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Cystatin C and Cardiac Measures in Children and Adolescents With CKD

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Abstract

Background—Cardiovascular disease (CVD) is highly prevalent among children with chronic kidney disease (CKD). Cystatin C is an established marker of kidney function and an emerging biomarker for CVD events. We quantified the relationship between cystatin C and cardiac structure and function over time among children with CKD and assessed whether cystatin C and diastolic function retained an association after accounting for kidney function.

Study Design—Prospective cohort study.

Setting & Participants—678 children and adolescents with mild-moderate CKD enrolled in the Chronic Kidney Disease in Children (CKiD) study with 1228 echocardiographically obtained cardiac structure and function measures.

Predictor—Serum cystatin C (mg/L) measured annually.

Outcomes—Cardiac structure (left ventricular mass index [$\text{g}/\text{m}^2.7$]) and cardiac function (shortening fraction; E/A , E'/A' , E/E' ratios) measured every other year.

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Supplementary Material

Table S1: Comparison of data from initial CKiD visit for 678 included and 213 excluded.

Note: The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

Supplementary Material Descriptive Text for Online Delivery

Supplementary Table S1 (PDF). Comparison of data from initial CKiD visit for 678 included and 213 excluded.

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Measurements—Demographics and anthropometrics, measured glomerular filtration rate (mGFR), heart rate, blood pressure, hemoglobin z score, serum albumin and calcium-phosphorus product.

Results—Independent of time, each 1-mg/L increase in cystatin C was independently associated with a concurrent 7.7% (95% CI, 5.3%–10.0%) increase in left ventricular mass index, a –4.7% (95% CI, –7.0% to –2.4%) change in E/A, a –6.6% (95% CI, –9.0% to –4.2%) change in E'/A', and a 2.5% (95% CI, 0.3–4.7) increase in E/E' ratio. mGFR was also independently associated with E'/A'. When cystatin C and mGFR were included in the same model, cystatin C remained independently associated with E'/A' while mGFR was not.

Limitations—24% of the cohort was missing data on outcomes of interest or measurements; study population only includes children and adolescents with mild to moderate CKD.

Conclusion—In this study of children and adolescents with mild-moderate CKD, cystatin C was independently associated with cardiac structure and diastolic function. Cystatin C remained able to predict diastolic function decline via E'/A' even after adjusting for mGFR, suggesting that cystatin C may have an independent role in CVD risk stratification among children and adolescents with CKD.

Index Words

biomarker; cardiovascular disease (CVD); left ventricular hypertrophy (LVH); left ventricular mass (LVM); pediatrics; children; adolescents; systolic function; cystatin C; chronic kidney disease (CKD); renal function; echocardiograph; cardiac structure; cardiac function

Sudden cardiac death remains the most common cause of mortality among children with chronic kidney disease (CKD)(1). Children with CKD manifest a multitude of cardiovascular disease (CVD) risk factors, and show early signs of heart disease including dilated cardiomyopathy and left ventricular hypertrophy (LVH). The progression of these abnormalities to cardiac death is thought to explain the markedly decreased lifespans of children with advanced CKD(1). While the increased CVD risk in youth with CKD has been well established(2, 3), the morbidity and mortality related to CVD has nevertheless increased over time(4). Identifying new biomarkers or clinical characteristics that could more easily identify those at increased CVD risk would help tailor treatment efforts.

Diastolic dysfunction is one of the earliest signs of cardiac disease among children with CKD(5) and it progresses as CKD advances(5, 6). Despite this, serum creatinine has not been shown to be a reliable biomarker of diastolic dysfunction(5, 7). Cystatin C, an endogenous proteinase inhibitor widely utilized as a surrogate marker of kidney function, is an emerging biomarker for CVD events. Cystatin C is associated with cardiac geometry, cardiac remodeling and CVD outcomes in adults(8–11). In this study, we aimed to quantify the independent relationship between cystatin C and both cardiac structure and function over time among children and adolescents with mild to moderate CKD. We also sought to determine how well other measures of kidney function predict diastolic function, and if any independent relationship between cystatin C and diastolic function existed after accounting for directly measured kidney function.

Methods

Study Population

As of December 2015, 891 children and adolescents (586 in April 2005–August 2009 and 305 between February 2011 and March 2014) with mild to moderate CKD were enrolled in the Chronic Kidney Disease in Children (CKiD) Study, a multicenter, prospective cohort study conducted at 49 pediatric nephrology centers across North America. The study design and conduct were approved by an observational study monitoring board appointed by the National Institute of Diabetes and Digestive and Kidney Diseases and by the internal review boards of each participating center. Data approval numbers are as follows: data coordinating center, H.34.03.09.05.A2; East Coast Clinical Coordinating Center, 10-007880; and Midwest Clinical Coordinating Center, 11120124. Each participating family provided informed consent.

