J Hum Genet* 2012;57:621-32.
  12. Lee H, Deignan JL, Dorrani N, Strom SP, Kantarci S, Quintero-Rivera F, et al. Clinical exome sequencing for genetic identification of rare Mendelian disorders. *JAMA* 2014;312:1880-7.
  13. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med* 2013;369:1502-11.
  14. Dixon-Salazar TJ, Silhavy JL, Udpa N, Schroth J, Bielas S, Schaffer AE, et al. Exome sequencing can improve diagnosis and alter patient management. *Sci Transl Med* 2012;4:138ra78.
  15. Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, Nickerson DA, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet* 2011;12:745-55.
  16. Goldstein DB, Allen A, Keebler J, Margulies EH, Petrou S, Petrovski S, et al. Sequencing studies in human genetics: design and interpretation. *Nat Rev Genet* 2013;14:460-70.
  17. Katsanis SH, Katsanis N. Molecular genetic testing and the future of clinical genomics. *Nat Rev Genet* 2013;14:415-26.
  18. Bierzynska A, McCarthy HJ, Soderquest K, Sen ES, Colby E, Ding WY, et al. Genomic and clinical profiling of a national nephrotic syndrome cohort advocates a precision medicine approach to disease management. *Kidney Int* 2017;91:937-47.
  19. Bekheirnia MR, Bekheirnia N, Bainbridge MN, Gu S, Coban Akdemir ZH, Gambin T, et al. Whole-exome sequencing in the molecular diagnosis of individuals with congenital anomalies of the kidney and urinary tract and identification of a new causative gene. *Genet Med* 2017;19:412-20.
  20. Seo GH, Kim T, Choi IH, Park JY, Lee J, Kim S, et al. Diagnostic yield and clinical utility of whole exome sequencing using an automated variant prioritization system, EVIDENCE. *Clin Genet* 2020;98:562-70.
  21. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-24.
  22. Boger CA, Chen MH, Tin A, Olden M, Kottgen A, de Boer IH, et al. CUBN is a gene locus for albuminuria. *J Am Soc Nephrol* 2011;22:555-70.
  23. Manickam K, McClain MR, Demmer LA, Biswas S, Kearney HM, Malinowski J, et al. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2021;23:2029-37.
  24. Deignan JL, Chung WK, Kearney HM, Monaghan KG, Rehder CW, Chao EC, et al. Points to consider in the reevaluation and reanalysis of genomic test results: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2019;21:1267-70.
  25. Hoekstra T, Hemmelder MH, van Ittersum FJ. RENINE annual report 2015. Utrecht: Nederlandse Federatie voor Nefrologie; 2017.
  26. Lata S, Marasa M, Li Y, Fasel DA, Groopman E, Jobanputra V, et al. Whole-exome sequencing in adults with chronic kidney disease: a pilot study. *Ann Intern Med* 2018;168:100-9.
  27. van der Ven AT, Connaughton DM, Ityel H, Mann N, Nakayama M, Chen J, et al. Whole-exome sequencing identifies causative mutations in families with congenital anomalies of the kidney and urinary tract. *J Am Soc Nephrol* 2018;29:2348-61.
  28. Baum MA, Ho M, Stablein D, Alexander SR; North American Pediatric Renal Transplant Cooperative Study. Outcome of renal transplantation in adolescents with focal segmental glomerulosclerosis. *Pediatr Transplant* 2002;6:488-92.
  29. Malone AF, Phelan PJ, Hall G, Cetinçelik U, Homstad A, Alonso AS, et al. Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis. *Kidney Int* 2014;86:1253-9.
  30. Deltas C, Savva I, Voskarides K, Papazachariou L, Pierides A. Carriers of autosomal recessive Alport syndrome with thin basement membrane nephropathy presenting as focal segmental glomerulosclerosis in later life. *Nephron* 2015;130:271-80.
  31. Gast C, Pengelly RJ, Lyon M, Bunyan DJ, Seaby EG, Graham N, et al. Collagen (COL4A) mutations are the most frequent mutations underlying adult focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 2016;31:961-70.

# Delta neutrophil index as a predictor of vesicoureteral reflux in children with febrile urinary tract infection

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**Purpose:** Delta neutrophil index (DNI) indicates immature granulocytes in peripheral blood and has been confirmed to be effective as a prognostic factor for neonatal sepsis. Also, it has been reported to have diagnostic value in acute pyelonephritis and in predicting vesicoureteral reflux (VUR) in the infant. We conducted the study to verify whether DNI is also helpful in the entire pediatric age group with febrile urinary tract infection (UTI).

**Methods:** Medical records of children hospitalized for febrile UTIs were analyzed retrospectively. All subjects underwent kidney ultrasound and voiding cystourethrography. In the group with and without VUR, we compared sex and age, and the following laboratory values: the white blood cell count, neutrophil, polymorphonuclear leucocyte, eosinophil, hemoglobin, platelet count, C-reactive protein, DNI value, and the finding of ultrasound.

**Results:** A total of 315 patients (163 males and 152 females; range, 0–127 months) were eligible, and 41 patients (13%) had VUR. As a result of univariate analysis, the white blood cell count, neutrophil, DNI, and ultrasonic abnormalities were high in the reflux group, and the hemoglobin and lymphocyte fraction values were low. The value of DNI and the abnormal ultrasound were significantly higher in the reflux group on the multivariate analysis. The area under the curve value of the receiver operating curve was higher in DNI (0.640; 95% confidence interval, 0.536–0.744;  $P=0.004$ ), and the DNI cutoff value for VUR prediction was 1.85%.

**Conclusions:** We identified that ultrasound findings and DNI values were helpful predictors of VUR in pediatric febrile UTIs.

**Keywords:** Child; Urinary tract infections; Vesico-ureteral reflux

## Introduction

Urinary tract infection (UTI) is a common bacterial disease in childhood. If appropriate diagnosis and treatment are not performed early, chronic renal failure or high blood pressure may occur due to renal scars [1,2]. In particular, in infants, about 30% to 50% of children with UTIs are known to have urinary tract abnormalities [3], and vesicoureteral reflux (VUR) is common. VUR is diagnosed in about 25% to 50% of children with febrile UTI and is well known to cause recurrent UTI and renal scar

[4]. It can be diagnosed through voiding cystourethrography (VCUG), but discussions on the indication of the test have continued because it has a disadvantage in radiation exposure and invasiveness. Previously, VCUG was recommended in children between the ages of 2 months and 2 years with febrile UTI [5], but the revised the American Academy of Pediatrics (AAP) guidelines in 2011 stated that VCUG should not be performed routinely after the first febrile UTI but performed in specific circumstances [6].

Therefore, young age, high C-reactive protein (CRP) concen-

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tration, family history of urinary tract diseases, and neurosurgical abnormalities have been suggested as risk factors for VUR to predict VCUG implementation [7,8], but the association is not clearly established.

Meanwhile, immature granulocytes appear in peripheral blood in the process of promoting granulocyte production in the bone marrow during infection or systemic inflammatory reactions, and recently, these immature granules have been steadily suggested as predictors of infection or sepsis [9-11]. However, it was difficult to apply clinical trials because manual calculation is required to identify immature granules, and accuracy depends on the examiner. Delta neutrophil index (DNI) was recently proposed as a new indicator to reflect the circulating fraction of immature granules [9]. DNI is an index calculated in leukocyte identification in ADVIA 2120 (Siemens Healthineers, Erlangen Germany), one of the types of automatic blood cell analyzer [12], which has the advantage of obtaining results quickly because it is automatically calculated during complete blood cell count. Because DNI reflects the number of immature granulocytes, it has been reported to help determine the severity of patients suspected of sepsis or systemic inflammation [9,13,14], and recent studies have also reported diagnostic value in young infants with febrile UTI [15]. Lee et al. [15] identified that DNI showed a moderate specificity and low sensitivity for predicting the presence of VUR, especially in younger infants. So, the authors conducted this study to confirm the clinical usefulness of DNI as a predictor of VUR in the entire pediatric age group with febrile UTI.

## Methods

From December 2002 to April 2007, the medical records of children hospitalized for febrile UTI at Konyang University Hospital in Daejeon were analyzed retrospectively. Because VCUG was selectively implemented since 2011 after the changed AAP guideline, we selected patients with febrile UTI who were treated before 2011 who underwent both kidney ultrasound (USG) and VCUG to reduce selection bias.

Among them, 315 people (163 males and 152 females) who underwent both kidney USG and VCUG were included as the study subjects. Total white blood cell count, polymorphonuclear leukocyte, total neutrophil count, CRP, hemoglobin, platelet count, DNI value, and abnormalities of USG were compared in both groups with or without VUR. Those who did not perform either test or had congenital deformities such as single kidney, bladder

diverticulum, or polycystic kidney disease were excluded.

We used the blood test results performed on the hospitalization date, and the total blood cell calculation value and DNI were automatically calculated by the automatic blood cell analyzer (ADVIA 2120; Siemens Healthineers). The DNI values calculated through the ADVIA 2120 automated blood cell analyzer are as follows:  $DNI = (\text{the leukocyte subfraction assayed in the myeloperoxidase channel by cytochemical reaction}) - (\text{the leukocyte subfraction counted in the nuclear lobularity channel by the reflected light beam})$ .

USG-positive was defined as cases whose renal USG performed by radiologist confirmed one-sided or both-sided pelviectasia or hydronephrosis, and VUR was defined as cases reflux from VCUG was confirmed.

SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and R package 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analysis. Through univariate and multivariate analysis and area under the curve value of the receiver operating characteristic (ROC) curve, we compared the effectiveness of the variables and obtained the cutoff value of DNI. The case of  $P < 0.05$  was judged to be statistically significant.

## Results

### Baseline characteristics

Of the 315 patients, 163 (51.7%) were boys and 152 (48.3%) were girls, and the average age was  $14.0 \pm 22.9$  months (median, 4.7 months; range, 0–127 months). There were 209 (66.3%) under the age of 1 year, 81 (25.7%) from 1 to 5 years of age, and 25 (7.9%) over 5 years of age, respectively (Fig. 1). There were 65 patients (20.6%) with USG-positive, and 41 patients (13.0%) diagnosed VUR on one or both sides by VCUG (Table 1).

### Comparison of VUR positive and negative group

The mean age of reflux group was  $14.2 \pm 20.1$  months, which was not different from the group without VUR ( $14.0 \pm 23.3$  months). Girls ( $n=24$ , 58.5%) were more common in the VUR group, and boys ( $n=146$ , 53.3%) were more common in the group without VUR, but there was no statistically significant difference between the two groups. Blood tests showed no difference in eosinophil fraction ( $P=0.293$ ), polymorphonuclear cell fraction ( $P=0.233$ ), and platelet count ( $P=0.380$ ), but the total white blood cell count ( $18.0 \pm 6.5 \times 10^3/\mu\text{L}$  vs.  $15.3 \pm 6.7 \times 10^3/\mu\text{L}$ ,  $P=0.013$ ), neutrophil fraction ( $57.1\% \pm 19.3\%$  vs.  $50.7\% \pm 18.5\%$ ,  $P=0.039$ ), and CRP ( $2.6 \pm 1.6$  mg/dL vs.  $4.0 \pm 2.0$  mg/dL,  $P=0.003$ ) showed higher

values in the VUR group (Table 1).

The rate of abnormal findings on USG was 36.6% (15/41) in the reflux group and 18.2% (50/274) in the group without VUR ( $P=0.007$ ). Even in the group with VUR, as many as 65.3% (32/49) patients showed normal findings on renal USG. In our study, the detection sensitivity of renal USG for VUR was only 34.5%. It is because VUR grades 1 and 2 do not have hydronephrosis, and grade 3 may not have hydronephrosis, depending on the status of the bladder. The DNI value was also statistically significantly higher in the reflux group ( $3.4\pm 10.0\%$  vs.  $-0.2\pm 5.6\%$ ,  $P=0.031$ ) (Table 1).

**Possible predictive factors for VUR**

As a result of multivariate logistic regression analysis, the vari-

ables that differed significantly between the group with and without VUR were CRP, kidney USG, and DNI. It means that these indices can be used to predict the existence of VUR. The odds ratio (OR) of USG-positive, CRP, and DNI were 2.744 (95% confidence interval [CI], 1.249–6.026;  $P=0.012$ ), 1.383 (95% CI, 1.193–1.604;  $P<0.001$ ) and 1.079 (95% CI, 1.016–1.147;  $P=0.014$ ), respectively (Table 2).

We used ROC analysis to compare the predictive capabilities of these indices. The area under the curve value of the ROC curve of the USG-positive was 0.408 (95% CI, 0.309–0.507;  $P=0.058$ ), CRP was 0.631 (95% CI, 0.540–0.721;  $P=0.005$ ), and that of DNI was 0.640 (95% CI, 0.536–0.744;  $P=0.004$ ), the highest at DNI (Table 3, Fig. 2).

**The cutoff value of DNI for the prediction of VUR**

The cutoff value of the DNI for VUR prediction was statistically significant when set to 1.85 ( $P<0.05$ ). The sensitivity was 68.2%, and the specificity was 66.7%. The positive predictive value was 23.5%, and the negative predictive value was 93.4%.

**Discussion**

UTI in children can lead to renal scars, chronic renal failure, and high blood pressure due to damage to the renal parenchyma if diagnosed early and not appropriately treated. VUR is diagnosed in about 25% to 50% of children with UTI, which can cause recurrence risk and renal scar [4], so continuous prophylactic antibiotic use or surgical correction through early detec-

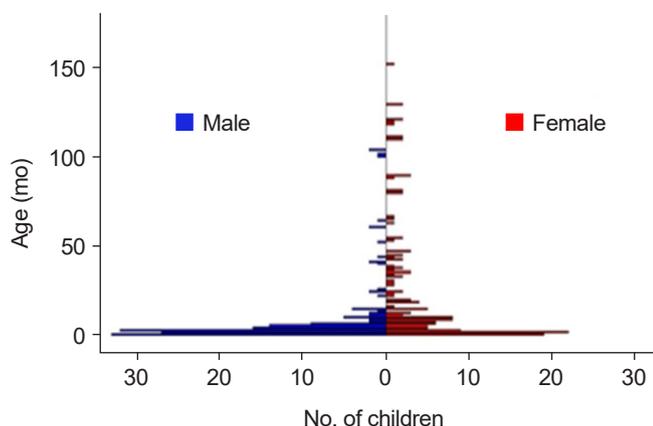


Fig. 1. Age distribution of children with febrile urinary tract infection.

