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Interactions between FGF23 and Genotype in Autosomal Dominant Polycystic Kidney Disease

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Abstract

Background Higher serum intact fibroblast growth factor 23 (iFGF23) was associated with disease progression in participants with autosomal dominant polycystic kidney disease (ADPKD) in the HALT-PKD Studies. PKD mutation is also an important determinant of progression. We hypothesized that serum levels of iFGF23 and vitamin D metabolites (1,25-dihydroxyvitamin D [1,25$(OH)_2$D] and 25-hydroxyvitamin D [25[OH]D]) differ according to ADPKD mutation and differentially predict clinical end points according to genotype (significant interaction between genotype and mineral metabolites).

Methods A total of 864 individuals with ADPKD who participated in the HALT-PKD Study A or B and had measurements of mineral metabolites (1,25$[OH]_2$D, 25[OH]D, iFGF23) were categorized by PKD mutation (PKD1 truncating, PKD1 nontruncating, PKD2, or no mutation detected [NMD]). The association of the interactions of genotype × iFGF23, genotype × 1,25(OH)2D, and genotype × 25(OH)D with (*1*) annualized change in eGFR; (*2*) mean annualized percentage change in height-corrected total kidney volume (Study A only); and (*3*) time to a composite of 50% reduction in eGFR, ESKD, or death were evaluated using linear regression and Cox proportional hazards regression.

Results Median (interquartile range) iFGF23 differed (PKD1 truncating, 55.8 [40.7–76.8]; PKD1 nontruncating, 49.9 [37.7–71.0]; PKD2, 49.0 [33.8–70.5]; NMD, 50.3 [39.7–67.4] pg/ml; $P=0.03$) and mean±SD 1,25$(OH)_2$D differed (PKD1 truncating, 32.8±12.8; PKD1 nontruncating, 33.4±12.5; PKD2, 34.1±13.1; NMD, 38.0±14.6 pg/ml; $P=0.02$) according to PKD genotype. There was a significant interaction between iFGF23 and genotype ($P=0.02$) for the composite end point in fully adjusted models, but no significant interaction between 1,25$(OH)_2$D or 25(OH)D and genotype for clinical end points.

Conclusions ADPKD genotype interacts significantly with FGF23 to influence clinical end points. Whereas the worst outcomes were in individuals with a PKD1-truncating or -nontruncating mutation and the highest iFGF23 tertile, risk of the composite end point differed according to iFGF23 the most in the PKD1-nontruncating and PKD2 groups.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic and potentially fatal disease, affecting 1 in 400–1000 individuals (1). ADPKD is characterized by the progressive development and enlargement of renal cysts, which eventually destroy the normal parenchyma, leading to ESKD in the majority of afflicted patients (1,2). The disease is genetically heterogeneous, with the majority of cases resulting from mutations in one of two genes, *PKD1* (approximately 78%) and *PKD2* (approximately 15%) (3).

Fibroblast growth factor 23 (FGF23) is a protein synthesized by bone and bone marrow cells that acts as a phosphaturic hormone, and also suppresses renal synthesis of 1,25-dihydroxyvitamin D (1,25$[OH]_2$D) (4,5). We recently demonstrated that higher serum intact FGF23 (iFGF23) concentration was independently associated with adverse clinical end points in participants in the Halt Progression of Polycystic Kidney Disease (HALT-PKD) Studies (6). PKD mutation group (*PKD1* truncating, *PKD1* nontruncating, *PKD2*, or no mutation detected [NMD]) was also an important predictor of adverse clinical end points in HALT-PKD Study participants (7). Thus, we sought to determine whether genotype may modify the effects of iFGF23 (as well as vitamin D metabolites) on clinical end points,

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which is of clinical relevance regarding the utility of such biomarkers in predicting ADPKD progression.

Given the more severe course of disease in individuals with *PKD1*-truncating mutations, we hypothesized that serum levels of iFGF23, 1,25(OH)$_2$D, and 25-hydroxyvitamin D (25[OH]D) differed in HALT-PKD Study participants according to ADPKD genotype. We also hypothesized that mineral metabolites (iFGF23, 1,25[OH]$_2$D, and 25[OH]D) would differentially predict adverse clinical outcomes across the study according to genotype (*i.e.*, a significant interaction between genotype and mineral metabolites).

Materials and Methods

Study Design and Participants

The design of the HALT-PKD Studies A and B have been described in detail previously (8–10). Briefly, the study was two concurrent, prospective, randomized, double-blind, placebo-controlled, multicenter trials. Eligible participants were enrolled across seven clinical sites between February 2006 and June 2009. Study A used a 2×2 factorial design and evaluated the effect of (*1*) multilevel renin-angiotensin-aldosterone system blockade with an angiotensin-converting enzyme inhibitor (ACEi) plus angiotensin receptor blocker compared with ACEi plus placebo, and (*2*) low (95–110/60–75 mm Hg) compared with standard (120–130/70–80 mm Hg) BP control. Study B evaluated only the effect of ACEi plus angiotensin receptor blocker compared with ACEi plus placebo.