Study participants attended annual follow-up visits after their initial baseline visit. Children and adolescents were included in our analyses if they had echocardiography data from one or more visits and complete data on the exposure of interest and all covariates. There were 1,401 visits, across 733 participants, with full echocardiography data that had occurred by December 2015. At 173 of these visits, the exposure of interest or at least one covariate was missing, leaving 1,228 visits from 678 children and adolescents (median follow-up, 3.0 years) in our primary analysis. The 678 participants contributed one to four visits to the analysis (334, one visit; 188, two visits; 106, three visits; and 50, four visits [Figure 1]).

Echocardiography

At even-numbered annual CKiD visits (or once every two years) participants undergo local standardized protocol echocardiography. Images are read centrally by a study cardiologist where measurements are taken in triplicate and averaged. Left ventricular mass (LVM) is obtained by American Society of Echocardiography criteria(12, 13) using 2D M-mode, and the LVM index (LVMI) is calculated as $LVM/height^{2.7}$ where LVM is in grams and height is in meters(12, 14). To assess systolic function, shortening fraction (SF) was obtained: $[(LV \text{ end-diastolic diameter} - LV \text{ end-systolic diameter})/LV \text{ end-diastolic diameter}]$. Pulsed-wave tissue Doppler in the apical view is used to obtain the maximal early filling (E-wave) and late diastolic filling (A-wave) velocities, the early diastolic annular velocity (E') and the late diastolic annular velocity (A')(15). These measurements are then used to obtain the measures of diastolic function described below.

Primary Outcomes

The LVMI was used to determine cardiac structure, with values at or greater than the age-/sex-specific 95th percentile indicative of LVH(16). Systolic function was ascertained by SF. Values <25% indicate abnormally low SF. Diastolic function was assessed via three ratios: E/A ratio (early-to-atrial LV filling ratio), E'/A' ratio (early-to-late septal mitral annular peak velocity) and E/E' ratio (estimate of LV filling pressure). Normal E/A and E'/A' ratios are >1.0; lower values denote stiffer ventricular walls and diminished ability for adequate LV relaxation and fill. The E/E' ratio is an estimate of LV filling pressures, and normal values are <8.0(15); higher values indicate worse LV compliance.

Primary Exposure

Cystatin C was measured at each annual visit. Serum specimens were analyzed by standardized methods to produce measurements in mg/L(17). The cystatin C value taken at the time of echocardiography was used in the analyses.

Covariates

The following variables were included in all multivariable analyses: baseline age, sex, race; average age-/sex-specific body mass index z score from current (visit with echocardiography) and previous visit (visit immediately prior to echocardiography); average age-/sex-/height-specific systolic and diastolic blood pressure z scores from current and previous visit (measured via manual auscultation); and years with CKD at the time of echocardiography. Multivariable models for cardiac structure and diastolic function were additionally adjusted for the average serum calcium-phosphorous product from current and previous visit. The model for systolic function was further adjusted for average heart rate from 24-hour ambulatory blood pressure monitoring (ABPM); average race-/age-/sex-specific hemoglobin z score from current and previous visit; average serum albumin (g/dL) from current and previous visit, and β -blocker, angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker use (not used at prior nor current visit versus use at prior visit only versus use at current visit only versus use at both visits). Due to incomplete ABPM measurements, the model for systolic function only includes the 953 visits from 581 participants with a valid mean heart rate from 24-hour ABPM.

In our second set of analyses, we determined the effect other time-varying measures of kidney function had on both E'/A' and E/E' in comparison to cystatin C. These kidney function measures included serum creatinine (Scr)-based estimated GFR, or $eGFR_{cr} = (0.413 \times \text{height in cm})/\text{Scr}$; serum urea nitrogen (SUN)-, Scr-, and cystatin C-based eGFR, or $eGFR_{SUN-cr-cys} = 39.8 \times (\text{height in m}/\text{Scr})^{0.456} \times (1.8/\text{Cystatin C})^{0.418} \times (30/\text{SUN})^{0.079} \times 1.076$ (if male) $\times (\text{height in m}/1.4)^{0.179}$, serum creatinine, GFR measured by the plasma disappearance of iohexol [mGFR]).

