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Prognostic Value of Fibroblast Growth Factor 23 in Autosomal Dominant Polycystic Kidney Disease

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive cyst growth and a loss of functioning renal mass, but a decline in glomerular filtration rate (GFR) and onset of end-stage renal disease (ESRD) occur late in the disease course. There is therefore a great need for early prognostic biomarkers in this disorder.

Methods: We measured baseline serum fibroblast growth factor 23 (FGF23) levels in 192 patients with ADPKD from the Consortium for Radiologic Imaging Studies of PKD (CRISP) cohort that were followed for a median of 13 years and tested the association between FGF23 levels and change over time in height-adjusted total kidney volume (htTKV), GFR, and time to the composite endpoints of ESRD, death, and doubling of serum creatinine.

Results: Patients in the highest quartile for baseline FGF23 level had a higher rate of increase in htTKV (0.95% per year, $P = 0.0016$), and faster rate of decline in GFR (difference of −1.03 ml/min/1.73 m^2 per year, $P = 0.005$) compared with the lowest quartile, after adjusting for other covariates, including htTKV and genotype. The highest quartile of FGF23 was also associated with a substantial increase in risk for the composite endpoint of ESRD, death, or doubling of serum creatinine (hazard ratio [HR] of 2.45 in the fully adjusted model, $P = 0.03$).

Conclusion: FGF23 is a prognostic biomarker for disease progression and clinically important outcomes in ADPKD, and has additive value to established imaging and genetic biomarkers.

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ADPKD affects 1 in 1000 individuals. It is characterized by exponential growth of cyst and total kidney volume, leading to loss of GFR, and eventually ESRD. However, GFR can be preserved for several decades, perhaps because of compensatory hyperfiltration, so that GFR decline occurs late in the natural history of the disease.[1] There is therefore a great need for early prognostic biomarkers in this disorder. Currently, the best biomarker is TKV, usually normalized to height (htTKV), as measured by magnetic resonance imaging scans,[2,3] or htTKV factored by age and categorized into prognostic classes by the method of Irazabal *et al.*[4] Genotype is also predictive, with mutations in *PKD1*, and particularly truncating mutations, conferring a poorer prognosis than patients with mutations in *PKD2* or those with no mutations detected.[5,6]

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FGF23 is a bone-derived phosphaturic hormone. Serum FGF23 levels are elevated at an early stage in patients with chronic kidney disease (CKD)[7] and is a strong risk factor for ESRD, cardiovascular morbidity, and mortality.[8] Recent studies in rats suggest that this is caused by decreased renal clearance and metabolism of FGF23.[9,10] Patients with ADPKD have markedly elevated serum FGF23 levels that are out of proportion to their level of kidney function.[11,12] Interestingly, FGF23 expression can be induced in the kidney with injury,[9,13] and it is ectopically expressed in the cyst-lining epithelium in rats with PKD.[14] These findings suggest the intriguing possibility that FGF23 might provide unique information about ADPKD disease progression or be additive with imaging or other conventional biomarkers. Klotho, the obligate coreceptor for FGF23, is a membrane protein expressed in the kidney and parathyroid gland, but its extracellular domain can be cleaved and released into the blood (soluble Klotho). Serum soluble Klotho levels show a reciprocal pattern to that of FGF23 and are suppressed in CKD.[15]

The association between FGF23 and outcomes has been examined in 1 study, using longitudinal data from the HALT-PKD trial.[16] This study found that high FGF23 levels were associated with an increase in the rate of htTKV growth and with a faster rate of decline in eGFR. However, FGF23 did not improve the prediction of eGFR decline when added to a model with baseline htTKV. HALT-PKD was conducted over a relatively short period of time (5 years) and few patients reached ESRD. The aim of the current study was to test whether FGF23 levels predict ADPKD outcomes over a longer follow-up period in the Consortium for Radiologic Imaging Studies of PKD (CRISP) cohort, and to test whether it predicts clinical outcomes, such as death, ESRD, or doubling of serum creatinine. We tested whether FGF23 had additive value to conventional prognostic markers such as baseline htTKV and genotype and compared them with other biochemical markers.

