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ORIGINAL ARTICLE

Creatinine-based equation to estimate the glomerular filtration rate in Japanese children and adolescents with chronic kidney disease

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Abstract
Background Renal inulin clearance is the gold standard for evaluation of kidney function, but cannot be measured easily in children. Therefore, we utilize the serum creatinine (Cr)-based estimated GFR (eGFR) measuring serum Cr by the enzymatic method, and we have reported simple serum Cr-based eGFR in Japanese children aged between 2 and 11 years old. Furthermore, we should use serum Cr-based eGFR in Japanese adolescents as well as children with chronic kidney disease for evaluation of renal function.
Methods The inulin clearance and serum Cr level determined by an enzymatic method were measured in 131 pediatric chronic kidney disease (CKD) patients between the ages of 2 and 18 years old with no underlying disease affecting renal function except CKD to determine the serum Cr-based eGFR in Japanese children and adolescents.
Results We offer the complex estimated GFR equation using polynomial formulae for reference serum creatinine levels with body length in Japanese children except infants, resulting in the following equation:

$$\text{eGFR} = 110.2 \times (\text{reference serum Cr}/\text{patient's serum Cr}) + 2.93$$

Reference serum Cr levels (y) are shown by the following two equations of body length (x):

$$\text{Males}: \quad y = -1.259x^5 + 7.815x^4 - 18.57x^3 + 21.39x^2 - 11.71x + 2.628$$

$$\text{Females}: \quad y = -4.536x^5 + 27.16x^4 - 63.47x^3 + 72.43x^2 - 40.06x + 8.778$$

Conclusion The new polynomial eGFR formula showing the relationship with body length and serum Cr level may be applicable for clinical screening of renal function in

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Japanese children and adolescents aged between 2 and 18 years.

Keywords Estimate glomerular filtration rate · Japanese children and adolescents · Creatinine-based equation · Chronic kidney disease

Introduction

Using renal inulin clearance to measure the glomerular filtration rate (GFR) directly is compromised by problems of collecting urine samples in children, and we should utilize the serum creatinine (Cr)-based estimated GFR (eGFR). Serum Cr levels are generally proportional to muscle mass and inversely proportional to renal function. Therefore, they are lower in infancy, and increase gradually with growth. Schwartz et al. [1] expressed the relations between body length, GFR, and serum Cr level as estimated GFR (eGFR; ml/min/1.73 m^2) = k × body length (cm)/serum Cr level (mg/dl). The coefficient k is 0.33 in preterm infants under 1 year old, 0.45 in full-term infants under 1 year old, 0.55 in children 2–12 years old, and 0.55 and 0.70 in females and males over 12 years old, respectively [1–4].

This formula is clinically useful as it allows estimation of the normal serum Cr level from the patient's body length. This equation utilizes the Jaffé method to measure Cr. However, enzymatic methods have recently been used to measure Cr, rendering the above formula no longer applicable. In 2009, the updated Schwartz formulae were reported as follows: eGFR (ml/min/1.73 m^2) = 0.413 × body length (cm)/serum Cr level (mg/dl) and eGFR (ml/min/1.73 m^2) = 39.1 × [body length (m)/s-Cr (mg/dl)]$^{0.516}$ × [1.8/cystatin C (mg/l)]$^{0.294}$ × [30/BUN (mg/dl)]$^{0.169}$ × [1.099]^{male} × [body length (m)]/1.4]$^{0.188}$ by enzymatic Cr determination in children 1–16 years old [5].

We doubt whether the new Schwartz equations can be used to estimate the GFR in Japanese children with chronic kidney disease (CKD), because there are differences in renal function and muscle mass between Japanese and American individuals. In addition, it is inconclusive whether one common "bedside" linear equation can be used in children from 1 to 16 years old, including the period of adolescence. Therefore, we attempted to derive formulae to estimate the glomerular filtration rate by enzymatic Cr determination in Japanese children with CKD.

We have determined reference serum Cr levels by an enzymatic method related to age, gender, and body length, and linear and polynomial equations showing the relationship between body length and the serum Cr level for screening of renal function in Japanese children [6, 7]. We intended to develop creatinine-based estimated GFR equations using these linear and polynomial equations, with serum creatinine levels being inversely proportional to renal function.

Initially, we developed an estimated GFR equation for Japanese children aged between 2 and 11 years old whose reference serum creatinine levels were thought to be proportional to body length as follows: eGFR (ml/min/1.73 m^2) = 0.35 × body length (cm)/serum Cr level (mg/dl) [8]. Here, we present a complex estimated GFR equation using polynomial formulae for reference serum creatinine levels with body length in Japanese children aged between 2 and 18 years old, i.e., all children and adolescents except infants.

Materials and methods

Study population

A total of 174 children (113 males and 61 females) between the ages of 1 month and 18 years old presenting at the facilities of the members for the Committee of Measures for Pediatric Chronic Kidney Disease (CKD) between 2008 and 2011 with chronic kidney disease were included. Nevertheless, excluding the cases we describe in detail later, a total of 131 patients (84 males and 47 females) were included in formulating the new eGFR. The study was approved by the local ethics boards of each institution, and written informed consent was obtained from the parents of each subject. The ethics committee approval number in Aichi Children's Health and Medical Center is 200810.

GFR and serum Cr measurements

Data regarding serum Cr levels, renal inulin clearance (Cin), and body length measured at the same time were reviewed. The glomerular filtration rate (GFR) was measured with inulin [9, 10]. Cin was measured from samples taken twice over 2 h under fasting and hydrated conditions by the continuous infusion method (Fig. 1). The children were fasted overnight and were allowed only water after waking up in the morning. First, they received an intravenous Ringer's solution load of 20 ml/kg body weight for 30 min to obtain good diuresis, followed by a load of 5 ml/kg/h until testing was completed. From 30 min after water loading, inulin was given intravenously in a priming dose of 40 mg/kg body weight for 30 min calculated to achieve an extracellular fluid (ECF) level of 20 mg/dl. After the priming dose, inulin was administered at a rate calculated to maintain a constant level in the blood [10]. For this purpose, the rate of inulin infusion must equal that of loss in the urine. To calculate inulin loss, GFR was estimated from serum creatinine by the old Schwartz

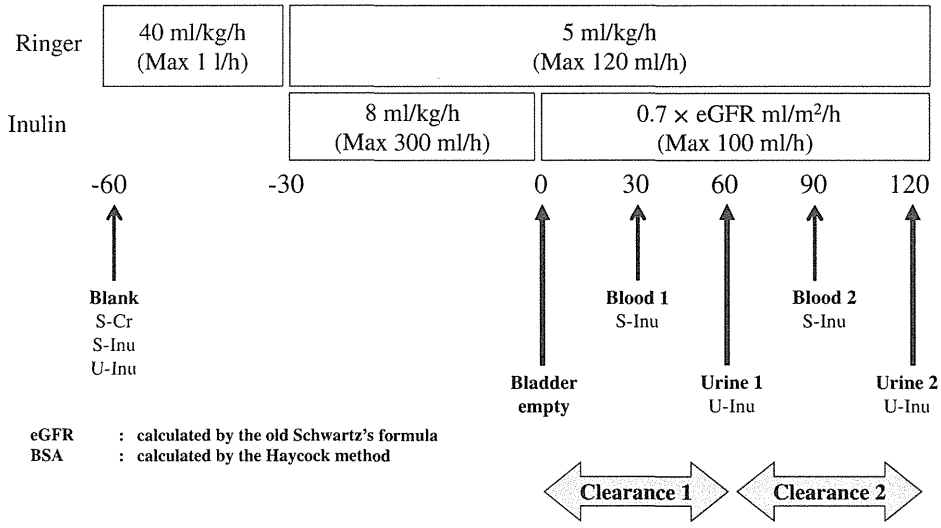

Fig. 1 Inulin clearance method standardized according to the Committee of Measures for Pediatric CKD. Inulin was given intravenously to achieve extracellular fluid levels of 20 mg/dl in testing. For this purpose, the rates of inulin infusion must equal the rates of loss in the urine, which were calculated using the Schwartz formulae based on the serum creatinine level

formulae [1–5]. Therefore, the patients received an inulin load of 0.7 × eGFR ml/m^2/h with calculation of body surface area by the Haycock method [11]. Urine samples were collected in two periods of 1 h each, and blood samples were obtained twice from an indwelling cannula in the middle of urine collection. We collected urine samples of children under 6 years old or with bladder dysfunction by indwelling catheters.

Serum samples were stored at −70 °C until serum Cr was measured by SRL, Inc. (Tokyo, Japan). The serum Cr level was determined by an enzymatic method using a Bio Majesty automated analyzer (JCA-BM8060; JEOL Ltd., Tokyo, Japan) with Pure Auto S CRE-L (Sekisui Medical Co., Ltd., Tokyo, Japan). The coefficient of variation was satisfactory (2.08 %). This method utilizes National Institute of Standards and Technology (NIST) Standard Reference Material 914a as calibration standards similar to isotope dilution mass spectroscopy (IDMS). Urine and serum samples were stored at 4 °C until urine and serum inulin were measured by SRL, Inc. The urine and serum levels of inulin were determined by an enzymatic method using an automated analyzer (Hitachi 7170; Hitachi Ltd., Tokyo, Japan) with Dia-color-inulin (Toyobo Co., Ltd., Tokyo, Japan). The coefficient of variation was satisfactory (<15 %).