Statistical Analyses

We conducted longitudinal analyses using the echocardiogram data from visits two, four, six and eight. Visit was treated as a categorical variable. Linear mixed models were used to determine the longitudinal association of cystatin C with cardiac structure (LVMi), systolic function (SF), and diastolic function (E/A , E'/A' and E/E' ratios). These models have the advantage that complete data are required only for a given visit, rather than for all visits for a given subject. Model parameters were estimated by the method of maximum likelihood, which produces unbiased estimates when data are missing at random. To account for the correlation among repeated observations on a given subject, all mixed models were adjusted for repeated visits with a random subject effect. Random subject effects were chosen because they are plausible as a reflection of individual characteristics of members of the population from which the study subjects were sampled. Furthermore, this approach is more parsimonious than treating 'subject' as a fixed effect, which is an approach that produces models with large numbers of parameters. Likelihood ratio statistics revealed no significant effects of cystatin C by visit, so for simplicity we present the main effects models without

cystatin C by visit interaction terms. All outcomes were log transformed to achieve normally distributed errors with constant variance; effects in this case are expressed multiplicatively as percent changes. Partial residual plots were used to graphically depict the relationship between cystatin C and the E/A , E'/A' and E/E' ratios after adjustment. A partial residual for a given predictor variable in a regression model is obtained by taking the raw residual from the model (observed minus predicted; in these mixed models, the raw residual included adjustment for the individual values of the random intercept using the best linear unbiased predictor, thus adjusting for repeated measurements of individual subjects), and adding back the product of the estimated regression coefficient and the value of the predictor variable (i.e., cystatin C) for each subject. Plots of the partial residuals versus the values of the independent variable are useful in visualizing the estimated effect of the predictor variable in a multivariable model.

To evaluate how other measures of kidney function predict diastolic function, we chose E'/A' and E/E' as our outcomes, as these measures are more widely used for assessment of diastolic function. We then investigated the relationship of the following measures of kidney function with diastolic function in separate models, adjusting for the same covariates: $eGFR_{cr}$; $eGFR_{SUN-cr-cys}$; serum creatinine (with height additionally added to this model); and $mGFR$. Lastly, our final model included both $mGFR$ and cystatin C to determine the longitudinal relationship each had with diastolic function, adjusting for the other, as well as $eGFR_{cr}$ and cystatin C in a separate model. Due to missing $mGFR$ measurements, this second set of analyses includes 1,163 visits from 655 children and adolescents. A two-sided p -value <0.05 was considered statistically significant. Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc, Cary NC, USA).

Results

Characteristics of the 678 children and adolescents from the CKiD Study included in our analyses at the first visit with echocardiography and cystatin C measured concurrently are presented in Table 1. Participants included in our analyses did not differ substantially from those excluded from the analysis at visit one (Table S1, available as online supplementary material). Twelve percent had LVH. Most had normal systolic function; only 1.5% had a SF $<25\%$. The diastolic function values were largely in the normal range (1st quartiles of E/A and E'/A' ratios, >1 ; median E/E' ratio, <8). Over time, the prevalence of SF $<25\%$ increased to 2.1%. The LVH prevalence and the E/A , E'/A' , E/E' ratios remained similar. The median values of cystatin C, serum creatinine, and $mGFR$ were 1.4 mg/L, 1.2 mg/dL, and 51.9 mL/min/1.73 m², respectively. The median of the age-/sex-/height-specific systolic and diastolic blood pressure z scores were 0.2 and 0.4, respectively. Sixty-five percent of participants reported use of β -blockers, angiotensin-converting enzyme inhibitors, or angiotensin II receptor blocker medications at either their prior or current visit. Seven percent reported corticosteroid use.

Cystatin C was independently associated with increasing LVMi over time using 1,228 visit pairs contributed by 678 participants: each 1-mg/L increase in cystatin C was associated with a 7.7% (95% confidence interval [CI], 5.3%–10.0%) increase in LVMi (Table 2). Cystatin C was not associated with systolic function, but was independently associated with

decreased E/A and E'/A' ratios, and increased E/E' ratio. Specifically, each 1-mg/L increase in cystatin C was associated with a -4.7% (95% CI, -7.0% to -2.4%) change in the E/A ratio and a -6.6% (95% CI, -9.0% to -4.2%) change in the E'/A' ratio. The same increase in cystatin C was also independently associated with a 2.5% (95% CI, 0.3%–4.7%) increase in the E/E' ratio. Figures 2–4 show the relationship between cystatin C and E/A ratio, E'/A' ratio, and E/E' ratio after adjusting for all covariates shown in Table 2. The slope of each regression line corresponds to the percentage change shown in Table 2. Furthermore, there is substantial heterogeneity in each outcome for a given value of cystatin C.