Table 1. Baseline characteristics of children in VUR positive or negative groups

Characteristics	VUR positive (n=41)	VUR negative (n=274)	Total (n=315)	P-value
Age (mo)	14.2±20.1	14.0±23.3	14.0±22.9	0.953
Sex				
Male	17 (41.5)	146 (53.3)	163 (51.7)	0.158
Female	24 (58.5)	128 (46.7)	152 (48.3)	-
WBC ( $\times 10^3/\mu\text{L}$ )	18.0±6.5	15.3±6.7	15.6±6.7	0.013
Segmented neutrophil (%)	57.1±19.3	50.7±18.5	51.5±18.7	0.039
Lymphocyte (%)	30.6±19.2	37.0±16.3	36.2±16.8	0.023
Eosinophil (%)	1.4±1.7	1.9±2.9	1.8±2.8	0.293
PMN (%)	56.2±17.4	53.0±15.6	53.5±15.9	0.233
Absolute neutrophil count (/UL)	11,334±6,523	8,383±5,533	8,767±5,747	0.002
CRP (mg/dL)	2.6±1.6	4.0±2.0	2.8±1.9	0.003
Hemoglobin (g/dL)	10.8±1.1	11.2±1.4	11.2±1.4	0.039
Platelet count ( $\times 10^3/\mu\text{L}$ )	479.4±155.7	447.1±227.6	451.3±219.7	0.380
Hydronephrosis	15 (36.6)	50 (18.2)	65 (20.6)	0.007
DNI (%)	3.4±10.0	-0.2±5.6	0.3±6.4	0.031

Values are presented as mean±standard deviation or number (%).

VUR, vesicoureteral reflux; WBC, white blood cell; PMN, polymorphonuclear leukocytes; CRP, C-reactive protein; DNI, delta neutrophil index.

**Table 2.** Multivariable logistic regression analysis of clinical and laboratory parameters for prediction of vesicoureteral reflux

Variable	$\beta$	SE	OR (95% CI)	P-value
Sex (male)	-1.054	0.407	0.348 (0.157–0.774)	0.010
White blood cells	-0.042	0.089	0.958 (0.805–1.141)	0.634
% Segmented neutrophil	-0.045	0.039	0.956 (0.887–1.031)	0.245
% Lymphocyte	0.025	0.042	0.975 (0.898–1.058)	0.547
Absolute neutrophil count	0.000	0.000	1.000 (1.000–1.000)	0.286
Hemoglobin	-0.208	0.149	0.812 (0.606–1.088)	0.163
C-reactive protein	0.324	0.076	1.383 (1.193–1.604)	<0.001
Hydronephrosis	1.009	0.401	2.744 (1.249–6.026)	0.012
Delta neutrophil index	0.076	0.031	1.079 (1.016–1.147)	0.014

SE, standard error; OR, odds ratio; CI, confidence interval.

**Table 3.** AUC values from ROC curve of factors for the prediction of vesicoureteral reflux

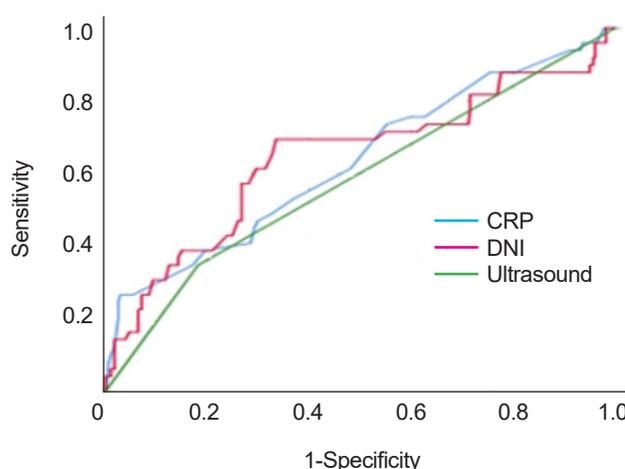
Variable	AUC	SE	95% CI	P-value
Ultrasound-positive	0.408	0.051	0.309–0.507	0.058
C-reactive protein	0.631	0.046	0.540–0.721	0.005
Delta neutrophil index	0.640	0.053	0.536–0.744	0.004

AUC, area under the ROC curve; ROC, receiver operating characteristic; SE, standard error; CI, confidence interval.

tion of VUR has been considered necessary in all children with UTI.

AAP recommended VCUG in all first febrile UTIs in guidelines of 1999 [5], but follow-up studies showed that the use of continuous prophylactic antibiotics or surgical correction did not reduce renal scars [16–19] in children with VUR. In addition, it was virtually impossible completely prevent renal scar in children with VUR [20–24] because most of the severe renal scars that cause hypertension and chronic renal failure, occurred prior to infection from the fetal period. Therefore, questions have been raised about the role of VCUG implemented after the first febrile UTI, and the changed guidelines recommend selective implementation. The 2007 British guidelines recommended VCUG only in atypical UTIs that did not respond well to treatment or recurrent UTIs in infants less than 6 months [25]. They suggested that VCUG was not required in children with uncomplicated UTIs over 6 months of age. The 2011 revised guidelines of AAP recommended VCUG only for in children with abnormalities on USG, atypical clinical course, or children with recurrent UTI, and no longer for all children with first febrile UTI [6].

So, many researchers studies have been making efforts to find the predictor for VUR because of the invasiveness, and the risk of radiation exposure VCUG. Oostenbrink et al. [7] suggested male, younger age, family history of urinary tract diseases, high level of CRP, and abnormal findings of USG findings as



**Fig. 2.** Receiver operating curve for predicting vesicoureteral reflux. CRP, C-reactive protein; DNI, delta neutrophil index.

independent factors of VUR. The authors aimed to identify the value of DNI in predicting the presence of VUR in children with febrile UTIs. Recently, Lee et al. [15] have identified the value of DNI with a comprehensive and more detailed study. In contrast, our study is a single institutional study and does not include dimercaptosuccinic acid (DMSA) scan. However, we verified that DNI was still worth predicting the presence of VUR even when the child with UTI is older than 12 months of age.

Our study confirmed that DNI and ultrasonography were predictors of VUR, which showed similar results to previous studies by Lee et al. [15] Especially, the area under the curve value of DNI in the ROC curve was the highest, so it was found to be more useful as VUR predictor than the finding of USG-positive recommended by most guidelines.

The cutoff value, which can predict VUR, was set at 1.85, which is low in usefulness as a screening test due to its sensi-

tivity at 68.2%. However, the negative predictive value is very high at 93.4%. It means the following facts; if the value of DNI is less than 1.85 in children with UTI, it is highly unlikely that VUR will be present. In this study, the DNI cutoff value was lower than that in other studies such as sepsis, which is thought to be because febrile UTI belongs to mild diseases compared to severe infectious diseases such as sepsis. The mean value of all patients in this study was 0.3 ( $\pm 6.4$ ). Previously, the value of DNI more than 6.5% was a significant indicator of sepsis or septic shock [26].

Contrary to most studies suggesting male sex as predictors of VUR, we found that boys had a lower risk of OR (0.348; 95% CI, 0.157–0.774), which seems unlikely to have a significant difference in sex between the group with and without VUR ( $P=0.158$ ). Our study has a few practical limitations. Above all, the subjects of the study are patients from the past. That is the limitation of this research being conducted retrospectively. Since 2011, VCUG was undertaken selectively. If the subjects were selected as recent patients, the selection bias would be significant. Also, there may be bias in patients whose DNI values are not measured or who have not undergone kidney USG or VCUG. Because it is a single institutional study, the number of data is relatively small and did not include the duration of fever or family history, which has been considered important in previous studies. In addition, we have not been able to compare DNI index with other reliable tools for VUR prediction, such as DMSA scan and urinary neutrophil gelatinase-associated lipocalin (NGAL). Traditionally, DMSA scan can find the possibility of a VUR very sensitively by finding a renal scar. However, there is a risk of radiation, and sedation for children of younger age. Recently, NGAL has been reported as a rapid and noninvasive method in the diagnosis of VUR [27]. Urinary NGAL-creatinine ratio is higher in children with VUR and may be used as a rapid and noninvasive method in diagnosing VUR. It is a sensitive and specific biomarker, but it still needs additional laboratory testing, and it is not covered by national health insurance.

Our study confirmed the usefulness of DNI as a predictor of VUR and also found that it was a better index than USG-positive, which was previously used as the best screening tool for VUR. We suggest that if the DNI value is lower than the cutoff, VCUG can be withheld because the negative predictive value of DNI is very high in children with febrile UTI. However, as previously described, since this study was conducted retrospectively in a single institution, additional large-scale prospective studies would be needed.

## Ethical statements

This study protocol was approved by Institutional Review Boards (IRB) of Konyang University Hospital (No. KYUH 2021-01-013-001). We were given exemption from getting informed consents by the IRB because the present study was a retrospective study, personal identifiers were completely removed, and the data were analyzed anonymously. Our study was conducted according to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

## Conflicts of interest

No potential conflict of interest relevant to this article was reported.

## Funding

None.

## Author contributions

Conceptualization: JEK, JSO, JMY, KOK, EJC  
 Data curation: JEK, JSO, JMY, KOK, EJC  
 Formal analysis: JEK, JSO, JMY, KOK, EJC  
 Investigation: JEK, JSO, JMY, KOK, EJC  
 Methodology: JEK, JSO, JMY, KOK, EJC  
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 Writing-original draft: JEK, JSO, JMY, KOK, EJC  
 Writing-review & editing: JEK, JSO, JMY, KOK, EJC  
 All authors read and approved the final manuscript.

## References

1. Jacobson SH, Eklof O, Lins LE, Wikstad I, Winberg J. Long-term prognosis of post-infectious renal scarring in relation to radiological findings in childhood: a 27-year follow-up. *Pediatr Nephrol* 1992;6:19-24.
2. Montini G, Tullus K, Hewitt I. Febrile urinary tract infections in children. *N Engl J Med* 2011;365:239-50.
3. Ginsburg CM, McCracken GH Jr. Urinary tract infections in young infants. *Pediatrics* 1982;69:409-12.
4. Yilmaz S, Ozcakar ZB, Kurt Sukur ED, Bulum B, Kavaz A, Elhan AH, et al. Vesicoureteral reflux and renal scarring risk in children after the

- first febrile urinary tract infection. *Nephron* 2016;132:175-80.
5. American Academy of Pediatrics. Committee on Quality Improvement. Subcommittee on Urinary Tract Infection. Practice parameter: the diagnosis, treatment, and evaluation of the initial urinary tract infection in febrile infants and young children. *Pediatrics* 1999;103(4 Pt 1):843-52.
  6. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management, Roberts KB. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;128:595-610.
  7. Oostenbrink R, van der Heijden AJ, Moons KG, Moll HA. Prediction of vesico-ureteric reflux in childhood urinary tract infection: a multivariate approach. *Acta Paediatr* 2000;89:806-10.
  8. Choi EJ, Lee MJ, Park SA, Lee OK. Predictors of high-grade vesicoureteral reflux in children with febrile urinary tract infections. *Child Kidney Dis* 2017;21:136-41.
  9. Nahm CH, Choi JW, Lee J. Delta neutrophil index in automated immature granulocyte counts for assessing disease severity of patients with sepsis. *Ann Clin Lab Sci* 2008;38:241-6.
  10. Ansari-Lari MA, Kickler TS, Borowitz MJ. Immature granulocyte measurement using the Sysmex XE-2100. Relationship to infection and sepsis. *Am J Clin Pathol* 2003;120:795-9.
  11. Mare TA, Treacher DF, Shankar-Hari M, Beale R, Lewis SM, Chambers DJ, et al. The diagnostic and prognostic significance of monitoring blood levels of immature neutrophils in patients with systemic inflammation. *Crit Care* 2015;19:57.
  12. Harris N, Kunicka J, Kratz A. The ADVIA 2120 hematology system: flow cytometry-based analysis of blood and body fluids in the routine hematology laboratory. *Lab Hematol* 2005;11:47-61.
  13. Lee SH, Ko KO, Lim JW, Yoon JM, Song YH, Lee JW, et al. Delta-neutrophil index: a potential predictor of coronary artery involvement in Kawasaki disease by retrospective analysis. *Rheumatol Int* 2019;39:1955-60.
  14. Koh ID, Jeon IS, Kim HM. Diagnostic significance of the delta neutrophil index and other conventional parameters in neonatal bacteremia. *Pediatr Infect Vaccine* 2017;24:1-6.
  15. Lee JW, Kim SH, Park SJ, Lee KH, Park JH, Kronbichler A, et al. The value of delta neutrophil index in young infants with febrile urinary tract infection. *Sci Rep* 2017;7:41265.
  16. Keren R, Carpenter MA, Hoberman A, Shaikh N, Matoo TK, Chesney RW, et al. Rationale and design issues of the Randomized Intervention for Children With Vesicoureteral Reflux (RIVUR) study. *Pediatrics* 2008;122 Suppl 5(Suppl 5):S240-50.
  17. Montini G, Rigon L, Zucchetta P, Fregonese F, Toffolo A, Gobber D, et al. Prophylaxis after first febrile urinary tract infection in children? A multicenter, randomized, controlled, noninferiority trial. *Pediatrics* 2008;122:1064-71.
  18. Pennesi M, Travan L, Peratoner L, Bordugo A, Cattaneo A, Ronfani L, et al. Is antibiotic prophylaxis in children with vesicoureteral reflux effective in preventing pyelonephritis and renal scars? A randomized, controlled trial. *Pediatrics* 2008;121:e1489-94.
  19. Hodson EM, Wheeler DM, Vimalchandra D, Smith GH, Craig JC. Interventions for primary vesicoureteric reflux. *Cochrane Database Syst Rev* 2007;(3):CD001532.
  20. Koyle MA, Elder JS, Skoog SJ, Mattoo TK, Pohl HG, Reddy PP, et al. Febrile urinary tract infection, vesicoureteral reflux, and renal scarring: current controversies in approach to evaluation. *Pediatr Surg Int* 2011;27:337-46.
  21. Lim R. Vesicoureteral reflux and urinary tract infection: evolving practices and current controversies in pediatric imaging. *AJR Am J Roentgenol* 2009;192:1197-208.
  22. Merguerian PA, Sverrisson EF, Herz DB, McQuiston LT. Urinary tract infections in children: recommendations for antibiotic prophylaxis and evaluation: an evidence-based approach. *Curr Urol Rep* 2010;11:98-108.
  23. Riccabona M, Avni FE, Blickman JG, Dacher JN, Darge K, Lobo ML, et al. Imaging recommendations in paediatric urology: minutes of the ESPR workgroup session on urinary tract infection, fetal hydronephrosis, urinary tract ultrasonography and voiding cystourethrography, Barcelona, Spain, June 2007. *Pediatr Radiol* 2008;38:138-45.
  24. Williams GJ, Hodson EH, Isaacs D, Craig JC. Diagnosis and management of urinary tract infection in children. *J Paediatr Child Health* 2012;48:296-301.
  25. National Collaborating Centre for Women's and Children's Health. Urinary tract infection in children: diagnosis, treatment and long-term management. London: RCOG Press; 2007.
  26. Park BH, Kang YA, Park MS, Jung WJ, Lee SH, Lee SK, et al. Delta neutrophil index as an early marker of disease severity in critically ill patients with sepsis. *BMC Infect Dis* 2011;11:299.
  27. Eskandarifar A, Rahehag R, Jafari M. Evaluating the measurement of urinary neutrophil gelatinase associated lipocalin for the diagnosis of vesicoureteral reflux in children. *Int J Pediatr* 2021;9:15015-21.

