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)2D] and 25-hydroxyvitamin D [25(OH)D]) differ according to ADPKD mutation and differentially predict clinical endpoints according to genotype (significant interaction between genotype and mineral metabolites).

Methods: 864 individuals with ADPKD who participated in the HALT-PKD Study A or B and had measurement of mineral metabolites (1,25(OH)2D, 25(OH)D, iFGF23) were categorized by PKD mutation (PKD1 truncating, PKD1 non-truncating, 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 estimated glomerular filtration rate; 2) mean annualized percent change in height-corrected total kidney volume (Study A only); 3) time to a composite of 50% reduction in eGFR, end-stage renal disease (ESRD), or death were evaluated using linear regression and Cox proportional hazards regression.

Results: Median [IQR] iFGF23 differed (PKD1 truncating: 55.8 [40.7, 76.8]; PKD1 non-truncating: 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±S.D. 1,25(OH)2D differed (PKD1 truncating: 32.8±12.8; PKD1 non-truncating: 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 endpoint in fully adjusted models, but no significant interaction between 1,25(OH)₂D or 25(OH)D and genotype for clinical endpoints.

**Conclusions:** ADPKD genotype interacts significantly with FGF23 to influence clinical endpoints. While the worst outcomes were in individuals with a PKD1 truncating or non-truncating mutation and the highest iFGF23 tertile, risk of the composite endpoint differed according to iFGF23 the most in the PKD1 non-truncating and PKD2 groups.
Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic and potentially fatal disease, affecting 1 in 400 to 1000 individuals. \(^{(1)}\) ADPKD is characterized by the progressive development and enlargement of renal cysts, which eventually destroy the normal parenchyma, leading to end-stage renal disease (ESRD) 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, \textit{PKD1} \((\text{approximately 78\%})\) and \textit{PKD2} \((\text{approximately 15\%})\). \(^{(3)}\)

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 \((1,25(OH)_2D)\). \(^{(4,5)}\) We recently demonstrated that higher serum intact FGF23 (iFGF23) concentration was independently associated with adverse clinical endpoints in participants in the Halt Progression of Polycystic Kidney Disease (HALT-PKD) Studies. \(^{(6)}\) PKD mutation group (\textit{PKD1} truncating, \textit{PKD1} non-truncating, \textit{PKD2}, or no mutation detected [NMD]) was also an important predictor of adverse clinical endpoints 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 endpoints, 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 \textit{PKD1} truncating mutations, we hypothesized that serum levels of iFGF23, 1,25(OH)_2D,
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)₂D, 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).

Material 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 employed a 2x2 factorial design and evaluated the effect of 1) multilevel renin angiotensin aldosterone system (RAAS) blockade with an angiotensin converting enzyme inhibitor (ACEi) + angiotensin receptor blocker (ARB) compared to ACEi + placebo and 2) low (95-110 / 60-75 mmHg) compared to standard (120-130 / 70-80 mmHg) blood pressure control. Study B evaluated only the effect of ACEi + ARB compared to ACEi + placebo.

All participants had a known diagnosis of ADPKD and either hypertension or high-normal blood pressure. Participants in Study A (n=558) were 15 to 49 years of age with an estimated glomerular filtration rate (eGFR) >60 ml/min/1.73m² using the four-variable Modification of Diet in Renal Disease (MDRD) equation. Participants in Study B (n=486) were 18 to 64 years of age...
with an eGFR of 25-60 ml/min/1.73m² (MDRD equation). Of these participants, n=1002 participated in an FGF23 ancillary study(6) and had measurement of mineral metabolite levels (iFGF23, 1,25(OH)₂D, 25(OH)D). An additional n=106 were missing PKD genotype, 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)₂D and 25(OH)D) and genotype as predictor variables. Mutation analysis was performed previously, with mutation class categorized as *PKD1* truncating mutations, *PKD1* non-truncating mutations, *PKD2* mutations, and no mutation detected (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 are 3.8% and 3.0%, respectively, for this assay. Vitamin D metabolites (1,25(OH)₂D 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, while the inter-assay 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, while the inter-assay CVs were 21.4% and 14.7% at 25 pg/mL and 56 pg/mL, respectively. Intact serum parathyroid hormone (PTH) concentrations were also measure by the University of Washington using an automated 2-site immunoassay (Beckman-Coulter, Inc., Brea, USA) (inter-assay CV between 3.4–6.1%).