We restricted our second set of analyses to 1163 visits with mGFR measurements and diastolic function measured concurrently to quantify the independent association between other markers of kidney function and diastolic function (Table 3). Of all the markers of kidney function, cystatin C was associated with the greatest percent decline in the E'/A' ratio: a 1-mg/L increase in cystatin C was associated with a -6.0% (95% CI, -8.4% to -3.5%) change in E'/A' ratio. In comparison, a -25-ml/min/1.73 m² change in mGFR was associated with a -3.7% (95% CI, -5.8% to -1.7%) change in E'/A' ratio. When cystatin C and mGFR were included in the model together, the association of cystatin C with diastolic function was slightly attenuated (-5.3% [95% CI, -8.6% to -1.8%] change) but there was no longer a significant association of mGFR with diastolic function (-0.9% [95% CI, -3.7% to 1.9%] change). When cystatin C and the eGFR_{cr} were included in the same model, the inferences are nearly identical, with slightly stronger associations (Model 7, Table 3). Cystatin C was associated with an increase in the E/E' ratio but to a much lesser degree (2.4% [95% CI, 0.2%–4.6%] increase) than the change seen with the E'/A' ratio. Neither cystatin C nor mGFR were associated with the E/E' ratio when both were included in the model.

Discussion

In this longitudinal, observational cohort study of children and adolescents with mild to moderate CKD, greater cystatin C was independently associated with increasing LVMI and worsening diastolic function. This serum marker, more commonly considered a surrogate marker of kidney function, was also able to predict decline in diastolic function as measured by E'/A' ratio even after adjusting for kidney function as assessed by the plasma disappearance of iothexol. This suggests that cystatin C may have an independent role in CVD risk stratification among children and adolescents with CKD, a particularly vulnerable group.

Cystatin C has several characteristics that make it an ideal biomarker for the estimation of kidney function. Specifically, it is steadily produced by all nucleated cells, freely filtered at the glomerulus, entirely reabsorbed and catabolized by the proximal tubules, and not renally secreted(9). Cystatin C is also biologically active as a cysteine protease inhibitor. Cysteine proteases such as cathepsins promote cardiovascular extracellular matrix degradation. When cathepsin activity is increased, cystatin C expression is similarly increased to counterbalance this activity. When there is an imbalance between this proteolytic activity and cystatin C,

myocardial remodeling occurs. This leads to alterations in left ventricular size, shape and function(9).

This biologic activity has prompted animal and human research into possible associations between cystatin C and CVD measures and outcomes. Several studies in adults have indeed described such an association among individuals with and without kidney disease(8, 9, 11). Because young people are free of many of the co-morbidities that can confound observed associations between cystatin C and CVD outcomes, studying this population is ideal, particularly when trying to tease out any contribution decreased kidney function may have on this relationship.

Prior studies in adults have led to consensus statements defining CKD as an independent risk factor for coronary heart disease(18, 19) making CVD risk prediction a priority in this population. In 2011, a large meta-analysis demonstrated that the hazard ratio for CVD mortality increased with decreasing kidney function(20). Even among children, CKD is considered a significant CVD risk factor. In fact, children with CKD are considered to be in the highest category of CVD risk, grouped along with children who are heart transplant recipients and who have diabetes, among others(21).

Clinically, at-risk children are screened for intermediate outcomes such as LVH to assess their CVD risk. In addition, diastolic function is also determined via echocardiography and can add to an individual's overall CVD risk assessment. Diastolic dysfunction is one of the main precursors to heart failure and CVD events in adults. The abnormal relaxation and decreased distensibility of the left ventricle that occurs in diastole and characterizes diastolic dysfunction leads to impaired LV filling. As a result, increased pressure in the left ventricle, left atrium and pulmonary vasculature can occur. Diastolic dysfunction is often asymptomatic, but patients with diastolic heart failure may have difficulty tolerating tachycardia and abrupt changes in blood pressure as these forms of hemodynamic stress can limit LV filling, impair preload and further limit cardiac relaxation. Early identification of those at risk for diastolic heart failure, particularly among individuals with CKD who are more prone to hemodynamic stress, is imperative for early treatment and prevention of CVD events.

While the association between CKD and CVD has been established for over a decade, several studies have called this into question and contending no increased CVD risk among those with mild to moderate CKD(22, 23). One explanation for these conflicting findings is that many studies with negative results utilized creatinine-based estimating equations for the definition of CKD in contrast to mGFR. In individuals with $GFR > 60 \text{ ml/min/1.72m}^2$, these equations are known to underestimate kidney function⁽²⁴⁾ and can therefore lead to misclassification bias.

With the development of more sophisticated cystatin C-based estimating equations, the association between CKD and CVD has been more strongly established among individuals with milder CKD. Recent studies also suggest that cystatin C may offer additional prognostic advantages to CVD risk stratification. Among elderly individuals followed up for as long as 9 years, cystatin C was a much stronger predictor of CVD events than creatinine

and was directly associated with all-cause death in a dose-response manner(25). In an observational study of adults with non-diabetic CKD stages 3–4, cystatin C was found to be at least as, if not more, strongly associated with CVD mortality than mGFR or creatinine(26).