Estimated GFR based on serum Cr

In Japanese children and adolescents, the reference serum Cr level (y) was expressed as a quintic equation of body length (x), and the regression equations were $y = -1.259x^5 + 7.815x^4 - 18.57x^3 + 21.39x^2 - 11.71x + 2.628$ in males and $y = -4.536x^5 + 27.16x^4 - 63.47x^3 + 72.43x^2 - 40.06x + 8.778$ in females [6]. As the reciprocal of serum Cr is generally correlated with GFR [1–5, 12], we utilized the equation for eGFR derived from serum Cr, eGFR (%) = (reference serum Cr/patient's serum Cr) × 100. Therefore, we derived the following two equations:

$$\text{Males} < 19 \text{ years old: eGFR (\%)} = [(-1.259x^5 + 7.815x^4 - 18.57x^3 + 21.39x^2 - 11.71x + 2.628)/\text{patient's serum Cr}] \times 100$$

$$\text{Females} < 19 \text{ years old: eGFR (\%)} = [(-4.536x^5 + 27.16x^4 - 63.47x^3 + 72.43x^2 - 40.06x + 8.778)/\text{patient's s serum Cr}] \times 100$$

With this report [6], we intend to develop the GFR (ml/min/1.73 m^2) estimation expression for Japanese children by examining relations of GFR (ml/min/1.73 m^2) and reference serum Cr/patient's serum Cr.

Exclusion criteria and cases excluded

In this study, the exclusion criteria were as follows: (1) severe obstructive uropathy; (2) infection during treatment; (3) inflammatory disease; (4) dehydration; (5) myopathy; (6) severe cardiac, hepatic, or pancreatic disease; (7) pregnancy or the possibility of pregnancy; (8) nursing; and (9) refusal or inability to give informed consent. Infants under 2 years old were excluded because of low GFR compared with adults [13]. Three cases (one case with no serum creatinine data and two cases with myopathy) were excluded because of violation of the protocol. In this study, doses of intravenously administered inulin were decided as blood concentrations were constant during testing by calculating the estimated GFR by the old Schwartz' formula. Therefore, cases in which the ratios of urine inulin excretion and intravenous inulin administration were <0.5 or >1.5 were excluded from

this study because this may have been due to failure to collect all urine. Pediatric patients with chronic kidney disease causing hyperfiltration such as diabetic nephropathy are rare, and we are interested in determining the eGFR of cases with GFR <120 ml/min/1.73 m^2. Therefore, we excluded cases with GFR >150 ml/min/1.73 m^2.

Statistical analyses

All analyses were conducted using Microsoft Excel 2010 and the JMP 10 statistical software package (SAS Institute Inc, Cary, NC, USA). Linear regression analyses were performed to evaluate relations between the ratios of patient's serum Cr/reference serum Cr and Cin in males and females. Differences in the bias (absolute value) of eGFRs were evaluated using paired *t* tests, and differences in accuracy (i.e., P_{30}) were evaluated using χ^2 tests, similar to the method of Horio et al. [14]. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results

Characteristics of the study population

Of the 174 children studied, 8 patients under 2 years old, 3 with violation of protocol, 27 whose ratios of urine inulin excretion and intravenous inulin administration were <0.5 or >1.5, and 5 with GFR >150 ml/min/1.73 m^2 were excluded from the study. Therefore, a total of 131 cases (84 males and 47 females) were included in this study (Table 1); 64 % were male, 41 % had congenital anomalies of the kidney and urinary tract (CAKUT), 5 % were posttransplant patients, and only 4 % had chronic glomerulonephritis. The median age was 10.8 years old, median height was 134.5 cm, and median weight was 30.9 kg. The median values of serum Cr, average inulin GFR, and maximum inulin GFR were 0.66 mg/dl, 66.6 ml/min/1.73 m^2, and 71.8 ml/min/1.73 m^2, respectively. As urine collection was suspected to become insufficient in children, we decided to use the maximum inulin GFR in the present study.

Serum Cr-based eGFR formula in pediatric CKD patients aged between 2 and 18 years old

Figure 2 shows scatter plots of maximum inulin GFR versus reference serum Cr/patient's serum Cr ratio in pediatric CKD patients aged between 2 and 18 years old, resulting in the following equation:

Table 1 Characteristics of 131 children included in this study

Characteristics	Median (IQR)	*n*
Total		131
Age (years)	10.8 (7.5–13.9)	
<6		17
≥6 and <12		59
≥12		55
Gender		
Male		84
Female		47
Renal abnormality		
Congenital anomalies of the kidney and urinary tract		54
Reflux nephropathy		15
Idiopathic nephrotic syndrome		13
Renal transplant		7
Chronic glomerulonephritis		5
Nephronophthisis		5
Neurogenic bladder		4
Polycystic kidney disease		3
Alport's syndrome		3
Miscellaneous		22
Height (cm) (years)	134.5 (112.6–152.2)	
<6	98.4 (91.6–110.0)	
≥6 and <12	122.4 (110.6–132.0)	
≥12	154.2 (145.4–162.8)	
Weight (kg) (yeras)	30.9 (19.6–41.9)	
<6	15.4 (12.3–17.7)	
≥6 and <12	24.6 (18.8–28.9)	
≥12	45.3 (37.9–50.7)	
BSA (m^2) (years)	1.04 (0.79–1.32)	
<6	0.65 (0.55–0.74)	
≥6 and <12	0.91 (0.76–1.03)	
≥12	1.38 (1.23–1.51)	
Serum creatinine (mg/dl) (years)	0.66 (0.51–0.90)	
<6	0.56 (0.38–0.66)	
≥6 and <12	0.69 (0.43–0.74)	
≥12	0.97 (0.63–1.05)	
Average inulin GFR (ml/min/1.73 m^2) (years)	66.6 (46.5–93.5)	
<6	58.8 (40.6–73.0)	
≥6 and <12	74.6 (50.0–95.5)	
≥12	71.7 (52.4–91.9)	
Maximum inulin GFR (ml/min/1.73 m^2) (years)	71.8 (53.0–97.4)	
<6	63.9 (46.0–74.4)	
≥6 and <12	80.0 (55.5–106.5)	
≥12	77.0 (53.9–93.6)	

IQR interquartile range

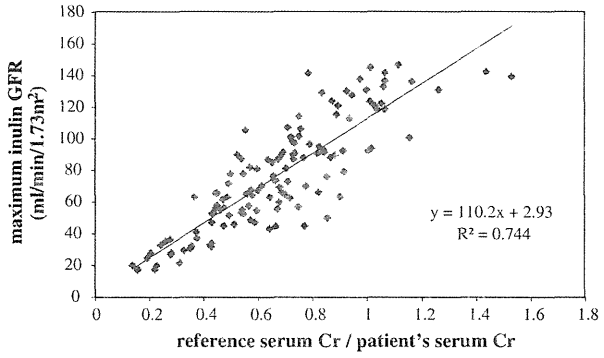

Fig. 2 Analysis of reference serum Cr/patient's serum Cr and maximum inulin GFR in pediatric CKD patients aged between 2 and 18 years old. The regression equation was $y = 110.2x + 2.93$. A significant positive correlation was observed in children with CKD aged between 2 and 18 years old, with a correlation coefficient of 0.863

$$\text{eGFR} = 110.2 \times (\text{reference serum Cr/patient's serum Cr}) + 2.93$$

Reference serum Cr levels (y) are shown by the following two equations of body length (x):

$$\text{Males}: \quad y = -1.259x^5 + 7.815x^4 - 18.57x^3 + 21.9x^2 - 11.71x + 2.628$$

$$\text{Female}: \quad y = -4.536x^5 + 27.16x^4 - 63.47x^3 + 72.43x^2 - 40.06x + 8.778$$

Correlation between two serum Cr-based eGFR formulae in pediatric CKD patients aged between 2 and 11 years old

We developed an estimated GFR equation for use in Japanese CKD patients aged between 2 and 11 years old as follows: eGFR (ml/min/1.73 m^2) = 0.35 × body length (cm)/serum Cr level (mg/dl) [6].

We compared our new formula with the formula in CKD patients of this age group. Figure 3 shows the correlation between these two serum Cr-based eGFR formulae in these patients. The eGFR using a quintic equation of body length (y) is shown as the eGFR using a linear equation of the body length (x) as follows: $y = 0.98x + 1.63$. In contrast, in CKD patients aged between 12 and 18 years old, the relation were shown as follows: $y = 1.06x + 6.56$.

Thus, the eGFR values derived from the two equations showed a good degree of accordance in Japanese CKD patients aged between 2 and 11 years old.