The outcomes of this analysis were decided *a priori* as 1) annualized change in eGFR in Study A and B; 2) mean annualized percent change in htTKV in Study A; 3) time to a) 50% reduction in eGFR, b) end-stage renal disease (ESRD), and c) a composite of 50% reduction in eGFR, ESRD (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 non-White, as determined by self-report. Systolic blood pressure (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 were between each measurement and the last two readings were repeated of there was a >10 mmHg
difference. The last two readings were averaged and reported. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) prediction equation 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. 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 ± standard deviation or median (interquartile range) for continuous variables and n (%) for categorical variables. Comparisons across categories were made using a Chi-square test for categorical 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 proportional hazards regression models. Analyses stratified by genotype were performed irrespective of the p-value of the interaction terms, as 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, ESRD, or death. Covariates that violated proportional
hazards assumptions were included with time interactions. iFGF23, 1,25(OH)₂D, 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. As 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 percent 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 percent 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 prior to participation.

Results

Participant characteristics at baseline

864 participants from HALT-PKD Study A and B with information on PKD genotype and mineral metabolite serum concentrations (iFGF23, 1,25(OH)2D, 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±S.D. age was 43±10 years, 94% (n=813) were White, the mean±S.D eGFR was 71±26 ml/min/1.73m2, median (interquartile range [IQR]) iFGF23 level was 52.5 (38.6, 73.2) pg/ml, and mean±S.D. 1,25(OH)2D and 25(OH)D were 33.5±12.8 pg/ml and 34.6±12.9 ng/ml, respectively. The median (interquartile range) baseline htTKV (n=458; Study A only) was 589 (407, 864) ml/m. Age, BMI, phosphorous, systolic blood pressure, urine albumin excretion, and htTKV differed across PKD genotypes at baseline (Table 1). 1,25(OH)2D 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 percent 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 percent change in htTKV in HALT-PKD Study A (p=0.82), indicating that the influence of iFGF23 on change in htTKV did not differ according to genotype. Results were similar for the interactions of 1,25(OH)₂D (p=0.97) and 25(OH)D (p=0.06) with genotype to predict annualized percent 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 that the influence of iFGF23 on change in eGFR did not differ according to genotype. Results were similar for the interactions of 1,25(OH)₂D (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 endpoints 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 endpoint (p=0.02). The risk of the composite endpoint was greatest in individuals with the highest level of iFGF23 and either a PKD1 truncating or PKD1 non-truncating mutation (Figure 1 and 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 endpoint (p=0.83). The influence of 1,25(OH)$_2$D on risk of each clinical endpoint did not differ according to genotype. (Figure 2 and 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 endpoint (p=0.77). The influence of 25(OH)D on risk of each clinical endpoint did not differ according to genotype. (Figure 3 and Supplemental Table 2).

Discussion

In participants in the HALT-PKD Studies, iFGF23 levels interacted significantly with genotype to influence a composite outcome of 50% decline in eGFR, ESRD or death. This indicates that the influence iFGF23 has upon clinical endpoints 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. While the worst outcomes were observed in individuals with a PKD1 truncating or non-truncating mutation and the highest tertile of iFGF23, risk of the composite endpoint differed according to iFGF23 concentrations more in the PKD1 non-truncating and PKD2 groups than the PKD1 truncating and no mutation detected groups. In contrast, although
1,25(OH)_2 levels also differed according to genotype (lowest in the PKD1 truncating group), neither 1,25(OH)_2 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, percent increase in hTKV, and death.(6) In the current analysis, we extended this work by examining the interaction with four genotype groups, an also examining the interaction of genotype with 1,25(OH)D and 25(OHD) as a predictor of progression.

25(OH)D is considered the best measure of vitamin D nutritional status because of its long half-life in the circulation of approximately three weeks. 25(OH)D is converted in the kidney by 1α-hydroxylase to 1,25(OH)_{2}D, the active form of vitamin D, although extrarenal conversion can also occur.(22) FGF23 inhibits 1-alpha hydroxylase and stimulates 24-hydroxylase, which decreases 1,25(OH)_{2}D 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)D and 25(OHD) 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 NPHP9), chronic vitamin D deficiency had adverse effects on proteinuria, inflammation, cardiovascular health, and renal function, despite mild inhibitory effects on kidney enlargement.(29) In the current study, we observed no significant interaction between either 1,25(OH)D or 25(OHD) 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 (CRISP), PKD1 truncating mutations were associated with lower eGFR, but no differences were observed between PKD1 truncating and PKD1 non-truncating mutations for the endpoint of htTKV.(7) Consistent with a more severe phenotype with PKD1 truncating mutations, an earlier observational study (Genkyst) also observed earlier ESRD with PKD1 truncating compared to PKD1 non-truncating 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 endpoint (which was of relevance to Study B), indicating a differential effect of iFGF23 upon clinical endpoints according to genotype. The survival plots indicate the worst outcomes for those the highest iFGF23 levels and a PKD1 truncating or non-truncating mutation. However, interestingly,
iFGF23 levels appeared to influence the composite endpoint more within the 
*PKD1* non-truncating and *PKD2* genotype than for the *PKD1* truncating and no mutation detected groups. For example, while the *PKD2* genotype was protective in all groups, less protection was observed with the highest iFGF23 concentrations. While iFGF23 levels tended to be higher and 1,25(OH)D 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 to 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.(31, 32) The primary cilium or the polycystin 1 and 2 complex may cause dysregulation of osteocyte FGF23 synthesis or tubular cell secretion.(31, 32) 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 the current study. Our findings are associative rather than causal and residual confounding may exist. The HALT-PKD studies had limited racial diversity thus the results may not be applicable to racial minorities. Only a single baseline measurement of mineral metabolites were available, and levels may have fluctuated over the follow-up period. The
composite endpoint 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 endpoint, 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 endpoints in APDKD. Our results have important clinical implications, as 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 endpoint in the fully adjusted models (significant interaction between iFGF23 and genotype). While the worst outcomes were observed individuals with a PKD1 truncating or non-truncating mutation and the highest tertile of iFGF23 (even after adjustment for baseline eGFR), iFGF23 concentrations may the greatest influence on outcomes in individuals with a PKD1 non-truncating 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