In our study, we show that among children and adolescents with mild to moderate CKD, cystatin C is an independent predictor of diastolic function. A prior study in adults attributed the association between cystatin C and CVD morbidity and mortality to the close correlation of cystatin C with kidney function(26). In fact, the addition of cystatin C to equations designed to estimate GFR has greatly added to our ability to stage and follow up children with CKD(27). However, we demonstrate here that increasing levels of cystatin C predicted diastolic function decline, independent of kidney function. So, while each commonly utilized method of eGFR among children and adolescents was significantly associated with diastolic function as ascertained by E'/A' ratio, cystatin C remained associated even after controlling for mGFR. Adding cystatin C to the panel of tests used to assess CVD risk could help risk stratify these young people and facilitate identification and treatment of additional risk factors.

LVH is a common precursor to diastolic dysfunction and is present in 17%–50% of children with CKD stages 2–4(27). We also demonstrated that increasing cystatin C was associated with an increase in LVMI over time. This is similar to findings described from a cross-sectional analysis in the Chronic Renal Insufficiency Cohort (CRIC) study: worsening kidney function as ascertained by cystatin C–based eGFR was strongly associated with higher LV mass, increased LVH, and abnormal LV geometry(28).

Systolic dysfunction was rare in our population. This is not surprising, as systolic function is thought to be relatively preserved in children with CKD, only becoming manifest once children reach end-stage disease(27). It is possible that we did not see an association between cystatin C and systolic function because so few participants in our cohort had dysfunction. This will need to be confirmed in future studies.

Interestingly, cystatin C did not have a significant interaction with time. This suggests that the relationship of cystatin C with diastolic function is constant—that it does not change (i.e., become stronger or weaker) with CKD duration. The finding that time did not modify the relationship between cystatin C and diastolic function lends further support that this serum marker may have a role in risk stratifying children and adolescents at any point in their course of kidney disease.

While we saw a strong independent association of cystatin C with E'/A' ratio, we did not see a similar association between cystatin C and E/E' ratio when mGFR was included in the multivariable model. This may be because the two ratios are assessing slightly different aspects of diastolic function: the E'/A' ratio assesses ventricular filling (lower ratios indicating a stiffer ventricular wall) and the E'/E ratio assesses ventricular compliance (greater ratios indicating worsening compliance). Future studies are needed to explore this further.

Our study has several limitations. Not all participants had all measures of interest, particularly when related to the 24-hour ABPM, so we were unable to benefit fully from the large population in the CKiD study. Interestingly, the median mGFR ascertained at the baseline visit of those included in our analyses compared to that of those not included because of missing data were nearly identical (52.3 versus 50.8 ml/min/1.73 m² [see Table S1]), which gives some assurance that we did not study a select group of healthier children and adolescents with respect to their stage of CKD. In addition, our population only included children and adolescents with mild to moderate CKD, with a relatively narrow range of GFR. Therefore, we cannot extend our observations to young people with normal kidney function or more advanced stages of CKD.

Our study also has several notable strengths. We present longitudinal data from a large, diverse group of children and adolescents with CKD, free of multiple risk factors confounding similar studies in adults. As examples, young people are typically free of many CVD risk factors related to lifestyle (smoking, alcohol use, diabetes) and have not had extended exposure to other traditional risk factors (dyslipidemia, hypertension, obesity, kidney failure). Another strength was that we were able to demonstrate a clear association of an easily obtained biomarker on a measured form of non-fatal CVD, providing an intermediate end point for targeted intervention. Finally, our data includes mGFR which allows for more accurate assessment of kidney function.

In summary, we were able to demonstrate that cystatin C independently predicts LVMi and diastolic function among children and adolescents with CKD. This presents an opportunity for improved risk stratification and more targeted therapies for children and adolescents with CKD, a group particularly at risk for CVD morbidity and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Contributions: Research idea and study design: TMB, KT, MFS, CC, SF, BW, MM; data acquisition: TMB, SF, BW, PM, TK, MM; data analysis/interpretation: TMB, KT, MFS, CC, PM, TK, SF, BW, MM; supervision or mentorship: TMB, MM. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. TMB takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Figure 1.

Flow chart outlining the number of visits and number of participants contributing data to the primary and subsequent analyses

Figure 2. Partial residual plot of adjusted E/A Ratio versus Cystatin C (mg/L)

^aAdjusted for baseline age, gender, African-American race, age-sex specific BMI z score, age-/sex-/height-specific systolic and diastolic blood pressure z scores, calcium-phosphorus product, and follow-up time. The outcome variable was log transformed for analysis.

Multiple points for each subject were included in the longitudinal analysis and are included in the plot, with adjustment for the individual values of the random intercept.

Figure 3. Partial residual plot of adjusted E'/A' Ratio versus Cystatin C (mg/L)

^aAdjusted for baseline age, gender, African-American race, age-sex specific BMI z score, age-/sex-/height-specific systolic and diastolic blood pressure z scores, calcium-phosphorus product, and follow-up time. The outcome variable was log transformed for analysis. Multiple points for each subject were included in the longitudinal analysis and are included in the plot, with adjustment for the individual values of the random intercept.

