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ORIGINAL ARTICLE

Clinical, pathological, and genetic characteristics of cases with asymptomatic proteinuria not manifesting nephrotic syndrome at onset: a single-center retrospective study

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Abstract

Background Cases with asymptomatic proteinuria (ASP) not manifesting nephrotic syndrome often pathologically show focal segmental glomerulosclerosis (FSGS). However, characteristics of those cases had not been intensively studied so far.

Methods We retrospectively reviewed clinical, pathological, and genetic characteristics of 37 children (median age, 9.3 years) who underwent renal biopsy for persistent isolated proteinuria (urine protein-to-creatinine ratio: UP/C, > 0.2 g/g) between 2003 and 2019. Targeted next-generation sequencing (NGS) was utilized for all patients with FSGS, excluding those with secondary FSGS.

Results At biopsy, all patients with FSGS ($N = 14$) had UP/C ≥ 0.5 g/g and the median UP/C was significantly higher in those with FSGS than those with minor glomerular abnormalities (MGA) ($N = 23$) (1.49 vs. 0.53 g/g, $P < 0.001$). Causative variants were found in seven patients with FSGS (*TRPC6*, *WT1*, *ACTN4*, and *INF2* in 3, 2, 1, and 1 patient, respectively): all gene variants were in genes manifesting autosomal dominant inheritance mode. The proportion of the perihilar variant was significantly higher in the genetic FSGS patients than in the non-genetic FSGS patients (4/7 vs. 0/7, $P < 0.05$). Kaplan–Meier analysis showed that the renal survival rate after ASP diagnosis was significantly lower in the genetic FSGS patients than in the non-genetic FSGS and the MGA patients ($P < 0.001$).

Conclusions UP/C was a simple and useful predictive parameter for the diagnosis of FSGS. APS without nephrotic syndrome at onset may be associated with autosomal dominant causes of FSGS, especially in those with the perihilar variant.

Keywords Asymptomatic proteinuria · Urine protein-to-creatinine ratio · Focal segmental glomerulosclerosis · Renal biopsy · Next-generation sequencing · Autosomal dominant variants · Perihilar variant

Introduction

Focal segmental glomerulosclerosis (FSGS), one of the most frequent causes of pediatric end-stage renal disease (ESRD) worldwide, comprises primary, secondary, and genetic types [1–3]. Abrupt-onset severe nephrotic syndrome develops in children with primary or autosomal recessive FSGS, whereas the majority of children with secondary or autosomal dominant FSGS present with asymptomatic proteinuria (ASP) at onset [1–3]. However, autosomal dominant FSGS is more likely to present as late-onset steroid-resistant nephrotic syndrome and more likely to progress to chronic kidney disease during adolescence or later in adulthood.

ASP, defined as isolated proteinuria without extrarenal symptoms, is detected by urine screening or during investigation for diseases unrelated to the kidneys and urinary tract [4]. Although ASP is transient and benign in most cases, studies previously reported the high likelihood of significant glomerular findings, including FSGS, in patients with persistent ASP [5–10]. We previously demonstrated that FSGS was present in 5 of 26 children with ASP (19%) and that all 5 patients with FSGS had a urine protein-to-creatinine ratio (UP/C) > 0.5 g/g on first morning void [11].

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There is currently no evidence regarding the utility of UP/C as a criterion to perform renal biopsy for confirmation of FSGS in children with ASP. Furthermore, there are no clear guidelines regarding the indications for genetic testing in patients with FSGS [12]. Therefore, we conducted a single-center retrospective study to clarify clinical, pathological, and genetic characteristics of ASP cases without nephrotic syndrome at onset.

Patients and methods

The present study included 37 consecutive Japanese children, including 21 boys and 16 girls, who underwent initial renal biopsy because of persistent isolated proteinuria (UP/C ≥ 0.2 g/g) for at least three months and were followed for at least one year between June 2003 and July 2019 at Saitama Children's Medical Center. Patients with orthostatic proteinuria, tubular proteinuria, hematuria (> 5 red blood cells/high-power field), glomerulonephritis, such as membranous nephropathy and IgA nephropathy, nephrotic syndrome (serum albumin ≤ 2.5 g/dL and UP/C ≥ 2.0 g/g), systemic disease, and congenital anomalies of the kidneys and urinary tract, such as hydronephrosis and hypoplastic kidney, were excluded. The indications of the renal biopsy for children with persistent proteinuria were as follows: UP/C ≥ 0.2–0.5 g/g for ≥ 6–12 months, or 0.5–1.0 g/g for ≥ 3–6 months, or ≥ 1.0 g/g for ≥ 3 months. Subsequent renal biopsies were performed in the patients with minor glomerular abnormalities (MGA) who had heavy persistent proteinuria (UP/C ≥ 1.0 g/g) despite the treatment with renin-angiotensin system blockers after the first renal biopsy.

