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 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 \(-1.03 \text{ ml/min/1.73 m}^2\) 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.

Kidney Int Rep (2021) 6, 953–961; https://doi.org/10.1016/j.ekir.2021.01.004
KEYWORDS: ADPKD; FGF23
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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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Received 6 October 2020; revised 8 December 2020; accepted 4 January 2021; published online 16 January 2021
FGF23 is a bone-derived phosphaturic hormone. Serum FGF23 levels are elevated at an early stage in patients with chronic kidney disease (CKD) and is a strong risk factor for ESRD, cardiovascular morbidity, and mortality. Patients with ADPKD have markedly elevated serum FGF23 levels that are out of proportion to their level of kidney function. Interestingly, FGF23 expression can be induced in the kidney with injury, and it is ectopically expressed in the cyst-lining epithelium in rats with PKD. 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.

The association between FGF23 and outcomes has been examined in 1 study, using longitudinal data from the HALT-PKD trial. This study found that high FGF23 levels were associated with an increase in the rate of hTKV 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 hTKV. 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 hTKV 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 ≥70 ml/min and serum creatinine ≤1.6 mg/dl in males and ≤1.4 mg/dl in females). Detailed descriptions of the CRISP study protocol and the baseline characteristics of the cohort have been published previously. 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 and corrected for height (hTKV, ml/m). Patients were classified into categories according to their baseline hTKV factored for age, using the method of Irazabal et al. Patients were re-enrolled in a 5-year follow-up study (CRISP II). 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 (Immuo-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 hTKV 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 χ² 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 urinary 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 m² 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 m² 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 m² 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 m² per year) was comparable.
to that for urine MCP-1 (−0.36 ml/min/1.73 m² per year, P = 0.004) and urine osmolality (−0.46 ml/min/1.73 m² 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.

### 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 m² | 99.2 (22.3)               | 102.1 (20.6)                    | 90.4 (22)                       | 95.9 (26)                       | 0.07    |
| CKD-EPI eGFR, ml/min/1.73 m² | 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 (0.2)                       | 1.1 (0.2)                       | 0.001   |
| htkTV, ml/m            | 505.6 (278.4)                   | 554.5 (324.4)                   | 720.9 (461)                     | 679.5 (337.4)                   | 0.01**  |
| Total cyst volume, ml  | 383.8 (363.8)                   | 452 (455.7)                     | 685.1 (630.4)                   | 597.3 (502.1)                   | 0.01**  |
| **Mayo Imaging Class** |                                 |                                 |                                 |                                 | 0.02**  |
| 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                   | 32 (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*   |
| 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**  |
| Urine phosphorus (24-hr), mg | 838 (385.4)                | 854.3 (293.8)                   | 1002.5 (698.8)                  | 1096.9 (427.7)                  | 0.007** |
| FEPhos, %              | 17.5 (7.9)                      | 14.9 (5.9)                      | 16.8 (5.8)                      | 16.8 (7.1)                      | 0.02**  |
| Soluble Klotho, pg/mL  | 903.5 (963.3)                   | 1082 (1448.4)                   | 877.3 (478.4)                   | 803.1 (403.1)                   | 0.57**  |
| Intact FGF23, pg/mL    | 34.5 (8.2)                      | 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*   |
| Urine sodium (24-hr), mg | 159.6 (78.6)                  | 184.9 (90.5)                    | 200.5 (90)                      | 205.6 (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*   |
| Urine MCP-1, pg/mg creatinine | 477.2 (344.7)          | 560.7 (518.8)                   | 682.4 (572.3)                   | 671.9 (566.4)                   | 0.20*   |

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; htkTV, height-adjusted total kidney volume; NMD, no mutation detected; PTH, parathyroid hormone; FEPhos, fractional excretion of phosphorus; MCP-1, monocyte chemoattractant protein 1.

*aNonparametric test used.

*bClass 2A/B cases omitted for this calculation because of low cell counts.
DISCUSSION