Figure 4. Partial residual plot of adjusted E/E' Ratio versus Cystatin C (mg/L)

^aAdjusted for baseline age, gender, African-American race, age-sex specific BMI *z* score, age-/sex-/height-specific systolic and diastolic blood pressure *z* scores, calcium-phosphorus product, and follow-up time. The outcome variable was log transformed for analysis. Multiple points for each subject were included in the longitudinal analysis and are included in the plot, with adjustment for the individual values of the random intercept.

Table 1

Characteristics of 678 CKiD Study participants at first visit with echocardiogram and cystatin C measured concurrently.

Characteristics	Overall		mGFR, mL/min/1.73m ²							
			90		60–89		45–59		30–44	
	(N=678)		(n=70)		(n=194)		(n=149)		(n=166)	(n=99)
LVMI, g/m ^{2.7}	30.7 (25.6, 37.2)		28.6 (24.8, 34.8)		28.5 (24.1, 34.8)		31.7 (26.5, 38.0)		30.8 (25.7, 37.3)	34.4 (28.7, 40.5)
LVH	12% (82)		7% (5)		7% (13)		14% (21)		12% (20)	23% (23)
Shortening Fraction, %	37% (34%, 41%)		36% (32%, 40%)		36% (33%, 40%)		38% (35%, 41%)		37% (33%, 41%)	39% (35%, 42%)
E/A Ratio	1.8 (1.5, 2.3)		2.0 (1.6, 2.3)		2.0 (1.6, 2.4)		1.8 (1.5, 2.2)		1.8 (1.5, 2.2)	1.7 (1.5, 2.0)
E'/A' Ratio	1.9 (1.5, 2.4)		2.0 (1.5, 2.5)		2.1 (1.7, 2.5)		2.0 (1.6, 2.3)		1.8 (1.5, 2.3)	1.8 (1.5, 2.1)
E/E' Ratio	7.3 (6.3, 8.6)		7.2 (5.9, 8.2)		7.2 (6.2, 8.4)		7.3 (6.3, 8.5)		7.4 (6.3, 8.4)	7.9 (6.3, 9.5)
Cystatin C, mg/L	1.4 (1.1, 2.0)		0.8 (0.7, 0.9)		1.1 (1.0, 1.3)		1.4 (1.3, 1.7)		1.9 (1.6, 2.3)	2.7 (2.4, 3.3)
Serum Creatinine, mg/dL	1.2 (0.9, 1.7)		0.7 (0.6, 0.9)		0.9 (0.7, 1.2)		1.1 (0.9, 1.3)		1.6 (1.3, 2.0)	2.6 (2.0, 3.7)
mGFR, mL/min/1.73m ²	51.9 (37.3, 72.4)		106.3 (100.7, 114.6)		73.1 (65.3, 79.3)		51.9 (49.0, 56.6)		38.8 (34.7, 41.9)	23.4 (19.1, 26.5)
eGFR _{cr} ^a	51.2 (34.5, 67.7)		88.5 (75.5, 103.4)		66.6 (58.3, 73.9)		52.3 (46.7, 59.9)		37.1 (30.7, 45.2)	23.5 (16.9, 29.2)
eGFR _{SUN-cr-cys} ^b	51.6 (36.8, 66.2)		91.9 (81.6, 99.1)		66.2 (60.8, 72.7)		51.7 (46.9, 56.0)		38.4 (33.4, 43.9)	25.4 (21.2, 30.1)
Length of time with CKD, y	9.3 (5.1, 13.5)		6.2 (2.5, 11.3)		8.3 (4.8, 12.4)		9.5 (5.5, 12.8)		10.1 (5.9, 13.7)	11.0 (6.2, 14.8)
Age, y	12.3 (9.0, 15.9)		14.2 (9.4, 17.1)		12.3 (8.3, 16.1)		11.8 (8.6, 14.7)		12.0 (9.2, 15.7)	13.9 (9.8, 16.1)
Female sex	39% (265)		41% (29)		31% (60)		44% (66)		45% (75)	35% (35)
African American	16% (109)		29% (20)		18% (35)		11% (16)		16% (26)	12% (12)
Heart Rate from 24-h ABPM ^c	82 (74, 89)		80 (72, 89)		81 (73, 87)		81 (75, 91)		85 (76, 89)	83 (74, 90)