Comparison of performance of our new eGFR formula and the other eGFR formulae including the updated Schwartz formula

We used a diagnostic test design to compare our new polynominal eGFR formula, our simple linear formula previously reported in CEN [8], and the original [1–4] and updated [5] Schwartz's formula in all 131 subjects and each age category, such as <12, and ≥12 years old; these are listed in Table 2. The new polynomial formula had significantly less bias than other eGFRs ($P < 0.001$). Accuracy was not significantly different between our simple linear formula and our polynomial formula, but significantly different between the two Schwartz's formulae and our polynomial formula. Root mean square error (RMSE) was lower for our new polynomial formula than for other eGFRs stratified by glomerular filtration rate measured by the inulin clearance method mGFR in all 131 subjects. In particular, Fig. 4 showed the RMSE between measured maximum inulin GFR and estimated GFR obtained using our polynomial formula in CKD patients aged between 2 and 16 years old was lower than the estimated GFR obtained using the updated Schwartz formula (17.2 vs. 18.3, respectively). The reason why we analyzed patients aged 2–16 years old was a limitation in updated Schwartz formula.

Discussion

The glomerular filtration rate is used to assess kidney function and is measured by monitoring renal clearance. Inulin clearance is the gold standard for evaluation of kidney function, but cannot be measured easily. Therefore, various methods have been used to determine GFR. The estimated GFR [eGFR (ml/min/1.73 m^2) = k × body length (cm)/serum Cr level (mg/dl)] determined by the Jaffé method devised by Schwartz has been used clinically [1]. Recently, however, enzymatic methods have been used to measure Cr rather than the Jaffé method, so it is not possible to use the formula in this form. Therefore, it was necessary to reevaluate the value of the coefficient k in the formula. Recently, Zappitelli et al. [15] revised the Schwartz formula relating the eGFR to the serum creatinine level determined enzymatically and reported that the value of k in the Schwartz equation decreased from 0.55 to 0.47 for children and adolescent girls. Schwartz et al. reported the updated formula, the so-called "bedside" version, as eGFR = 0.413 × body length (cm)/serum Cr level (mg/dl) by the enzymatic method showing a 25 % reduction in value of k from the previous value of 0.55 generated from Jaffé-based serum Cr measurements and eGFR (ml/min/1.73 m^2) = 39.1 × [body length (m)/s − Cr (mg/dl)]$^{0.516}$ × [1.8/cystatin C (mg/l)]$^{0.294}$ × [30/BUN (mg/dl)]$^{0.169}$ × [1.099]^{male} × [body length (m)]/1.4]$^{0.188}$ by enzymatic Cr determination in children 1–16 years old [5]. This was defined in a population of American children with chronic kidney disease, enriched for those with obstructive uropathy. They concluded that the formula can be used in children 1–16 years old.

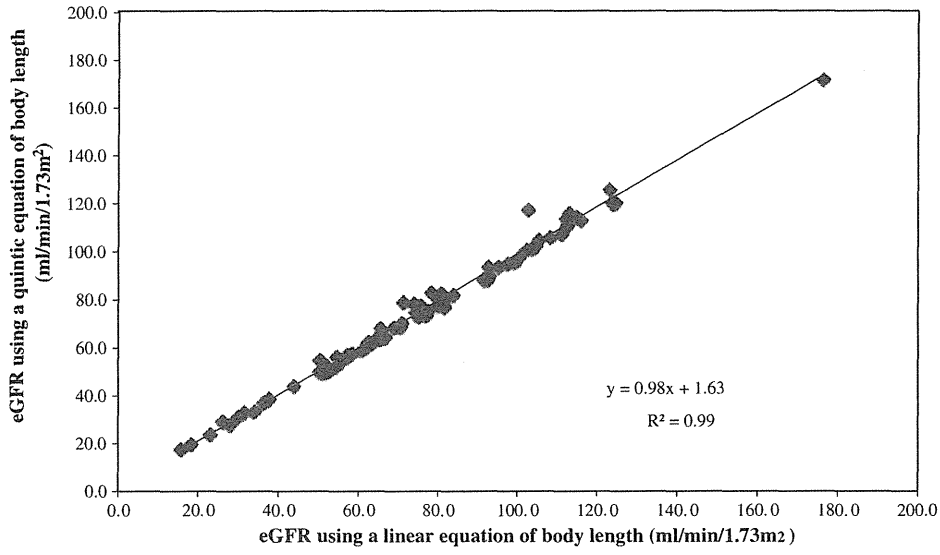

Fig. 3 Analysis of two eGFR equations in pediatric CKD patients aged between 2 and 11 years old. The two equations are eGFR using a linear and quintic equation of body length, respectively. The regression equation was $y = 0.976x + 1.63$. A significant positive correlation was observed in children with CKD aged between 2 and 11 years old, with a correlation coefficient of 0.995

Table 2 Performance of GFR-estimating equations in all 131 subjects

Variable and equation	All (n = 131)	Aged <12 years (n = 76)	Aged ≥12 years (n = 55)
Bias (ml/min/1.73 m^2)			
New polynomial formula	13.4 ± 10.7	12.9 ± 10.4	14.2 ± 11.1
Simple linear formula	14.4 ± 11.6***	13.3 ± 10.5***	15.8 ± 12.9***
Original Schwartz's formula	17.4 ± 13.2***	14.4 ± 11.1***	21.6 ± 14.8***
Updated Schwartz's formula	15.5 ± 15.5***	15.8 ± 15.3***	14.9 ± 11.2***
P_{30} (%)			
New polynomial formula	84 (77–90)	84 (74–92)	84 (76–90)
Simple linear formula	82 (75–89)^{ns}	83 (73–91)^{ns}	82 (74–89)^{ns}
Original Schwartz's formula	60 (51–68)***	67 (55–77)*	60 (51–69)***
Updated Schwartz's formula	69 (61–77)**	66 (54–76)**	69 (60–77)^{ns}
Root mean square error (ml/min/1.73 m^2)			
New polynomial formula	17.3	16.7	18.2
Simple linear formula	18.1	17.1	18.5
Original Schwartz's formula	17.6	16.2	16.5
Updated Schwartz's formula	18.1	17.1	18.5

Bias is the absolute value of mGFR minus eGFR and is reported as mean ± standard deviation; P_{30} refers to percentage of GFR estimates that are within 30 % of the mGFR, with 95 % confidence intervals given in parentheses

The new polynomial formula is the following equations: eGFR = 110.2 × (reference serum Cr/patient's serum Cr) + 2.93, and reference serum Cr levels (y) are shown by the following two equations of body length (x (m)): males: $y = -1.259x^5 + 7.815x^4 - 18.57x^3 + 21.39x^2 - 11.71x + 2.628$, and females: $y = -4.536x^5 + 27.16x^4 - 63.47x^3 + 72.43x^2 - 40.06x + 8.778$

The simple linear formula is the following equation: eGFR = 0.35 × body length (cm)/serum Cr level (mg/dl)

The original Schwartz's formula is the following equations: eGFR = k × body length (cm)/serum Cr level (mg/dl). The coefficient k is 0.55 in children 2–12 years old and 0.55 and 0.70 in females and males over 12 years old, respectively

The updated Schwartz's formula is the following equations: eGFR = 0.413 × body length (cm)/serum Cr level (mg/dl)

eGFR estimated glomerular filtration rate, *mGFR* glomerular filtration rate measured by the inulin clearance method

ns Not significant; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ show the statistical significance of the difference from our new formula

We reported that the new bedside Schwartz formula cannot be used when estimating GFR in Japanese children, especially between 1 and 16 years old, including the adolescent period, for reference serum Cr levels of our 1,074 subjects [6], showing a gradual significant decrease of eGFR with age [16]. There seems to be a large problem in that the ranges of the reference value for boys >12 years old and girls >14 years old overlap with the range for CKD

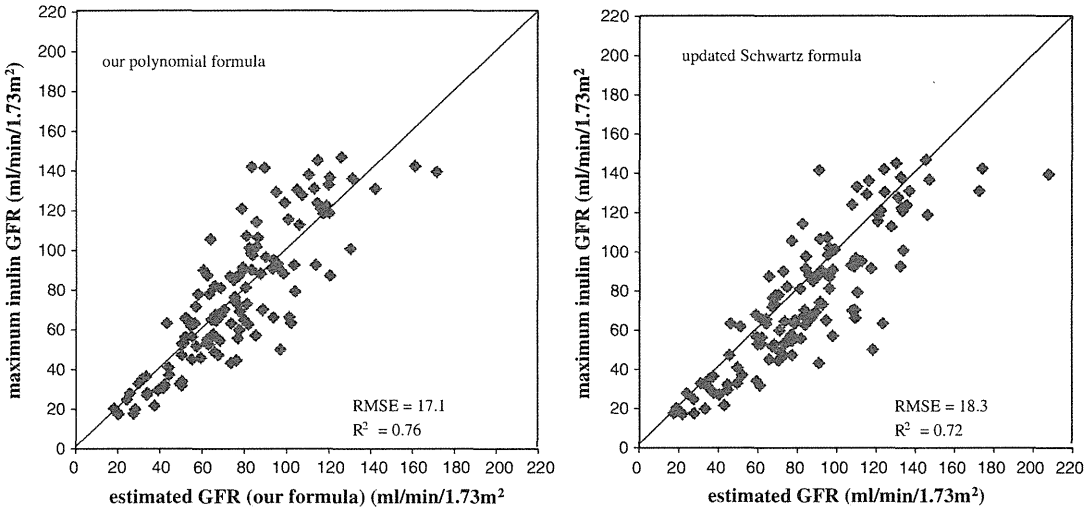

Fig. 4 Correlation between the estimated and measured maximum inulin GFR in CKD patients aged between 2 and 16 years old. *Left* Measured maximum inulin GFR versus the estimated GFR obtained using our polynomial formula. *Right* Measured maximum inulin GFR versus estimated GFR obtained using the updated Schwartz formula. *Smoothed lines* show the fit of the data

stage 2. Our results indicated that the eGFR value derived by the new bedside Schwartz formula decreased gradually with age in children with normal renal function. We doubt that the new "bedside" Schwartz formula cannot be used to estimate the GFR in Japanese pediatric CKD patients as well as in children with normal renal function.