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B Gitomer: Conceptualization; Data curation; Funding acquisition; Writing - review and editing
B McNair: Formal analysis; Methodology; Writing - original draft; Writing - review and editing
M Wolf: Conceptualization; Data curation; Funding acquisition; Writing - review and editing
P Harris: Data curation; Formal analysis; Methodology; Writing - review and editing
G Brosnahan: Data curation; Writing - review and editing
V Torres: Data curation; Formal analysis; Writing - review and editing
T Steinman: Data curation; Writing - review and editing
A Yu: Data curation; Writing - review and editing
A Chapman: Data curation; Writing - review and editing
M Chonchol: Conceptualization; Data curation; Funding acquisition; Writing - review and editing
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**Supplemental Material Table of Contents**

1. Supplemental Table 1
2. Supplemental Table 2
References


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<tr>
<th>Variable</th>
<th>PKD1 Truncating (n=440)</th>
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<th>PKD2 (n=132)</th>
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</tr>
<tr>
<td><strong>htTKV</strong>, ml/m *</td>
<td>634 (465, 954)</td>
<td>595 (440, 793)</td>
<td>470 (312, 654)</td>
<td>560 (309, 836)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are mean±S.D., n (%), or median (IQR). NMD, no mutation detected; % low, randomized to a blood pressure target of 95-110 / 60-75 mmHg; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate (CKD-EPI equation); BMI, body-mass index; FGF23, fibroblast growth factor 23; 1,25(OH)₂D, 1,25 dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; htTKV, height-adjusted total kidney volume.

* These variables only apply to participants in Study A (n=458)
### Table 2: Associations of Mineral Metabolite (FGF23, 1,25(OH)₂D, and 25(OH)D Concentrations) * Genotype with Annualized Percentage Change in Height-Adjusted Total Kidney Volume in HALT-PKD Study A

<table>
<thead>
<tr>
<th>FGF23 * Genotype</th>
<th>1,25(OH)₂D * Genotype</th>
<th>25(OH)D * Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-Estimate (95% CI)</strong></td>
<td><strong>β-Estimate (95% CI)</strong></td>
<td><strong>β-Estimate (95% CI)</strong></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>Adjusted</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>PKD1 Truncating * Mineral Metabolite</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>PKD1 Non-Truncating * Mineral Metabolite</td>
<td>-1.52 (-3.89, 0.85)</td>
<td>-0.72 (-2.93, 1.49)</td>
</tr>
<tr>
<td>PKD2 * Mineral Metabolite</td>
<td>-1.57 (-4.39, 1.26)</td>
<td>-1.20 (-3.82, 1.43)</td>
</tr>
<tr>
<td>NMD * Mineral Metabolite</td>
<td>-0.95 (-4.55, 2.65)</td>
<td>-0.74 (-4.08, 2.61)</td>
</tr>
</tbody>
</table>

FGF23, fibroblast growth factor 23; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; 95% CI, 95% confidence interval; NMD, no mutation detected

Adjusted model is adjusted for age, sex, body mass index, systolic blood pressure, randomization group, calcium, phosphorus, baseline estimated glomerular filtration rate, baseline height-adjusted total kidney volume, urinary albumin excretion
Table 3: Associations of Mineral Metabolite (FGF23, 1,25(OH)₂D, and 25(OH)D) and Genotype Interactions with Annualized Change in eGFR in HALT-PKD Study A and B