Characteristics	Overall	mGFR, mL/min/1.73m ²				
		90	60–89	45–59	30–44	15–29
	(N=678)	(n=70)	(n=194)	(n=149)	(n=166)	(n=99)
Age-/Sex-/Height-specific Systolic BP z score ^d	0.2 (−0.4, 0.9)	0.0 (−0.8, 0.9)	0.0 (−0.6, 0.7)	0.4 (−0.2, 0.8)	0.2 (−0.5, 0.8)	0.6 (−0.4, 1.4)
Age-/Sex-/Height-specific Diastolic BP z score ^d	0.4 (−0.2, 1.0)	0.2 (−0.3, 0.8)	0.3 (−0.2, 0.8)	0.4 (−0.1, 1.0)	0.5 (−0.2, 1.1)	0.6 (0.0, 1.3)
B-Blocker/ACE inhibitor/ARB use						
Prior Visit Only	6% (38)	3% (2)	3% (6)	6% (9)	6% (10)	11% (11)
Current Visit Only	8% (57)	6% (4)	5% (10)	9% (13)	11% (19)	11% (11)
Both Visits	51% (346)	57% (40)	51% (98)	48% (72)	53% (88)	48% (48)
Neither visit	35% (237)	34% (24)	41% (80)	37% (55)	30% (49)	29% (29)
Corticosteroid use	7% (47)	14% (10)	5% (10)	5% (7)	7% (11)	9% (9)
Race-/Age-/Sex-specific Hemoglobin z score ^d	−0.8 (−1.9, 0.5)	0.0 (−1.0, 1.1)	0.0 (−1.2, 0.8)	−0.4 (−1.6, 0.6)	−1.4 (−2.2, −0.1)	−2.3 (−3.4, −1.2)
Age-/Sex-specific BMI z score ^d	0.4 (−0.3, 1.2)	0.9 (0.1, 1.6)	0.5 (−0.3, 1.2)	0.5 (−0.2, 1.3)	0.3 (−0.5, 1.1)	0.1 (−0.6, 1.0)
Serum Albumin, g/dL ^d	4.4 (4.2, 4.6)	4.5 (4.2, 4.6)	4.5 (4.3, 4.7)	4.4 (4.2, 4.6)	4.3 (4.1, 4.5)	4.3 (4.1, 4.4)
Ca × P, mg/dL ^d	42.7 (38.3, 46.9)	39.6 (35.2, 44.2)	41.3 (37.6, 45.2)	42.3 (37.5, 47.0)	43.5 (40.3, 47.3)	47.2 (43.1, 53.3)

Note: Values for categorical variables are given as number (percentage); for continuous variables, as median [interquartile range]. Conversion factor for serum creatinine in mg/dL to μmol/L, ×88.4.

ABPM, ambulatory blood pressure monitoring; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; BP, blood pressure; Ca × P, calcium-phosphorus product; CKD, chronic kidney disease; CKiD, Chronic Kidney Disease in Children; mGFR, measured glomerular filtration rate; eGFR, estimated glomerular filtration rate; LVH, left ventricular hypertrophy; LVMi, left ventricular mass index; SUN, serum urea nitrogen; Scr, serum creatinine

^a eGFR_{Cr} = (0.413 × height in cm)/Scr

^b eGFR_{SUN-cr-cys} = 39.8 × (height in m/Scr)^{0.456} × (1.8/Cystatin C)^{0.418} × (30/SUN)^{0.079} × 1.076 (if male) × (height in m/1.4)^{0.179}

^c N=131 (19%) were missing mean heart rate from 24-hr ABPM

^d Average taken from visit immediately prior to first echocardiogram and visit of first echocardiogram

Longitudinal Association of Cystatin C with measures of Cardiac Structure and Function among Children and Adolescents with Mild-Moderate CKD

Table 2

Exposure	Cardiac Structure (LVMI; n=678; 1,228 visits)	Systolic Function (Shortening Fraction; n=581; 953 visits)		Diastolic Function (n=678; 1,228 visits)			
				E/A Ratio	E'/A' Ratio	E'/E' Ratio	E'/E' Ratio
Cystatin C, per 1-mg/L greater	7.7% (5.3% to 10.0%)	0.6	-1.1, 2.2	-4.7	-7.0, -2.4	-6.6	-9.0, -4.2
Covariates							
Baseline Age, per 1-y older	-0.7	-0.7	-1.0, -0.3	0.9	0.4, 1.5	-0.2	-0.8, 0.4
Female sex, vs Male sex	-2.3	0.02	-2.3, 2.4	-1.7	-5.2, 2.1	1.1	-3.0, 5.4
African American, vs non-African American	6.9	-1.3	-4.7, 2.3	5.5	0.1, 11.1	-1.4	-7.0, 4.4
Age-/Sex-specific BMI z score ^a , per 1-SD greater	6.4	0.3	-0.8, 1.3	-2.1	-3.6, -0.5	-4.3	-5.9, -2.6
Age-/Sex-/Height-specific SBP z score ^a , per 1-SD greater	5.0	2.9	1.4, 4.5	0.8	-1.3, 3.0	0.3	-1.9, 2.6
Age-/Sex-/Height-specific DBP z score ^a , per 1-SD greater	1.0	-2.3	-3.9, -0.7	-3.1	-5.3, -0.8	-5.7	-8.0, -3.4
Length of time with CKD, per 1-y longer	-0.4	0.3	-0.01, 0.6	0.3	-0.2, 0.7	0.3	-0.2, 0.8
Log(Ca ²⁺ P) ^a , mg ² /dL ²	-0.3	---	---	11.6	-1.4, 26.4	15.2	1.1, 31.2
Mean Heart Rate from 24-h ABPM, per 1 bpm greater	---	-0.05	-0.2, 0.1	---	---	---	---
Race-/Age-/Sex-specific Hemoglobin z score ^a , per 1-SD greater	---	-0.6	-1.4, 0.1	---	---	---	---
Serum Albumin, per 1-g/dL greater ^a	---	-1.1	-4.4, 2.3	---	---	---	---
β-Blocker, ACE inhibitor, ARB use ^b	---	0.7	-0.1, 1.6	---	---	---	---