METHODS

Study Population and Procedures

Participants in the CRISP study with an available baseline serum sample were eligible for inclusion. CRISP is a prospective, observational, longitudinal, multicenter study, begun in 2001, that enrolled 241 individuals 15-46 years of age with ADPKD and preserved kidney function (defined as creatinine clearance $\geq$70 ml/min and serum creatinine $\leq$1.6 mg/dl in males and $\leq$1.4 mg/dl in females). Detailed descriptions of the CRISP study protocol and the baseline characteristics of the cohort have been published previously.[2,17] A history of hypertension was defined as those previously diagnosed who were taking antihypertensive medications at baseline or those with systolic and diastolic blood pressures >140/90 mm Hg on 3 consecutive visits during participation in the study. Enrolled participants were followed between 2001 and 2005 (CRISP I) with yearly visits. At each visit, TKV was determined from magnetic resonance imaging scans using a stereologic method[17–19] and corrected for height (htTKV, ml/m). Patients were classified into categories according to their baseline htTKV factored for age, using the method of Irazabal *et al.*[4] (i.e., Mayo Imaging Class). GFR was measured by iothalamate clearance and indexed to body surface area (ml/min/1.73 m^2). Screening for *PKD1* and *PKD2* mutations was performed by denaturing high-performance liquid chromatography, followed by direct sequencing, together with screening for larger deletions or reverse transcription–polymerase chain reaction to test for abnormal splicing, as previously described.[20] A 24-hour urine sample was collected at baseline for determination of creatinine, urea nitrogen, protein, and electrolyte excretion. Urine osmolality averaged over 24 hours was estimated using the equation urine urea nitrogen in mg/L/28 + 2 $\times$ (urine sodium + potassium in mEq/L).[21] Urine monocyte chemoattractant protein 1 (MCP-1) concentration was assayed by enzyme-linked immunosorbent assay and normalized to urine creatinine concentration.[22] Two years after completion of the initial study, 201 participants that had not yet reached ESRD were re-enrolled in a 5-year follow-up study (CRISP II).[23] Finally, beginning in 2012, 165 participants were re-enrolled in CRISP III. In CRISP II and III, TKV and GFR were measured every 2 years.

Blood Measurements

Baseline stored serum samples from CRISP study participants were obtained from the National Institute of Diabetes and Digestive and Kidney Diseases Central Repository. Commercial enzyme-linked immunosorbent assay kits were used to assay intact FGF23 (Kainos, Kyoto, Japan), soluble Klotho (Immuno-Biological Laboratories, Gunma, Japan), and intact PTH 1-84 (Alpco, Salem, New Hampshire, USA).

Statistical Methods

We included all subjects with available data on FGF23, age, gender, hypertension, genotype, baseline GFR, and baseline htTKV for our primary analyses. Differences between groups in baseline characteristics were compared with either analysis of variance or Kruskal–Wallis test for continuous variables and the χ^2 test for categorical variables. Linear mixed models

were used to model the trajectories of htTKV and GFR. This allowed for repeated measures over time for each participant and the use of all available longitudinal data. Because kidney volume increases exponentially over time, htTKV was natural log-transformed. The intercept for each patient was allowed to vary randomly. All other covariates were modeled as fixed effects. The explanatory variable of interest was serum FGF23. This was discretized into quartiles, with the lowest quartile used as the reference, for the primary analysis. The interaction of each predictor variable with time was used to assess its influence on the rate of change ("slope") of ln(htTKV) and GFR. The base model was adjusted for baseline age and sex, and their interactions with time. Additional covariates that were included in the fully adjusted models were a history of hypertension, genotype (*PKD1* mutation, *PKD2* mutation, or no mutation detected), and (for GFR models) either htTKV or Mayo Imaging Class, together with their interactions with time. Composite time-to-event outcomes of death or ESRD (defined as the need for kidney transplantation or dialysis), both with and without doubling of serum creatinine, were estimated using the Kaplan–Meier method. Cox regression analysis was used to determine the effect of serum FGF23 on these endpoints after adjustment for potential confounders. All P values were 2-sided and considered statistically significant if < 0.05. Statistical analyses were performed using SAS software (version 9.3; SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Patient Baseline Characteristics

Of 241 patients enrolled in the CRISP study, 192 had baseline serum samples available for analysis and were included in this study. The baseline characteristics of the included patients were similar to the entire cohort (Supplementary Table S1). Patients were categorized into quartiles according to their baseline serum level of FGF23.

Patients with higher FGF23 levels were more likely to be male, have hypertension, and have a *PKD1* mutation (Table 1). They also had lower estimated GFR values, higher serum creatinine, higher htTKV and total cyst volume, higher hemoglobin, higher urine sodium and phosphorus excretion, and higher fractional excretion of phosphorus.

Association of FGF23 With Kidney Size and Growth Rate

The median duration of follow-up in this study was 13.0 years, during which htTKV for the entire study population grew at an annual rate of 7.1% ± 5.9% (mean ± SD). The association between FGF23 and kidney volume was assessed using linear mixed models. Because age and sex are known determinants of kidney volume and growth rate, they were included as covariates in the base model. Compared with the lowest quartile of serum FGF23 level, FGF23 levels in quartiles 3 and 4 were associated with greater htTKV at baseline, while FGF23 level in the highest quartile was associated with greater ln(htTKV) slope (Figure 1, A, and Table 2). After further adjustment for genotype and a history of hypertension, the association between the highest FGF23 quartile and ln(htTKV) slope remained statistically significant (growth rate difference of 0.95% per year compared with the lowest quartile, $P = 0.0016$). Higher hemoglobin level was associated with slower kidney growth rate, but adding it to the models did not alter the association of FGF23 with ln(htTKV) slope and did not improve model fit (Supplementary Tables S2 and S3).