Renal specimens were examined by light, immunofluorescence, and electron microcopy. Histologic variants of FSGS were assessed according to the Columbia classification [13]. All renal specimens were evaluated by a single pathologist (HM) blinded to the clinical profile of the patients. Comprehensive gene screening of patients with FSGS using targeted next-generation sequencing (NGS) was conducted at Kobe University Graduate School of Medicine [14]. The study was approved by the Ethics Committee of Saitama Children's Medical Center (approval no. 2019–04-003).

Statistical analyses

Categorical variables were presented as frequencies and percentages and compared using the chi-square or Fisher's exact test, as appropriate. Unless indicated otherwise, continuous variables were expressed as medians with interquartile ranges. Continuous variables were compared using the parametric two-sample *t* test or the non-parametric Mann–Whitney *U* test, as appropriate. Differences among three groups were compared using Kruskal–Wallis tests, with pairwise comparisons assessed using the Steel–Dwass test for multiple comparisons, The Kaplan–Meier method and log-rank test were used for analysis of the probability of renal survival without renal replacement therapy after the diagnosis of ASP. All analyses were performed using the JMP Pro statistical software version 15.0 (SAS Institute, Cary, NC, USA). The statistical significance level was set at a P value of < 0.05.

Results

The study cohort is summarized in Fig. 1. In all patients in the current study, ASP was incidentally detected in a school urine screening test or during investigation for other diseases such as gastroenteritis and bronchitis. The median duration from ASP diagnosis to the first biopsy was 10 (interquartile range, 3.0–32.0) months. At the time of first renal biopsy, the median age and median UP/C were 9.3 (interquartile range, 6.7–13.3) years and 0.82 (interquartile range, 0.48–1.82) g/g, respectively. The initial histological diagnoses were FSGS, and MGA in 11, and 26 patients, respectively. In the initial FSGS group ($N = 11$), 1 patient achieved spontaneous remission of proteinuria and 3 patients were diagnosed with secondary FSGS, including 2 patients with preterm low birthweight and 1 patient with obesity. The remaining 7 patients with FSGS were evaluated by targeted NGS, which revealed *TRPC6* and *WT1* variants in 3 and 1 patient, respectively, whereas the remaining 3 patients had undetectable disease-causing variants. In the initial MGA group ($N = 26$), 3 patients underwent sequential renal biopsies because of persistent heavy proteinuria after the first biopsy and were reclassified from MGA to FSGS. Comparison of the clinical characteristics between patients with FSGS ($N = 14$) and those with MGA ($N = 23$) are shown in Table 1. Fourteen of the 26 children

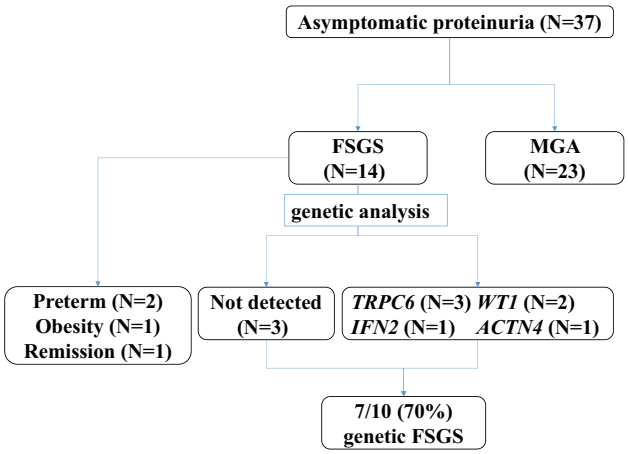

Fig. 1 Study cohort

Table 1 Comparison of clinical characteristics between FSGS and MGA groups at renal biopsy

