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Original Article

Does criterion of renal biopsy in asymptomatic isolated proteinuria children has already been identified?

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Abstract: Background: Asymptomatic isolated proteinuria (ASP) is uncommon in children and the pathological studies are not much. By now there is no consensus on the indications of renal biopsy. Purpose: To explore the optimal cut-off point of ratio of urinary protein to creatinine as an indication of renal biopsy in children with persistent ASP. Methods: 1,519 children who underwent renal biopsy from 2007 to 2014 in The Children's Hospital of Zhejiang University School of Medicine were retrospectively reviewed to screen the patients with ASP. Both the methods of minimum *P* value and receiver operating characteristics (ROC) analysis were used to determine the optimal urinary protein/creatinine ratio (uP/uCr) for separation of minor glomerular abnormalities from other significant glomerular changes. Results: 37 children with persistent ASP were identified, of them, 26 cases were male and 11 cases were female. Pathological results of renal biopsy suggested that 24 cases were minor glomerular abnormalities (MGA) and 13 cases were other significant glomerular changes. With the minimum *P* value approach and ROC analysis, we obtained the optimal cut-off points of uP/uCr ratio, 0.49 g/g and 0.50 g/g, respectively, to separate MGA from other significant glomerular changes. Both the cut-off values led to the same recognition of the renal outcomes. Follow-up results suggested that the patients with low proteinuria (< optimal cut-off value) exert better prognosis than the patients with high proteinuria (≥ optimal cut-off value). Conclusions: A uP/uCr ratio of ≥ 0.50 g/g might a reasonable criterion of renal biopsy for ASP children. More independent and larger scale studies should be performed to confirm our results.

Keywords: Asymptomatic isolated proteinuria, children, pathology, renal biopsy

Introduction

There are about 1-5% of asymptomatic children exerting a degree of proteinuria [1]. Persistent asymptomatic isolated proteinuria (ASP) is used when abnormal protein excretion is the only finding in urine and no abnormal findings of the kidney or urinary tract disease are found in the history or physical or laboratory data [2, 3]. Although ASP is usually a benign condition, it may be the early sign of developing a serious illness such as chronic kidney disease (CKD) in patients with ASP [4, 5]. Thus, it is critical to develop a reliable method to identify ASP before onset of CKD. For this purpose, renal biopsy is usually used. Recently, the urinary protein/creatinine ratio (uP/uCr) has been widely used to assess proteinuria and the children with an uP/uCr value less than 0.2 g/g are treated as normal [6, 7]. However, utility of 0.2 g/g as standard of renal biopsy is controversial due to it may bring unnecessary renal biopsy for some patients. Up to now, only two studies are performed in Japanese children with ASP to determine optimal uP/uCr value as the criteria of renal biopsy and both of them suggested that the use of a uP/uCr ≥ 0.5 g/g may be a reasonable criterion for renal biopsy aimed at distinguishing renal outcomes in patients with persistent isolated ASP [3, 8].

In the present study, we screened 1,519 children underwent renal biopsy in a China center and identified 37 patients with ASP. Then we determined an optimal cut-off point for (uP/uCr) as an indication for renal biopsy.

Table 1. Clinical characteristics of patients with ASP in the whole cohort, and in low and high proteinuria groups divided by optimal cut-off point of urinary protein to creatinine ratio (uP/uCr)

Parameter	Whole cohort (n=37)	Low proteinuria (n=10)	High proteinuria (n=27)	*P* value
Gender (Boy/Girl)	26/11	6/4	20/7	0.442
Age at discovery (year)	5 (0.67-11.67)	8.79 (3.4-11.67)	4.33 (0.67-10.83)	0.053
Age at initial biopsy (year)	7.17 (1-13)	8.79 (3.5-11.67)	6.33 (1-13)	0.483
Time from discovery to initial biopsy (year)	0.17 (0-11.42)	0.09 (0-0.8)	0.33 (0-11.42)	0.053
uP/uCr at initial biopsy (g/g)	0.9 (0.11-5.46)	0.38 (0.11-0.48)	1.68 (0.5-5.46)	0.000
Estimated GFR at initial biopsy (ml/min/1.73 m^2)	126.7 (72.36-172.2)	136.9 (112-160.1)	123 (72.36-172.2)	0.400
albumin (g/L)	43.9±4.9	46.7±3.9	42.9±4.8	0.032
Creatinine (μmol/L)	44.1±14.8	44.0±8.6	44.1±16.7	0.986
Blood urea nitrogen (mmol/L)	4.8±1.5	4.0±0.8	5.1±1.6	0.070
Cholesterol (mmol/L)	4.4±0.8	4.4±0.8	4.3±1.0	0.776

Note: ASP: Asymptomatic isolated proteinuria; GFR, Glomerular filtration rate.