Association of FGF23 With GFR

During the study, GFR declined at a rate of 2.87 ± 3.89 ml/min/1.73 m^2 per year (mean ± SD). In the base model, the highest quartile of FGF23 was associated with a faster decline in GFR slope, but not with baseline GFR (Figure 1, B, and Table 3). After further adjustment for genotype, a history of hypertension, and baseline htTKV, the association between FGF23 and GFR slope remained statistically significant (difference of −1.03 ml/min/1.73 m^2 per year for the highest compared with the lowest quartile of FGF23, $P = 0.005$). The findings were similar when the model was adjusted for Irazabal class instead of htTKV (Table 3), and when creatinine-based eGFR using the Chronic Kidney Disease Epidemiology Collaboration equation was used as the outcome instead of measured GFR (Supplementary Table S4). Higher hemoglobin level was associated with slower rate of GFR decline but adding it to the models did not alter the association of FGF23 with GFR slope and did not improve model fit (Supplementary Tables S2 and S3).

To compare the effect size of different biomarkers, FGF23 was treated as a continuous variable in the adjusted model. FGF23 level was negatively associated with GFR slope (−0.009 ml/min/1.73 m^2 per year for each 1-pg/ml increase in serum FGF23, $P = 0.03$). The difference in GFR slope associated with an increase in biomarker level spanning the interquartile range (IQR) for the study cohort (IQR shift) was used as a measure of standardized effect size (Table 4). The IQR shift for FGF23 (−0.24 ml/min/1.73 m^2 per year) was comparable

Table 1. Baseline characteristics of study population, stratified by quartiles of FGF23

Baseline characteristic	Quartile 1, <42.9 pg/ml, $n = 48$	Quartile 2, 42.9-52.0 pg/ml, $n = 48$	Quartile 3, 52.0-67.6 pg/ml, $n = 48$	Quartile 4, >67.6 pg/ml, $n = 48$	*P* value
Age, yr	33.3 (8.6)	30.6 (8.5)	34.2 (8.3)	32.6 (8.2)	0.2
Female sex, *n* (%)	38 (79.2)	32 (66.7)	27 (56.3)	21 (43.8)	0.003
Race/ethnicity, *n* (%)					0.27
White	43 (89.6)	39 (81.3)	42 (87.5)	46 (95.8)	
African American	4 (8.3)	6 (12.5)	6 (12.5)	2 (4.2)	
Hispanic	0 (0)	2 (4.2)	0 (0)	0 (0)	
Asian	1 (2.1)	1 (2.1)	0 (0)	0 (0)	
Hypertension, *n* (%)	26 (54.2)	23 (47.9)	35 (72.9)	34 (70.8)	0.03
ACEI or ARB use, *n* (%)	18 (37.5)	19 (40.4)	29 (60.4)	27 (56.3)	0.06
Kidney function					
Iothalamate GFR, ml/min/1.73 m^2	99.2 (22.3)	102.1 (20.6)	90.4 (22)	95.9 (26)	0.07
CKD-EPI eGFR, ml/min/1.73 m^2	92.3 (24.3)	98.7 (20)	90.3 (20.1)	85.8 (21.6)	0.03
Serum creatinine, mg/dl	0.9 (0.2)	0.9 (0.2)	1 (0.2)	1.1 (0.2)	0.001
htTKV, ml/m	505.6 (278.4)	554.5 (324.4)	720.9 (461)	679.5 (337.4)	0.01[a]
Total cyst volume, ml	363.8 (363.8)	452 (455.7)	685.1 (630.4)	597.3 (502.1)	0.01[a]
Mayo Imaging Class					0.02[b]
1A	7 (14.58)	2 (4.2)	3 (6.3)	0 (0)	
1B	10 (20.8)	18 (37.5)	10 (20.8)	9 (18.8)	
1C	20 (41.7)	9 (18.8)	17 (35.4)	16 (33.3)	
1D	8 (16.7)	11 (22.9)	10 (20.8)	10 (20.8)	
1E	3 (6.3)	7 (14.6)	7 (14.6)	12 (25)	
2A	0 (0)	1 (2.1)	1 (2.1)	1 (2.1)	
2B	0 (0)	0 (0)	0 (0)	0 (0)	
PKD mutation					0.05
PKD1	33 (68.8)	37 (77.1)	37 (77.1)	44 (91.7)	
PKD2 or NMD	15 (31.3)	11 (22.9)	11 (22.9)	4 (8.3)	
Mineral bone parameters					
Serum calcium, mg/dL	9.2 (0.4)	9.1 (0.4)	9.2 (0.4)	9.3 (0.4)	0.22
Serum phosphorus, mg/dL	3.4 (0.5)	3.6 (0.6)	3.6 (0.5)	3.6 (0.6)	0.23
Serum magnesium, mg/dL	1.8 (0.2)	1.8 (0.3)	1.8 (0.2)	1.8 (0.2)	0.81
Alkaline phosphatase, U/L	88.1 (48.3)	71.5 (39.6)	86.1 (65.6)	83.3 (50.1)	0.45[a]
Intact PTH, pg/mL	33.3 (14.5)	30.1 (19.2)	38.3 (25.7)	35.5 (19)	0.26
Urine calcium (24-hr), mg	151.8 (111.8)	170.5 (96.6)	159 (97.3)	153.4 (120.7)	0.37[a]
Urine phosphorus (24-hr), mg	838 (385.4)	854.3 (293.8)	1002.5 (698.8)	1069.9 (427.7)	0.007[a]
FEphos, %	17.5 (7.9)	14.9 (5.9)	16.8 (5.8)	18.6 (7.1)	0.02[a]
Soluble Klotho, pg/mL	903.5 (963.3)	1082 (1448.4)	877.3 (478.4)	803.1 (403.1)	0.57[a]
Intact FGF23, pg/mL	34.5 (6.2)	47.5 (2.5)	59.3 (4.7)	91.8 (39.2)	
Hemoglobin, g/dL	12.9 (1.2)	13.3 (1.3)	13.6 (1.2)	13.7 (1.3)	0.007
Urine protein (24-hr), mg	213.2 (211.6)	200 (157)	222.3 (140.5)	271.9 (239.1)	0.54[a]
Urine sodium (24-hr), mg	159.6 (78.6)	184.9 (90.5)	200.5 (90)	205.5 (74.7)	0.04
Urine osmolality (24-hr), mOsm/kg	342.9 (137)	358.9 (142.8)	367.7 (152.4)	379.8 (158.3)	0.73[a]
Urine MCP-1, pg/mg creatinine	477.2 (344.7)	560.7 (518.8)	682.4 (572.3)	671.9 (566.4)	0.20[a]