All participants had a known diagnosis of ADPKD and either hypertension or high-normal BP. Participants in Study A (n=558) were 15–49 years of age with an eGFR >60 ml/min per 1.73 m^2 using the four-variable Modification of Diet in Renal Disease (MDRD) equation. Participants in Study B (n=486) were 18–64 years of age with an eGFR of 25–60 ml/min per 1.73 m^2 (MDRD equation). Of these participants, n=1002 participated in an FGF23 ancillary study (6) and had measurements of mineral metabolite levels (iFGF23, 1,25[OH]$_2$D, 25[OH]D). An additional n=106 were missing PKD genotype and n=32 were missing other covariates; thus n=864 were included in this analysis.

Study Variables

The primary exposure variable for this analysis was the interaction between serum iFGF23 and PKD genotype. Additionally, we examined the interaction between vitamin D metabolites (1,25[OH]$_2$D and 25[OH]D) and genotype as predictor variables. Mutation analysis was performed previously, with mutation class categorized as *PKD1*-truncating mutations, *PKD1*-nontruncating mutations, *PKD2* mutations, and NMD (7). All participants provided stored serum samples at their baseline visit, which were stored in a central repository at −80°C until they were shipped to the University of Washington for measurement of mineral metabolites. Serum iFGF23 was measured in duplicate using the Kainos immunoassay, which detects the full-length, biologically intact FGF23 molecule *via* midmolecule and distal epitopes, as described previously (6). The intra- and interassay coefficients of variability (CVs) are 3.8% and 3.0%, respectively, for this assay. Vitamin D metabolites (1,25[OH]$_2$D and 25[OH]D) were measured using immunoaffinity purification and liquid chromatography–tandem mass spectrometry. The analytical measurement range for the 25(OH)D assay was 7–150 ng/ml. The intra-assay CVs were 5.6% and 4.5% at 11 and 28 ng/ml, respectively; whereas the interassay CVs were 9.1% and 5.6% at 16 and 51 ng/ml, respectively. For 1,25(OH)$_2$D, the range of the assay was 5–200 pg/ml. The intra-assay CVs were 12.6% and 9.7% at 13 and 45 pg/ml, respectively; whereas the interassay CVs were 21.4% and 14.7% at 25 and 56 pg/ml, respectively. Intact serum parathyroid hormone concentrations were also measure by the University of Washington using an automated two-site immunoassay (Beckman-Coulter, Inc., Brea, CA) (interassay CV between 3.4% and 6.1%).

The outcomes of this analysis were decided *a priori* as (*1*) annualized change in eGFR in Study A and B; (*2*) mean annualized percentage change in height-adjusted total kidney volume (htTKV) in Study A; (*3*) time to (*a*) 50% reduction in eGFR, (*b*) ESKD, and (*c*) a composite of 50% reduction in eGFR, ESKD (initiation of dialysis or preemptive transplant), or death. Classifications of outcomes were made at the clinical centers and then reviewed by an outcome committee composed of the HALT-PKD Study investigators who were blinded to randomized treatment assignments (8).

Confounders related to the predictor variables and the primary outcomes, all measured at baseline, were selected *a priori* as potential covariates, and all were measured at baseline. Race was categorized as white and nonwhite, as determined by self-report. Systolic BP (SBP) was measured in the clinical research clinics with the participant seated quietly in a chair for at least 5 minutes, feet on the floor, and arm supported at heart level. Three measurements were taken with at least 30 seconds between each measurement, and the last two readings were repeated if there was a >10 mm Hg difference. The last two readings were averaged and reported. (8) eGFR was calculated with the CKD Epidemiology Collaboration (CKD-EPI) prediction equation (11) using serum creatinine measured by a centralized HALT-PKD Study laboratory at baseline, 4 months, 12 months, and every 6 months thereafter. Body mass index (BMI) was calculated using baseline-adjusted body weight in kilograms divided by baseline height in meters squared (measured at clinical research clinics) and rounded to the nearest tenth. Urinary albumin excretion was determined from 24-hour urine collections (8). Serum calcium and phosphorus were measured at the Cleveland Clinic central laboratory using standard techniques.

Statistical Analyses

Baseline characteristics were summarized by PKD genotype and presented as mean±SD or median (interquartile range [IQR]) for continuous variables and n (%) for categoric variables. Comparisons across categories were made using a chi-squared test for categoric data and ANOVA or Kruskal–Wallis tests for continuous variables. iFGF23 was log-transformed in analyses due to the skewed distribution of the data.