# Predictors of renal scars in infants with recurrent febrile urinary tract infection: a retrospective, single-center study

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**Purpose:** To determine predictive factors for detecting renal parenchymal damages (RPDs) in infants with recurrent febrile urinary tract infection (fUTI).

**Methods:** From January 2015 to December 2021, 102 infants with recurrent fUTI and who underwent <sup>99m</sup>Tc-dimercaptosuccinic acid (DMSA) renal scan in our hospital were included in this study. Controls included infants with normal DMSA results performed 3 months apart from the 2nd episode of fUTI. DMSA-positive group included infants with positive DMSA results performed 3 months apart from the 2nd episode of fUTI or at the 3rd episode of fUTI. The recurrence rate, causative bacteria, renal size discrepancy of both kidneys, and laboratory findings including C-reactive protein (CRP) and spot urine sodium-to-potassium ratio (uNa/K) were compared between both groups.

**Results:** Only 3.8% of 79 infants with a 2nd episode of fUTI showed positive DMSA results. fUTI recurred more frequently within 12 months of follow-up in the DMSA-positive group than in the control group (69% vs. 13%,  $P<0.001$ ). CRP values were significantly higher in the DMSA-positive group than in the control group (7.3 mg/dL vs. 3.7 mg/dL,  $P<0.001$ ). Spot uNa/K were significantly lower in the DMSA-positive group than in the control group (0.6 vs. 1.1,  $P<0.001$ ).

**Conclusions:** Congenital renal scar and RPDs on the DMSA scan were more frequently found in infants with recurrent fUTI than those in the control group. High CRP values and low spot uNa/K in acute infections were helpful in predicting the presence of RPD in infants with recurrent fUTI.

**Keywords:** C-reactive protein; Urinalysis; Urinary tract infection

## Introduction

Febrile urinary tract infection (fUTI) is common in infants who are immunocompromised and lack a defense mechanism against bacterial ascending infection [1]. The fUTI imaging strategies used worldwide include a top-down approach (<sup>99m</sup>Tc-di-

mercaptosuccinic acid [DMSA] renal scan is the first imaging study) and a bottom-up approach (voiding cystourethrography [VCUG] is the imaging study prior to DMSA scan) [2].

Currently, routine DMSA renal scan and VCUG are not recommended during the 1st episode of fUTI because of radiation exposure [3]. However, despite differing opinions, renal ultra-

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sonography (US) may be beneficial during the 1st episode of fUTI in order to exclude renal abscess or other accompanying congenital anomalies of the kidney and urinary tract (CAKUT) if no economic hindrance exists [4].

Therefore, a less invasive and less expensive urinary tract imaging strategy that can detect high-risk patients possessing lower glomeruli number than normal whose urinary tract infection (UTI) is susceptible to progress into chronic renal injury (CRI) during their lifetime would be ideal. Furthermore, the fewer the number of practiced imaging studies, the more ideal that strategy would be.

Congenital hypoplastic kidney or congenital renal scar (cRS) is likely to progress into CRI due to recurrent pyelonephritis and thus should be diagnosed as early as possible [5]. Fortunately, the incidence of simple renal hypoplasia is very low (0.27%) compared with that of fUTI [6]. Most patients with cRS also have high-grade vesicoureteral reflux (VUR) [7].

High-grade VUR, a common anomaly of CAKUT causing recurrent fUTI, is a main target of the bottom-up approach in the fUTI imaging strategy mainly because it is an anomaly that needs early surgical management in children with fUTI who have renal scars [8]. Nonetheless, if further fUTI does not recur, an acquired renal scar (aRS) is less likely to develop in the future. Among children with fUTI, pyelitis that does not cause renal scarring is more common than pyelonephritis that causes renal scarring [9]. If renal scarring does not occur in children with fUTI, immediate surgical treatment is not needed, and if eventually needed, it could be postponed for a later time. Hence, we prefer performing a DMSA scan before a VCUG during the 2nd episode of fUTI.

Renal scarring can lead to renal complications like proteinuria or hypertension, and progress into CRI through recurrent pyelonephritis and so on [10].

There have been many reports studying the factors predicting renal scarring in fUTI children. Procalcitonin, high-grade VUR, previous renal scarring, urine pentraxin-3, plasma neutrophil gelatinase-associated lipocalin, and urinary interleukin-6 or interleukin-8 positively correlated with the presence of renal scarring in fUTI children [11-15]. Although procalcitonin was a better predictor of renal scarring, it was more expensive than C-reactive protein (CRP). The other predicting factors were clinically impractical methods in the general clinical field and correlated with the presence of VUR.

Some authors have published a few reports pertaining to spot urine sodium-to-potassium ratio (uNa/K) as a useful pre-

dictor of acute pyelonephritis in fUTI children excluding pyelitis or lower UTI with other fever focus [16-18]. In these studies, authors paid attention whether spot uNa/K could be useful for predicting renal parenchymal damages (RPDs) leading into renal scar.

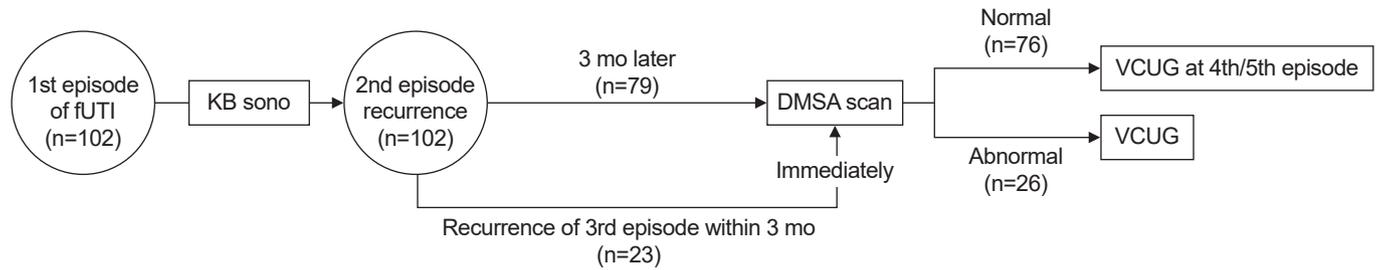
The aim of this study was to retrospectively analyze the laboratory and radiological findings in children with fUTI who had undergone a DMSA scan in our hospital according to our urinary tract imaging strategy and to determine the factors that predict the presence of RPDs including spot uNa/K.

## Methods

This study included a total of 134 children who had been admitted to our hospital with a past history of more than two episodes of fUTI and had performed a DMSA scan in our hospital between January 2015 and December 2021. Of the 134 children, 32 were excluded: five patients underwent DMSA scans during the acute phase of infection during their 2nd episode of fUTI, nine patients had accompanying CAKUT, except hydronephrosis on US, and 18 patients had no CRP, urine Na, urine K data. Finally, this study enrolled 102 children, with 26 patients in the DMSA-positive group and 76 patients in the control group.

fUTI was defined as high fever ( $\geq 38^{\circ}\text{C}$ ), pyuria ( $>5$  white blood cells [WBCs]/high-power field), positive leukocyte esterase on urinalysis, positive serum CRP values, and no other fever focus at admission.

Our urinary tract imaging strategy was as follows: for the 1st episode of fUTI, an US was performed. Then, a DMSA scan was performed 3 months after the acute infection in cases with either a suspicion of hypoplastic kidney (small kidney, less than 3 percentile of normal range) on the US during the 1st episode or in cases with a 2nd episode of fUTI (Fig. 1). In cases where fUTI recurred within 3 months from the 2nd episode, a DMSA scan was performed during the acute phase of infection at the 3rd episode (Fig. 1). VCUG was recommended in patients whose DMSA scan revealed positive findings. Significant bacteriuria was defined as 100,000 colony-forming units/mL of a single strain isolate. Urine samples were collected with a clean catch bag. At admission, blood samples (CRP, WBC, etc.) and spot urine samples (urinalysis, urine Na, urine K, etc.) were collected. All blood and urine samples from the 1st and 2nd episodes were included in the study. Urine electrolytes sampled over 24 hours after the start of intravenous hydration were excluded because those values change to a great extent with increased



**Fig. 1.** Schematic view of our hospital's diagnostic approach to febrile urinary tract infection (fUTI). KB, kidney and bladder; DMSA,  $^{99m}\text{Tc}$ -dimercaptosuccinic acid; VCUG, voiding cystourethrography.

intravascular volume. CRP was measured by turbidimetry. DMSA scans were performed with planar technique and were interpreted by both a nuclear medicine consultant and a pediatric nephrologist.

Patients whose DMSA scan showed a small kidney (less than 3 percentile of normal range) with either diffuse cortical defects with deformative renal contour or with a relative renal uptake of less than 35% in a kidney were classified as having cRS and were included in the DMSA-positive group. Mild cortical defects were defined as the presence of one or two cortical defects (photopenia) with normal renal contour line. Moderate to severe cortical defects were defined as the presence of multiple focal cortical defects ( $\geq 3$ ) on the DMSA scan. Patients with normal DMSA findings were included in the control group. We compared clinical characteristics, CRP, WBC, uNa/K, urine culture, and renal US between the DMSA-positive and control groups. Mean values of blood and urine samples from the 1st and 2nd episodes in each patient were calculated. All data were retrospectively analyzed.

All variables are presented as mean  $\pm$  standard deviation, and Student *t*-test was used when factors were compared between two independent groups. Continuous variables were analyzed using Wilcoxon-Mann-Whitney test. Statistical significance was defined as  $P \leq 0.05$ .

## Results

Among 79 infants with the 2nd episode of fUTI, only three (3.8%) showed positive DMSA results on the scan performed 3 months after the last episode, including an infant who had cRS. Among 27 infants with a 3rd episode of fUTI, four (14.8%) had normal renal cortices, eight (29.6%) had mild cortical defects, eight (29.6%) had cRS, and seven (25.9%) had moderate to severe cortical defects without cRS (with a possibility of aRS developing in the future) on the acute DMSA scan. Four infants with a 3rd

episode of fUTI were excluded from the DMSA-positive group because of missing data.

The DMSA-positive group included three infants who underwent DMSA scanning 3 months after the 2nd episode of fUTI and 23 infants who underwent acute DMSA scanning during the 3rd episode of fUTI.

fUTI recurred more frequently in the DMSA-positive group than in the control group within 12 months of follow-up after their DMSA scans were performed (34.6% vs. 13.2%,  $P=0.01$ ) (Table 1). The DMSA-positive group had a male predominance compared with the control group (92.3% vs. 72.4%,  $P=0.03$ ).

The mean interval period between the 1st and 2nd episodes of fUTI, culture-negative pyelonephritis, non-*Escherichia coli* bacteria (bacteria causing fUTI except *E. coli*), and the presence of extended-spectrum beta-lactamase-producing *E. coli* as causative bacteria were not significant between the DMSA-positive and control groups (Table 1).

Serum CRP values (normal range  $\leq 0.03$  mg/dL) were significantly higher in the DMSA-positive group than in the control group (7.3 mg/dL vs. 3.7 mg/dL,  $P < 0.001$ ). The differences in serum WBC and procalcitonin values were not significant between the two groups ( $P=0.12$  and  $P=0.09$ , respectively). However, procalcitonin was not sampled in some of the patients in this study.

Spot uNa/K in urine samples collected within 24 hours after admission was significantly lower in the DMSA-positive group than in the control group (0.6 vs. 11,  $P < 0.001$ ).

Renal US revealed that only three infants with cRS had a significantly large size discrepancy in both kidneys (1.9, 1.9, and 2.7 cm), whereas the others with cRS had no significant difference in the size discrepancy of both kidneys compared with the control group ( $P=0.24$ ).

VCUG was recommended to all infants enrolled in the DMSA-positive group. However, the parents of 13 infants at our hospital objected to VCUG, including two infants with cRS, four

**Table 1.** Comparison of data between the DMSA-positive group and controls according to urinary tract imaging strategy

Variable	DMSA-positive (n=26)	Control (n=76)	P-value <sup>a)</sup>
Age (mo)	4.2±2.1	3.5±1.8	0.16
Sex, male:female	24:2	55:21	0.03
Follow-up (mo)	10.5±7.6	12.2±10.2	
Recurrent APN after DMSA scan	9 (35)	10 (13)	0.01
Interval between the 1st and 2nd episode (mo)	2.3±2.7	3.3±3.1	0.13
Urine culture-negative fUTI	2 (8)	3 (4)	0.45
Same bacteria as that in the 1st episode on urine culture	14 (54)	52 (68)	0.16
Non- <i>Escherichia coli</i> cause	5 (20)	8 (10)	0.29
ESBL-positive	9 (35)	20 (26)	0.38
Congenital scar	9 (35)	0	
<b>Blood tests</b>			
C-reactive protein (mg/dL)	7.3±7.7	3.7±3.3	<0.001
White blood cell (/μL)	18,309±7,744	16,043±5,526	0.12
Procalcitonin (ng/mL)	6.1±9.0	1.9±2.1	0.09
<b>Urine tests</b>			
Protein/creatinine ratio	14±19	1.8±1.9	0.33
Urine Na (mEq/L)	22.3±16.0	28.1±24.6	0.07
Urine K (mEq/L)	34.6±14.0	19.0±16.0	0.07
Urine Na/K ratio	0.6±0.4	1.1±1.5	<0.001
Both kidney size discrepancy on US (cm)	0.7±0.8	0.4±0.3	0.07

Values are presented as mean±standard deviation or number (%).