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; htTKV, height-adjusted total kidney volume; NMD, no mutation detected; PTH, parathyroid hormone; FEphos, fractional excretion of phosphorus; MCP-1, monocyte chemoattractant protein 1.
[a]Nonparametric test used.
[b]Class 2A/B cases omitted for this calculation because of low cell counts.

to that for urine MCP-1 (−0.36 ml/min/1.73 m^2 per year, $P = 0.004$) and urine osmolality (−0.46 ml/min/1.73 m^2 per year, $P = 0.04$). By contrast, serum Klotho showed a small and nonsignificant positive association with GFR slope.

Association of FGF23 With Time to Doubling of Creatinine, ESRD, or Death

During the follow-up period, 64 (33.3%) patients reached the composite endpoint of doubling of serum creatinine, ESRD, or death. Time to event was significantly associated with quartiles of FGF23 levels ($P = 0.0001$ by log-rank test, Figure 2, A). In the fully adjusted Cox model (Table 5), the highest quartile of FGF23 level was significantly associated with an increased risk of the composite endpoint (HR 2.45, $P = 0.03$). FGF23 level was also associated with renal death (death or ESRD, $P = 0.004$ by log-rank test, Figure 2B), but this was not statistically significant in the adjusted models.

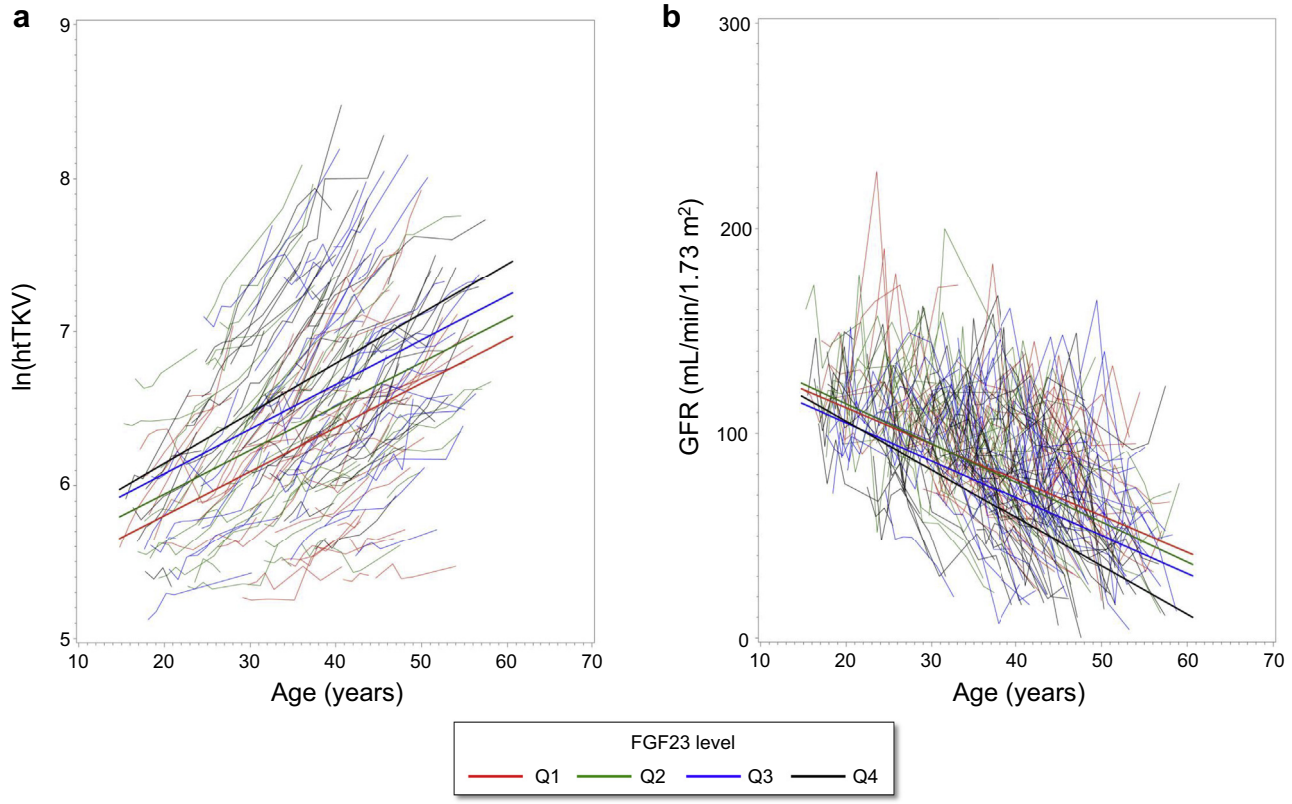

Figure 1. Linear mixed modeling of autosomal dominant polycystic kidney disease progression by fibroblast growth factor 23 (FGF23) level. (A) Log of height-adjusted total kidney volume (htTKV) in ml/m. (B) Glomerular filtration rate (GFR) measured by iothalamate clearance. The spaghetti plots show individual trajectories for the 192 study participants. Bold straight lines show the modeled trajectories color-coded for each quartile of FGF23 level (Q1–Q4), adjusted for baseline age and sex.

DISCUSSION