The associations of the interaction terms with clinical outcomes were assessed using linear regression and Cox

Table 1. Demographics and clinical characteristics of HALT-PKD Study A and B participants included in the FGF23 ancillary study according to ADPKD genotype

Variable	PKD1 Truncating (n=440)	PKD1 Nontruncating (n=229)	PKD2 (n=132)	NMD (n=63)	P Value
Age, yr	41±10	43±10	46±10	44±10	<0.001
Sex, n (%) male	217 (49%)	111 (49%)	69 (52%)	31 (49%)	0.92
Race, n (%) white	409 (93%)	216 (94%)	129 (98%)	59 (94%)	0.24
Study BP target randomization group, n (%) low[a,b]	98 (22%)	64 (28%)	(45 34%)	20 (32%)	0.09
Study BP medication randomization group, n (%) ACEi/ARB	225 (51%)	116 (49%)	64 (52%)	30 (52%)	0.96
SBP, mm Hg	128±14	129±15	125±14	129±15	0.048
eGFR, ml/min per 1.73 m^2[c]	69±27	72±26	74±26	73±24	0.13
BMI, kg/m^2	27.0±5.0	27.9±4.6	27.8±5.0	29.3±7.0	0.002
Calcium, mg/dl	9.4±0.5	9.3±0.4	9.4±0.4	9.4±0.4	0.41
Phosphate, mg/dl	3.5±0.6	3.3±0.5	3.4±0.5	3.3±0.5	0.001
Urine albumin excretion, mg/24 h	26 (16–55)	21 (14–49)	15 (11–32)	17 (11–45)	<0.001
FGF23, pg/ml	55.8 (40.7–76.8)	49.9 (37.7–71.0)	49.0 (33.8–70.5)	50.3 (39.7–67.4)	0.03
1,25$(OH)_2$D, pg/ml	32.8±12.5	33.4±12.5	34.1±13.1	38.0±14.6	0.02
25(OH)D, ng/ml	35.6±13.8	33.8±13.1	34.1±10.7	32.0±9.5	0.11
PTH, pg/ml	45.2±25.1	44.0±24.1	40.6±23.6	45.7±22.7	0.27
htTKV, ml/m^b	634 (465–954)	595 (440–793)	470 (312–654)	560 (309–836)	<0.001

Data are mean±SD, n (%), or median (interquartile range). HALT-PKD, Halt Progression of Polycystic Kidney Disease; FGF23, fibroblast growth factor 23; ADPKD, autosomal dominant PKD; NMD, no mutation detected; ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; SBP, systolic BP; BMI, body mass index; 1,25$(OH)_2$D, 1,25 dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; htTKV, height-adjusted total kidney volume.
[a]Randomized to a BP target of 95–110/60–75 mm Hg.
[b]These variables only apply to participants in Study A (n=458).
[c]Measured by CKD Epidemiology Collaboration equation.

proportional hazards regression models. Analyses stratified by genotype were performed irrespective of the P value of the interaction terms, because this was the *a priori* goal of this study. In the Cox proportional hazards analysis, the dependent variable was a composite of 50% decline in eGFR, ESKD, or death. Covariates that violated proportional hazards assumptions were included with time interactions. iFGF23, 1,25$(OH)_2$D, and 25(OH)D were modeled across each genotype, and the hazard ratios for the interactions were calculated at each tertile for each mineral metabolite. Due to the small number of events within each category, we were unable to test the interaction of the tertiles for each metabolite with genotype. The final Cox proportional hazards models were adjusted for age; sex; race/ethnicity; randomization group; and baseline measurements of SBP, eGFR (CKD-EPI), BMI, urinary albumin excretion, serum calcium, and serum phosphorus. Because htTKV was only measured in HALT-PKD Study A, it was not possible to adjust for htTKV as a covariate.

In the linear regression models, the dependent variables were annualized change in eGFR and mean annualized percentage change in htTKV, as calculated previously (6,9,12). The final multivariable linear models were adjusted for age; sex; race/ethnicity; study (A or B); randomization group; and baseline measurements of SBP, eGFR (CKD-EPI), BMI, urinary albumin excretion, serum calcium, and serum phosphorus. Baseline htTKV was also included as a covariate in the analysis where mean annualized percentage change in htTKV was the dependent variable.

Two-tailed values of P<0.05 were considered statistically significant for all analyses without adjustment for multiple comparisons due to the hypothesis-generating nature of this study. All statistical analyses were performed using SAS version 9.4.

Study Approval

All procedures were approved by the Institutional Review Board of the University of Colorado Anschutz Medical Campus, and adhere to the Declaration of Helsinki. The nature, benefits, and risks of the study were explained to the volunteers and their written informed consent was obtained before participation.