DMSA, <sup>99m</sup>Tc-dimercaptosuccinic acid; APN, acute pyelonephritis; fUTI, febrile urinary tract infection; ESBL, extended-spectrum beta-lactamase; Na, sodium; K, potassium; US, ultrasonography.

<sup>a)</sup>Student t-test.

infants with moderate to severe cortical defects, and seven infants with mild cortical defects on the DMSA scan. Thirteen infants in the DMSA-positive group underwent VCUG in this study. All enrolled infants had VUR, with one, three, four, and five infants having VUR grades 2, 3, 4, and 5, respectively. Bilateral VUR was detected in four infants (31%) (Table 2). Seven infants with cRS, with one, one, three, and two infants having VUR grades 2, 3, 4, and 5, respectively. Nine infants with VUR grades 4 and 5 included five infants with cRS, one infant with mild cortical defects, and three infants with moderate to severe cortical defects (Table 2).

## Discussion

The recurrence of fUTI has already been known as a strong predisposing factor of aRS regardless of age of occurrence [19].

This study also showed that infants with positive DMSA results had more frequent episodes of fUTI than controls; however, the follow-up period was short. In this study, only 3.8% of infants with a 2nd episode of fUTI had positive result on the DMSA scan performed 3 months after the acute infection. In

**Table 2.** The results of 13 infants who received voiding cystourethrography

VUR grade	Overall	cRS	Focal defect	Multifocal defects
No VUR	-	-	-	-
1	-	-	-	-
2	1	1	-	-
3	3	1	2	-
4	4	3	1	-
5	5	2	-	3

VUR, vesicoureteral reflux; cRS, congenital renal scar.

this study, the detection rate of cRS at the 2nd episode of fUTI was 2.5%, but it increased to 29.6% at the 3rd episode. Similar to high-grade VUR, cRS seems to be a strong independent predictive factor of recurrent fUTI. Previous renal scarring has already been known as a predictive factor for new renal scar formation [12]. Although a study reported that non-*E. coli* UTI was associated with the development of renal scars, this study contradicted that result [20].

This study supported the result of a report that CRP could predict renal scarring in children with fUTI [21]. Some studies also reported on the association between serum procalcitonin

and development of renal scarring [11,22]. However, the present study could not confirm such an association because blood samples of procalcitonin were not obtained from some patients enrolled in this study.

A study previously reported that the CRP values and uNa/K in infants could be useful tools for discriminating between pyelonephritis and other fUTI (pyelitis, lower UTI with other fever focus) [16]. According to the study, high CRP values and low uNa/K among infants with fUTI had suggested the increased possibility of the presence of cortical defects on their acute DMSA scan. Another study summarized the hypothetical pathogenesis of acute pyelonephritis related to the changes of CRP or uNa/K [18]. Renal parenchymal inflammation due to ascending bacterial infection can cause changes in the diameter of glomerular capillaries, which can activate the intrarenal renin-angiotensin-aldosterone pathway, thus resulting in the antinatriuretic phenomenon [18]. Similarly, the present study shows that the DMSA-positive group had significantly higher CRP and lower uNa/K than the control group (Table 1). Hence, this study suggests that high CRPs and low uNa/K in infants with repetitive fUTI may suggest an increased possibility of the presence of RPDs, whereas low CRPs and high uNa/K may suggest a decreased possibility of the presence of RPDs. The cutoff values of those factors in this study were not calculated because of the small sample size. In the previous report [16], the values deduced to discriminate acute pyelonephritis from other fUTI were CRP level of 3.0 mg/dL and uNa/K of 1.015.

Obstructive uropathy and congenital hypoplastic kidney are the common diseases that cause CRI in children [23]. Therefore, children with congenital hypoplastic kidney should be monitored throughout their lifetime. It is usually discovered in children in their early life through an US or DMSA scan of fUTI or recurrent fUTI. Thus, a DMSA scan should be done regardless of previous VCUG, particularly in children with recurrent fUTI. We believe that early detection and treatment of children with recurrent fUTI who have the potential risk of CRI would be helpful in preventing the progression into CRI.

This study has some limitations. The number of enrolled patients is small, and the follow-up period is very short. The retrospective nature of the study has many statistical limitations and biases that preclude a concrete conclusion. Although most of the DMSA scans after the 3rd episode of fUTI showed RPD, there were difficulties in differentiating whether this was due to acute inflammation or due to renal scarring. A follow-up scan could not be performed because most patients continued

treatment at a tertiary hospital.

In conclusion, RPDs on the DMSA scan were found more frequently in infants with recurrent fUTI than in the control group. High CRP values and low uNa/K sampled at the acute phase of infection were helpful in predicting the presence of RPDs in infants with recurrent fUTI.

## Ethical statements

The CHA University Institutional Review Board approved this study and the consent procedure (No. CHA 2021-09-043). The informed consent was waived because of the retrospective nature of this study.

## Conflicts of interest

No potential conflict of interest relevant to this article was reported.

## Funding

None.

## Author contributions

Conceptualization: JHL, JHH

Data curation: JHH

Formal analysis: JHL, JHH

Investigation: JHH

Methodology: JHL

Visualization: JHH

Writing-original draft: JHL, JHH, SR

Writing-review & editing: JHL, JHH, SR

All authors read and approved the final manuscript.

## References

1. Chang SL, Shortliffe LD. Pediatric urinary tract infections. *Pediatr Clin North Am* 2006;53:379-400.
2. Scott Wang HH, Cahill D, Panagides J, Logvinenko T, Nelson C. Top-down versus bottom-up approach in children presenting with urinary tract infection: comparative effectiveness analysis using RIVUR and CUTIE data. *J Urol* 2021;206:1284-90.
3. O'Reilly SE, Plyku D, Sgouros G, Fahey FH, Ted Treves S, Frey EC, et al. A risk index for pediatric patients undergoing diagnostic imaging

- with (99m)Tc-dimercaptosuccinic acid that accounts for body habitus. *Phys Med Biol* 2016;61:2319-32.
4. Subcommittee on Urinary Tract Infection; Steering Committee on Quality Improvement and Management, Roberts KB. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;128:595-610.
  5. Cain JE, Di Giovanni V, Smeeton J, Rosenblum ND. Genetics of renal hypoplasia: insights into the mechanisms controlling nephron endowment. *Pediatr Res* 2010;68:91-8.
  6. Bonsib SM. Renal hypoplasia, from grossly insufficient to not quite enough: consideration for expanded concepts based upon the author's perspective with historical review. *Adv Anat Pathol* 2020;27:311-30.
  7. Abdelhalim A, Khoury AE. Critical appraisal of the top-down approach for vesicoureteral reflux. *Investig Clin Urol* 2017;58(Suppl 1):S14-22.
  8. Al Qahtani W, Sarhan O, Al Otay A, El Helaly A, Al Kawai F. Primary bilateral high-grade vesicoureteral reflux in children: management perspective. *Cureus* 2020;12:e12266.
  9. Lee JH, Rhie S. Reconsideration of urine culture for the diagnosis of acute pyelonephritis in children: a new challenging method for diagnosing acute pyelonephritis. *Korean J Pediatr* 2019;62:433-7.
  10. Park YS. Renal scar formation after urinary tract infection in children. *Korean J Pediatr* 2012;55:367-70.
  11. Leroy S, Fernandez-Lopez A, Nikfar R, Romanello C, Bouissou F, Gervaix A, et al. Association of procalcitonin with acute pyelonephritis and renal scars in pediatric UTI. *Pediatrics* 2013;131:870-9.
  12. Soylu A, Demir BK, Turkmen M, Bekem O, Saygi M, Cakmakci H, et al. Predictors of renal scar in children with urinary infection and vesicoureteral reflux. *Pediatr Nephrol* 2008;23:2227-32.
  13. Becerir T, Yuksel S, Evrengul H, Ergin A, Enli Y. Urinary excretion of pentraxin-3 correlates with the presence of renal scar following acute pyelonephritis in children. *Int Urol Nephrol* 2019;51:571-7.
  14. Lee JH, Yim HE, Yoo KH. Associations of plasma neutrophil gelatinase-associated lipocalin, anemia, and renal scarring in children with febrile urinary tract infections. *J Korean Med Sci* 2020;35:e65.
  15. Gokce I, Alpay H, Biyikli N, Unluguzel G, Dede F, Topuzoglu A. Urinary levels of interleukin-6 and interleukin-8 in patients with vesicoureteral reflux and renal parenchymal scar. *Pediatr Nephrol* 2010;25:905-12.
  16. Jung SJ, Lee JH. Prediction of cortical defect using C-reactive protein and urine sodium to potassium ratio in infants with febrile urinary tract infection. *Yonsei Med J* 2016;57:103-10.
  17. Lee JH. Discrimination of culture negative pyelonephritis in children with suspected febrile urinary tract infection and negative urine culture results. *J Microbiol Immunol Infect* 2019;52:598-603.
  18. Lee JH, Jang SJ, Rhie S. Antinatriuretic phenomena seen in children with acute pyelonephritis may be related to the activation of intrarenal RAAS. *Medicine (Baltimore)* 2018;97:e12152.
  19. Kosmeri C, Kalaitzidis R, Siomou E. An update on renal scarring after urinary tract infection in children: what are the risk factors? *J Pediatr Urol* 2019;15:598-603.
  20. Shaikh N, Craig JC, Rovers MM, Da Dalt L, Gardikis S, Hoberman A, et al. Identification of children and adolescents at risk for renal scarring after a first urinary tract infection: a meta-analysis with individual patient data. *JAMA Pediatr* 2014;168:893-900.
  21. Rodriguez LM, Robles B, Marugan JM, Suarez A, Garcia Ruiz de Morales JM. Do serum C-reactive protein and interleukin-6 predict kidney scarring after urinary tract infection? *Indian J Pediatr* 2013;80:1002-6.
  22. Bressan S, Andreola B, Zucchetta P, Montini G, Burei M, Perilongo G, et al. Procalcitonin as a predictor of renal scarring in infants and young children. *Pediatr Nephrol* 2009;24:1199-204.
  23. Warady BA, Chadha V. Chronic kidney disease in children: the global perspective. *Pediatr Nephrol* 2007;22:1999-2009.

# Recurrent hemolytic uremic syndrome caused by *DGKE* gene mutation: a case report

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Atypical hemolytic uremic syndrome (aHUS) is a rare disease characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury without any association with preceding diarrhea. Dysregulation of the complement system is the most common cause of aHUS, and monoclonal humanized anti-C5 antibodies are now recommended as the first-line treatment for aHUS. However, if the complement pathway is not the cause of aHUS, C5 inhibitors are ineffective. In this study, we report the second reported case of aHUS caused by *DGKE* mutations in Republic of Korea. The patient was an 11-month-old infant who presented with prodromal diarrhea similar to typical HUS, self-remitted with conservative management unlike complement-mediated aHUS but recurred with fever. While infantile aHUS often implies genetic dysregulation of the complement system, other rare genetic causes, such as *DGKE* mutation, need to be considered before deciding long-term treatment with C5 inhibitors.

**Keywords:** Atypical hemolytic uremic syndrome; Complement factor H; DGK epsilon; Eculizumab; Thrombotic microangiopathies

## Introduction

Hemolytic uremic syndrome (HUS) is a form of thrombotic microangiopathy (TMA), characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury [1]. In children, enterohemorrhagic *Escherichia coli* (EHEC) producing Shiga toxin is the most common cause of HUS (typical HUS), accounting for 90% of pediatric cases. However, some cases are associated with inherent dysregulation of the complement system (atypical HUS, aHUS), commonly caused by mutations in components of the complement system, including factor H (CFH), factor I, factor B, or complement 3 [2]. In the

past, plasma therapy (plasma exchange or plasma infusion) was applied to ameliorate the dysregulation of complement activation, which is often insufficient to prevent permanent damage to the kidneys. Currently, monoclonal humanized anti-C5 antibodies, such as eculizumab, which block activation of the complement pathway, are the first-line treatment for aHUS with excellent renal outcomes [3]. However, C5 blockade is not always safe because the complement system plays a crucial role in the immune system, as indicated by the fatal outcome of meningococcal infection in patients who were treated with eculizumab, the first monoclonal humanized anti-C5 antibody approved for the treatment of aHUS [4]. Therefore, C5 blockade

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is only indicated when dysregulated complement activation is involved in the pathophysiology of aHUS.

While *CFH* mutations are the most common cause of aHUS, especially in children [5,6], genes other than those involved in the complement system have also been implicated in aHUS. *DGKE*, encoding diacylglycerol (DAG) kinase epsilon (*DGKE*), is one such gene. Lemaire et al. [7] identified this gene using whole-exome sequencing of a patient with infantile aHUS. *DGKE* is found in the endothelium, platelets, and podocytes. In endothelial cells, arachidonic acid-containing DAG activate protein kinase C, promoting thrombosis, and *DGKE* normally inactivates DAG signaling [7]. Therefore, *DGKE* mutations result in a thrombogenic status, which is not related to complement pathway activation. *DGKE* mutations are known to cause steroid-resistant nephrotic syndrome or membranoproliferative glomerulonephritis [8]. In Republic of Korea, an aHUS case caused by a *DGKE* mutation has been reported previously [6], but the details of the clinical course are not well documented.