Our study confirms that serum FGF23 concentration in patients with ADPKD is associated with the rate of increase in htTKV and the rate of decline in GFR. This association appeared to be nonlinear and only significant for the highest quartile of FGF23 (>67.6 pg/ml). Elevated FGF23 level was also associated with increased risk of doubling of serum creatinine, ESRD, or death. This is of particular interest as these are the hard clinical endpoints or surrogate endpoints that are used in clinical trials of chronic kidney diseases.[24]

Chonchol *et al.*[16] made similar observations in an earlier analysis of the HALT-PKD study. We tried to adjust for the minimum number of covariates, focusing on potential confounders or important alternative biomarkers. In the analysis by Chonchol *et al.*,[16] they adjusted for a larger number of variables, some of which may have had a complex relationship with the predictor and the outcome. Importantly though, in both their and our current analyses, the association of FGF23 with outcome was either preserved or minimally attenuated after adjusting for covariates. An important difference is that in the HALT-PKD study, FGF23 did not substantially improve prediction of kidney function decline over htTKV. By contrast, we can now show that FGF23 is an independent and robust predictor of kidney function decline even after adjusting for the most strongly predictive biomarkers currently available, which are either htTKV or Mayo Imaging Class, combined with PKD genotype. Although CRISP was a much smaller study, the substantially longer follow-up (13 years compared with 5.4 years for HALT-PKD) may have given it more power to detect temporal differences in renal outcomes.

In both our study and that of Chonchol *et al.*,[16] the magnitude of the association of FGF23 with htTKV was relatively small, whereas the effect size of its association with decline in GFR and renal events was fairly large and (in our analysis) largely independent of htTKV. Previous studies have shown that the association of FGF23 with loss of kidney function is a general phenomenon in CKD, regardless of etiology.[8,25–27] In ADPKD, FGF23 could be playing a role in nephron loss independent of its effect on cyst and kidney volume growth.