Materials and methods

Patients

We studied 38 children with ASP screened from 1,519 children who underwent renal biopsy from 2007 to 2014 in The Children's Hospital of Zhejiang University School of Medicine were retrospectively reviewed and 38 children with ASP were screened. The diagnosis was based upon the presence of an uP/uCr ratio > 0.2 g/g in an early-morning specimen. One of them was excluded due to the missing of follow-up information. Renal biopsy was carried out when mild-to-moderate proteinuria (0.2 g/g < uP/uCr ≤ 1.0 g/g) lasted for at least six months or severe proteinuria (uP/uCr > 1.0 g/g) for one months after the discovery. None of the patients had hematuria, edema, or azotemia. Before a renal biopsy, each patient was given special check to exclude orthostatic proteinuria, non glomerular proteinuria, or glomerulonephritis secondary to some diseases such as systemic lupus erythematosus and hepatitis B virus infection. Each patient's family gave a written informed consent for a renal biopsy. Renal tissue was obtained by needle biopsy under ultrasound guidance. Renal biopsy specimens were investigated by routine light, immunofluorescence, and electron microscopy. Clinical data such as age at discovery or renal biopsy, uP/uCr, kidney function, albumin, estimated GFR, ultrasonography of kidney, and follow-up information were obtained from medical records. All patients were naive to angiotensin-converting enzyme inhibitors (ACE-Is) and angiotensin II receptor blockers (ARBs) prior to their biopsy.

Statistical analysis

The data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The continuous data were expressed as mean ± standard deviation ($\bar{x}\pm s$) or median based on the data distribution and the difference between two groups were compared using the t-test or Mann-Whitney U test. The categorical data were analyzed using the χ^2 test. To estimate the "optimal" cut-off point of uP/uCr, the minimum *P*-value approach was firstly used to dichotomize the continuous uP/uCr measurements at an appropriate level for the diagnosis of glomerular disease [9]. The prevalence of minor glomerular abnormalities (MGA) or other significant glomerular changes among the individuals with an uP/uCr value more or less than the cut-off value was compared with the χ^2 test. The cut-off value with a minimum *P* value was treated as optimal cut-off point. Then, receiver operating characteristic (ROC) was also performed to determine the relationship between uP/uCr and the glomerular changes (minor glomerular abnormalities and other significant glomerular changes). The optimal cut-off point was calculated by Youden index [10]. A *P*-value of less than 0.05 was considered significant.

Results

Patients

37 (26 boys, 11 girls) patients with persistent isolated ASP were finally identified from 1,519 patients underwent renal biopsy from 2007 to 2014 in The Children's Hospital of Zhejiang University School of Medicine. The characteris-

Table 2. Pathological results and IgM+ deposition in patients with persistent isolated asymptomatic proteinuria stratified by optimal cut-off point of urinary protein to creatinine

Parameters	Whole cohort (n=37)	Low proteinuria (n=10)	High proteinuria (n=27)
Pathology			
MGA	24	9	15
FSGS	7	0	7
MsPGN	1	0	1
IgAN	4	1	3
Focal glomeruloscerosis	1	0	1
IgM+ deposition			
Yes	11	0	11
No	26	10	16

Note: MGA: Minor glomerular abnormality; FSGS: Focal segmental glomerulosclerosis; MsPGN: Mesangial proliferative glomerulonephritis; IgAN: IgA nephropathy.

tics of the patients were shown in **Table 1** and Table S1. The kidney function, albumin, estimated GFR and ultrasonography of kidney of all the patients were normal. The onset age ranged from 0.7 to 11.7 years old with an average value of 5.5±3.3. The proteinuria of 40.5% patients (15/37) was found in physical examination while 59.5% patients (22/37) presented with symptoms such as upper respiratory tract infection or intestinal infection before proteinuria diagnosis. The mean period from discovery to initial biopsy was 1.5±2.5 year. According the renal biopsy results, 24 patients were diagnosed as minor glomerular abnormalities while 13 patients were other significant glomerular changes (7 FSGS, 1 MsPGN, 4 IgAN, and 1 focal glomeruloscerosis) (**Table 2**). 29.7% patients (11/37) exhibited IgM deposition. The median duration of follow-up was 57 months (24-93). During the follow-up, 18 of 37 children (48.6%) got remission (**Table 2**).