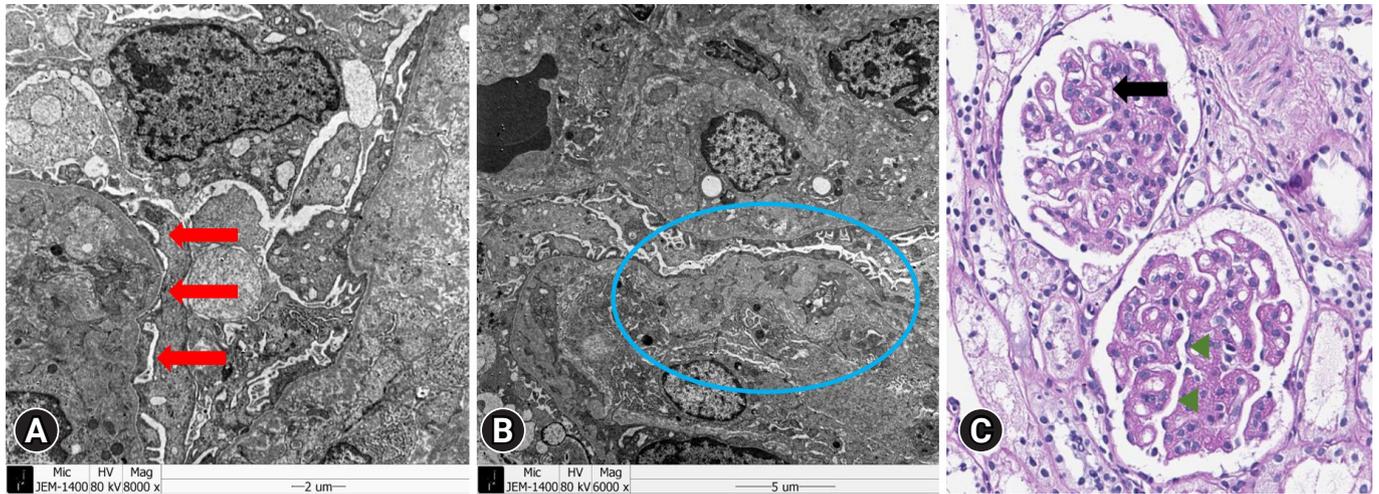
Here, we report the second case of aHUS caused by *DGKE* mutations in Republic of Korea.

## Case report

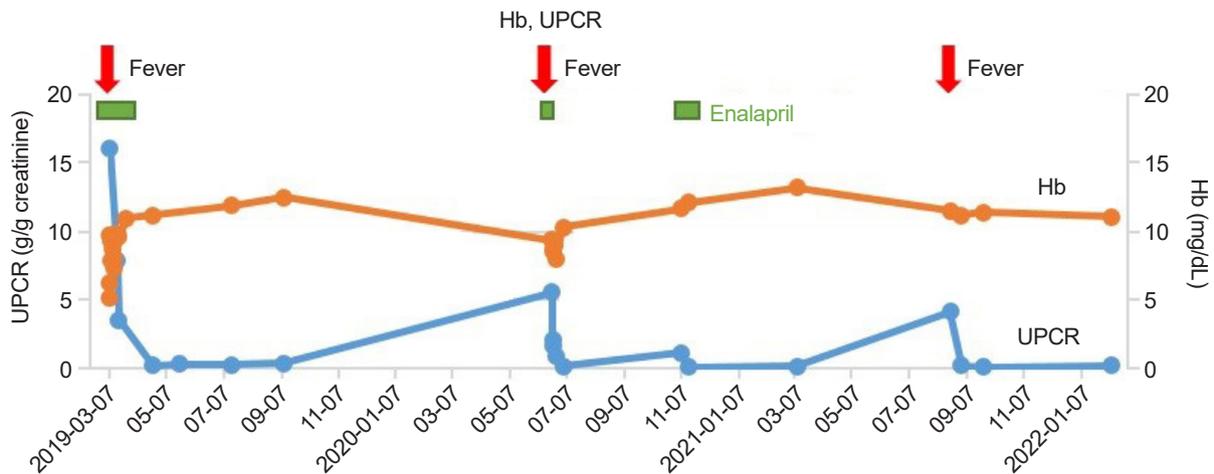
An 11-month-old male patient presented to a local pediatric clinic with a fever. Antibiotics were prescribed for the presumptive diagnosis of acute pharyngitis. A few days later, he developed diarrhea followed by vomiting and hematuria and was transferred to our institution. At our hospital, he had hypertension with a systolic blood pressure above 140 mmHg; he looked acutely ill and anemic. His tongue and lips were dehydrated, and he was edematous, especially in his extremities and eyelids. Laboratory workup showed anemia (hemoglobin [Hb], 6.3 mg/dL), thrombocytopenia (79,000/ $\mu$ L), high blood urea nitrogen (94 mg/dL), high creatinine (1.99 mg/dL), high lactate dehydrogenase (3,297 IU/L), hyperuricemia (14.2 mg/dL), hyperphosphatemia (7.2 mg/dL), high urine protein/creatinine ratio (UPCR; 16.14 g/g creatinine), and hematuria (50–99 red blood cells per high-power field [RBC/HPF]). His complement 3 level (112 mg/dL) and 4 level (18 mg/dL) were within normal limits. EHEC test result was negative and there was no stool Hb. On kidney ultrasonography, the parenchymal echogenicity of both kidneys increased without hydronephrosis. He had oliguria (80 mL/day, 7.8 mL/kg/day); thus, hemodialysis was administered for 1 day. Amlodipine, allopurinol, and calcium carbonate were prescribed for hypertension, hyperuricemia, and hyperphos-

phatemia, respectively. There was no laboratory evidence of EHEC infection, but the presumptive diagnosis was typical HUS because he had prodromal diarrhea. After seven days, almost all laboratory results improved with conservative management, similar to typical HUS. However, he was very young and did not have a history of raw food intake that could have caused EHEC infection. Therefore, the patient was discharged with a warning of recurrence and suspected aHUS. When he was 26-month-old, 15 months after the first episode, he had a fever of up to 39°C and melena. The fever subsided after 2 days, but he looked pale after 4 days, so he visited a local pediatric clinic. Hematuria, proteinuria, and anemia (Hb, 9.8 mg/dL) were found; therefore, he was transferred to our institution. In laboratory workup, mild anemia (Hb, 9.4 mg/dL) and mild creatinine elevation (0.42 mg/dL, baseline 0.35 mg/dL) were noted along with elevated plasma Hb (14.6 mg/dL) and lactate dehydrogenase (585 IU/L), suggesting hemolysis. Hematuria (>100 RBC/HPF) and proteinuria (UPCR, 5.60 g/g creatinine) were evaluated by urine analysis. Both stool polymerase chain reaction and culture were negative for EHEC. After admission, the patient's general condition and laboratory abnormalities improved without treatment for several days. Suspecting an aHUS relapse, a kidney biopsy was performed. The glomeruli were mildly increased in size and had focal mild hypercellular endothelial cells and tram-track appearance. Two global sclerotic glomeruli were noted among the 57 glomeruli. Slight focal infiltration of mononuclear cells was observed in the tubules. Diffuse thickened glomerular basement membrane, slight focal effacement of the foot process, subendothelial widening and mesangial interposition were observed by electron microscopy. In immunofluorescence staining, C3 and Lambda were reported as +/-, and IgM and C4d were reported as positive in the glomerular capillary loops and peritubular capillaries (Fig. 1). Therefore, the pathological findings were consistent with chronic TMA. The TMA gene panel revealed a homozygous nonsense mutation (c.1498C>T in exon11 (p.Arg500\*)) in *DGKE*. This gene panel covers 25 genes associated with TTP and HUS (*ADAMTS13*, *C1S*, *C2*, *C3*, *C5*, *C8A*, *C9*, *CD55*, *CD59*, *CFB*, *CFD*, *CFH*, *CFHR5*, *CFI*, *CR2*, *DGKE*, *F12*, *INF2*, *MASP1*, *MASP2*, *MMACHC*, *MMUT*, *PLG*, *THBD*, *WT1*).

Proteinuria was monitored during follow-up. When he was 31-month-old, his proteinuria increased to 1.17; therefore, enalapril was prescribed. After 2 weeks, proteinuria disappeared and he did not recur despite discontinuation of medications. At the age of 40 months, 29 months after the first episode, he experienced a third episode of aHUS along with a fever of up



**Fig. 1.** Pathologic findings. (A) Electron microscopy image. Diffuse thickened glomerular basement membrane and focal slight effacement of foot process were marked with red arrows. (B) Electron microscopy image. Subendothelial widening and mesangial interposition were marked with blue circle. (C) Periodic acid-Schiff staining image (×500). Endothelial cells were mildly hypercellular in the glomerulus and glomerular size was mildly increased (marked with black arrow). Some glomerulus showed tram-track appearance (marked with green arrowheads).



**Fig. 2.** Laboratory data. Fever events are annotated with red arrows. Hb, hemoglobin; UPCR, urine protein/creatinine ratio.

to 40°C and hematuria. Spontaneous remission was achieved within 1 month without medication. At the last follow-up at the age of 46 months, his blood pressure and laboratory findings were unremarkable, without proteinuria. Hb and UPCR levels during follow-up are displayed in Fig. 2.

**Discussion**

This is a case of recurrent HUS that showed spontaneous remission with supportive care. Because of the infantile-onset and relapse history, aHUS was suspected, and a *DGKE* muta-

tion was identified by a genetic test. The homozygous nonsense mutation of this patient (c.1498C>T in exon11 (p.Arg500\*)) has not been reported before. However, as it is a truncating mutation, the mutation is considered as pathogenic in this patient. Similar to previous reports on *DGKE* mutations, our case presented at a very early onset (median age <1 year) with aHUS with a self-limiting disease course [9,10]. His initial presentation was accompanied by diarrhea; therefore, typical HUS was suspected at first. However, aHUS is often triggered by infection, and the first episode in our case was triggered by gastrointestinal infection.

In general, HUS in young children is typically followed by bloody diarrhea due to EHEC infection. Typically, they have a history of ingesting raw or undercooked food. Otherwise, aHUS should be suspected, especially in very young infants. Unlike typical HUS, aHUS does not spontaneously remit and often relapses. Since it can be fatal, aggressive management is necessary, previously with plasma and now with C5 inhibitors, if aHUS is associated with complement dysregulation. The *CFH* mutation was first suspected in a case of very young aHUS. For aHUS with a *CFH* mutation, C3 levels often decrease, and approximately 60% to 70% of patients lose renal function if not properly managed [11]. However, our patient had normal C3 levels and a self-remitting course, which was not consistent with aHUS associated with complement dysregulation. In such cases, a *DGKE* defect must be suspected. Currently, correct genetic diagnosis is more important because of the availability of C5 inhibitors, the treatment of choice for complement-related aHUS.

Eculizumab, the currently available C5 inhibitor, is an antibody targeting the complement pathway; it is unrelated to the *DGKE* mutation, which is related to the coagulation pathway. There have been some case reports of *DGKE* mutation-associated aHUS in which eculizumab was effective [12]. However, these cases might have recovered even without eculizumab, since *DGKE*-associated aHUS is usually self-remitting. Eculizumab has been proven to be relatively safe and very effective for aHUS, but it is regularly administered to prevent relapse of aHUS once indicated. Therefore, even when aHUS is suspected, causes other than complement system dysregulation must be considered before deciding to administer C5 inhibitors. Other than *DGKE* mutations, secondary causes of aHUS include medication, malignancy, infection, autoimmune diseases, and genetic causes, such as cobalamin C defect or G6PD deficiency.

Despite the self-remitting course of aHUS caused by *DGKE* defects, the long-term outcome of *DGKE* defects is not favorable. Chronic kidney disease stages 4 and 5 are common in patients with *DGKE* mutation [7]. Until the last follow-up, our patient showed third relapse. Chronic relapse of aHUS or development of membranoproliferative glomerulonephritis and/or steroid-resistant nephrotic syndrome might occur in this patient in the future. Therefore, careful long-term follow-up was indicated in this case.

HUS in infants is not common, mandating the suspicion of aHUS. While *CFH* or other complement-related aHUS is more prevalent, *DGKE* mutations need to be suspected in cases with

normal complement levels and spontaneously recovering courses. Although aHUS related to *DGKE* may recur, a C5 inhibitor is not indicated. However, close follow-up is necessary, because other glomerulopathies may have occurred in this case.

## Ethical statements

This study was approved by the Institutional Review Board of Seoul National University Hospital (No. H-2011-048-1171). Informed consent from patient was obtained.

## Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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## Author contributions

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All authors read and approved the final manuscript.

## References

- Loirat C, Noris M, Fremeaux-Bacchi V. Complement and the atypical hemolytic uremic syndrome in children. *Pediatr Nephrol* 2008;23:1957-72.
- Geerdink LM, Westra D, van Wijk JA, Dorresteijn EM, Lilien MR, Davin JC, et al. Atypical hemolytic uremic syndrome in children: complement mutations and clinical characteristics. *Pediatr Nephrol*

- 2012;27:1283-91.
3. Loirat C, Fakhouri F, Ariceta G, Besbas N, Bitzan M, Bjerre A, et al. An international consensus approach to the management of atypical hemolytic uremic syndrome in children. *Pediatr Nephrol* 2016;31:15-39.
  4. McNamara LA, Topaz N, Wang X, Hariri S, Fox L, MacNeil JR. High risk for invasive meningococcal disease among patients receiving eculizumab (Soliris) despite receipt of meningococcal vaccine. *MMWR Morb Mortal Wkly Rep* 2017;66:734-7.
  5. Nester CM, Thomas CP. Atypical hemolytic uremic syndrome: what is it, how is it diagnosed, and how is it treated? *Hematology Am Soc Hematol Educ Program* 2012;2012:617-25.
  6. Lee JM, Park YS, Lee JH, Park SJ, Shin JI, Park YH, et al. Atypical hemolytic uremic syndrome: Korean pediatric series. *Pediatr Int* 2015;57:431-8.
  7. Lemaire M, Fremeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, et al. Recessive mutations in *DGKE* cause atypical hemolytic-uremic syndrome. *Nat Genet* 2013;45:531-6.
  8. Bezdicka M, Pavlicek P, Blahova K, Hacek J, Zieg J. Various phenotypes of disease associated with mutated *DGKE* gene. *Eur J Med Genet* 2020;63:103953.
  9. Azukaitis K, Simkova E, Majid MA, Galiano M, Benz K, Amann K, et al. The phenotypic spectrum of nephropathies associated with mutations in diacylglycerol kinase  $\epsilon$ . *J Am Soc Nephrol* 2017;28:3066-75.
  10. Mele C, Lemaire M, Iatropoulos P, Piras R, Bresin E, Bettoni S, et al. Characterization of a new *DGKE* intronic mutation in genetically unsolved cases of familial atypical hemolytic uremic syndrome. *Clin J Am Soc Nephrol* 2015;10:1011-9.
  11. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med* 2009;361:1676-87.
  12. Husain D, Barron B, Barron AG, Sandokji I, Marsenic O, Warejko JK. Atypical hemolytic uremic syndrome due to *DGKE* mutation and response to eculizumab: lessons for the clinical nephrologist. *J Nephrol* 2021;34:1331-5.