The source of the early FGF23 elevation in ADPKD is unknown. We find that FGF23 level is not associated with serum phosphorus level but is positively associated with urinary phosphorus excretion. This suggests

Table 2. Linear model for the outcome of ln(htTKV) with FGF23 by quartiles as the explanatory variable

	Base model[a]				Adjusted model[b]			
	Estimate	SE	Exp(estimate)[c]	*P* value	Estimate	SE	Exp(estimate)[c]	*P* value
Intercept	5.304	0.177	201	<0.0001	4.922	0.172	137	<0.0001
Time, yr	0.0586	0.004338	1.060	<0.0001	0.0581	0.0050	1.060	<0.0001
FGF23, pg/ml								
<42.9	Reference				Reference			
42.9-52.0	0.143	0.1125	1.153	0.21	0.113	0.097	1.119	0.25
52.0-67.6	0.270	0.113	1.310	0.017	0.155	0.099	1.168	0.12
>67.6	0.321	0.1153	1.379	0.005	0.134	0.102	1.143	0.19
FGF23 × time, pg/ml								
<42.9	Reference				Reference			
42.9-52.0	−0.0007	0.0029	0.999	0.82	−0.0012	0.0028	0.999	0.68
52.0-67.6	0.0011	0.0030	1.001	0.70	−0.0021	0.0030	0.998	0.48
>67.6	0.0120	0.0030	1.012	<0.0001	0.0095	0.0030	1.009	0.002
Age, yr	0.0238	0.0047	1.024	<0.0001	0.0192	0.0044	1.019	<0.0001
Age × time	−0.0006	0.0001	0.999	<0.0001	−0.0008	0.0001	0.999	<0.0001
Sex								
Female	Reference				Reference			
Male	−0.003	0.084	0.997	0.97	0.038	0.073	1.039	0.60
Sex × time								
Female	Reference				Reference			
Male	0.0231	0.0023	1.023	<0.0001	0.0236	0.0022	1.024	<0.0001
Hypertension					0.389	0.076	1.475	<0.0001
Hypertension × time					0.0120	0.0022	1.012	<0.0001
Genotype								
PKD2 or NMD					0			
PKD1					0.460	0.086	1.585	<0.0001
Genotype × time								
PKD2 or NMD					0			
PKD1					0.0005	0.0026	1.001	0.85

FGF23, fibroblast growth factor 23; htTKV, height-adjusted total kidney volume; NMD, no mutation detected; SE, standard error.
[a]Base model includes baseline age and sex as covariates.
[b]Adjusted model is base model, plus adjustment for hypertension and genotype.
[c]For the intercept, this represents the htTKV in ml/m at age 0, with all of the other variables set to the reference category. For categorical variables, this represents the ratio of htTKV for the indicated category relative to the reference category. For time, age, and interaction with time variables, this represents the proportional increment in htTKV per year.

that FGF23 in ADPKD is not stimulated by phosphate retention with decreasing renal function but by some other mechanism. Studies have shown elevation in circulating FGF23 levels after uninephrectomy in rats[9,10] and after living kidney donation in humans,[28] independent of any change in GFR. Interestingly, it has been postulated that patients with ADPKD have compensatory glomerular hyperfiltration, based on the observation that GFR in ADPKD can be preserved for several decades.[1,29] We speculate that the reason serum FGF23 levels are elevated in ADPKD out of proportion to the decrease in overall GFR may be because FGF23 is a biomarker for loss of functioning nephrons that is masked early on by hyperfiltration.

Although Klotho is generally regulated reciprocally to FGF23, and low levels are found in patients with ADPKD, we found that FGF23 is a much better prognostic biomarker. Indeed, using IQR shift as a standardized effect size to compare to known biomarkers, FGF23 was found to be comparable to urine MCP-1 and urine osmolality.

The main limitation of our study is the relatively small sample size and statistical power. This may have limited the ability to detect additional associations after adjusting for multiple covariates. Because the CRISP cohort included a young population of patients with well-preserved GFR, there were relatively few ESRD events and deaths during the follow-up period, so it was underpowered to assess the risk of these outcomes in adjusted models. Finally, because this is an observational study, we cannot determine whether FGF23 levels are causal in the patient outcomes.

A major strength of the study is the long duration of follow-up. CRISP is the longest observational cohort study in patients with ADPKD. This allowed us to accurately ascertain GFR slopes. We were able to use both directly measured and estimated GFR as outcomes. Genotype and htTKV, the other major predictors of GFR trajectory, were assessed in all patients in the cohort and so we were able to determine that FGF23 had additive predictive value even when these were included in the model.