Optimal cut-off point for renal biopsy criteria

The minimum *P* value approach was firstly used. Of the 30 candidate cut-off values in the present study, the minimum *P* value was 0.0655 and its corresponding uP/uCr level was 0.5 g/g (**Figure 1A**). In addition, ROC analysis was also performed to estimate the optimal cut-off point and the results suggested the optimal cut-off point to separate MGA from other significant glomerular disease was 0.49 g/g (**Figure 1B**). The area under the ROC curve (AUC) was 0.623 with sensitivity as 0.923 and specificity as 0.375.

Clinical validation of the optimal cut-off point

The patients were divided into low-proteinuria and high-proteinuria groups according to the optimal cut-off points derived from the minimum *P* value approach and ROC analysis. Interestedly, either by the value of 0.5 g/g or by the value of 0.49 g/g, the grouping results were same (**Tables 1**, **2**). Ten patients were low-proteinuria and 27 patients were high-proteinuria. As shown in **Table 1**, there was no significant difference between the two groups with regard to gender, age at discovery or initial biopsy, time from discovery to initial renal biopsy, estimated GFR, creatinine, blood urea nitrogen, and cholesterol except albumin. And the uP/uCr in low-proteinuria group was significant low than that in high group (0.3±0.1 g/g vs. 1.8±1.1 g/g, P=0.000). The pathological diagnosis based on the renal biopsy was shown in **Table 1**. Of the ten patients with low proteinuria, nine was MGA and one was IgAN (Lee II). And, of the 27 patients in high proteinuria group, the patients with MGA, FSGS, MsPGN, IgAN, and Focal glomeruloscerosis were 15, seven, one, three, and one, respectively. Five cases were showed in **Figure 2**. The diagnosis of the renal biopsy of them was MGA (**Figure 2A**), FSGS (**Figure 2B**), MsPGN (**Figure 2C**), IgAN (**Figure 2D**), and Focal glomeruloscerosis (**Figure 2E**), respectively. The case with MGA had low proteinuria and the others had high proteinuria. Immunofluorescence staining indicated that sole IgM+ was the main deposition and all the patients with IgM+ deposition (n=10) were located in high proteinuria group (**Table 2**).

Therapy and follow-up

All the patients in low proteinuria group received the angiotension-converting enzyme inhibitor (ACEI) alone. In high proteinuria group, six patients received ACEI alone, 11 patients received ACEI combined with other drugs such as angiotensin II receptor blockers (ARB), prednisone, immunosuppressor and so on, eight patients received other treatments, mainly pred-