# Idiopathic infantile hypercalcemia with severe nephrocalcinosis, associated with *CYP24A1* mutations: a case report

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Nephrocalcinosis often occurs in infants and is caused by excessive calcium or vitamin D supplementation, neonatal primary hyperparathyroidism, and genetic disorders. Idiopathic infantile hypercalcemia (IIH), a rare cause of nephrocalcinosis, results from genetic defects in *CYP24A1* or *SLC34A1*. Mutations in *CYP24A1*, which encodes 25-hydroxyvitamin D 24-hydroxylase, disrupt active vitamin D degradation. IIH clinically manifests as failure to thrive and hypercalcemia within the first year of life and usually remits spontaneously. Herein, we present a case of IIH with *CYP24A1* mutations. An 11-month-old girl visited our hospital with incidental hypercalcemia. She showed failure to thrive, and her oral intake had decreased over time since the age of 6 months. Her initial serum parathyroid hormone level was low, 25-OH vitamin D and 1,25(OH)<sub>2</sub> vitamin D levels were normal, and renal ultrasonography showed bilateral nephrocalcinosis. Whole-exome sequencing revealed compound heterozygous variants in *CYP24A1* (NM\_000782.4:c.376C>T [p.Pro126Ser] and c.1310C>A [p.Pro437His]). Although her hypercalcemia and poor oral intake spontaneously resolved in approximately 8 months, we suggested that her nephrocalcinosis and renal function be regularly checked in consideration of potential asymptomatic renal damage. Hypercalcemia caused by IIH should be suspected in infants with severe nephrocalcinosis, especially when presenting with failure to thrive.

**Keywords:** Case reports; Failure to thrive; Hypercalcemia; Nephrocalcinosis; Vitamin D3 24-hydroxylase

## Introduction

Nephrocalcinosis, a possible cause of chronic kidney disease (CKD), is rarely identified during infancy. Because it may lead to kidney damage [1,2], the underlying causes need to be identified and managed, if possible. Nephrocalcinosis is commonly caused by primary hyperparathyroidism, long-term use of loop diuretics or vitamin D, distal renal tubular acidosis, and hered-

itary disorders, such as Bartter syndrome [1,3]. Therefore, evaluation of childhood nephrocalcinosis includes urine analysis of hematuria, protein excretion, pH, calcium excretion, and other minerals such as uric acid, oxalic acid, phosphate, and citrate. Analyses of serum calcium, phosphorus, magnesium, uric acid, alkaline phosphatase, pH, bicarbonate, and creatinine levels are also required. Additional studies including parathyroid hormone (PTH), vitamin D metabolites, and molecular genetic

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testing should be considered [4]. When nephrocalcinosis is associated with hypercalcemia and/or hypercalciuria, clinicians should consider the possibility of a genetic disorder, such as Williams syndrome, Jansen's metaphyseal chondrodysplasia, or blue diaper syndrome [5].

Idiopathic infantile hypercalcemia (IIH) is a rare disorder caused by a genetic defect in the key enzymes involved in calcium and vitamin D metabolism. The incidence of IIH is approximately 1 in 33,000 live births, and until now, two causative genes have been identified for IIH: *CYP24A1* (IIH type 1) and *SLC34A1* (IIH type 2) [6]. *CYP24A1* encodes 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), a component of the mitochondrial inner membrane P450. When vitamin D<sub>3</sub>, cholecalciferol, is administered to the human body through the skin and diet, it is metabolized through 25-hydroxylation in the liver and then via 1 $\alpha$ -hydroxylation in the kidney to produce biologically active 1,25-dihydroxyvitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>D<sub>3</sub>). This active 1,25-(OH)<sub>2</sub>D<sub>3</sub> is catabolized by CYP24A1, making it a water-soluble metabolite of calcitriol [7]. CYP24A1 also catabolizes 25-hydroxyvitamin D<sub>3</sub> (25-(OH)D<sub>3</sub>), the precursor of active vitamin D. Thus, *CYP24A1* plays a critical role in regulating active vitamin D levels, and defective *CYP24A1* increases the concentration of 1,25-(OH)<sub>2</sub>D<sub>3</sub> in the blood. *SLC34A1* encodes the Na<sup>+</sup>/phosphate (Pi) cotransporter NaPi-IIa, a transmembrane cotransporter in the proximal renal tubule, which plays an essential role in absorbing Pi from primary urine [6]. Defective absorption of Pi due to dysfunctional NaPi-IIa in IIH type 2 stimulates inappropriate synthesis of 1,25-(OH)<sub>2</sub>D<sub>3</sub>. Therefore, both IIH types 1 and 2 present with an inappropriately high concentration of 1,25-(OH)<sub>2</sub>D<sub>3</sub>, calcitriol. Calcitriol regulates serum ionized serum calcium levels by stimulating intestinal calcium reabsorption and renal calcium reabsorption at the distal tubule (along with PTH) or activating PTH when serum calcium levels are low. PTH stimulates osteoclast formation and differentiation, which in turn induces calcium mobilization from bones [8]. Therefore, in IIH, abnormally elevated active calcitriol levels eventually cause hypercalcemia via its action on the intestine, kidneys, and bones.

In patients with IIH, the classic manifestations include vomiting, anorexia, polyuria, polydipsia, hypotonia, and failure to thrive within 1 year of life, most commonly within 3 to 7 months of age. Unexplained fever, constipation, and hypertension can also occur. Laboratory evaluations usually reveal suppressed serum PTH levels, mildly elevated 25-(OH)D<sub>3</sub> and 1,25-(OH)<sub>2</sub>D<sub>3</sub> levels, and hypercalciuria, often accompanied by nephrocalci-

nosis [7,9,10]. The management of IIH is mainly conservative, with a recommendation of low-calcium diet and avoiding excessive vitamin D. When necessary, intravenous fluid hydration can be used to alleviate hypercalcemia and dehydration. If symptomatic hypercalcemia persists, glucocorticoids to prevent renal calcium reabsorption and inhibition of 1,25-(OH)<sub>2</sub>D<sub>3</sub> activity, bisphosphonates for inhibition of osteoclast activity, orazole agents (e.g., ketoconazole) for inhibition of P450 enzymes can be considered. Pi supplementation is necessary in case of defective NaPi-IIa in IIH type 2 [6]. In rare cases, rapid hemodialysis management is required to treat life-threatening hypercalcemia [6,9,11].

Herein, we present a case of severe nephrocalcinosis, wherein genetic analysis revealed IIH type 1 with pathogenic compound heterozygous variants of *CYP24A1*. This report has its significance in that this case is the first report of IIH type 1 patient of South Korea, among whom genetic analysis had been done.

## Case report

An 11-month-old girl was transferred to our hospital for a second opinion on incidentally discovered hypercalcemia. Hypercalcemia was found at a primary hospital during laboratory workup for fever. Initially, her calcium level was 13.4 mg/dL (reference: 8.6–10.2 mg/dL), ionized calcium level was 1.45 mmol/L (reference: 1.15–1.33 mmol/L), and spot urine calcium/creatinine was 2.19 mg/mg (reference:  $\leq$ 0.6 mg/mg creatinine), respectively. Serum phosphate level was 5.0 mg/dL, and blood urea nitrogen and creatinine were 23.8 mg/dL and 0.44 mg/dL, respectively. The albumin level was normal and intact PTH level was low, less than 0.7 pg/dL. The urine analysis at the primary hospital showed hypercalciuria, but there was no hematuria nor proteinuria. Urine electrolytes (sodium, potassium, uric acid, phosphorus, and magnesium) were not abnormally elevated.

When she was initially brought to our hospital, physical examination results were nonspecific and unremarkable. The fever had subsided, but poor oral intake, which was noticed at the age of 6 months, persisted. At that time, her serum calcium and phosphorus levels were 12.2 mg/dL (reference: 8.8–10.5 mg/dL) and 4.7 mg/dL (reference: 4.1–6.2 mg/dL), respectively. Ionized calcium level was 1.62 mmol/L (reference: 1.05–1.35 mmol/L), and creatinine level was 0.54 mg/dL. Serum albumin level was 4.7 g/dL (reference: 3.3–5.2 g/dL). Urine analysis revealed no hematuria or proteinuria, and urine calcium to creatinine ratio also improved so there was no hypercalciuria. Her spot urine

calcium to creatinine ratio was 0.36 mg/mg.

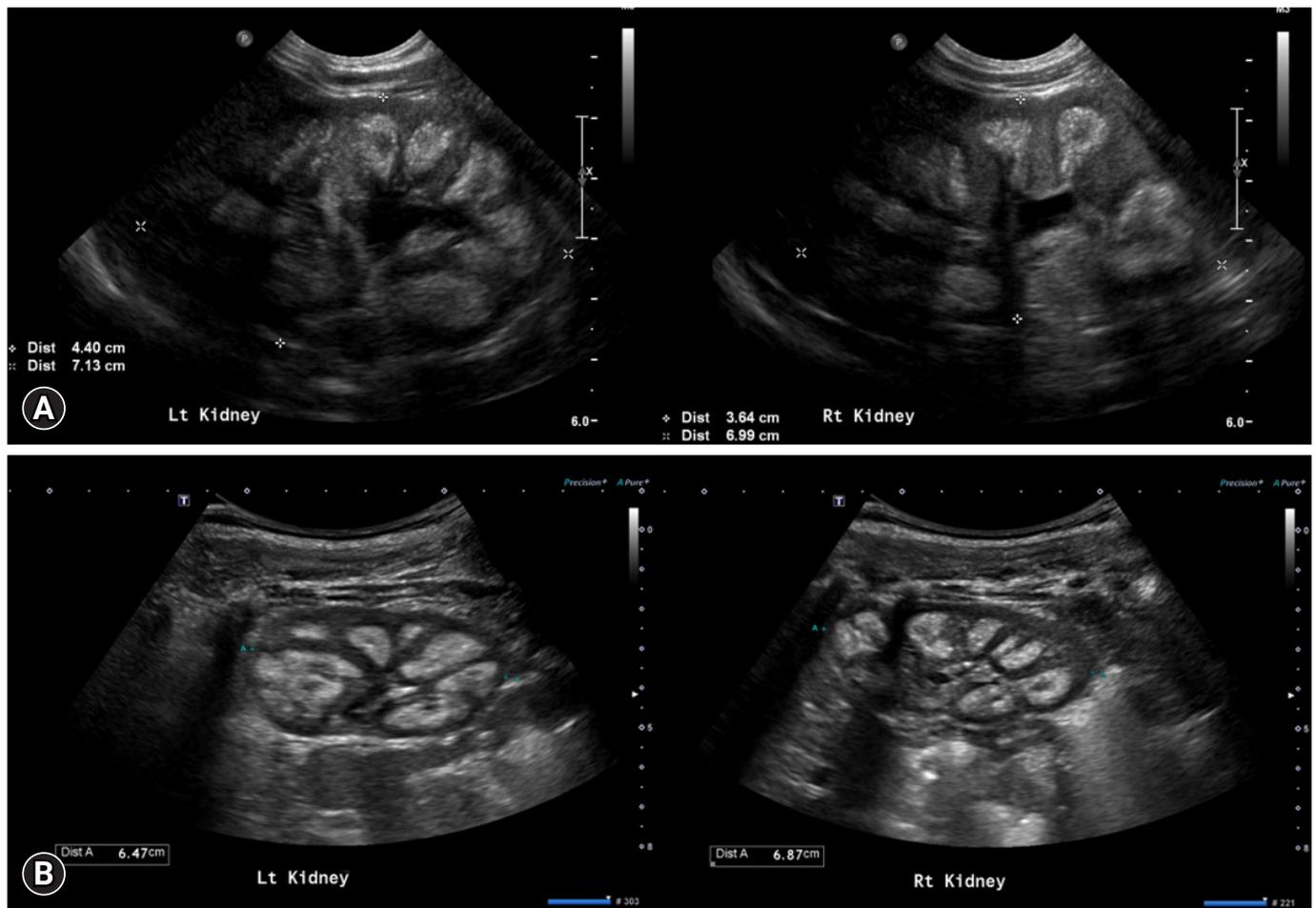
She was born after 37 weeks and 4 days of gestation, with a birth weight of 3.2 kg (62nd percentile). She had no developmental delays. She had been fed approximately 600 mL/day of powdered milk and 200 mL/day of weaning food. She was receiving 5 mL/day of multivitamin supplementation containing cholecalciferol (4,000 IU/100 mL).

Further evaluations for hypercalcemia revealed low serum PTH levels (0.7 pg/mL, reference: 8–76 pg/mL) excluding hyperparathyroidism, normal PTH-related peptide (less than 1.1 pmol/L), 25(OH) vitamin D (46.7 ng/mL, reference: 30–100 ng/mL), and 1,25(OH)<sub>2</sub> vitamin D levels (23.4 pg/mL, reference: 19.6–54.3 pg/mL). Kidney ultrasonography revealed diffusely increased echogenicity of the medullary pyramids, indicating nephrocalcinosis (Fig. 1A).

Echocardiography was performed to rule out Williams syn-

drome; however, the findings were unremarkable. Whole-exome sequencing was carried out from genomic DNA extracted from buccal swab sample, by company named “3 billion.” The test result revealed compound heterozygous variants of uncertain significance in *CYP24A1* (NM\_000782.4), c.376C>T (p.Pro126Ser), and c.1310C>A (p.Pro437His), which were predicted in silico to be pathogenic, and likely damaging to the protein structure or function. These variants were reported to have an extremely low frequency in both the gnomAD v2.1.1 and v3 datasets.