When we performed the nationwide, population-based survey of children with pre-dialysis CKD in Japan, we used the new diagnostic criteria for CKD in children [17]. Then stage 3–5 CKD was classified as serum Cr more than twice, four times, and eight times the median reference serum Cr levels matched for age and sex, which were previously determined in Japanese children [6]. However, with those diagnostic criteria, we were not able to determine the numerical eGFR in a CKD patient. In a similar way of thinking, Pottel et al. [18] reported the simple height-independent eGFR equation in children.

At any rate, the new bedside Schwartz formula has an inherent problem in that it uses the same coefficient between the ages of 1 and 16 years old. In addition, we assumed that renal function and muscle mass show ethnic differences. Therefore, it is necessary to establish a specialized estimated GFR equation for use in Japanese children and adolescents.

We developed an estimated GFR equation for use in Japanese children aged between 2 and 11 years old whose reference serum creatinine levels were thought to be proportional to body length as follows: eGFR (ml/min/1.73 m^2) = 0.35 × body length (cm)/serum Cr level (mg/dl) [8]. In the present study, we presented the complex estimated GFR equation using polynomial formulae for reference serum creatinine levels with body length in Japanese children aged between 2 and 18 years old, i.e., all children and adolescents except infants.

Our polynomial formula had lower bias ($P < 0.001$) than our simple linear formula [8] as well as the original [1–4] and updated [5] Schwartz's formula in all 131 subjects and each age category, such as <12 and ≥12 years old. In addition, the % accuracy of our polynomial formula was superior to the original and updated Schwartz's formula ($P < 0.001$ and $P < 0.01$, respectively). Ultimately, our new formula derived from the body length and serum Cr in Japanese children aged between 2 and 18 years old will be useful for estimating their renal function despite the complicated formula as computerization of medical care simplifies their application. Although the polynominal eGFR equation seems complex, we use the quintic equation to estimate the GFR of children of all ages except infants by one expression. Especially the equation will be useful because we were able to use it even in adolescents. Limitations of our study include the small number of subjects, especially females, and having no other data set to validate the equations. Actually, the prevalence of pre-dialysis stage 3–5 CKD was about 3 cases/100000 Japanese children, which was lower than that reported in the ItalKid [19] and REPIR II Projects [20], and the number of Japanese children with stage 3–5CKD was estimated to be about 500 [17]. Therefore, further studies are required to validate our equations using a different data set. However, we consider that the new polynomial eGFR formula showing the relationship with the body length and serum Cr level may be applicable for clinical screening of renal function in Japanese children and adolescents aged between 2 and 18 years, and these methods of evaluation of renal function in children will be useful worldwide.

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Original Article

Pre-dialysis chronic kidney disease in children: results of a nationwide survey in Japan

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Keywords: child, creatinine, epidemiology, Japan, kidney diseases

ABSTRACT

Background. Chronic kidney disease (CKD) in children is a progressive and intractable condition that may severely impair the child's growth, development and quality of life. Epidemiological information on pediatric CKD, particularly in Asians, is scant.

Methods. We conducted a nationwide, population-based survey of Japanese children aged 3 months to 15 years with pre-dialysis CKD to examine the prevalence of pediatric CKD in Japan. CKD was classified according to newly established criteria derived from reference serum creatinine levels in Japanese children. Surveys were sent to 1190 institutions across Japan to report on cases of pediatric CKD managed as of 1 April 2010.

Results. A total of 925 institutions (77.7%) responded. Information on 447 children was collected. When subdivided according to our diagnostic criteria, 70.5% of children had stage 3 CKD, 23.9% stage 4 and 5.6% stage 5. The estimated prevalence of Japanese children with CKD was 2.98 cases/100 000 children. Of 407 CKD cases with non-glomerular disease, 278 (68.3%) had congenital anomalies of the kidney and urinary tract (CAKUT). The newly established criteria showed good validity compared with existing criteria, including the abbreviated Schwartz equation.

Conclusions. Findings from the first nationwide survey of pre-dialysis CKD in Asian children indicate that the prevalence of stage 3–5 CKD in children in Japan aged 3 months to 15 years is 2.98 cases/100 000 children. Most children with CKD presented with non-glomerular disease, most frequently CAKUT.

Improved management of CAKUT, including renoprotective treatment and urological intervention, is required.

INTRODUCTION

Chronic kidney disease (CKD) in children is a progressive and intractable condition, with devastating effects on the patient's growth, development and quality of life. If left untreated, pediatric CKD eventually progresses to end-stage renal disease (ESRD), which requires long-term dialysis or repeated renal transplantation. The mortality rate for children with ESRD on dialysis is estimated to be 30–150 times that of the general pediatric population [1, 2]. Therefore, it is particularly important to detect CKD as early as possible, possibly by applying simple but accurate screening of at-risk children. Early identification of these children can then allow the physician to promptly introduce appropriate therapy that can prevent or slow the progression of CKD to ESRD, reducing the incidence of stage 5 CKD and to control comorbidity.

Epidemiological information on CKD in children is currently limited, but this sort of information is necessary to understand the extent of the problem, to identify populations at risk and to determine the efficacy of current therapeutic interventions. Although several studies have described the epidemiology of pre-dialysis CKD in children in Western countries [3–10], very few have focused on Asian children. It is also important to consider that there may be differences in the epidemiology of CKD among countries that may be due to racial differences, variations in screening methods among medical institutions and differences in in-school screening programs. To address this problem of limited information in Asian children and to assist subsequent population-based surveys, we previously determined reference serum creatinine (SCr) levels in Japanese children [11].

Our first objective in this study was to determine the prevalence of pre-dialysis CKD in a cross-sectional, nationwide survey of Japanese children aged 3 months to 15 years with pre-dialysis CKD. Stage 3–5 CKD was detected and classified using newly established criteria derived from normal SCr levels of age- and sex-matched Japanese children. Because CKD is defined as a glomerular filtration rate (GFR) of <60 mL/min/1.73 m^2 (less than half of normal GFR) in the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines [12, 13] and the Kidney Disease: Improving Global Outcomes (KDIGO) position statement [14] (corresponding to stage 3 or worse), we focused on patients meeting this criterion and who had not yet received dialysis or renal transplantation. Our second objective was to determine the etiology of CKD as well as the method of detection of CKD and the treatment modalities used in routine clinical practice in Japan.

MATERIALS AND METHODS

Establishment of new diagnostic criteria for CKD in children

The new diagnostic criteria for stage 3–5 CKD were based on previously established reference SCr levels of Japanese children [11]. Briefly, in that study, body length and SCr levels were determined in 1151 healthy children aged 1 month to 18 years who presented at the facilities of the Committee of Measures for Pediatric CKD and Tokyo Health Service Association between 2008 and 2009. Reference intervals of SCr against age were calculated in children aged 3 months to 11 years, and those against age and sex were calculated in children aged 12–15 years.

According to the K/DOQI guidelines [12, 13] and KDIGO position statement [14] for CKD, stage 3–5 CKD was classified as GFR 30–59, 15–29 and <15 mL/min/1.73 m^2, respectively (<1/2, <1/4 and <1/8 of normal GFR, respectively), whereas normal GFR was considered to be ~120 mL/min/1.73 m^2. Given that the GFR is inversely proportional to SCr for a given body type and age [15], we classified stage 3–5 CKD as SCr more than twice, four times and eight times, the median normal SCr levels matched for age alone in children aged 3 months to 11 years (Table 1), or matched for age and sex in children aged 12–15 years (Table 2).

Study design and population

This was a cross-sectional, nationwide, population-based survey conducted by the Pediatric CKD Study Group in Japan in conjunction with the Committee of Measures for Pediatric CKD of the Japanese Society for Pediatric Nephrology (JSPN). Two surveys were sent in August 2010 to a total of 1190 institutions in Japan, including all institutions that are members of the JSPN, all university and children's hospitals and all general hospitals with >200 beds, inviting them to report cases of pediatric CKD that were managed as of 1 April 2010. We selected these types of hospitals because children with apparent CKD were usually referred to institutions meeting one of these criteria. The deadlines for the first and second surveys were October 2010 and November 2010, respectively.