Results

Participant Characteristics at Baseline

A total of 864 participants from HALT-PKD Study A and B with information on PKD genotype and mineral metabolite serum concentrations (iFGF23, 1,25[OH]$_2$D, and 25 [OH]D) were included in the linear regression analysis of annualized change in eGFR and the Cox proportional hazards models. Among these participants, the mean±SD age was 43±10 years, 94% (n=813) were white, the mean±SD eGFR was 71±26 ml/min per 1.73 m^2, median (IQR) iFGF23 level was 52.5 (38.6–73.2) pg/ml, and mean±SD 1,25$(OH)_2$D and 25(OH)D were 33.5±12.8 pg/ml and 34.6±12.9 ng/ml, respectively. The median (IQR) baseline htTKV (n=458; Study A only) was 589 (407–864) ml/m. Age, BMI, phosphorous, SBP, urine albumin excretion, and

Table 2. Associations of mineral metabolite (FGF23, 1,25[OH]$_2$D, and 25[OH]D concentrations) × genotype with annualized percentage change in height-adjusted total kidney volume in HALT-PKD Study A

Association	β-Estimate (95% CI)					
	FGF23 × Genotype		1,25(OH)$_2$D × Genotype		25(OH)D × Genotype	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
PKD1 truncating × mineral metabolite	Reference	Reference	Reference	Reference	Reference	Reference
PKD1 nontruncating × mineral metabolite	−1.52 (−3.89 to 0.85)	−0.72 (−2.93 to 1.49)	0.03 (−0.06 to 0.12)	0.02 (−0.07 to 0.10)	0.05 (−0.04 to 0.14)	0.06 (−0.03 to 0.14)
PKD2 × mineral metabolite	−1.57 (−4.39 to 1.26)	−1.20 (−3.82 to 1.43)	−0.01 (−0.11 to 0.09)	0.02 (−0.07 to 0.12)	0.15 (0.03 to 0.28)	0.16 (0.04 to 0.28)
NMD × mineral metabolite	−0.95 (−4.55 to 2.65)	−0.74 (−4.08 to 2.61)	0.02 (−0.11 to 0.14)	0.01 (−0.10 to 0.13)	0.09 (−0.10 to 0.28)	0.09 (−0.09 to 0.27)

Adjusted model is adjusted for age, sex, body mass index, systolic BP, randomization group, calcium, phosphorus, baseline eGFR, baseline height-adjusted total kidney volume, and urinary albumin excretion. FGF23, fibroblast growth factor 23; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; HALT-PKD, Halt Progression of Polycystic Kidney Disease; NMD, no mutation detected.

htTKV differed across PKD genotypes at baseline (Table 1). 1,25(OH)$_2$D levels differed according to genotype (lowest in the *PKD1*-truncating mutation group), and iFGF23 levels also differed according to genotype (highest in the *PKD1*-truncating mutation group).

Relation between the Mineral Metabolite and Genotype Interactions and Annualized Percentage Change in htTKV in HALT-PKD Study A

In the fully adjusted linear regression model, the interaction of iFGF23 × genotype was not a significant predictor of annualized percentage change in htTKV in HALT-PKD Study A (P=0.82), indicating the influence of iFGF23 on change in htTKV did not differ according to genotype. Results were similar for the interactions of 1,25(OH)$_2$D (P=0.97) and 25(OH)D (P=0.06) with genotype to predict annualized percentage change in htTKV (Table 2).

Relation between the Mineral Metabolite and Genotype Interactions and Annualized Change in eGFR in HALT-PKD Study A and B

In the fully adjusted linear regression model, the interaction term of iFGF23 × genotype was not a significant predictor of annualized change in eGFR in HALT-PKD Study A and B (P=0.47), indicating the influence of iFGF23 on change in eGFR did not differ according to genotype. Results were similar for the interactions of 1,25(OH)$_2$D (P=0.28) and 25(OH)D (P=0.97) with genotype to predict annualized change in eGFR (Table 3).

Relation between Mineral Metabolite and Genotype Interactions and Clinical End Points in HALT-PKD Study A and B

In the fully adjusted Cox proportional hazards models, the interaction between iFGF23 as a continuous variable and genotype was significantly associated with the composite

Table 3. Associations of mineral metabolite (FGF23, 1,25[OH]$_2$D, and 25[OH]D) and genotype interactions with annualized change in eGFR in HALT-PKD study A and B

Association	β-Estimate (95% CI)					
	FGF23 × Genotype		1,25(OH)$_2$D × Genotype		25(OH)D × Genotype	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
PKD1 truncating × mineral metabolite	Reference	Reference	Reference	Reference	Reference	Reference
PKD1 nontruncating × mineral metabolite	−0.47 (−1.63 to 0.69)	−0.51 (−1.66 to 0.63)	0.05 (0.00 to 0.10)	0.05 (−0.00 to 0.09)	−0.01 (−0.05 to 0.04)	−0.00 (−0.05 to 0.04)
PKD2 × mineral metabolite	0.85 (−0.57 to 2.26)	0.65 (−0.75 to 2.05)	0.01 (−0.05 to 0.07)	0.01 (−0.05 to 0.06)	−0.02 (−0.09 to 0.05)	−0.01 (−0.08 to 0.05)
NMD × mineral metabolite	−0.38 (−2.37 to 1.61)	−0.49 (−2.46 to 1.49)	0.04 (−0.03 to 0.11)	0.04 (−0.03 to 0.11)	0.00 (−0.11 to 0.11)	0.02 (−0.09 to 0.12)

Adjusted model is adjusted for age, sex, body mass index, systolic BP, randomization group, calcium, phosphorus, baseline eGFR, baseline height-adjusted total kidney volume, and urinary albumin excretion. FGF23, fibroblast growth factor 23; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; HALT-PKD, Halt Progression of Polycystic Kidney Disease; NMD, no mutation detected.