	FSGS group (*N* = 14)		MGA group (N = 23)		*P* value
Sex (male/female)	8/6		13/10		1.00
ASP detected by urine screening test (yes/no)	13/1		17/6		0.22
UP/C at biopsy (>0.5/≤0.5 g/g)	14/0		12/11		0.0022
	Median	(IQR)	Median	(IQR)	
Age at ASP diagnosis (years)	7.9	(3.7–11.9)	7.8	(4.8–11.1)	0.94
Duration from ASP diagnosis to biopsy (months)	12.5	(3.0–44.5)	14.0	(5.0–36.0)	0.79
Age at biopsy (years)	10.5	(6.7–13.7)	8.8	(6.8–12.4)	0.65
Serum albumin at biopsy (g/dL)	3.8	(3.6–4.2)	4.1	(3.9–4.5)	0.034
eGFR at biopsy (mL/min/1.73 m^2)	112.5	(87.8–128.2)	120.1	(113.7–141.5)	0.19
UP/C at biopsy	1.49	(0.96–2.25)	0.53	(0.39–1.03)	0.0007
Number of glomeruli in biopsy sample	20	(14–24)	16	(10–27)	0.66

FSGS, focal segmental glomerulosclerosis; *MGA;* minor glomerular abnormalities, *ASP*, asymptomatic proteinuria; *eGFR*, estimated glomerular filtration rate; *UP/C*, urinary protein-to-creatinine ratio

with a UP/C > 0.5 g/g had FSGS, whereas none of 11 children with a UP/C ≤ 0.5 g/g had FSGS ($P = 0.0022$). The median UP/C was significantly higher in the FSGS group than in the MGA group ($P < 0.001$) (Fig. 2a), but was not significantly different between the genetic FSGS group and the non-genetic FSGS group (Fig. 2b). In the 3 patients with late FSGS diagnosis, targeted NSG revealed *ACTN4*, *WT1*, and *INF2* variants in 1 patient each. In summary, 7 of the 10 patients with FSGS (70%) evaluated by genetic testing were diagnosed with autosomal dominant FSGS (Table2). According to the Columbia classification for FSGS, genetic FSGS patients were classified the following variants: perihilar ($N = 4$) and not otherwise specified ($N = 3$). However, the histologic variant of all patients with non-genetic FSGS showed not otherwise specified. The proportion of the perihilar variant was significantly higher in the genetic FSGS patients than in the non-genetic FGSG patients (4/7 vs. 0/7, $p < 0.05$).

Although immunosuppressive treatment with cyclosporine and high-dose steroids was initiated in 4 of the 7 patients with genetic FSGS when nephrotic syndrome subsequently developed, none of the patients responded to therapy and all progressed to ESRD. In contrast, the remaining 3 patients with genetic FSGS and 7 patients with non-genetic FSGS who were treated with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers did not develop ESRD (Table 2). At the last visit (median observation period, 5.3 years), 23 patients with MGA also did not develop ESRD and the median UP/C was 0.2 g/g (interquartile range, 0.06–0.45). Kaplan–Meier analysis showed that the renal survival rate after ASP diagnosis was significantly lower in the genetic FSGS patients than in the MGA and the non-genetic FSGS patients (log-rank test, $P < 0.001$) (Fig. 3).