The first questionnaire was designed to record the presence and approximate number of children with stage 3–5 CKD in each institution. The second questionnaire recorded data for each case, including age, date of birth, sex, height, SCr level, primary renal diagnosis and associated diseases, method of detection, comorbidities and prescribed treatment. For the purpose of this survey, only data recorded within 6 months of 1 April 2010 were included. The patient's age was calculated from the date of birth and the date of each measurement. This questionnaire also recorded information for each institution, including the SCr assay method used, and prescribed treatment strategies. The respondents were asked to search their medical records for patients with a confirmed diagnosis of CKD or for patients with an abnormal SCr.

The inclusion criteria were as follows: (i) children with CKD aged 3 months to 15 years at the time of 1 April 2010; (ii) stage 3–5 CKD, as determined by the newly established diagnostic criteria and (iii) no prior treatment with dialysis or renal transplantation. Only cases with kidney dysfunction that had lasted for >3 months were included and cases with transient increases in creatinine were excluded.

The study was conducted in accordance with the ethical principles set out in the Declaration of Helsinki, and with the

Table 1. Diagnostic criteria for stage 3–5 chronic kidney disease based on reference serum creatinine levels (mg/dL) of Japanese children aged 3 months to 11 years

Age	2.5th percentile	50th percentile	97.5th percentile	CKD stage 3	CKD stage 4	CKD stage 5
<2 years						
3–5 months	0.14	0.20	0.26	0.41–0.80	0.81–1.60	≥1.61
6–8 months	0.14	0.22	0.31	0.45–0.88	0.89–1.76	≥1.77
9–11 months	0.14	0.22	0.34	0.45–0.88	0.89–1.76	≥1.77
1 year	0.16	0.23	0.32	0.47–0.92	0.93–1.84	≥1.85
2–11 (years)						
2	0.17	0.24	0.37	0.49–0.96	0.97–1.92	≥1.93
3	0.21	0.27	0.37	0.55–1.08	1.09–2.16	≥2.17
4	0.20	0.30	0.40	0.61–1.20	1.21–2.40	≥2.41
5	0.25	0.34	0.45	0.69–1.36	1.37–2.72	≥2.73
6	0.25	0.34	0.48	0.69–1.36	1.37–2.72	≥2.73
7	0.28	0.37	0.49	0.75–1.48	1.49–2.96	≥2.97
8	0.29	0.40	0.53	0.81–1.60	1.61–3.20	≥3.21
9	0.34	0.41	0.51	0.83–1.64	1.65–3.28	≥3.29
10	0.30	0.41	0.57	0.83–1.64	1.65–3.28	≥3.29
11	0.35	0.45	0.58	0.91–1.80	1.81–3.60	≥3.61

Values were matched for age alone. Values for the 2.5, 50 and 97.5th percentiles are as presented in Uemura *et al.* [11]. Table reproduced with the permission of the Japanese Society of Nephrology.

Table 2. Diagnostic criteria for stage 3–5 chronic kidney disease based on reference serum creatinine levels (mg/dL) of Japanese male and female children aged 12–15 years

Age	2.5th percentile	50th percentile	97.5th percentile	CKD stage 3	CKD stage 4	CKD stage 5
Males						
(years)						
12	0.40	0.53	0.61	1.07–2.12	2.13–4.24	≥4.25
13	0.42	0.59	0.80	1.19–2.36	2.37–4.72	≥4.73
14	0.54	0.65	0.96	1.31–2.60	2.61–5.20	≥5.21
15	0.48	0.68	0.93	1.37–2.72	2.73–5.44	≥5.45
Females						
(years)						
12	0.40	0.52	0.66	1.05–2.08	2.09–4.16	≥4.17
13	0.41	0.53	0.69	1.07–2.12	2.13–4.24	≥4.25
14	0.46	0.58	0.71	1.17–2.32	2.33–4.64	≥4.65
15	0.47	0.56	0.72	1.13–2.24	2.25–4.48	≥4.49

Values were matched for age and sex. Values for the 2.5, 50 and 97.5th percentiles are as presented in Uemura *et al.* [11]. Table reproduced with the permission of the Japanese Society of Nephrology

ethical guidelines for epidemiological studies issued by the Ministry of Health, Labour and Welfare in Japan. The study was approved by the JSPN ethics board and a central ethics board (the institution of the Principal Investigator, KI) before study commencement. Because, data were reported retrospectively using patient charts, informed consent was not obtained in accordance with the above guidelines.

Statistical analyses

Estimation of the number of patients with stage 3–5 CKD in Japan from the reported number of patients in our survey was conducted as follows. The estimates were derived as the reported number divided by the response rate. Because the response rate tends to be lower in institutions with fewer patients, simple estimates can overestimate the true prevalence. Therefore, the reported patients were stratified according to institution type (i.e. university hospital, children's hospital and general hospital) and the number of beds (<200, 200–500 and ≥500), based on the assumption that the response rate is independent of the number of patients in each stratified category [16]. Then, the number of reported patients in each category was divided by the response rate and summed to calculate the total estimated number of patients in Japan. The total estimated number of patients was divided by the size of the population at risk in Japan reported by the Statistics Bureau of the Ministry of Internal Affairs and Communications of Japan (http://www.stat.go.jp/english/index.htm) to calculate the prevalence as of 1 April 2010. Weighted κ with 95% confidence interval (CI) was calculated to compare the CKD classification used here with the abbreviated Schwartz equation. All statistical analyses were carried out using SAS system version 9 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Subject characteristics

A total of 925 of 1190 institutions (77.7%) responded to the first questionnaire. A total of 479 children were identified in the second questionnaire. Of these, 447 children (272 males and 175 females) with stage 3–5 CKD who had not been treated with dialysis/renal transplantation fulfilled the eligibility criteria and were included in this study. Their characteristics are summarized in Table 3. Most of the children (315; 70.5%) had stage 3 CKD, whereas 107 (23.9%) had stage 4 and 25 (5.6%) had stage 5. The number of Japanese children with stage 3–5 CKD was estimated to be 542.5 (95% CI: 497.5–587.5) as of 1 April 2010. On the basis of this, the prevalence of stage 3–5 CKD was calculated to be 2.98 cases/100 000 Japanese children aged 3 months to 15 years. Figure 1 shows the SCr values for males and females according to CKD stage. All of the responding institutions used enzyme immunoassays to determine SCr levels for the assessment of CKD stage; none used other methods, such as the Jaffe method.

Figure 2 shows the frequencies of CKD stage according to the estimated GFR (eGFR) of 412 children in whom height was measured. Stage 3–5 CKD was classified using our diagnostic criteria derived from SCr levels of age- and sex-matched Japanese children, while the eGFR was determined using the abbreviated Schwartz equation, which was recently revised from the original Schwartz equation [17]. This figure also shows the distribution of children classified in each CKD stage determined using both methods. These data indicate that the distribution of CKD stages determined using population-based reference values is comparable with the distribution derived using a method based on the abbreviated Schwartz

ORIGINAL ARTICLE

Table 3. Patient characteristics according to chronic kidney disease stage

	All subjects	Stage 3	Stage 4	Stage 5
n	447	315	107	25
Age (years)	8.7 ± 4.5	8.7 ± 4.6	8.5 ± 4.3	10.0 ± 4.5
Serum creatinine (mg/dL)	1.6 ± 1.2	1.1 ± 0.4	2.2 ± 0.8	5.3 ± 2.0
Height (cm)	119.8 ± 28.9	121.1 ± 28.7	118.8 ± 27.4	107.8 ± 35.6
Height SDS[a]	−1.6 ± 1.8	−1.3 ± 1.5	−2.2 ± 2	−3.5 ± 3
BUN (mg/dL)	35.6 ± 18.8	28.4 ± 9.8	48.6 ± 18.2	74.9 ± 31.5
CysC (mg/L)	2.1 ± 0.8	1.9 ± 0.5	3.1 ± 1.0	4.1 ± 0.9
eGFR–abbreviated (mL/min/1.73 m^2)[b]	39.5 ± 16	47.2 ± 11.2	22.6 ± 5.5	9.6 ± 3.2
eGFR–complete (mL/min/1.73 m^2)[c]	39.6 ± 12.3	43.7 ± 9.7	24.9 ± 5.3	11.6 ± 4.1

Values are means ± standard deviation.
SDS, standard deviation score; BUN, blood urea nitrogen; CysC, cystatin C.
[a]Height SDS was calculated using data recorded by the Japanese Society for Pediatric Endocrinology in 2000 (http://jspe.umin.jp/ipp_taikaku.htm).
[b]Determined using the abbreviated Schwartz equation.
[c]Determined using the complete Schwartz equation.