Table 3. Linear model for the outcome of GFR with FGF23 by quartiles as the explanatory variable

	Base model[a]			Adjusted model (including htTKV)[b]			Adjusted model (including MIC)[c]		
	Estimate	SE	*P* value	Estimate	SE	*P* value	Estimate	SE	*P* value
Intercept	138.3	7.6	<0.0001	135.8	7.7	<0.0001	147.7	9.3	<0.0001
Time, yr	1.05	0.53	0.048	1.64	0.58	0.005	3.47	0.71	<0.0001
FGF23, pg/ml									
<42.9	Reference			Reference			Reference		
42.9-52.0	2.9	4.8	0.55	3.9	4.3	0.36	1.6	4.5	0.71
52.0-67.6	−6.9	4.8	0.15	−2.7	4.4	0.54	−5.5	4.4	0.21
>67.6	−3.4	4.9	0.49	−0.8	4.5	0.86	−2.3	4.6	0.61
FGF23 × time, pg/ml									
<42.9	Reference			Reference			Reference		
42.9-52.0	−0.54	0.35	0.13	−0.11	0.34	0.74	−0.32	0.36	0.37
52.0-67.6	−0.24	0.36	0.50	0.35	0.36	0.32	0.11	0.36	0.76
>67.6	−1.87	0.36	<0.0001	−1.03	0.36	0.005	−1.09	0.37	0.003
Age, yr	−1.11	0.20	<0.0001	−0.79	0.20	<0.0001	−1.31	0.21	<0.0001
Age × time	−0.09	0.01	<0.0001	−0.05	0.02	0.001	−0.13	0.02	<0.0001
Sex									
Female	Reference			Reference			Reference		
Male	−0.8	3.6	0.81	−0.2	3.2	0.95	0.0	3.2	0.99
Sex × time									
Female	Reference			Reference			Reference		
Male	−0.02	0.27	0.94	−0.03	0.26	0.90	−0.01	0.26	0.96
Hypertension				−0.7	3.5	0.85	−1.2	3.6	0.73
Hypertension × time				−0.78	0.28	0.005	−0.70	0.29	0.01
Genotype									
PKD2 or NMD				Reference			Reference		
PKD1				3.6	4.0	0.36	4.6	4.2	0.28
Genotype × time									
PKD2 or NMD				Reference			Reference		
PKD1				−0.48	0.32	0.13	−0.37	0.33	0.26
htTKV, ml/m				−0.0198	0.0050	<0.0001			
htTKV × time				−0.0028	0.0005	<0.0001			
MIC									
1A							Reference		
1B							2.3	7.0	0.74
1C							−5.3	7.0	0.45
1D							−14.6	7.5	0.05
1E							−15.5	8.3	0.06
MIC × time									
1A							0		
1B							−0.14	0.54	0.80
1C							−0.78	0.53	0.14
1D							−1.34	0.59	0.02
1E							−3.05	0.65	<0.0001

FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; htTKV, height-adjusted total kidney volume; MIC, Mayo Imaging Class; NMD, no mutation detected; SE, standard error.
[a]Base model includes baseline age and sex as covariates.
[b]Adjusted for baseline age, sex, hypertension, genotype, and htTKV.
[c]Adjusted for baseline age, sex, hypertension, genotype, and MIC.

In conclusion, we find that high serum FGF23 level (≥68 pg/ml) is associated with faster growth in htTKV, faster decline in GFR independent of htTKV, and earlier onset of ESRD, death, or doubling of serum creatinine. FGF23 improves prediction of GFR even in models adjusted for htTKV and genotype and is comparable to urine MCP-1 and urine osmolality. We speculate that FGF23 may be uniquely informative of functioning renal mass in ADPKD. Further studies are necessary to determine whether the association of FGF23 with outcomes represent causal effects.

Table 4. Comparison of biomarker prediction of GFR slope

Biomarker	Estimate (95% CI)	*P* value
Serum FGF23	−0.24 (−0.47 to −0.02)	0.03
Serum Klotho	0.002 (−0.007 to 0.011)	0.61
Urine MCP-1	−0.36 (−0.61 to −0.12)	0.004
Urine protein	0.01 (−0.29 to 0.27)	0.93
Urine sodium	−0.17 (−0.47 to 0.12)	0.24
Urine osmolality	−0.46 (−0.89 to −0.03)	0.04

CI, confidence interval; MCP-1, monocyte chemoattractant protein 1.
Estimates represent the difference in GFR slope (ml/min/1.73 m^2 per year) for a shift in biomarker level from the 25th to 75th percentile for the study population (interquartile range shift). Each biomarker was individually assessed in a fully adjusted model with age, sex, hypertension, genotype, baseline height-adjusted total kidney volume, and their interactions with time.

Table 5. Adjusted Cox model for risk of ESRD, death, or doubling of serum creatinine

	ESRD, death, or doubling of serum creatinine[a] (64 events)		ESRD or death[a] (37 events)	
	HR	*P* value	HR	*P* value
FGF23, pg/ml				
<42.9	Reference		Reference	
42.9-52.0	0.97	0.95	0.58	0.43
52.0-67.6	1.19	0.69	1.05	0.94
>67.6	2.45	0.03	1.85	0.27
Age, yr	0.99	0.50	1.02	0.56
Sex, male	1.11	0.69	1.01	0.97
Baseline GFR, ml/min/1.73 m^2	0.97	<0.0001	0.95	0.0002
Hypertension	2.46	0.02	2.77	0.07
PKD1 genotype	1.55	0.31	1.65	0.38
HtTKV, mL/m	1.002	<0.0001	1.002	<0.0001

ESRD, end-stage renal disease; FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; HR, hazard ratio; htTKV, height-adjusted total kidney volume.
[a]Adjusted for baseline age, sex, baseline GFR, hypertension, genotype, and htTKV.