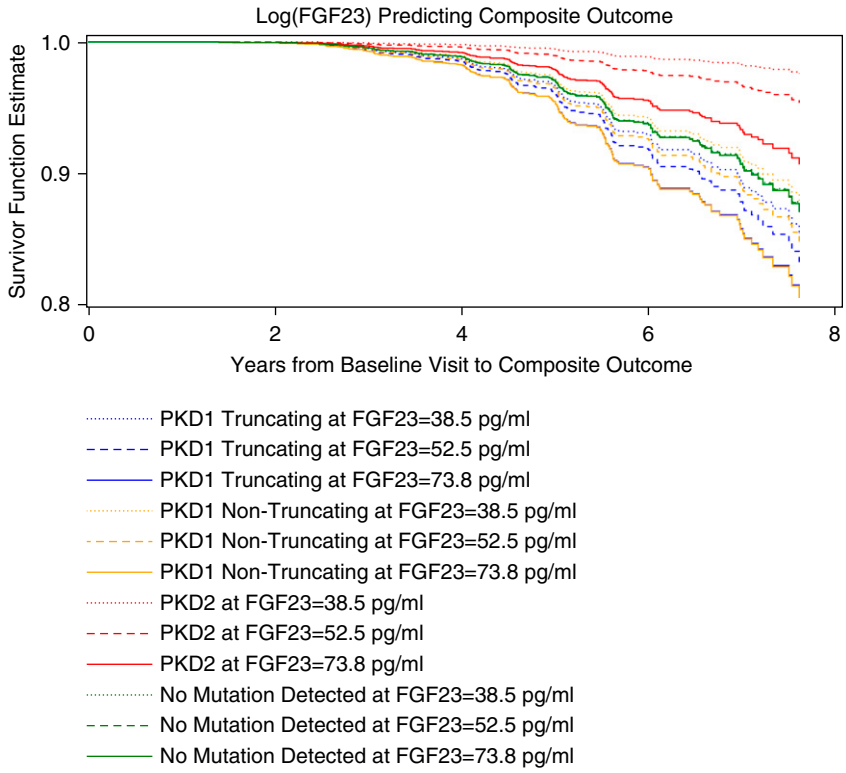

Figure 1. | The interaction between intact fibroblast growth factor 23 and genotype was signficantly associated with the composite end point. Cox proportional hazards curves for the composite end point of 50% decline in eGFR, ESKD, or death, according to polycystic kidney disease (PKD) genotype and fibroblast growth factor 23 (FGF23) tertile**.** The figure is adjusted for age, sex, body mass index, systolic BP, randomization group, calcium, phosphorus, baseline eGFR, and urinary albumin excretion.

end point (P=0.02). The risk of the composite end point was greatest in individuals with the highest level of iFGF23 and either a PKD1-truncating or PKD1-nontruncating mutation (Figure 1, Table 4).

In the fully adjusted Cox proportional hazards models, the interaction between 1,25$(OH)_2$D and genotype was not significantly associated with the composite end point (P=0.83). The influence of 1,25$(OH)_2$D on risk of each clinical end point did not differ according to genotype (Figure 2, Supplemental Table 1).

In the fully adjusted Cox proportional hazards models, the interaction between 25(OH)D and genotype was not significantly associated with the composite end point (P=0.77). The influence of 25(OH)D on risk of each clinical end point did not differ according to genotype (Figure 3, Supplemental Table 2).

Table 4. Associations of FGF23 and genotype interactions with the composite end point (50% decline in eGFR, ESKD, or death) in HALT-PKD Study A and B

FGF23 × Genotype Groups	HR (95% CI)	
	Unadjusted	Adjusted
PKD1 nontruncating, FGF23 tertile 1	1.37 (0.85 to 2.21)	0.82 (0.43 to 1.54)
PKD1 nontruncating, FGF23 tertile 2	1.02 (0.70 to 1.50)	0.90 (0.57 to 1.43)
PKD1 nontruncating, FGF23 tertile 3	0.74 (0.53 to 1.02)	1.01 (0.71 to 1.42)
PKD2, FGF23 tertile 1	0.19 (0.06 to 0.58)	0.16 (0.05 to 0.50)
PKD2, FGF23 tertile 2	0.25 (0.11 to 0.59)	0.26 (0.11 to 0.62)
PKD2, FGF23 tertile 3	0.35 (0.19 to 0.64)	0.46 (0.24 to 0.86)
NMD, FGF23 tertile 1	1.15 (0.48 to 2.76)	0.87 (0.31 to 2.44)
NMD, FGF23 tertile 2	0.81 (0.41 to 1.59)	0.75 (0.36 to 1.57)
NMD, FGF23 tertile 3	0.55 (0.29 to 1.02)	0.64 (0.32 to 1.26)