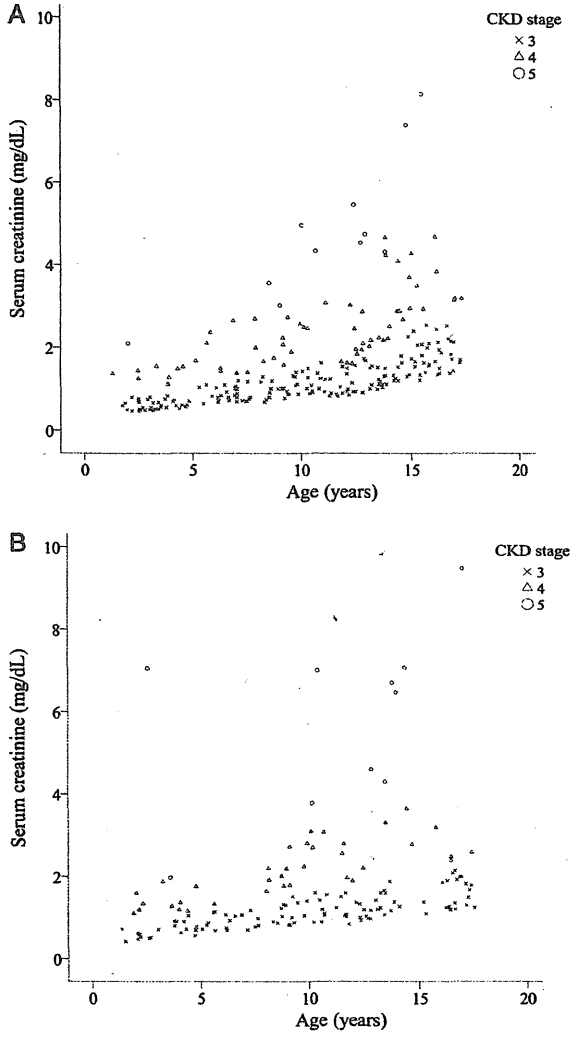


FIGURE 1. Serum creatinine levels according to age and CKD stage. Actual serum creatinine levels according to age and CKD stage are plotted separately for males (**A**) and females (**B**).

equation. The weighted κ-value for this comparison was 0.71 (95% CI: 0.65–0.77). For 198 children with cystatin C data, similar distributions were obtained when we compared our new classification with the complete Schwartz equation, which requires cystatin C-values [17] (data not shown).

Primary etiologies of pediatric CKD in Japan

The primary etiologies of CKD in the study population are presented in Table 4. Non-glomerular disease (407/447; 91.1%) was the most common primary cause of CKD, whereas glomerular disease accounted for 7.8% (35/447) of all cases.

Among those with non-glomerular diseases, 278 (68.3%) children had congenital anomalies of the kidney and urinary tract (CAKUT), of which 60 (21.6% of those with CAKUT) had obstructive urological malformations comprising posterior urethral valve, stricture of the urethra, hydronephrosis, hydroureter and cloacal anomaly (Table 4). The three most common causes of glomerular diseases were Alport's syndrome, focal segmental glomerulosclerosis and chronic glomerulonephritis ($n = 8$ each). No children presented with definitively diagnosed IgA nephropathy. Figure 3 shows the distribution of CAKUT and non-CAKUT diseases by age.

The diseases included recognizable syndrome [$n = 46$ (10.3%)] as follows: Down syndrome (OMIN, #190685, $n = 6$); VATER association (#192350, $n = 4$); Kabuki syndrome (#147920); Wolf–Hirschhorn syndrome (#194190) and Townes–Brocks syndrome (#107480, $n = 3$ each); prune belly syndrome (#100100) and branchio-oto-renal syndrome (#113650, 2 each) and others.

Methods of detecting Stage 3–5 CKD

Table 5 summarizes the methods and reasons for the detection of children with stage 3–5 CKD. Table 5 also presents the age at diagnosis for each of the methods. Fetal and perinatal ultrasonography was the most common method, followed by analysis by chance and urinary tract infection. As might be expected, CKD was generally detected at an earlier age in children with CAKUT than in children with other forms of CKD, particularly for analysis by chance (3.9 versus 5.8 years), urinary tract infection (0.7 versus 1.8 years) and failure to thrive (0.3 versus 2.2 years). Annual urinalysis at school detected CKD in 27 children (9.7%; median age, 8.9 years) with CAKUT and 12 children (7.1%; median age, 8.3 years) with other forms of CKD.

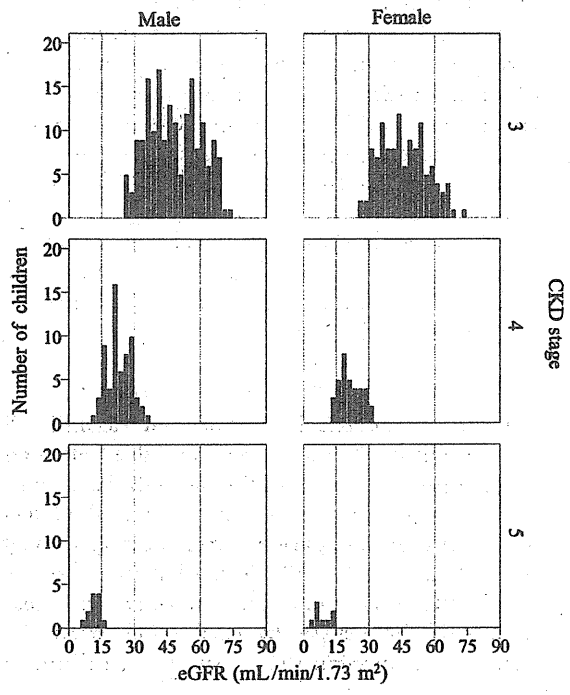
Treatment modalities for pediatric CKD

The treatment modalities for all patients included in this survey, and for patients with CAKUT and those with other forms of CKD, are summarized in Table 6. The most common treatments for CAKUT were angiotensin II receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs) which were used in approximately one-quarter of the patients. Both ARBs and ACEIs together were used in 11 and 23 patients with CAKUT and other forms of CKD, respectively (data not shown). Carbon adsorbents (e.g. AST-120), which are approved as renoprotective agents adsorbing uremic toxins in the gastrointestinal tract [18] and calcium antagonists, were used in 13.0 and 7.2% of patients, respectively.

DISCUSSION

Our findings revealed that the prevalence of stage 3–5 CKD in children in Japan aged 3 months to 15 years is 2.98 cases/100 000 children. Out of 447 CKD cases surveyed, 407 (91.1%) had non-glomerular disease; among them, 278 (68.3%) had CAKUT. To our knowledge, this is the first cross-sectional, nationwide, population-based survey of children with pre-dialysis CKD in Asia. Several reports to date have described the epidemiology of pre-dialysis CKD in children; however, these studies were restricted to Western countries [3–10].

SCr levels were frequently used to estimate the GFR and screen for CKD. The original Schwartz equation has been used extensively in clinical practice for estimating the GFR in children, where GFR (mL/min/1.73 m^2) = age-dependent



Our classification	CKD classification determined using the abbreviated Schwartz equation				
	2	3	4	5	Total
Males					
3	35 (19.7%)	135 (75.8%)	8 (4.5%)	0 (0.0%)	178 (100.0%)
4	0 (0.0%)	6 (9.5%)	53 (84.1%)	4 (6.3%)	63 (100.0%)
5	0 (0.0%)	0 (0.0%)	1 (8.3%)	11 (91.7%)	12 (100.0%)
Females					
3	13 (11.2%)	99 (85.3%)	4 (3.4%)	0 (0.0%)	116 (100.0%)
4	0 (0.0%)	2 (5.7%)	30 (85.7%)	3 (8.6%)	35 (100.0%)
5	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (100.0%)	8 (100.0%)

FIGURE 2. Distribution of CKD stage in males and females. Stage 3–5 CKD was classified using our newly established diagnostic criteria derived from normal SCr levels of age- and sex-matched Japanese children. The eGFR was determined using the abbreviated Schwartz equation [17]. Stage 3–5 CKD was classified as GFR 30–59, 15–29 and <15 mL/min/1.73 m^2, respectively (<1/2, <1/4 and <1/8 of normal GFR, respectively). Only subjects in whom height was measured were included in this analysis. Values in the table are *n* (%).

coefficient $\kappa \times$ height (cm)/SCr (mg/dL) [15]. This equation was recently modified because of the increasing use of enzymatic methods to determine SCr levels, replacing the Jaffe method [17]. However, there are some possible limitations of the original Schwartz equation. First, it requires the patient's height, which is not always measured in routine clinical practice. Secondly, the GFR was reported to be lower in Asian adults than in Caucasians [19], which may have led us to overestimate the GFR when using the Schwartz equation in Asian children. To overcome these perceived limitations, several research groups have sought to establish reference levels in large populations of children [11, 20], which may be more practical and relevant for screening purposes in a specific country. Accordingly, in our present study, we evaluated renal function by comparison with established reference values [11]. In this way, CKD was determined based on SCr, rather than relying on equations adjusted for height and mathematical constants. As a result, children aged <2 years, to whom the normal CKD classification could not be applied, could be included. Similarly, Pottel *et al.* [20] proposed and validated a height-independent, population-normalized equation derived from the patient's SCr and the median SCr for age-matched healthy children. Based on their results, population-based reference levels for renal function and CKD may provide a valid approach to determine CKD stage for screening purposes, as in the present study. Indeed, our newly established CKD classification showed good validity compared with the abbreviated and complete Schwartz equations.