DISCLOSURE

ASLY is a consultant for Regulus Therapeutics, Calico Life Sciences, and Navitor Pharmaceuticals, and has served on an advisory board for Otsuka Pharmaceuticals. VET is a member of the steering committees for the TEMPO and REPRISE clinical trials, has received research support from Otsuka Pharmaceuticals, and is a consultant for Sanofi, Reata, Palladio, Regulus Therapeutics, Mironid, and Blueprint Medicines. PCH has received research funding from Otsuka Pharmaceuticals. MM is a consultant for Otsuka, Sanofi, Chinook Therapeutics, and Natera, and has received research support from Otsuka Pharmaceuticals, Sanofi, and Chinook Therapeutics. FFR has served as a consultant and an unbranded speaker bureau member for Otsuka, is a consultant for Keryx, Kadmon, and Sanofi, and has received research funding from Otsuka, Kadmon, Sanofi, and Reata. ABC is a consultant for Otsuka, Reata, and Sanofi, and has received research funding from Boston Scientific, Kadmon, and Otsuka. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

MET, ASLY, JDM, JRS, and JJG conceived and designed this study; SZ and JRS performed serum immunoassays; JJG, VET, ABC, MM, FFR, and DPL recruited the patient cohort and collected the data; KTB analyzed the images; PCH performed the genotyping; PL and JDM performed the statistical analysis of the data; MET, ASLY, and JDM wrote the initial draft of the manuscript; all authors contributed to the interpretation of the data and the discussion, revised the manuscript, and approved the final version.

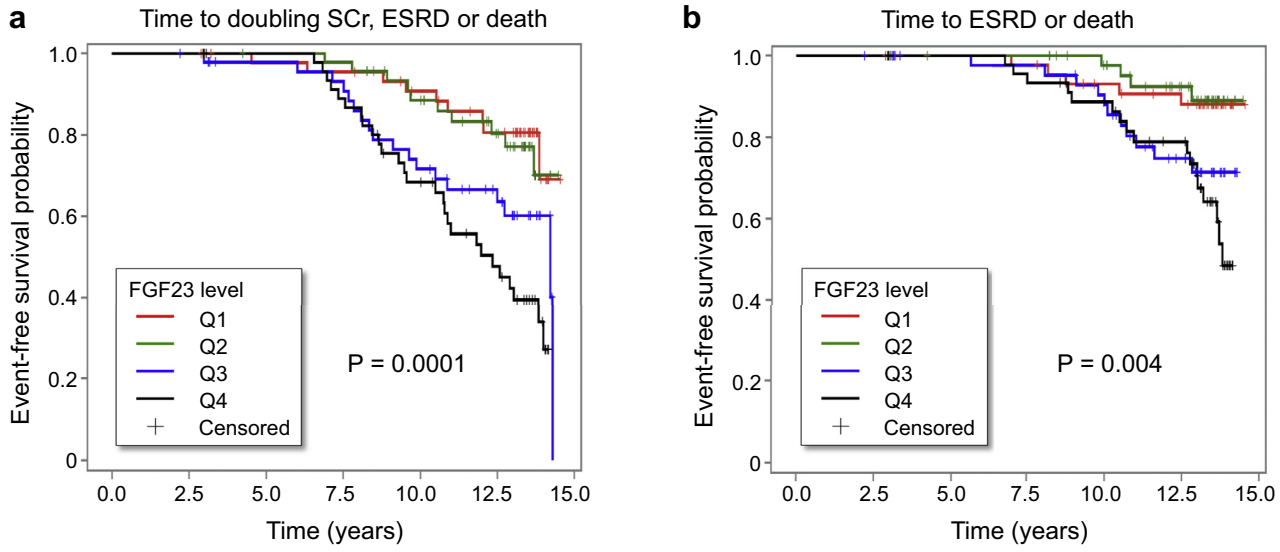

Figure 2. Kaplan–Meier analysis of the probability of survival free of renal events, according to quartiles of fibroblast growth factor 23 (FGF23) level (Q1–Q4). (A) Doubling of serum creatinine (SCr), end-stage renal disease (ESRD), or death. (B) ESRD or death.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Baseline characteristics of study patients, compared to the entire CRISP cohort.

Table S2. Linear models for the ln(htTKV) and GFR with inclusion of hemoglobin as a covariate.

Table S3. Effect of including hemoglobin as a covariate on model fit statistics.

Table S4. Linear model for the outcome of eGFR in fully adjusted model including htTKV.

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