Note: Values are given as % change (95% confidence interval). Each analysis is adjusted for the covariates listed in each respective column

^a Average taken from previous and current visits

^b Ordinal valued β-blocker, ACE inhibitor, or ARB use at neither prior nor current visit, prior visit only, current visit only, or both prior and current visits

Abbreviations and definitions: ABPM – ambulatory blood pressure monitoring; ACE – angiotensin-converting enzyme; ARB – angiotensin II receptor blocker; BMI – body mass index; CKD – chronic kidney disease; DBP – diastolic blood pressure; LVMI, left ventricular mass index; SBP – systolic blood pressure; SD, standard deviation; E/A ratio (early-to-atrial LV filling ratio), E'/A' ratio (early-to-late septal mitral annular peak velocity) and E'/E' ratio (estimate of LV filling pressure).

Table 3

Longitudinal Analyses of Correlates of Diastolic Function among Children and Adolescents with Mild-Moderate CKD

	E'/A' Ratio		E/E' Ratio	
Model 1: Cystatin C, per 1 mg/L greater	-6.0% (-8.4% to -3.5%)		2.4	0.2, 4.6
Model 2: eGFR_{cr}^b, per 25-mL/min/1.73 m² decrease	-3.5	-5.9, -1.1	0.6	-1.4, 2.6
Model 3: eGFR_{SUN-cr-cys}^c, per 25-mL/min/1.73 m² decrease	-5.0	-7.6, -2.3	1.6	-0.6, 3.8
Model 4: Serum Creatinine^d, per 1-mg/dL greater	-3.3	-5.2, -1.4	0.5	-1.1, 2.1
Model 5: mGFR, per 25-mL/min/1.73 m² decrease	-3.7	-5.8, -1.7	1.9	0.2, 3.6
Model 6: mGFR + Cystatin C				
mGFR, per 25-mL/min/1.73 m ² decrease	-0.9	-3.7, 1.9	1.2	-1.1, 3.5
Cystatin C, per 1-mg/L greater	-5.3	-8.6, -1.8	1.3	-1.6, 4.3
Model 7: eGFR_{cr} + Cystatin C				
eGFR _{cr} , per 25-mL/min/1.73 m ² decrease	-0.7	-4.0, 2.6	-1.6	-4.2, 1.1
Cystatin C, per 1-mg/L greater	-6.6	-9.9, -3.1	3.6	0.6, 6.7

Note: Values are given as %change (95% confidence interval). Models are adjusted for baseline age, sex, race; length of time with CKD; average of concurrent and prior age-/sex-specific body mass index *z* score, log(calcium-phosphorus product), Systolic Blood Pressure *z* score, Diastolic Blood Pressure *z* score

$$^b \text{eGFR}_{\text{cr}} = (0.413 \times \text{height in cm}) / \text{Scr}$$

$$^c \text{eGFR}_{\text{SUN-cr-cys}} = 39.8 \times (\text{height in m} / \text{Scr})^{0.456} \times (1.8 / \text{Cystatin C})^{0.418} \times (30 / \text{SUN})^{0.079} \times 1.076 \text{ (if male)} \times (\text{height in m} / 1.4)^{0.179}$$

$$^d \text{Multivariable model further adjusted for height in cm}$$

Abbreviations and definitions: CKD, chronic kidney disease; CKiD – Chronic Kidney Disease in Children study; eGFR – estimated glomerular filtration rate; mGFR – glomerular filtration rate measured by the plasma disappearance of iohexol. E'/A' ratio (early-to-late septal mitral annular peak velocity); E/E' ratio (estimate of LV filling pressure); SUN, serum urea nitrogen; Scr, serum creatinine