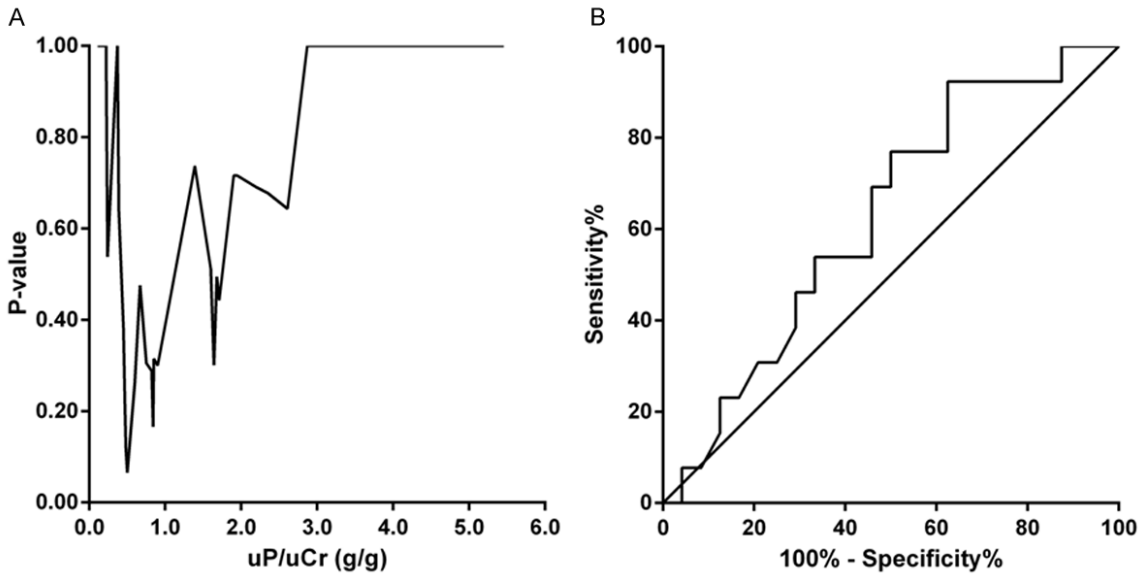

Figure 1. Optimal urinary protein-to-creatinine ratio (uP/uCr) cut-off points for separating minor glomerular abnormalities from significant glomerular changes in patients with persistent isolated asymptomatic proteinuria (ASP) using the minimum *p*-value approach (A) and receiver operating characteristics (ROC) analysis (B).

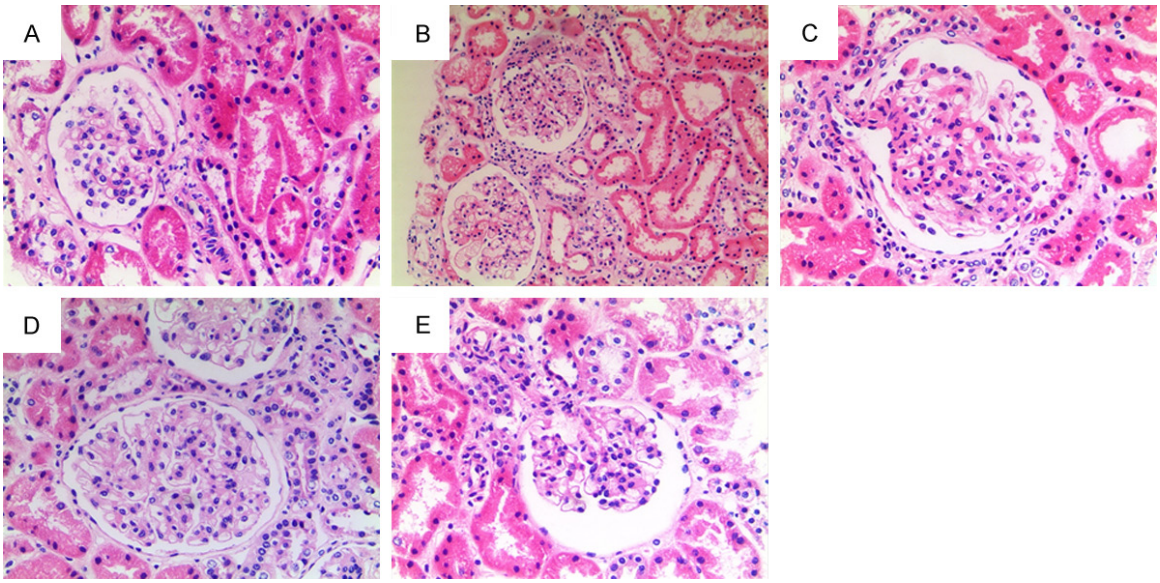

Figure 2. Five patients with different pathology type indicated by renal biopsy and their proteinuria level. A. A nine years old boy with low proteinuria and MGA (400×). B. A five years old boy with high proteinuria and FSGS (400×). C. A twelve years old boy with high proteinuria and MsPGN (400×). D. A twelve years old girl with high proteinuria and IgAN. E. A seven years old girl with high proteinuria and focal glomeruloscerosis (400×).

nisone containing therapy, and two patients received no treatments (**Table 3**). The duration of follow-up ranged from 24 to 93 months. The median follow up was 58 (37-93) months in low proteinuria group and 55 (24-93) months in high proteinuria group, and no significant difference of follow up duration was observed between the two groups (P=0.350). Complete remission (CR), mild proteinuria, moderate proteinuria, and severe proteinuria were defined by the uP/Cr values, < 0.2, 0.2 to < 0.5, 0.5 to < 1.0, and ≥ 1.0 g/g, respectively. The results suggested that CR was achieved in 9 of the 10 patients in the low-proteinuria group (90%)

Table 3. Therapy of the patients with persistent isolated asymptomatic proteinuria and their clinical status at the latest follow up

Parameters	Whole cohort (n=37)	Low proteinuria (n=10)	High proteinuria (n=27)
Therapy			
ACEI alone	16	10	6
ACEI combined with other drugs	11	0	11
Other treatments	8	0	8
No treatment	2	0	2
Clinical status at latest follow up			
Complete remission	17	9	8
Mild proteinuria	2	0	2
Moderate proteinuria	5	1	4
Severe proteinuria	13	0	13

while CR was achieved in only seven of the 27 patients in high proteinuria group (25.9%). The patients with mild, moderate, or severe proteinuria in high proteinuria group were two, four, and 13, respectively.