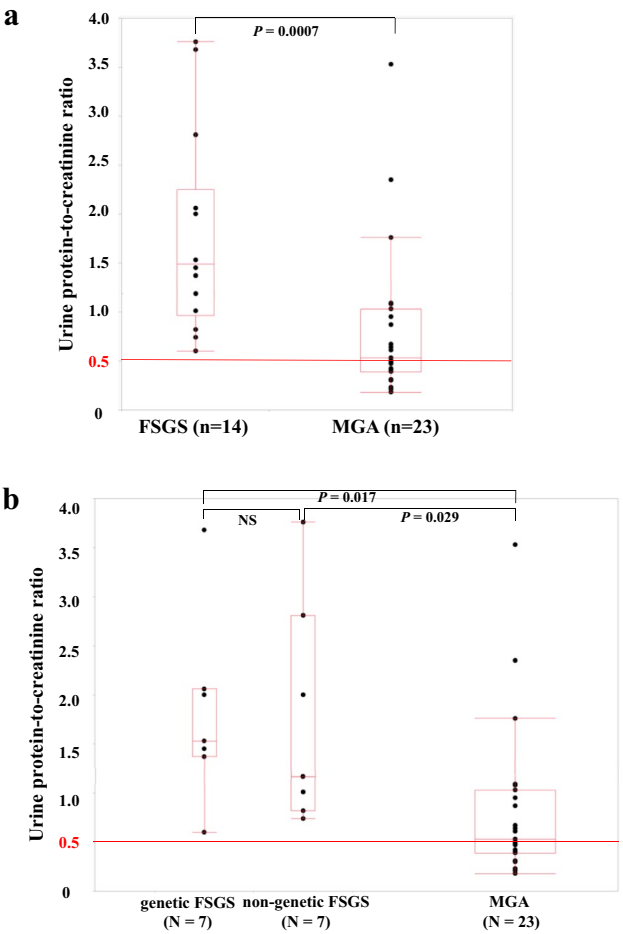

Fig. 2 **a** Scatter plots showing urine protein-to-creatinine ratio at renal biopsy in FSGS and MGA groups (each box shows the median and the 25th and 75th percentiles). **b** Scatter plots showing urine protein-to-creatinine ratio at renal biopsy in genetic FSGS, non-genetic FSGS and MGA groups (each box shows the median and the 25th and 75th percentiles)

Table 2 Baseline characteristics and clinical courses of 14 patients with FSGS

	Sex	Age at ASP diagnosis (y)	UP/C at first biopsy (g/g)	eGFR at first biopsy (mL/min/1.73 m^2)	Age at FSGS diagnosis (y)	Columbia classification	Gene (genome)	Mode of inheritance	Treatment (Age at nephrotic syndrome)	Age at last observation (y)	UP/C at last observation (g/g)	Prognosis (duration from ASP diagnosis to ESRD)
1	F	12.4††	1.45	86.7	16.6	NOS	*TRPC6* (c.2624 A > T)	AD (father)	ARB, ACEI, PSL, CsA (16.7 y)	23.9		ESRD (11.5 years) Hemodialysis
2†	M	10.2††	0.69	120.3	12.1	NOS	*IFN2* (c.550 G > A)	AD (de novo)	ARB, ACEI, PSL, CsA (12.8 y)	15.3		ESRD (5.1 years) Transplantation
3†	M	7.0††	1.88	113.2	9.3	Perihilar	*ACTN4* (c.671 T > C)	AD (de novo)	ARB, ACEI, PSL, CsA (9.5 y)	13.4		ESRD (6.4 years) Transplantation
4†	F	3.8††	2.17	119.9	8.4	Perihilar	*WT1* (c.1178G > A)	AD (de novo)	ARB, ACEI, PSL, CsA (5.9 y)	14.2		ESRD (10.4 years) Peritoneal dialysis
5	F	1.9	2.06	99.2	2.2	NOS	*WT1* (c.1432 + 5G > A)	AD (de novo)	ARB, ACEI	8.4	0.26	Normal renal function
6	M	13.5††	0.60	135.7	14.1	Perihilar	*TRPC6* (c.2624 A > T)	AD (mother)	ARB, ACEI	17.7	1.70	Normal renal function
7	M	3.0††	2.00	113.7	6.6	Perihilar	*TRPC6* (c.523 C > T)	AD (de novo)	ARB, ACEI	8.8	0.67	Normal renal function
8	M	11.8††	0.74	111.3	13.1	NOS	Not detected		ARB, ACEI	23.0	0.55	CKD G3
9	M	3.4††	2.00	88.2	3.7	NOS	Not detected		ARB, ACEI	5.8	0.89	CKD G2
10	F	6.5††	2.81	129.6	6.8	NOS	Not detected		ARB, ACEI	12.8	0.04	Normal renal function
11	F	11.1††	1.01	163.4	11.3	NOS	Not done Remission of proteinuria		ARB	18.8	0.03	Normal renal function
12	F	8.8††	0.82	127.7	9.7	NOS	Not done Severe obesity (obesity index 66%)		ARB	22.8	0.13	CKD G2
13	M	7.8††	1.17	58.1	8.1	NOS	Not done Preterm infant (GA 23 weeks, 594 g)		ARB, ACEI	12.1	0.48	CKD G3
14	M	7.1††	3.76	66.6	14.5	NOS	Not done Preterm infant (GA 35 weeks, 1562 g)		ARB, ACEI	21.3	0.13	CKD G3

FSGS, focal segmental glomerulosclerosis; *ASP*, asymptomatic proteinuria; *UP/C*, urinary protein-to-creatinine ratio; *eGFR,* estimated glomerular filtration rate; *AD*, autosomal dominant; *ESRD*, end-stage renal disease; *NOS*, not otherwise specified; *ARB*, angiotensin II receptor blockers; *ACEI,* angiotensin-converting enzyme inhibitors; *PSL*, prednisolone; *CsA*, cyclosporine; *CKD*, chronic kidney disease; *GA*, gestational age † late FSGS diagnosis, †† urine screening