To classify stage 3–5 CKD, we used new diagnostic criteria based on previously determined SCr reference levels in age- and sex-matched Japanese children [11]. In that study, SCr was determined using enzymatic methods; in our current study, the participating institutes only used the enzymatic method to determine SCr. Therefore, our current results are

Table 4. Primary etiologies of stage 3–5 chronic kidney disease in Japanese children aged 3 months to 15 years

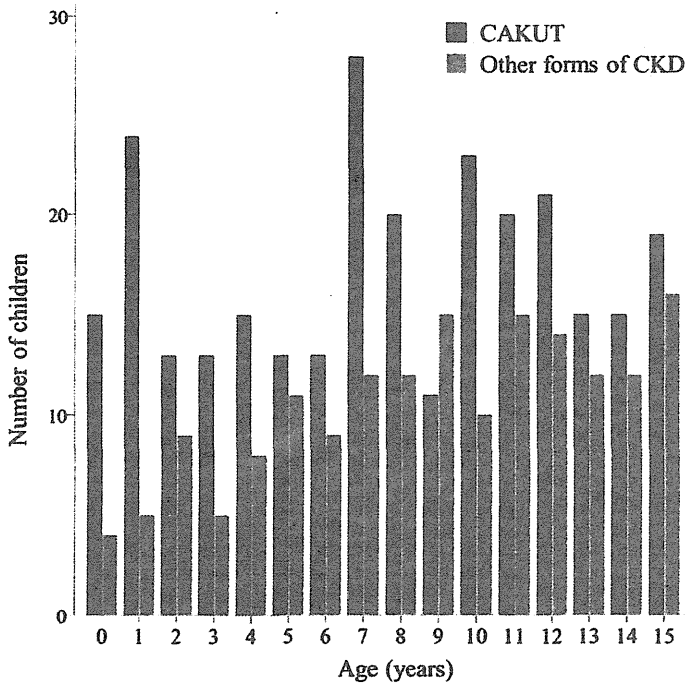
Primary disease	Non-glomerular kidney disease (n = 407, 91.1%)	Glomerular kidney disease (n = 35, 7.8%)	Unclassified (n = 5, 1.1%)
	n (%)	n (%)	n (%)
CAKUT	278 (68.3)	0 (0.0)	0 (0.0)
CAKUT with obstructive urological malformations[a]	60 (21.6)	0 (0.0)	0 (0.0)
CAKUT without obstructive urological malformations	218 (78.4)	0 (0.0)	0 (0.0)
Cortical necrosis (perinatal period)	40 (9.8)	0 (0.0)	0 (0.0)
Polycystic kidney disease	20 (4.9)	0 (0.0)	0 (0.0)
Nephronophthisis	19 (4.7)	0 (0.0)	0 (0.0)
Drug induced	17 (4.2)	0 (0.0)	1 (20.0)
Other inherited kidney damage	10 (2.5)	1 (2.9)	0 (0.0)
Acute kidney injury	10 (2.5)	0 (0.0)	0 (0.0)
Neurogenic bladder	6 (1.5)	0 (0.0)	0 (0.0)
Other non-inheritable character	4 (1.0)	2 (5.7)	0 (0.0)
Alport's syndrome	0 (0)	8 (22.9)	0 (0.0)
Cystinosis	1 (0.2)	0 (0.0)	0 (0.0)
Wilms tumor	1 (0.2)	0 (0.0)	0 (0.0)
Chronic tubulointerstitial nephritis	1 (0.2)	0 (0.0)	0 (0.0)
Focal segmental glomerulosclerosis	0 (0.0)	8 (22.9)	0 (0.0)
Chronic glomerulonephritis	0 (0.0)	8 (22.9)	0 (0.0)
Congenital nephrotic syndrome	0 (0.0)	3 (8.6)	0 (0.0)
Hemolytic uremic syndrome	0 (0.0)	3 (8.6)	0 (0.0)
Systemic lupus erythematosus	0 (0.0)	2 (5.7)	0 (0.0)
Unknown	0 (0.0)	0 (0.0)	4 (80.0)

[a]Posterior urethral valve, stricture of the urethra, hydronephrosis, hydroureter, and cloacal anomaly.

not subject to confounding because of the use of multiple assay types.

The prevalence of pre-dialysis stage 3–5 CKD was estimated to be 2.98 cases/100 000 Japanese children, which was lower than that reported in the ItalKid and REPIR II Projects (7.47 and 7.106 cases/100 000 children, respectively). The reason for this lower prevalence of CKD in Japan in comparison with Western countries is unclear, but differences in the age of the cohort and the method of case definition may account for some of the difference. For example, the ItalKid Project [5] included children aged <20 years, used the original Schwartz equation to determine GFR and included children with eGFR <75 mL/min/1.73 m^2. Similarly, the REPIR II study [4] included children aged <19 years with stage 2 CKD, which accounted for 42% of their cases. Nevertheless, the estimated prevalence of stage 3–5 CKD in Spain, based on data from the REPIR II study, is 4.12 cases per 100 000 children (7.106 × 58%), which is slightly higher than that estimated in our study. The low frequency of pre-dialysis CKD in our study is consistent with the low frequency of children with ESRD in Japan [7].

A number of factors, such as differences in racial and ethnic distributions, primary cause of CKD and quality of medical care, may contribute to the difference in reported prevalence estimates between Japan and Western countries. Additionally, the prevalence of obstructive uropathy is low in Japan, being detected in just 21.6% of patients with CAKUT; by contrast, in Western countries, obstructive uropathy accounts for many cases of non-glomerular disease in children with CKD [21, 22]. Several factors may explain the differences in the prevalence of CAKUT with obstructive uropathy, including (i) genetic differences that affect the distribution of



Age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Population (thousands)[a]	1,078	1,092	1,084	1,072	1,050	1,055	1,111	1,145	1,160	1,180	1,179	1,193	1,188	1,183	1,206	1,208	18,217
CAKUT[b]	15	24	13	13	15	13	13	28	20	11	23	20	21	15	15	19	278
Other forms of CKD[b]	4	5	9	5	8	11	9	12	12	15	10	15	14	12	12	16	169

FIGURE 3. Age distribution of children with stage 3–5 CKD in Japan. Children with CAKUT are shown in dark gray bars, while those with other forms of CKD are shown in light gray bars. [a]Total numbers of children of each age in Japan derived from national census data (1 April 2010) published by the Statistics Bureau of Ministry of Internal Affairs and Communications in Japan (http://www.stat.go.jp/english/index.htm). [b]Number of children with CAKUT or other forms of CKD reported in the survey. CKD, chronic kidney disease; CAKUT, congenital anomalies of the kidney and urinary tract.

obstructive diseases (e.g. prune-belly syndrome) and (ii) the diagnosis of these congenital diseases may be difficult, resulting in underestimation of obstructive uropathies. However, despite the lower frequency of obstructive uropathy in Japan, appropriate urological interventions are still an indispensable part of the management of children with CKD, because they are one of very few treatments that can change the outcome of CKD [23].

Despite the lower prevalence of CKD in our study compared with European cohorts, we believe that our data accurately represent the current situation in Japan because 1190 institutes, including all institutes belonging to the JSPN, were included in the survey and there was a very high response rate (77.7%). We also stratified institutions by hospital type and the number of beds to improve the accuracy of the estimated prevalence. Because the response rate tended to be lower for institutions with fewer patients, estimates of CKD prevalence that do not take strata (hospital size and type) into account are possibly overestimates. For example, a simple estimate without stratification in the present study would have been 599.0 children rather than the 542.5 estimated with strata taken into account. Thus, the stratified estimation method should correct for a bias between response rates and hospital type/size. Nevertheless, it is possible that some patients with stage 3–5 CKD were treated at other types of institutions not included in this survey.

The majority of Japanese children with CKD presented with non-glomerular disease. CAKUT was the primary cause of CKD (i.e. 62.2% of all CKD cases). This observation was expected. Unlike in adults, in whom diabetes and hypertension are the primary cause of CKD, congenital causes are responsible for majority of pediatric CKD cases [1, 7]. The prevalence of CAKUT in our study is also consistent with that reported in the ItalKid and REPIR II studies (67.5 and 59%, respectively) [3, 4].