Our study confirms that serum FGF23 concentration in patients with ADPKD is associated with the rate of increase in hTKV 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 hTKV. 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 hTKV 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 hTKV 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 hTKV. 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
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.
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 3. Linear model for the outcome of GFR with FGF23 by quartiles as the explanatory variable

|                        | Base model | Adjusted model (including htTKV) | Adjusted model (including MIC) |
|------------------------|------------|---------------------------------|--------------------------------|
|                        | 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 |        |         |
|                        | 3.9        | 4.3    | 0.36    | 1.6      | 4.5    | 0.71    |          |        |         |
| 42.9-52.0              | 2.9        | 4.8    | 0.55    |          |        |         |          |        |         |
|                        | Reference  |        |         | Reference |        |         | Reference |        |         |
|                        | -2.7       | 4.4    | 0.54    | -5.5     | 4.4    | 0.21    |          |        |         |
| 52.0-67.6              | -6.9       | 4.8    | 0.15    |          |        |         |          |        |         |
|                        | Reference  |        |         | Reference |        |         | Reference |        |         |
|                        | -0.8       | 4.5    | 0.86    | -2.3     | 4.6    | 0.61    |          |        |         |
| >67.6                  | -3.4       | 4.9    | 0.49    |          |        |         |          |        |         |
|                        | Reference  |        |         | Reference |        |         | Reference |        |         |
| 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 |        |         | Reference |        |         |
| PKD1                   | 3.6        | 4.0    | 0.36    | 4.6      | 4.2    | 0.28    |          |        |         |
| Genotype × time        |            |        |         |          |        |         |          |        |         |
| PKD2 or NMD            | Reference  |        |         | Reference |        |         | Reference |        |         |
| PKD1                   | -0.48      | 0.32   | 0.13    | -0.37    | 0.33   | 0.26    |          |        |         |
| htTKV, ml/m            | -0.0198    | 0.0050 | <0.0001 | -0.0028  | 0.0005 | <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.

*Base model includes baseline age and sex as covariates.
*Adjusted for baseline age, sex, hypertension, genotype, and htTKV.
*Adjusted for baseline age, sex, hypertension, genotype, and MIC.

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 m² 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 (64 events) | ESRD or death* (37 events) |
|----------------|--------------------------------------------------------|----------------------------|
| **FGF23, pg/ml** | **HR** | **P value** | **HR** | **P value** |
| <42.9          | 0.97   | 0.95        | 0.58   | 0.43        |
| 42.9–52.0      | 1.19   | 0.69        | 1.05   | 0.94        |
| 52.0–67.6      | 2.45   | 0.03        | 1.86   | 0.27        |
| >67.6          | 0.99   | 0.50        | 1.02   | 0.56        |
| Age, yr        | 1.11   | 0.69        | 1.01   | 0.97        |
| Sex, male      | 0.97   | <0.0001     | 0.95   | 0.0002      |
| Baseline GFR, ml/min/1.73 m² | 2.46   | 0.02        | 2.77   | 0.07        |
| Hypertension   | 1.55   | 0.31        | 1.65   | 0.38        |
| PKD1 genotype  | 1.002  | <0.0001     | 1.002  | <0.0001     |
| HTNKV, ml/m    | 0.97   | <0.0001     | 1.07   | <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.

*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, and 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.

**ACKNOWLEDGEMENTS**

Supported by a Clinical and Translational Science award from the National Center for Advancing Translational Sciences to the University of Kansas Medical Center for Frontiers: The Heartland Institute for Clinical and Translational Research (UL1TR000001), by the University of Kansas Medical Center Research Institute, and by National Institute of Diabetes and Digestive and Kidney Diseases grants to the Kansas PKD Research and Translation Core Center (P30 DK106912 and U54 DK126126). The CRISP study is supported by cooperative agreements from the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health (DK056943, DK056956, DK056957, and DK056961), and by R01 DK113111. The investigators are indebted to the study coordinators in CRISP.

**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(hTKV) 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 hTKV.

**REFERENCES**

1. Yu ASL, Shen C, Landsittel DP, et al. Long-term trajectory of kidney function in autosomal-dominant polycystic kidney disease. *Kidney Int*. 2019;95:1253–1261.

2. Grantham JJ, Torres VE, Chapman AB, et al. Volume progression in polycystic kidney disease. *N Engl J Med*. 2006;354:2122–2130.

3. Yu ASL, Shen C, Landsittel DP, et al. Baseline total kidney volume and the rate of kidney growth are associated with chronic kidney disease progression in autosomal dominant polycystic kidney disease. *Kidney Int*. 2017;93:691–699.

4. Irazabal MV, Rangel LJ, Bergstralh EJ, et al. Imaging classification of autosomal dominant polycystic kidney disease: a simple model for selecting patients for clinical trials. *J Am Soc Nephrol*. 2015;26:160–172.

5. Cornec-Le Gall E, Audrezet MP, Chen JM, et al. Type of PKD1 mutation influences renal outcome in ADPKD. *J Am Soc Nephrol*. 2013;24:1006–1013.