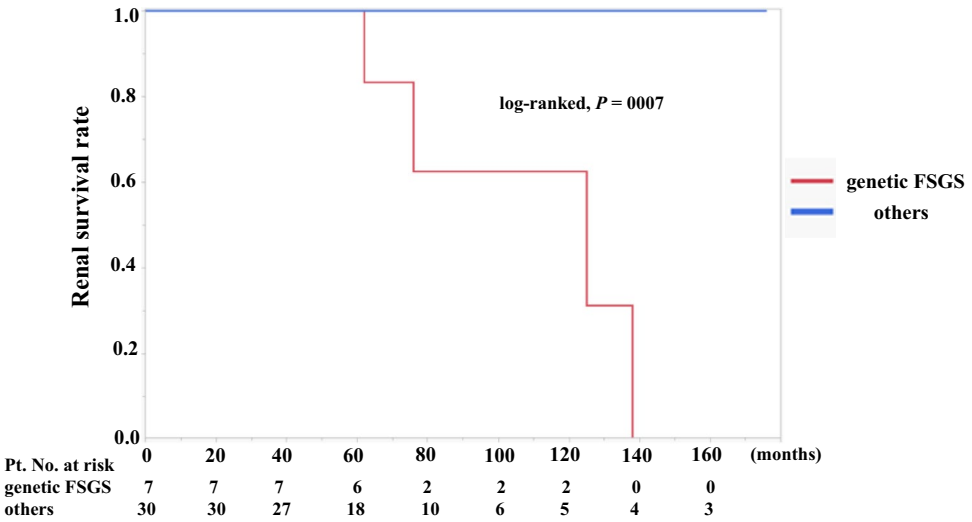

Fig. 3 Kaplan–Meier curves showing the renal survival rate after ASP diagnosis. The probability of renal survival without renal replacement therapy after ASP diagnosis was significantly lower in the genetic FSGS patients than in the non-genetic FSGS and the MGA patients (log-ranked, $P < 0.001$)

Discussion

In this retrospective study, we found that 14 of the 37 Japanese children with persistent isolated proteinuria who underwent renal biopsy had FSGS. We also showed that the median UP/C was significantly higher in those with FSGS than those with MGA. We detected autosomal dominant variants in 7 of 10 patients with FSGS evaluated by targeted NGS. Furthermore, we demonstrated that the perihilar variant may be specific for the patients with autosomal dominant FSGS. This study clarified clinical, pathological, and genetic characteristics of ASP cases without nephrotic range proteinuria at onset for the first time.

Although the positive rate of isolated proteinuria in school urine screenings for children was not high [15], persistent proteinuria may be the initial manifestation of severe glomerular disease. In a review article of urine screenings for 3-year-old Japanese children, Yanagihara et al. reported that serious illness including FSGS and nephrotic syndrome was found in children with isolated proteinuria at a comparatively high rate [10]. The authors concluded that children with persistent proteinuria should be referred to nephrologist because the prevalence of those requiring treatment was relatively high in this cohort. In a retrospective study of mass school urine screening test for Korean children, Park et al. also reported that the incidence of abnormal pathological findings such as FSGS in those with persistent isolated proteinuria was high [8]. In our study, we found that 13 of the 14 patients with FSGS (93%) were detected by urine screenings for preschool or schoolchildren. Based on these findings, we believe that the importance of proteinuria screening for children was confirmed.

In a retrospective study of 44 Japanese children who underwent renal biopsy because of persistent ASP without nephrotic syndrome (UP/C > 0.2 g/g for at least 3 months), including those with hematuria, Hama et al. reported that the optimal UP/C cutoff to discriminate significant glomerular findings such as FSGS and IgA nephropathy from MGA was 0.5 g/g [5]. Furthermore, the authors found that 5 of the 29 children with a UP/C ≥ 0.5 g/g had FSGS and that only 1 of the 15 children with a UP/C < 0.5 g/g had FSGS. The proportion of patients with FSGS in that study was lower than that in the present study (14% vs 38%); in the current study we did not include patients with chronic glomerulonephritis such as IgA nephropathy and membranous nephropathy due to the lack of hematuria. However, in another study from their same institution, which included 53 Japanese ASP children without nephrotic syndrome in the absence of hematuria, Yoshikawa et al. reported that FSGS was detected in 15 patients (28%), which is comparable to the current study cohort [6]. Furthermore, in a retrospective study of 31 APS children without nephrotic syndrome, Trachtman et al. reported that FSGS was the most common lesion, accounting for 47% of the pathological findings [7]. The authors concluded that renal biopsy should be performed to determine the nature of any significant glomerular disease in patients with persistent ASP for more than one year. However, their analysis failed to reveal an optimal cutoff value for proteinuria that differentiated patients with and without significant glomerular findings probably because the decision for renal biopsy was reached on a case-by-case basis in patients with ASP. Although proper assessment of ASP without hematuria in children is controversial, we suggest that renal biopsy should be considered in patients with ASP who have a UP/C of > 0.5 g/g because of the high rate of FSGS observed in the present study cohort.