Interestingly, there were very few cases of glomerular disease, such as focal segmental glomerulosclerosis, and no confirmed cases of IgA nephropathy (one case was suspected, but diagnosis was not confirmed). In a Japanese registry of pediatric ESRD patients conducted in 1998, 19% of patients had focal segmental glomerulosclerosis and 3% had IgA nephropathy [24]. The present analysis is likely to have underestimated the prevalence of these diseases for several reasons. First, these diseases progress more rapidly than non-

Table 5. Method of detection of Stage 3–5 CKD

Screening method	CAKUT (n = 278)	Age at which CKD was detected (years)		Other formsof CKD (n = 169)	Age at whichCKD was detected (years)	
	n (%)	Median	IQR	n (%)	Median	IQR
Fetal ultrasonography/ultrasonography in the neonatal period	88 (31.7)	0.0	0.0–0.0	19 (11.2)	0.0	0.0–0.0
Analysis by chance	38 (13.7)	3.9	1.2–6.1	32 (18.9)	5.8	1.7–9.4
Urinary tract infection	38 (13.7)	0.7	0.3–2.0	4 (2.4)	1.8	0.3–3.6
Annual urinalysis at school	27 (9.7)	8.9	7.0–10.3	12 (7.1)	8.3	7.1–10.9
Blood analysis in the neonatal period, asphyxia, neonatal shock and other events	25 (9.0)	0.0	0.0–0.1	31 (18.3)	0.0	0.0–0.0
Failure to thrive, weight loss and general fatigue	25 (9.0)	0.3	0.1–1.0	7 (4.1)	2.2	0.2–12.3
Urinalysis at 3 years	9 (3.2)	3.2	3.0–3.4	7 (4.1)	3.1	3.0–3.6
Routine health check (infants/toddlers)	7 (2.5)	0.3	0.1–1.7	4 (2.4)	2.8	0.4–5.1
Symptoms of glomerulonephritis (edema, oliguria or gross hematuria)	5 (1.8)	3.8	1.0–5.0	13 (7.7)	5.3	2.7–8.7
Analysis because of anomalies and syndromal stigmata	3 (1.1)	0.0	0.0–0.1	1 (0.6)	1.7	1.7–1.7
Detected during the management of other diseases(e.g. heart disease and malignancy)	2 (0.7)	5.3	5.3–5.3	18 (10.7)	3.2	0.2–8.2
Dysuria, including neurogenic bladder and nocturia	2 (0.7)	4.9	4.9–4.9	4 (2.4)	5.7	1.2–9.5
Analysis because of family history	0 (0.0)	—	—	3 (1.8)	6.2	4.5–9.7
Sepsis	0 (0.0)	—	—	3 (1.8)	0.0	0.0–0.1
Others	0 (0.0)	—	—	2 (1.2)	2.2	0.8–3.7
Unknown (not available)	9 (3.2)	—	—	9 (5.3)	—	—

CKD, chronic kidney disease; CAKUT, congenital anomalies of the kidney and urinary tract.

glomerular diseases and could have been missed in the survey. Secondly, we restricted our analysis to those aged <16 years, but chronic glomerulonephritis frequently affects patients aged 16–20 years. Furthermore, these diseases respond well to novel treatment regimens that are well established in Japan, including combination therapy for IgA nephropathy [25] and cyclosporine in combination with steroids for steroid-resistant nephrotic syndrome, including focal segmental glomerulosclerosis [26].

Fetal/neonatal ultrasonography was the most frequently used method to detect CAKUT, followed by blood analyses by chance and urinary tract infection. Only 27 children with CAKUT and 12 with other forms of CKD were detected following annual urinalysis at school. Patients with CKD, particularly children with CAKUT, do not necessarily show abnormal urinalysis, and are missed by the screening. It is also possible that CKD (particularly non-CAKUT forms of CKD) could be detected in the earlier stages (earlier than stage 3) and patients could then receive appropriate intervention to treat the underlying disease. The treatment strategies for CAKUT and other forms of CKD in each institution were generally similar, although the responding institutions more often reported using carbon absorbents for CAKUT and ACEIs in other forms of CKD (data not shown).

Some limitations of the study merit consideration. First, only 77.7% of the surveyed institutions responded to the questionnaire, which may limit the accuracy of the estimate. Secondly, although the classification system used for CKD staging in the present study was based on reference SCr levels determined via enzymatic methods from Japanese children, these diagnostic criteria have not been validated globally and other reference values would be needed for other populations. Height could have also been determined to estimate GFR via the Schwartz equation; however, because the GFR is inversely

Table 6. Treatment strategies for CAKUT and other forms of CKD for individual patients

	CAKUT (n = 278)	Other forms of CKD (n = 169)	All patients (n = 447)
	n (%)	n (%)	n (%)
ARBs			
No	201 (72.3)	115 (68.0)	316 (70.7)
Yes	74 (26.6)	53 (31.4)	127 (28.4)
NA	3 (1.1)	1 (0.6)	4 (0.9)
ACEIs			
No	209 (75.2)	108 (63.9)	317 (70.9)
Yes	66 (23.7)	60 (35.5)	126 (28.2)
NA	3 (1.1)	1 (0.6)	4 (0.9)
Carbon absorbents			
No	237 (85.3)	144 (85.2)	381 (85.2)
Yes	34 (12.2)	24 (14.2)	58 (13.0)
NA	7 (2.5)	1 (0.6)	8 (1.8)
Calcium antagonists			
No	264 (94.9)	147 (87.0)	411 (91.9)
Yes	11 (4.0)	21 (12.4)	32 (7.2)
NA	3 (1.1)	1 (0.6)	4 (0.9)

CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; ARB, angiotensin II receptor blocker; ACEI, angiotensin-converting enzyme inhibitor; NA, not available.

proportional to SCr in age- and sex-matched individuals, and because we used age- and sex-matched reference SCr levels established in a previous study with 1151 children, our measurements should be accurate enough and more practical for screening purposes. Indeed, our CKD staging showed good agreement with CKD staging based on the abbreviated Schwartz equation (Figure 2). Because, our CKD staging method is based on the SCr level, CKD may be missed in children with small muscle mass, such as those with spina bifida, neuromuscular disease and short stature.

To our knowledge, this is the first nationwide, population-based survey of children with pre-dialysis CKD in Asia and applied reference levels for CKD derived from a large cohort of Japanese children. This method showed good agreement with the abbreviated Schwartz equation and is practical for screening purposes, including children aged <2 years, as current methods are not appropriate for estimating CKD in this age group. The estimated prevalence of stage 3–5 CKD in Japan was 2.98 cases/100 000 children, which is lower than that in Western countries. Most cases presented with non-glomerular disease, and CAKUT was the most common cause of CKD. Improved management of CAKUT in children with CKD, including renoprotective treatment and urological interventions, is required. We are planning randomized and longitudinal studies to improve the management of pediatric CKD, and better understand its long-term prognosis.

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CONFLICT OF INTEREST STATEMENT

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Short clinical report

Concurrent deletion of *BMP4* and *OTX2* genes, two master genes in ophthalmogenesis

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ABSTRACT

BMP4 and *OTX2* are master genes in ophthalmogenesis. Mutations of *BMP4* and *OTX2* often lead to eye defects, including anophthalmia–microphthalmia. A significant degree of variable expressivity has been reported in heterozygous individuals with *BMP4* or *OTX2* mutation. Interestingly, both *BMP4* and *OTX2* reside on 14q22, being only 2.8 Mb apart. Previous studies reported that among three patients with 14q22 deletion involving *BMP4* and *OTX2*, all had severe eye defects. The minimal degree of variable expressivity among these individuals who were doubly deleted for *BMP4* and *OTX2* could be attributed to the combinatorial relationship of the two genes observed in animal models. We herein report a patient with a concurrent deletion of *BMP4* and *OTX2* who exhibited bilateral microphthalmia, more specifically, anterior segment dysgenesis with microcornea. Evolutionarily conserved physical linkage of *Bmp4* and *Otx2* loci may suggest an advantage of the proximal alignment of the two genes. Another striking feature in the propositus was the progressive white matter loss observed by serial neuroimaging. A review of twelve previously reported patients with 14q22 microdeletion revealed decreased white matter volume in half of the patients. It remains to be elucidated whether the white matter lesion is age-dependent and progressive. In conclusion, anterior segment defects of the eyes, especially when accompanied by decreased white matter volume on neuroimaging, should raise the clinical suspicion of 14q22 microdeletion.

1. Introduction

14q22 microdeletion syndrome has been proposed as a recognizable contiguous gene deletion syndrome, mainly characterized by anophthalmia–microphthalmia (AM) and developmental delay [1]. In terms of AM, it is noteworthy that the 14q22 region contains two master genes for ophthalmogenesis, *BMP4* and *OTX2*, the two genes being only 2.8 Mb apart. *Bmp4* belongs to the *Tgfb1* superfamily and plays a pivotal role in ocular development as well as in the development of the teeth, limbs and bones [2]. *Otx2* is a critical gene for tissue specification in the forebrain and its derivative, eyes [3].

Mutations of *BMP4* lead to eye defects, including AM [4]. Similarly, mutations of *OTX2* can also cause an indistinguishable phenotype [5]. In both humans and mice, with heterozygous mutation of *BMP4* [4,6], and in those with heterozygous mutations of *OTX2* [5,7], a significant degree of variable expressivity of the ocular phenotype is the rule. In mice, *Bmp4* +/− causes anterior segment dysgenesis, but the penetrance and severity of the ocular phenotype is strongly influenced by the genetic background. On the C57BL/6J background, most of *Bmp4* +/− mice exhibit a bilateral severe ocular phenotype, whereas on other genetic backgrounds, few heterozygous mice show clinically detectable ocular phenotypes [6]. Similarly, in humans, the severity of the ocular phenotype varies significantly among affected patients, and truncating mutation of *OTX2* identified in children with bilateral AM have been detected in unaffected parents, providing evidence for incomplete penetrance [5].

Among three patients with 14q22 deletion involving *BMP4* and *OTX2*, all had severe eye defects. The minimal degree of variable

Abbreviations: AM, anophthalmia–microphthalmia.

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