The reference group is *PKD1* truncating for the same tertile of FGF23 in any given comparison. FGF23 tertile 1 is at 38.5 pg/ml; FGF23 tertile 2 is at 53.0 pg/ml; FGF23 tertile 3 is at 73.7 pg/ml. Adjusted model is adjusted for age, sex, body mass index, systolic BP, randomization group, calcium, phosphorus, baseline eGFR, and urinary albumin excretion. FGF23, fibroblast growth factor 23; HALT-PKD, Halt Progression of Polycystic Kidney Disease; HR, hazard ratio; NMD, no mutation detected.

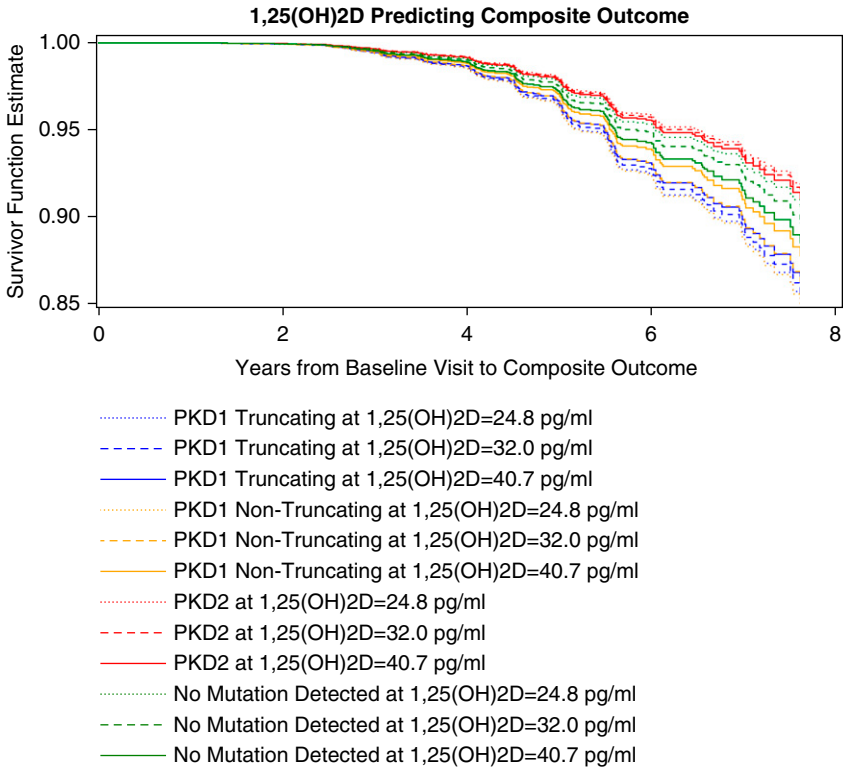

Figure 2. | The interaction between 1,25-dihydroxyvitamin D and genotype was not signficantly associated with the composite end point. Cox proportional hazards curves for the composite end point of 50% decline in eGFR, ESKD, or death, according to PKD genotype and 1,25-dihydroxyvitamin D (1,25[OH]2D) tertile**.** The figure is adjusted for age, sex, body mass index, systolic BP, randomization group, calcium, phosphorus, baseline eGFR, and urinary albumin excretion.

Discussion

In participants in the HALT-PKD Studies, iFGF23 levels interacted significantly with genotype to influence a composite outcome of 50% decline in eGFR, ESKD, or death. This indicates the influence iFGF23 has upon clinical end points in ADPKD depends upon an individual's genotype, with particular relevance to individuals with late-stage disease (Study B). Additionally, levels tended to differ according to genotype, with the highest levels in the PKD1-truncating group. Although the worst outcomes were observed in individuals with a PKD1-truncating or -nontruncating mutation and the highest tertile of iFGF23, risk of the composite end point differed according to iFGF23 concentrations more in the PKD1-nontruncating and PKD2 groups than the PKD1-truncating and NMD groups. In contrast, although $1,25(OH)_2D$ levels also differed according to genotype (lowest in the PKD1-truncating group), neither $1,25(OH)_2D$ nor 25(OH)D levels interacted with genotype to predict clinical outcomes.