Discussion

In the present, we used the minimum *P* value approach and ROC analysis to explore the optimal cut-off value of uP/uCr ratio for the criteria of renal biopsy. The minimum *P* value approach indicated a uP/uCr ratio ≥ 0.49 g/g as the optimal cut-off value while ROC analysis suggested a uP/uCr ratio ≥ 0.50 g/g. Both then could separate minor glomerular abnormalities from other significant glomerular changes and achieved the same results. In the low-proteinuria group (uP/uCr < 0.50 g/g), 90% (9/10) patients were MGA while only one was FSGS. And in the high-proteinuria group (uP/uCr ≥ 0.50 g/g), 15 of 27 patients were MGA and the others presented significant glomerular changes.

ASP accounts for a small portion of proteinuria in children. In Korea, a mass urine screening test of over 7 million school children found 1,044 children with hematuria and/or proteinuria, of which, only 163 cases were diagnosed as isolated proteinuria [11]. ASP is dormant and presents no clinical manifestations including edema, hypertension, and renal function damage, and has not received enough attention in the past. Recently, it is gradually recognized that ASP maybe an early sign of kidney diseases. Researches on asymptomatic haematuria and/or proteinuria showed that the most common pathological type of microhaematuria with asymptomatic proteinuria was IgA nephropathy (18/23, 79%), while the common pathological type of isolated microhaematuria (45/80, 56%) and ASP (5/9, 56%) was minor glomerular abnormalities [12]. Other related studies have also revealed the pathology difference among the three clinical symptoms [11, 13]. Although most the ASP patients are normal during follow-up, a small proportion of ASP patients may have significant glomerular disease and poor prognosis. This brings difficulties for the nephrologists to decide whether to perform renal biopsy because that the rate of biopsy-related morbidity is 8-10% [14]. Thus, it is beneficial to develop a criterion to avoid unnecessary renal biopsy.

Up to now there is no consensus on the indication of renal biopsy in ASP children. Howard et al. [15] found the level of proteinuria could not predict the presence of kidney disease in ASP patients, but if proteinuria persisted for more than 1 year, renal biopsy needed to be considered. However, Hama et al. [3] performed ROC analysis in 44 children with ASP screened from 1,167 patients who underwent renal biopsy and identified an uP/uCr ≥ 0.5 g/g as reasonable cut-off for the decision to conduct renal biopsy in children with persistent isolated ASP. This value is the same in the reports of Hirano et al. [8], who used the minimum *P* value approach to identify the value in 32 patients with persistent isolated ASP. The optimal cut-off point of uP/uCr ratio (0.49 or 0.5 g/g) derived from 37 patients in our study with minimum *P* value approach and ROC analysis was similar with the results in above studies. As the limitation of sample number in this study, greater sample sizes are needed to fully determine the indication of renal biopsy.

Glomerular IgM deposition has been described in many types of kidney diseases such as focal segmental glomerulosclerosis (FSGS), minimal change disease, mesangial proliferative glomerulonephritis [16, 17]. A study on mouse model of non-sclerotic glomerular disease indi-

cated IgM binded to glomerular epitopes and might contribute to the progression of glomerular damage in complement induced glomerulopathy [18]. In a clinical study, children with steroid-dependent or resistant minimal change nephrotic syndrome had a high prevalence of IgM+ (23/55, 41.8%) [19]. Interestingly, we found diffuse sole IgM+ deposition was all in high proteinuria group in the present study. It suggested that IgM deposition might correlated with the renal pathology of the ASP patients.

In this study, we also compared the clinical status of these ASP children at the latest follow up, which ranged from 2.0 to 7.7 years. Most of patients in low proteinuria group had good prognosis with 9/10 patients as complete remission and 1/10 patient as mild proteinuria. In the high proteinuria group, only 8/27 patients obtained complete remission and 13/27 patients were severe proteinuria.