Prior to the advent of NGS methods, several studies showed that the prognosis was poor in children with FSGS who presented with ASP not manifesting nephrotic syndrome compared to those with nephrotic syndrome at the

time of initial diagnosis [16, 17]. Yoshikawa et al. found that all ASP patients without nephrotic syndrome who subsequently progressed to ESRD had FSGS and that the treatment for FSGS was unsatisfactory [6]. Furthermore, in a retrospective study of Korean children with steroid-resistant FSGS, Paik et al. reported that none of the 7 ASP patients without nephrotic syndrome at onset responded to immunosuppressive agents and that 3 patients progressed to ESRD. Therefore, in the NGS era, a genetic cause rather than a primary cause should be considered in patients with asymptomatic FSGS in the absence of nephrotic syndrome at onset, because the misclassification of FSGS may lead to inappropriate and harmful immunosuppressive therapy in these patients. Over 60 monogenic causes of FSGS that have been identified to date reveal dysfunction in podocyte-associated proteins as the pathogenesis of proteinuria. In particular, autosomal dominant FSGS may exhibit ASP without nephrotic syndrome at onset and develop into steroid-resistant nephrotic syndrome later in adolescence or adulthood. Therefore, early confirmation of genetic diagnosis in the patients with FSGS before the development of nephrotic syndrome would prevent unnecessary treatments with steroids and calcineurin inhibitors. However, the indication for genetic testing in children with ASP remains unclear because the rate of causative monogenic variants in these patients is unknown. In a retrospective study of 230 Japanese patients who were evaluated by NGS, including those with steroid-resistant nephrotic syndrome, FSGS, and ASP, Nagano et al. reported that monogenic disease-causing variants were present in 69 patients (30%) [14]. Additionally, the authors found that the rates of nephrotic syndrome, edema, and remission were significantly lower in patients with these variants than in those without variants. In the present study only including Japanese children with ASP, the proportion of causal variants was very high (70%) among the patients with FSGS evaluated by NGS. Based on these findings, ASP without nephrotic syndrome at onset may be associated with genetic disease in Japanese children with FSGS.

Although the perihilar variant is commonly observed with secondary FSGS associated with altered hemodynamics such as obesity and hypertensive nephrosclerosis [18], it remains unclear whether the histologic form can be induced by genetic FSGS. Surprisingly, we found that the perihilar variant was observed only in 4 of the 7 patients with genetic FSGS. Recently, Ishizuka et al. also reported that 3 of 8 patients with genetic FSGS had finding of the perihilar variant, whereas none of 8 patients with primary FSGS revealed the perihilar from [19]. Therefore, we suggest that NGS be performed for ASP patients with perihilar FSGS who did not have secondary causes of reduced nephron number.

Our study has several limitations. First, the present study is limited by the retrospective design including a small number of patients from a single center. Second, although the number of glomeruli evaluated between FSGS and MGA groups was not significantly different, the small sample size in the biopsy (i.e., < 20 glomeruli) may lead to misclassification of the histological diagnosis [20]. Third, we did not perform NGS in secondary FSGS and MGA patients. Finally, all patients in the present study were Japanese, and the high proportion of monogenic disease-causing variants in ASP children with FSGS cannot be generalized to cohorts from different ethnicities such as Caucasian cohorts.

In conclusion, UP/C may be a useful predictive parameter for the diagnosis of FSGS in children with persistent isolated proteinuria. Cases with ASP not manifesting nephrotic syndrome at onset may be associated with autosomal dominant causes of FSGS, especially in those with the perihilar variant. We propose that early genetic testing should be performed in ASP patients with perihilar FSGS in the absence of clinical evidence of secondary causes of FSGS. Future prospective studies are warranted to determine the optimal timing for renal biopsy and genetic testing in children with persistent isolated proteinuria.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Research Committee and/or National Research Committee at which the study was conducted with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was not required in view of the retrospective study design and the anonymity of the patient records reviewed.

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