We advised the patient’s parents that the multivitamin supplementation be discontinued and additional calcium supplementation be avoided for her. We decided to observe her clinical progression without any other additional management, and her serum calcium levels spontaneously decreased over time, improving oral intake. After 2 months, her serum calcium levels



**Fig. 1.** Renal ultrasonography of the index patient, showing diffuse echogenicity filled in both renal medullary pyramids. (A) Initial examination. (B) Follow-up examination (after 9 months).

were 10.2 mg/dL with ionized calcium levels at 1.39 mmol/L. At the last visit, which was after 8 months from the first visit to our hospital, serum calcium levels and ionized calcium levels were 10.1 mg/dL and 1.33 mmol/L, respectively. Her body weight at the initial visit to our hospital was 7.1 kg, which was less than the 3rd percentile for her age. After 2 months, her body weight was 9.3 kg, which was in the 25th to 50th percentile for her age, and her height was 73 cm, which was in the 5th to 10th percentile. During the last follow-up at the age of 21 months, the patient's body weight and height were 12 kg (75th–90th percentile) and 85.3 cm (50th–75th percentile), respectively. Follow-up kidney ultrasonography was performed 9 months after the visit, and bilateral kidney nephrocalcinosis was found to be still present (Fig. 1B).

## Discussion

Herein, we have discussed a typical case of IIH type 1, presenting with severe nephrocalcinosis associated with hypercalcemia and normophosphatemia. Other case reports of IIH in South Korea were those of IIH type 2 (*SLC34A1* mutation) [12,13]. In one report, nephrocalcinosis was prenatally detected at 28 weeks of gestation. The patient was treated with intravenous hydration, furosemide, and corticosteroids for hypercalcemia (12.8 mg/dL), which improved within 7 days. The patient was fed a low-calcium, vitamin D-free formula, and at the age of 7 months, renal echogenicity also improved [12]. This case report differs from our index patient in that hypercalcemia and nephrocalcinosis improved more rapidly with more aggressive treatment. Additionally, the genetic causes were different. Theoretically, serum phosphorus levels are expected to be low in IIH type 2, as shown in a study using a mouse model, wherein the serum phosphorus levels were not normalized by vitamin D restriction, but only by Pi load [6]. However, one of the previous Korean reports of IIH and our case involved normophosphatemia, making a differential diagnosis between the two types of IIH challenging, as previously reported. The 1,25-(OH)<sub>2</sub>D<sub>3</sub> level in our case was within the normal range, similar to that in previous reports. We suppose that it should have been lower than normal if our patient's *CYP24A1* enzyme was not defective since she had severe hypercalcemia, which would have reduced the 1,25-(OH)<sub>2</sub>D<sub>3</sub> level through negative feedback.

In addition to the incidental fever, the chief complaint in our case was poor oral intake. This is a common symptom of IIH. As hypercalcemia impairs gastrointestinal motility via reduction

in the contractility of smooth muscles, this may contribute to delay of gastric emptying and subsequent reduced appetite [14]. Furthermore, hypercalcemia promotes gastrin secretion, which may contribute to nausea and poor oral intake [15]. Thus, if infants with nephrocalcinosis exhibit poor oral intake, hypercalcemia should be considered, of which IIH is a rare cause. Other more common causes of infantile hypercalcemia include vitamin D overdose and Williams syndrome. Therefore, workup of serum vitamin D metabolites and echocardiography screening are necessary to clinically exclude these etiologies and suspect IIH. Since IIH remits spontaneously in many cases, radical management might not be necessary, and close follow-up is indicated, as shown herein. Nonetheless, long-term follow-up, including kidney function, is necessary because there is a chance of ongoing subclinical metabolic abnormalities in these patients.

The natural history and long-term outcomes of IIH are not well known. Clinical symptoms seem to disappear spontaneously with the normalization of serum calcium levels. However, one study involving long-term follow-up of 18 patients with IIH showed that the renal prognosis of survivors of IIH tended to be poorer than that of the general population, demonstrating a high prevalence of CKD, with CKD II in 77% and two cases of end-stage kidney failure despite avoidance of vitamin D or calcium supplementation and sun exposure [10].

A limitation of this report is that phasing of the variants was not possible in this case. Thus, we are not sure whether the two variants exist as cis or trans isomers. In addition, these mutations are variants of unknown significance and have not been classified to be pathogenic or likely pathogenic according to ACMG (American College of Medical Genetics and Genomics) guidelines. Although, the whole-exome sequencing result of the patient met 1 criterion of “moderate evidence of pathogenicity,” and 2 criteria of “supporting evidence of pathogenicity.” Firstly, these variants have been reported with an extremely low frequency from gnomAD dataset. Secondly, in silico prediction tools and conservation analysis supported deleterious effects on the gene product. Thirdly, the patient's phenotype is highly specific for IIH. The patient's clinical presentation and course are compatible with those of IIH type 1, suggesting that this rare disease needs to be considered.

We reported a case of IIH caused by *CYP24A1* mutations that presented with fever, failure to thrive, and severe nephrocalcinosis. When idiopathic hypercalcemia and poor oral intake or nephrocalcinosis are present, consideration of

IIH as a causative disease is necessary. A proper and timely diagnosis of this disease can help in the correct management. Although the patient's hypercalcemia resolved, her nephrocalcinosis persisted, implying that careful regular check-ups for her kidney status are needed as subclinical renal injury may still progress.

## Ethical statements

The Institutional Review Board of Seoul National University Hospital approved this study (IRB No. 2011-048-1171). The study received informed consent for the report from the parents of the patient.

## Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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Project administration: HGK, YHA

Visualization: JY, HGK

Writing-original draft: JY, HGK

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All authors read and approved the final manuscript.

## References

- Vaidya SR, Yarrarapu SNS, Aeddula NR. Nephrocalcinosis [Internet]. Treasure Island: StatPearls Publishing; 2021 Aug [cited 2022 Apr 27]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK537205/>.
- Ronnefarth G, Misselwitz J; Members of the Arbeitsgemeinschaft für pädiatrische Nephrologie. Nephrocalcinosis in children: a retrospective survey. *Pediatr Nephrol* 2000;14:1016-21.
- Habbig S, Beck BB, Hoppe B. Nephrocalcinosis and urolithiasis in children. *Kidney Int* 2011;80:1278-91.
- Hoppe B, Kemper MJ. Diagnostic examination of the child with urolithiasis or nephrocalcinosis. *Pediatr Nephrol* 2010;25:403-13.
- Lietman SA, Germain-Lee EL, Levine MA. Hypercalcemia in children and adolescents. *Curr Opin Pediatr* 2010;22:508-15.
- De Paolis E, Scaglione GL, De Bonis M, Minucci A, Capoluongo E. CYP24A1 and SLC34A1 genetic defects associated with idiopathic infantile hypercalcemia: from genotype to phenotype. *Clin Chem Lab Med* 2019;57:1650-67.
- Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, et al. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N Engl J Med* 2011;365:410-21.
- Gil A, Plaza-Diaz J, Mesa MD. Vitamin D: classic and novel actions. *Ann Nutr Metab* 2018;72:87-95.
- Marks BE, Doyle DA. Idiopathic infantile hypercalcemia: case report and review of the literature. *J Pediatr Endocrinol Metab* 2016;29:127-32.
- Janiec A, Halat-Wolska P, Obrycki L, Ciara E, Wojcik M, Pludowski P, et al. Long-term outcome of the survivors of infantile hypercalcemia with CYP24A1 and SLC34A1 mutations. *Nephrol Dial Transplant* 2021;36:1484-92.
- Vogiatzi MG, Jacobson-Dickman E, DeBoer MD; Drugs, and Therapeutics Committee of The Pediatric Endocrine Society. Vitamin D supplementation and risk of toxicity in pediatrics: a review of current literature. *J Clin Endocrinol Metab* 2014;99:1132-41.
- Kang SJ, Lee R, Kim HS. Infantile hypercalcemia with novel compound heterozygous mutation in SLC34A1 encoding renal sodium-phosphate cotransporter 2a: a case report. *Ann Pediatr Endocrinol Metab* 2019;24:64-7.
- Lee GM, Moon JE, Kim HA, Lee SJ, Ko CW. A novel variant of SLC34A1 gene in an infant with idiopathic infantile hypercalcemia. *ESPE Abstr* 2018;89:P2-047.
- Barnett ML. Hypercalcemia. *Semin Oncol Nurs* 1999;15:190-201.
- Moza D, Dasgupta K, Daskalopoulou S. Hypercalcemia and upper GI symptoms. *Can J Gen Int Med* 2018;13:51-2.

## GENERAL INFORMATION

*Childhood Kidney Diseases* (*Child Kidney Dis*, *ChiKD*) is a peer-reviewed open-access journal of medicine published in English. The Journal is published twice per year (the last day of June and December). It is the official publication of the Korean Society of Pediatric Nephrology (KSPN). *ChiKD* covers clinical and research works relevant to all aspects of pediatric nephrology. The journal aims to serve researchers engaged in pediatrics, nephrology, urology, genetics and laboratory medicine, and related fields through the prompt publication of significant advances in pediatric nephrology and to rapidly disseminate recently updated knowledge to the public. Additionally, it will initiate dynamic, international, academic discussions concerning the major topics related to pediatric nephrology.

Manuscripts submitted to *ChiKD* should be prepared according to the following instructions. For issues not addressed in these instructions, authors are referred to “Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals” from the International Committee of Medical Journal Editors (ICMJE; <http://www.icmje.org>). It also adheres completely to the “Principles of Transparency and Best Practice in Scholarly Publishing” from Committee on Publication Ethics (COPE), the Directory of Open Access Journals (DOAJ), the World Association of Medical Editors (WAME), and the Open Access Scholarly Publishers Association (OASPA) (<http://doaj.org/bestpractice>) if not otherwise described below.

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The journal adheres to the ethical guidelines for research and publication described in the Guidelines on Good Publication (<http://publicationethics.org/resources/guidelines>) and the ICMJE Guidelines (<http://www.icmje.org>).

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An author is considered as an individual who has made substantive intellectual contributions to a published study and whose authorship continues to have important academic, social, and financial implications. The ICMJE has recommended the following criteria for authorship: (1) substantial contributions to conception and design, acquisition, analysis, and

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### 2. Originality, plagiarism, and duplicate publication

Submitted manuscripts should be original and should not be under consideration by other scientific journals for publication. No part of an accepted manuscript should be duplicated in any other scientific journal without the permission of the Editorial Board, although the figures and tables can be used freely if the original source is acknowledged according to the Creative Commons Attribution License. It is mandatory for all authors to resolve any copyright issues when citing a figure or table from another journal that is not open-access. The cover letter must include the corresponding author's name, address, telephone number, and e-mail address, and state that the manuscript is not under consideration for publication and has not been published elsewhere. Similarity Check is used to screen submitted manuscripts for possible plagiarism or duplicate publication upon arrival. If plagiarism or duplicate publication is detected, the manuscript will be rejected, the authors will be announced in the journal, and their institutions will be informed. There will also be penalties for the authors. If the author(s) wishes to obtain a duplicate or secondary publication for various other reasons, such as for readers of a different language, he/she should obtain approval from the editors-in-chief of both the first and second journals.

### 3. Conflict of interest statement

A conflict of interest exists when an author (or the author's institution), reviewer, or editor has financial or personal rela-

tionships that inappropriately influence his/her actions (such relationships are also known as dual commitments, competing interests, or competing loyalties). All authors should disclose their conflicts of interest, such as (1) financial relationships (such as employment, consultancies, stock ownership, honoraria, paid expert testimony), (2) personal relationships, (3) academic competition, and (4) intellectual beliefs. We define “people with personal connections” as minors (age under 18) or researchers’ family members (spouse, offspring, relatives, and so on). These conflicts of interest must be included after the discussion section. Each author should certify the disclosure of any conflict of interest with his/her signature. If no conflict exists, please state that “The author(s) declare(s) that there is no conflict of interest.”

#### **4. Statement of privacy, confidentiality, and written informed consent**

The ICMJE has recommended the following statement for the protection of privacy, confidentiality, and written informed consent: The rights of patients should not be infringed without written informed consent. Identifying details should not be published in written descriptions, photographs, and pedigrees unless it is essential for scientific purposes and the patient (or his/her parents or guardian) provides written informed consent for publication. However, complete patient anonymity is difficult to achieve; therefore, informed consent should be obtained in the event that anonymity of the patient is not assured. For example, masking the eye region of patients in photographs is not adequate to ensure anonymity. If identifying characteristics are changed to protect anonymity, authors should provide assurance that alterations do not distort scientific meaning and editors should take note of this. When informed consent has been obtained, it should be indicated in the published article.

#### **5. Statement of human and animal rights**

While reporting experiments that involve human subjects, it should be stated that the study was performed according to the Declaration of Helsinki (<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>) and approved by the Research Ethics Committee (REC) or the Institutional Review Board (IRB) of the institution where the experiment was performed. Written informed consent should

be obtained from all subjects. In animal studies, a statement should be provided indicating that the experimental process, such as the breeding and the use of laboratory animals, was approved by the REC of the institution where the experiment was performed or that it did not violate the rules of the REC of the institution or the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (<https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>). The authors should preserve raw experimental study data for at least 1 year after the publication of the paper and should present this data if required by the Editorial Board.

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Any research that deals with a clinical trial should be registered with the primary national clinical trial registration site, such as <http://cris.nih.go.kr/cris/index.jsp>, <https://clinicaltrials.gov/> or other sites accredited by World Health Organization (<https://www.who.int/clinical-trials-registry-platform>).

#### **7. Process for managing research and publication misconduct**

When the journal faces suspected cases of research and publication misconduct such as redundant (duplicate) publication, plagiarism, fraudulent or fabricated data, changes in authorship, an undisclosed conflict of interest, ethical problems with a submitted manuscript, a reviewer who has appropriated an author’s idea or data, complaints against editors, and so on, the resolution process will follow the flowchart provided by the COPE (<http://publicationethics.org/resources/flowcharts>). The REC of *ChiKD* will carry out the discussion and decision for suspected cases. We will not hesitate to publish errata, corrigenda, clarifications, retractions, and apologies when needed.