In conclusion, we identified the optimal cut-off point of uP/uCr as an indication of renal biopsy in 37 children with ASP screened from 1,519 cases underwent renal biopsy using minimum *P* value method and ROC approach and the optimal value was similar with the results in previous studies. Follow up suggested that high proteinuria was associated with poor prognosis in ASP children. More independent studies with large scale samples should be performed to confirm the results.

Disclosure of conflict of interest

None.

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Table S1. Clinical information of the patients with persistent isolated asymptomatic proteinuria

Gender	GFR (ml/min/1.73 m^2)	Cr (μmol/L)	BUN (mmol/L)	CHO (mmol/L)	uP/uCr (g/g)	Albumin (g/L)	Biopsy age (yr)	Onset age (yr)	Kidney pathology
Boy	116	49.4	4.94	3.84	0.11	47.3	8.83	8.83	MGA
Girl	140	56.8	3.77	4.28	0.22	47.3	10.17	10	MGA
Boy	118	56.8	3.5	4.58	0.22	49	10.30	10.3	MGA
Boy	160.1	41	4.54	3.87	0.24	42.4	8.75	8.75	IgAN
Boy		43.8	5.71	5.44	0.37	42	4.00	3.92	MGA
Girl	157	45	2.89	3.79	0.39	48	11.67	11.67	MGA
Girl	142	41	3.8	3.79	0.39	48	10.00	9.2	MGA
Boy	112	30.7	3.56	5.47	0.45	49	3.50	3.4	MGA
Girl	133.8	33.7	4.13	3.44	0.47	40.5	4.00	3.83	MGA
Boy		42	3.5	5.79	0.48	53.4	4.33	3.58	MGA
Boy		33.6	3.56	4.37	0.50	48.3	5.92	5.92	FSGS
Girl		79	8.3	3.32	0.60	42	7.83	7.83	FSGS
Boy	155	26	4.13	3.44	0.67	39	4.00	3.8	MGA
Boy		95.6	9.39	3.32	0.75	43.9	9.08	8.83	MGA
Girl		26.4	5.03	3.8	0.82	45.1	3.92	3.75	MGA
Boy	147.6	35	3.19	4.86	0.84	41.8	9.83	9.75	IgAN
Boy		56	6.01	6.33	0.85	47	12.42	1	MGA
Boy	144.7	40.6	4.34	4.33	0.90	41	12.83	9	MsPGN
Boy	147	49	5.9	2.7	0.90	49	6.00	3.75	FSGS
Boy	142	48	4	5.6	1.39	41	4.67	1.67	MGA
Girl		55.5	4.96	4.42	1.60	43.8	2.17	2	MGA
Boy	77.4	40.7	5.43	3.21	1.60	47.5	1.00	1	MGA
Boy	72.36	60.4	3.3	4.18	1.64	48.3	11.33	10.83	Focal glomeruloscerosis
Boy	172.2	33	4.12	2.66	1.68	40.3	6.33	5.67	MGA
Boy	107	21.9	3.16	6.2	1.71	35.9	7.83	1	FSGS
Boy	112.98	43.8	5.71	5.44	1.90	36.9	7.17	1	MGA
Girl	90	54	5.9	3.4	1.90	34	9.00	5.3	FSGS
Girl	145.1	32.3	4.8	3.91	1.95	38.9	8.00	5	MGA
Girl	126.7	41.6	6.17	4.16	2.20	38.5	3.00	2.67	IgAN
Boy	81.4	37	5	5.6	2.20	44	1.00	1	MGA
Boy	130	33.6	3.56	4.37	2.35	46.5	7.83	7.67	MGA
Boy	118.8	46.6	7.49	4.76	2.61	48.5	11.75	6	FSGS
Boy	123	52	4.36	3.24	2.87	44.9	4.33	4.33	MGA
Girl	100	23	3.1	4.5	2.87	46	13.00	9.8	IgAN
Boy	151	26	2.97	4.58	3.06	44.5	1.75	0.67	MGA
Boy	105.7	51	5.79	5.51	3.93	31.6	5.83	5.75	FSGS
Boy	92	49.6	6.74	4.61	5.46	49.7	3.75	3.75	MGA

Note: Cr: Creatinine; BUN: Blood urea nitrogen; CHO: Cholesterol; MGA: Minor glomerular abnormality; FSGS: Focal segmental glomerulosclerosis; MsPGN: Mesangial proliferative glomerulonephritis; IgAN: IgA nephropathy.