FGF23 is a known independent predictor of cardiovascular and all-cause mortality in CKD (13–16), as well as kidney disease progression and/or incident CKD in some (14,15,17) but not all studies (14,18). Increased FGF23 levels have been observed in adults with ADPKD, even when renal function is normal (19,20), as well as in kidneys of Pkd1 knockout mice (21). In our previous analysis of the association of FGF23 levels with progression in the HALT-PKD Studies, we found that iFGF23 was independently associated with kidney function decline, percentage increase in htTKV, and death (6). In this analysis, we extended this work by examining the interaction with four genotype groups, and also examining the interaction of genotype with $1,25(OH)_2D$ and 25(OH)D as a predictor of progression.

25(OH)D is considered the best measure of vitamin D nutritional status because of its long $t_{1/2}$ in the circulation of approximately 3 weeks. 25(OH)D is converted in the kidney by 1α-hydroxylase to $1,25(OH)_2D$, the active form of vitamin D, although extrarenal conversion can also occur (22). FGF23 inhibits 1α-hydroxylase and stimulates 24-hydroxylase, which decreases $1,25(OH)_2D$ levels (23). Several observational studies have shown inverse associations between vitamin D metabolites and adverse outcomes in patients with CKD, including all-cause mortality (24,25) and kidney disease progression (26,27). However, very little is known about vitamin D levels as a predictor of progression in ADPKD. A recent, small, cross-sectional study observed no independent association of either $1,25(OH)_2D$ and 25(OH)D levels with total kidney volume (28). In Lewis PKD rats (a hypertensive rodent model of PKD which phenotypically resembles ARPKD but is a genetic ortholog of human nephrocystin protein 9), chronic vitamin D deficiency had adverse effects on proteinuria, inflammation, cardiovascular health, and renal function, despite mild inhibitory effects on kidney enlargement (29). In this study, we observed no significant interaction between either

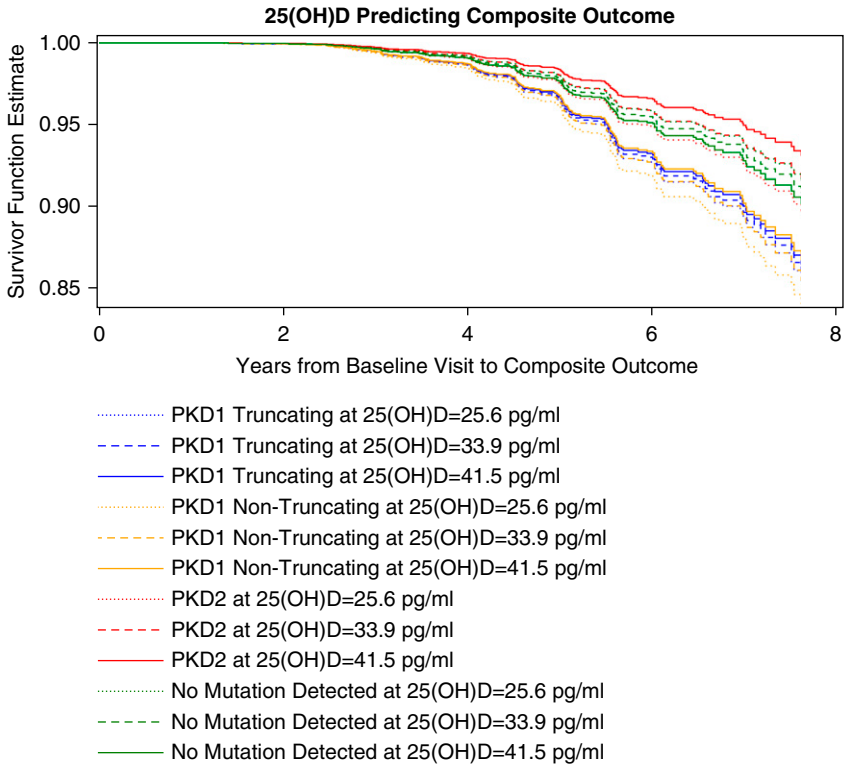

Figure 3. | The interaction between 25-hydroxyvitamin D and genotype was not signficantly associated with the composite end point. Cox proportional hazards curves for the composite end point of 50% decline in eGFR, ESKD, or death, according to PKD genotype and 25-hydroxyvitamin D (25[OH]D) tertile. The figure is adjusted for age, sex, body mass index, systolic BP, randomization group, calcium, phosphorus, baseline eGFR, and urinary albumin excretion.

$1,25(OH)_2D$ or 25(OH)D and genotype as predictors of clinical outcomes.

Genotype is the major contributor to ADPKD progression (3,7). In a combined analysis of the HALT-PKD Studies and Consortium for Radiologic Imaging Studies of PKD, *PKD1*-truncating mutations were associated with lower eGFR, but no differences were observed between *PKD1*-truncating and *PKD1*-nontruncating mutations for the end point of htTKV (7). Consistent with a more severe phenotype with *PKD1*-truncating mutations, an earlier observational study (Genkyst) also observed earlier ESKD with *PKD1*-truncating compared with *PKD1*-nontruncating mutations (30). We have extended this work by examining the interaction of genotype with mineral metabolite levels.