#### **8. Complaints and appeals policy**

The policies of *ChiKD* are principally intended to protect the authors, reviewers, editors, and the publisher of the journal. The process of handling complaints and appeals follows the guidelines of the COPE (<https://publicationethics.org/guidance/Guidelines>).

#### **9. Editorial responsibilities**

The Editorial Board will continuously work to monitor and

safeguard publication ethics, including guidelines for retracting articles; maintenance of the integrity of the academic record; preclusion of business needs from compromising intellectual and ethical standards; publishing corrections, clarifications, retractions, and apologies when needed; and excluding plagiarism and fraudulent data. The editors maintain the following responsibilities: responsibility and authority to reject and accept articles; avoiding any conflict of interest with respect to articles they reject or accept; promoting publication of corrections or retractions when errors are found; and preservation of the anonymity of reviewers.

## COPYRIGHT, OPEN ACCESS POLICY, AND CLINICAL DATA SHARING POLICY

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The full-text of *ChiKD* is archived in the Korea Citation Index (KCI; <https://www.kci.go.kr/kciportal/main.kci>), and the National Library of Korea (NLK; <https://seoji.nl.go.kr/archive>). *ChiKD* provides an electronic backup and preservation of access to the journal content in the event the journal is no longer published by archiving in KCI and NLK.

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Open data policy: *ChiKD* does not mandate data sharing. In the interest of transparency and in support of FAIR data principles (<https://www.go-fair.org/fair-principles/>), however, authors are

requested to provide the data and other artefacts supporting the results in the paper by archiving them in an appropriate public repository. Authors may provide a data availability statement, including a link to the repository they have used, in order that this statement can be published in their paper. Shared data should be cited.

Clinical data sharing policy: *ChiKD* accepts the ICMJE Recommendations for a data sharing statement policy (<http://icmje.org/icmjerecommendations.pdf>). Authors may refer to the editorial, "Data Sharing Statements for Clinical Trials: A Requirement of the International Committee of Medical Journal Editors," in *JKMS* vol. 32, no. 7:1051-1053 (<https://doi.org/10.3346/jkms.2017.32.7.1051>).

## MANUSCRIPT SUBMISSION AND PEER REVIEW PROCESS

### 1. Online submission

All manuscripts should be submitted via the e-submission system available at <http://submit.chikd.org/>. Manuscripts should be submitted by the corresponding author, who should indicate the address, phone number, and e-mail address for correspondence on the title page of the manuscript. The revised manuscript is to be submitted through the same web system under the same identification numbers. Once an author has registered and logged into your account, the online system will lead the user through the steps of the submission process in order. All articles submitted to the journal must comply with these instructions. Failure to do so will result in the return of the manuscript and possible delay in publication. For assistance, please contact us via e-mail ([chikd@chikd.org](mailto:chikd@chikd.org)) or telephone (+82-10-4391-0788).

### 2. Online submission

*ChiKD* reviews all received materials. All papers are evaluated by a double-blind, peer-review process. Manuscripts are sent to the two (or more) most relevant investigators, who review the content. The acceptance criteria for all papers are based on the quality and originality of the research and its clinical and scientific significance. An initial decision will normally be made within 2 weeks after the reviewers agree to review a manuscript, and the reviewers' comments will then be sent to the corresponding authors. Revised manuscripts must be submitted online by the corresponding author. Failure to resubmit

the revised manuscript within 4 weeks of the editorial decision is regarded as a withdrawal. The editorial office should be notified if additional time is needed or if an author chooses not to submit a revision. The editorial committee makes decisions concerning editing, revision, and acceptance or rejection, and editing may include shortening an article, reducing the number of illustrations or tables, or changing the paper's format or the order of the manuscript. The editor selects referees, and the results of reviews will be classified as follows:

- Accepted: The manuscript will be forwarded to the publisher without further corrections.
- Minor revision: The author should address the comments from the reviewers, which will be confirmed by the reviewers.
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All manuscripts from editors, employees, or members of the editorial board are processed in the same way as other unsolicited manuscripts. During the review process, submitters will not engage in the selection of reviewers or the decision process. Editors will not handle their own manuscripts even if the manuscripts are commissioned.

### 4. Conditions of publication

All authors are required to affirm the following statements prior to their manuscript being considered:

- (1) If the manuscript does not have a new result or conclusion, then it should not have the same title as a previously published article.
- (2) Once a case has been published in an original paper, it may not be reproduced as a case report. However, the Editorial Board may consider making an exception and accepting a report in circumstances in which a novel diagnostic method, a novel therapeutic trial, or a previously unknown accompanying condition is found.
- (3) Clinical trials on drugs with commercial implications will be evaluated by the proper subcommittee before being reviewed for publication.

- (4) Case reports of previously published cases will not be accepted. The editorial board will make an exception only if the case is very rare. The index of *ChiKD* should be reviewed before submitting a case report.
- (5) Rejected manuscripts may not be resubmitted.
- (6) If the author does not address the comments made by the reviewer or if the manuscript does not follow the guidelines provided, it will be rejected.

## MANUSCRIPT PREPARATION

The details of manuscript preparation differ according to the publication type, including reviews, original articles, case reports, editorials, and correspondence. Other types can be discussed with the Editorial Board.

### 1. Publication type

*ChiKD* publishes review articles, original articles, case reports, editorial, and correspondence.

- 1) **Reviews:** This type of article offers concise reviews of important topics in pediatric nephrology. Review articles are accepted after peer review. They should have the following structure: title page, unstructured abstract of no more than 200 words and keywords, main text (introduction, body text, conclusion), references, tables, figures, and figure legends. The length of the text excluding references, tables, and figures should not exceed 5,000 words. The number of references is limited to 100.
- 2) **Original articles:** These are papers containing the results of clinical or laboratory investigations, which are sufficiently well documented to be acceptable to critical readers. The original articles should be organized in the following order: title page, structured abstract of no more than 250 words and keywords, main text (introduction, methods, results, discussion), references, tables, figures, and figure legends. Maximum length: 4,000 words of text (not including the abstract, tables, figures, and references). A maximum of 6 tables or 6 figures is allowed. The number of references should not exceed 40.
- 3) **Case reports:** Case reports should be organized in the following order: title page, unstructured abstract of no more than 200 words) and keywords, main text (introduction, case report, discussion), references, tables, figures, and figure legends. The length of the text, excluding referenc-

es, tables, and figures, should not exceed 2,500 words. A maximum total of 6 tables and figures may be included. The number of references is limited to 15.

- 4) **Editorials:** Editorials should be commentaries on articles published recently in the journal. Editorial topics could include active areas of research, fresh insights, and debates. The order of the submitted manuscript should include a title page, discussion, conflict of interest, acknowledgments (if applicable) and references. The text should be limited to 1,500 words and 10 references. A maximum total of 2 tables and figures may be included.
- 5) **Correspondence:** Correspondence (letters to the editor) may be in response to a published article, or a short, free-standing piece expressing an opinion. A brief case report can be published as a letter to the editor. Correspondence should be no longer than 1,000 words of text and 10 references. Letters can be edited by the Editorial Board. Responses by the author of the subject paper may be provided in the same issue or next issue of the journal. Replies by authors should not exceed 500 words of text and 5 references. A maximum total of 2 tables and figures may be included.

Table shows the recommended maximums of manuscripts according to publication type.

**Table 1.** Recommended maximums for articles submitted to ChiKD

Type of article	Abstract (words)	Text (words)	References	Tables & figures
Review	200	5,000	100	No limits
Original article	250	4,000	40	6 tables, 6 figures
Case report	200	2,500	15	Total 6
Editorials	No	1,500	10	Total 2
Correspondence	No	1,000	10	Total 2
In reply	-	500	5	Total 2

## 2. General guidelines

- Manuscripts must be written in English. Authors (particularly non-native English speakers) who submit a manuscript should have it checked by a professional editing service prior to submission and must submit proof of English editing.
- The manuscript must be submitted in MS Word format (doc or docx).
- The text of the manuscript, including tables and their footnotes and figure legends, must be double-spaced and in

standard 12-point font on an A4 size page. All pages should be numbered consecutively starting with the title page.

- Drug and chemical names should be stated in standard chemical or generic nomenclature. For medicine, use generic names. If a brand name should be used, insert it in parentheses after the generic name.
- Units of measure should be presented according to the International System (SI) of units. All units must be preceded by one space except percentage (%) and temperature (°C).
- Descriptions of genes or related structures in a manuscript should include the names and official symbols provided by the US National Center for Biotechnology Information (NCBI) or the HUGO Gene Nomenclature Committee.
- The terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors) should be correctly used. The sex and/or gender of study participants and the sex of animals or cells should be reported, and the methods used to determine sex and gender should be described. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., ovarian cancer).
- Statistical expression: mean and standard deviation should be described as mean±SD, and mean and standard error as mean±SE. *P*-values should be described as *P*<0.05 or *P*=0.003.

## 3. Reporting guidelines for specific study designs

For specific study designs, such as randomized controlled studies, studies of diagnostic accuracy, meta-analyses, observational studies, and nonrandomized studies, authors are encouraged to also consult the reporting guidelines relevant to their specific research design. A good source of reporting guidelines is the EQUATOR Network (<https://www.equator-network.org/>) and the National Library of Medicine ([https://www.nlm.nih.gov/services/research\\_report\\_guide.html](https://www.nlm.nih.gov/services/research_report_guide.html)).

## FORMAT OF MANUSCRIPTS

We recommend using the template provided at <http://www.chikd.org/authors/authors.php> to format the manuscript.

### 1. Title page

The title page should include: (1) the concise and informative title of the article; (2) the full name(s) of the author(s); (3) the in-

stitutional affiliation(s) of the author(s); (4) the running title, of 10 words or less; (5) the e-mail address, telephone number of the corresponding author; and (6) notes. If several authors and institutions are listed, it should be made clear with which department and institution each author is affiliated. For a multicenter study, each individual's affiliation should be indicated using a superscript Arabic number 1,2,3... The corresponding author or first author should be clearly designated. In a separate paragraph, an address for correspondence including the name of the corresponding author and his/her degree, address (institutional affiliation, city, zip code, and country), telephone numbers, and e-mail address should be given. The running title should not be a declarative or interrogative sentence. Notes (disclaimers) include ethics approval and consent to participate, conflict of interest, funding, authors' contributions, additional contributions, and ORCID of all authors. All contributors who do not meet the criteria for authorship as defined above should be listed in an additional contribution section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

## 2. Abstract and keywords

1) **Abstract:** Original articles provide a structured abstract of less than 250 words, which should be divided into the following sections:

- **Purpose:** A single sentence describing why the study was done and the type of study carried out. Clinical studies should include the setting (e.g., practice or hospital).
- **Methods:** The total number of species of animals or subjects, with (where relevant) the method of selection. For in vitro studies, specify the cell and tissue used, the assays or assessments carried out, and the statistical tests applied.
- **Results:** The main results obtained, providing means ( $\pm$ SD or SE) or medians (with ranges) and significance levels, where necessary. Clinical data should include any withdrawals.
- **Conclusions:** Implications based on the methods and results presented.

Abbreviations, if needed, should be kept to an absolute minimum, and given with proper identifications.

2) **Keywords:** Authors should provide, and identify as such, up to 5 keywords or short phrases that will assist indexers in cross-indexing the article and can be published with the abstract. Use terms from the Medical Subject Headings (MeSH) list of Index Medicus; if suitable MeSH terms are not yet available for recently introduced terms, present terms may be used. The first letter of a keyword should be capitalized (e.g., Nephrotic syndrome; Hematuria).

## 3. Main text

1) **Introduction:** The introduction should contain enough references to the most pertinent papers to inform readers and describe others' relevant findings. It also includes the specific question driving the authors' particular investigation.

2) **Methods:** We endorse the principles articulated in the Declaration of Helsinki and expect that all investigations involving human materials have been performed in accordance with these principles. Animal experiments must be reviewed and approved by an appropriate committee (Institutional Animal Care and Use Committee) for the care and use of animals. Studies involving pathogens requiring a high degree of biosafety should pass the review of a relevant Institutional Biosafety Committee. The approval of the experimental protocol should be described in the Methods section. An explanation of the experimental methods should be concise and sufficient for repetition by other qualified investigators. Procedures that have been published previously should not be described in detail; however, new or significant modifications of previously published procedures need full descriptions. The sources of special chemicals or preparations should be given (i.e., name of company, city and state, and country). The methods of statistical analyses and the criteria used to determine statistical significance (i.e., the significance level) should be described. Case reports, case histories, or case descriptions do not contain separate Methods or Results sections.

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### Journal article:

1. Choe JY, Jang KM, Hwang YJ, Choi BS, Park JK, Yoon YR, et al. Effects of age and sex on the pharmacokinetics of tacrolimus during pediatric kidney transplantation: a single center study. *Child Kidney Dis* 2014;18:18-23.
2. Aier A, Pais P, Raman V. Psychosocial aspects of children with chronic kidney disease and their families. *Clin Exp Pediatr* 2021 Nov 10 [Epub]. <https://doi.org/10.3345/cep.2021.01004>.

### Book or book chapter:

3. Volpe JJ. *Neurology of the newborn*. 5th ed. Philadelphia: Saunders/Elsevier; 2008.
4. Hong CE. *Textbook of pediatrics*. 9th ed. Seoul: Korea Textbook Publishing Co.; 2008.
5. Pan ES, Cole FS, Weintrub PS. Viral infections of the fetus and newborn. In: Taeusch HW, Ballard RA, Gleason CA, editors. *Avery’s diseases of the newborn*. 8th ed. Philadelphia: Elsevier Saunders; 2005. p. 495–529.

### Website

6. International Committee of Medical Journal Editors. Read the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals[Internet]. Philadelphia: International Committee of Medical Journal Editors; 2021 [cited 2022 Jan 10]. Available from: <http://www.icmje.org/recommendations/>.

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Tables should be typed double-spaced on separate pages within the manuscript, and they should be titled and numbered in Arabic numerals in the order of their first citation in the text. Each column should be given a short heading. Only the first letter of the first word in each row and column should be capital letters. If numerical measurements are given, the unit of measurement should be included in the each heading. The statistical significance of observed differences in the data should be indicated by the appropriate statistical analysis. All abbreviations should be defined in footnotes. For special remarks, superscripts a), b), c)... should be used. No more than 6 tables are needed. Tables should follow the references on separate pages.

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