<table>
<thead>
<tr>
<th>Mineral Metabolite</th>
<th>FGF23 * Genotype</th>
<th>1,25(OH)₂D * Genotype</th>
<th>25(OH)D * Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-Estimate (95% CI)</td>
<td>β-Estimate (95% CI)</td>
<td>β-Estimate (95% CI)</td>
</tr>
<tr>
<td>PKD1 Truncating * Mineral Metabolite</td>
<td>Unadjusted</td>
<td>Adjusted</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>PKD1 Non-Truncating * Mineral Metabolite</td>
<td>-0.47 (-1.63, 0.69)</td>
<td>-0.51 (-1.66, 0.63)</td>
<td>0.05 (0.00, 0.10)</td>
</tr>
<tr>
<td>PKD2 * Mineral Metabolite</td>
<td>0.85 (-0.57, 2.26)</td>
<td>0.65 (-0.75, 2.05)</td>
<td>0.01 (-0.05, 0.07)</td>
</tr>
<tr>
<td>NMD * Mineral Metabolite</td>
<td>-0.38 (-2.37, 1.61)</td>
<td>-0.49 (-2.46, 1.49)</td>
<td>0.04 (-0.03, 0.11)</td>
</tr>
</tbody>
</table>

95% CI; 95% confidence interval; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D

Adjusted model is adjusted for age, sex, body mass index, systolic blood pressure, randomization group, calcium, phosphorus, baseline estimated glomerular filtration rate, baseline height-adjusted total kidney volume, urinary albumin excretion
### Table 4: Associations of FGF23 and Genotype Interactions with the Composite Endpoint (50% Decline in eGFR, ESRD or Death) in HALT-PKD Study A and B

<table>
<thead>
<tr>
<th>FGF23 * Genotype Groups</th>
<th>HR (95% CI)</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PKD1</strong> Non-Truncating, FGF23 Tertile 1</td>
<td>1.37 (0.85, 2.21)</td>
<td>0.82 (0.43, 1.54)</td>
<td></td>
</tr>
<tr>
<td><strong>PKD1</strong> Non-Truncating, FGF23 Tertile 2</td>
<td>1.02 (0.70, 1.50)</td>
<td>0.90 (0.57, 1.43)</td>
<td></td>
</tr>
<tr>
<td><strong>PKD1</strong> Non-Truncating, FGF23 Tertile 3</td>
<td>0.74 (0.53, 1.02)</td>
<td>1.01 (0.71, 1.42)</td>
<td></td>
</tr>
<tr>
<td><strong>PKD2</strong>, FGF23 Tertile 1</td>
<td>0.19 (0.06, 0.58)</td>
<td>0.16 (0.05, 0.50)</td>
<td></td>
</tr>
<tr>
<td><strong>PKD2</strong>, FGF23 Tertile 2</td>
<td>0.25 (0.11, 0.59)</td>
<td>0.26 (0.11, 0.62)</td>
<td></td>
</tr>
<tr>
<td><strong>PKD2</strong>, FGF23 Tertile 3</td>
<td>0.35 (0.19, 0.64)</td>
<td>0.46 (0.24, 0.86)</td>
<td></td>
</tr>
<tr>
<td><strong>NMD</strong>, FGF23 Tertile 1</td>
<td>1.15 (0.48, 2.76)</td>
<td>0.87 (0.31, 2.44)</td>
<td></td>
</tr>
<tr>
<td><strong>NMD</strong>, FGF23 Tertile 2</td>
<td>0.81 (0.41, 1.59)</td>
<td>0.75 (0.36, 1.57)</td>
<td></td>
</tr>
<tr>
<td><strong>NMD</strong>, FGF23 Tertile 3</td>
<td>0.55 (0.29, 1.02)</td>
<td>0.64 (0.32, 1.26)</td>
<td></td>
</tr>
</tbody>
</table>

FGF23, fibroblast growth factor 23; HR, hazard ratio; 95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; NMD, no mutation detected.

Adjusted model is adjusted for age, sex, body mass index, systolic blood pressure, randomization group, calcium, phosphorus, baseline estimated glomerular filtration rate, urinary albumin excretion.

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.
Figure 1. Cox proportional hazards curves for the composite endpoint of 50% decline in eGFR, ESRD or death, according to PKD genotype and fibroblast growth factor 23 (FGF23) tertile. The figure is adjusted for age, sex, body mass index, systolic blood pressure, randomization group, calcium, phosphorus, baseline estimated glomerular filtration rate, urinary albumin excretion.
Figure 2. Cox proportional hazards curves for the composite endpoint of 50% decline in eGFR, ESRD 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 blood pressure, randomization group, calcium, phosphorus, baseline estimated glomerular filtration rate, urinary albumin excretion.
Figure 3. Cox proportional hazards curves for the composite endpoint of 50% decline in eGFR, ESRD 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 blood pressure, randomization group, calcium, phosphorus, baseline estimated glomerular filtration rate, urinary albumin excretion.