We observed a significant interaction between genotype and iFGF23 levels for the composite end point (which was of relevance to Study B), indicating a differential effect of iFGF23 upon clinical end points according to genotype. The survival plots indicate the worst outcomes for those with the highest iFGF23 levels and a *PKD1*-truncating or -nontruncating mutation. However, interestingly, iFGF23 levels appeared to influence the composite end point more within the *PKD1*-nontruncating and *PKD2* genotype than for the *PKD1*-truncating and NMD groups. For example, although the *PKD2* genotype was protective in all groups, less protection was observed with the highest iFGF23 concentrations. Although iFGF23 levels tended to be higher and $1,25(OH)_2D$ levels tended to be lower in those with PKD1 mutations, it is not possible to distinguish whether the mutation type could directly influence mineral metabolite regulation or whether this is a reflection of severity of disease. However, interestingly, iFGF23 levels are higher in patients with ADPKD as compared with eGFR-matched individuals with CKD due to different etiologies (19). Additionally, we adjusted for baseline eGFR in our models, suggesting that the differential effect of iFGF23 upon clinical outcomes according to genotype is not solely a reflection of differences in eGFR. As we discussed previously (6), FGF23 can exert profibrotic or toxic effects on the kidney tubules or other structures (21,31). The primary cilium or the polycystin 1 and 2 complex may cause dysregulation of osteocyte FGF23 synthesis or tubular cell secretion (21,31). Thus, it is possible that these structures may influence FGF23 synthesis, perhaps through altered mechanosensing, and in turn influence vitamin D levels.

There are several limitations to this study. Our findings are associative rather than causal and residual confounding may exist. The HALT-PKD Studies had limited racial diversity and thus the results may not be applicable to racial minorities. Only a single baseline measurement of mineral metabolites was available, and levels may have fluctuated over the follow-up period. The composite end point is of relevance to the late-stage population in Study B rather than Study A (early stage), thus limiting sample size and power, particularly when considering multiple genotypes and tertiles of metabolites. Accordingly, it was not possible to

examine the individual components of the composite end point due to a small number of events in each of the groups. However, there are also notable strengths. Most importantly, this is the first study to consider the interaction between genotype and mineral metabolites to influence clinically relevant end points in APDKD. Our results have important clinical implications because it is important to understand how biomarkers perform across the spectrum of disease, including according to genotype. We included a relatively large cohort with comprehensive covariates. Additionally, iFGF23 and vitamin D measurements were performed at a single reference laboratory.

In conclusion, iFGF23, but not $25(OH)_2D$ or 25(OH)D, differentially predicted the composite end point in the fully adjusted models (significant interaction between iFGF23 and genotype). Although the worst outcomes were observed individuals with a *PKD1*-truncating or -nontruncating mutation and the highest tertile of iFGF23 (even after adjustment for baseline eGFR), iFGF23 concentrations may confer the greatest influence on outcomes in individuals with a *PKD1*-nontruncating or *PKD2* mutation. Consequently, biomarkers of mineral metabolism and strategies to potentially lower iFGF23 may be of greater clinical value in individuals with these genotypes. Whether ADPKD genotype is directly influencing regulation of mineral metabolism is currently unknown and is an important future direction.

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Author Contributions

A. Chapman, M. Chonchol, G. Brosnahan, B. Gitomer, P. Harris, T. Steinman, V. Torres, M. Wolf, and A. Yu were responsible for data curation; M. Chonchol, B. Gitomer, K. Nowak, and M. Wolf conceptualized the study; M. Chonchol, B. Gitomer, and M. Wolf were responsible for funding acquisition; L. Grau was responsible for validation; L. Grau, P. Harris, B. McNair, and K. Nowak were responsible for methodology; L. Grau, P. Harris, B. McNair, and K. Nowak, were responsible for formal analysis; L. Grau and K. Nowak wrote the original draft; K. Nowak provided supervision; and all authors reviewed and edited the manuscript.

Disclosures

M. Chonchol reports grants from Kadmon, Otsuka, and Sanofi, outside the submitted work. P. Harris reports grants from Otsuka Pharmaceuticals, other from Mitobridge, other from Otsuka Pharmaceuticals, other from Regulus, and other from Vertex Pharmaceuticals, outside the submitted work. V. Torres reports grants and other from Acceleron Pharma Inc., grants and other from Blueprint Medicines, grants and other from Mironid, grants and other from Otsuka Pharmaceuticals, grants and other from Palladio Biosciences, other from Reata, other from Sanofi Genzyme, and other from Vertex Pharmaceuticals, outside the submitted work. M. Wolf reports personal fees and other from Akebia, personal fees from Ardelyx, personal fees from Astrazeneca, and personal fees from Pharmacosmos, outside the submitted work. All remaining authors have nothing to disclose.

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Supplemental Material

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Supplemental Table 1.

Supplemental Table 2.

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