6. Heyer CM, Sundsbak JL, Abebe KZ, et al. Predicted mutation strength of nontruncating PKD1 mutations aids genotype-phenotype correlations in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2016;27:2782–2784.

7. Isakova T, Wahl P, Vargas GS, et al. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int*. 2011;79:1370–1378.

8. Isakova T, Xie H, Yang W, et al. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA*. 2011;305:2432–2439.

9. Mace ML, Gravesen E, Nordholm A, et al. Kidney fibroblast growth factor 23 does not contribute to elevation of its circulating levels in uremia. *Kidney Int*. 2017;92:165–178.

10. Mace ML, Gravesen E, Hofman-Bang J, Olgaard K, Lewin E. Key role of the kidney in the regulation of fibroblast growth factor 23. *Kidney Int*. 2015;88:1304–1313.

11. Pavik I, Jaeger P, Kistler AD, et al. Patients with autosomal dominant polycystic kidney disease have elevated fibroblast growth factor 23 levels and a renal leak of phosphate. *Kidney Int*. 2011;79:234–240.

12. Pavik I, Jaeger P, Ebner L, et al. Soluble klotho and autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2012;7:248–257.

13. Zanchi C, Locatelli M, Benigni A, et al. Renal expression of FGF23 in progressive renal disease of diabetes and the effect of ACE inhibitor. *PLoS One*. 2013;8:e70775.

14. Spichtig D, Zhang H, Mohebbi N, et al. Renal expression of FGF23 and peripheral resistance to elevated FGF23 in rodent models of polycystic kidney disease. *Kidney Int*. 2014;85:1340–1350.

15. Pavik I, Jaeger P, Ebner L, et al. Secreted Klotho and FGF23 in chronic kidney disease stage 1 to 5: a sequence suggested from a cross-sectional study. *Nephrol Dial Transplant*. 2013;28:352–359.

16. Chonchol M, Gitomer B, Isakova T, et al. Fibroblast growth factor 23 and kidney disease progression in autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2017;12:1461–1469.

17. Chapman AB, Guay-Woodford LM, Grantham JJ, et al. Renal structure in early autosomal-dominant polycystic kidney disease (ADPKD): The Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) cohort. *Kidney Int*. 2003;64:1035–1045.

18. Bae KT, Commean PK, Lee J. Volumetric measurement of renal cysts and parenchyma using MRI: phantoms and patients with polycystic kidney disease. *J Comput Assist Tomogr*. 2000;24:614–619.

19. Bae KT, Tao C, Zhu F, et al. MRI-based kidney volume measurements in ADPKD: reliability and effect of gadolinium enhancement. *Clin J Am Soc Nephrol*. 2009;4:719–725.

20. Rossetti S, Consugar MB, Chapman AB, et al. Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2007;18:2143–2160.

21. Torres VE, Grantham JJ, Chapman AB, et al. Potentially modifiable factors affecting the progression of autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2011;6:640–647.

22. Zheng D, Wolfe M, Cowley BD Jr, Wallace DP, Yamaguchi T, Grantham JJ. Urinary excretion of monocyte chemotactic protein-1 in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2003;14:2588–2595.

23. Chapman AB, Bost JE, Torres VE, et al. Kidney volume and functional outcomes in autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2012;7:479–486.

24. Bakris GL, Whelton P, Weir M, Mimran A, Keane W, Schiffirin E. The future of clinical trials in chronic renal disease: outcome of an NIH/FDA/Physician Specialist Conference. Evaluation of Clinical Trial Endpoints in Chronic Renal Disease Study Group. *J Clin Pharmacol*. 2000;40:815–825.

25. Titan SM, Zatz R, Gracioli FG, et al. FGF-23 as a predictor of renal outcome in diabetic nephropathy. *Clin J Am Soc Nephrol*. 2011;6:241–247.

26. Kendrick J, Cheung AK, Kaufman JS, et al. FGF-23 associates with death, cardiovascular events, and initiation of chronic dialysis. *J Am Soc Nephrol*. 2011;22:1913–1922.

27. Fliser D, Kollerits B, Neyer U, et al. Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (MMKD) Study. *J Am Soc Nephrol*. 2007;18:2600–2608.

28. Tan SJ, Hewitson TD, Hughes PD, Holt SG, Toussaint ND. Changes in markers of mineral metabolism after living kidney donation. *Transplant Direct*. 2017;3:e150.

29. Grantham JJ, Mulamalla S, Swenson-Fields Kl. Why kidneys fail in autosomal dominant polycystic kidney disease. *Nat Rev Nephrol*. 2011;7:556–566.