Erythropoietin and fibroblast growth factor 23 in autosomal dominant polycystic kidney disease patients

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Supplementary Material:

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Supplementary Table S1: Multiple linear regression modeling, dependent variable: log-transformed C-terminal (total) FGF23 (n=78)

Model	Independent Variables	Standardized Coefficient	P-value	Variance Inflation Factor	Adjusted R^2
1	**EPO**	**0.35**	**0.002**	1.00	0.11
2	**EPO** Age Sex eGFR Height Adjusted TKV Liver Volume	**0.37** 0.07 -0.11 -0.05 0.08 -0.08	**0.002** 0.59 0.39 0.73 0.51 0.49	1.06 1.39 1.27 1.50 1.23 1.17	0.08
3	**EPO** Age Sex eGFR Height Adjusted TKV Liver Volume Phosphate Calcium PTH 1,25D Hemoglobin	**0.32** 0.09 -0.13 0.02 0.13 -0.12 0.17 -0.36 -0.11 -0.28 0.04	**0.009** 0.48 0.39 0.89 0.29 0.31 0.12 0.003 0.33 0.023 0.76	1.36 1.59 2.21 1.54 1.42 1.25 1.14 1.33 1.25 1.40 2.16	0.22

EPO: erythropoietin, eGFR: estimated glomerular filtration rate, TKV: total kidney volume, PTH: parathyroid hormone, 1,25D: 1,25-dihydroxyvitamin D.

Supplementary Table S2: Multiple linear regression modeling, dependent variable: log-transformed intact FGF23 (n=78)

Model	Independent Variables	Standardized Coefficient	P-value	Variance Inflation Factor	Adjusted R^2
1	EPO	-0.04	0.73	1.00	0.00
2	EPO Age Sex eGFR Height Adjusted TKV Liver Volume	-0.01 0.00 -0.22 -0.06 0.12 0.17	0.96 1.00 0.07 0.64 0.34 0.15	1.06 1.39 1.27 1.50 1.23 1.17	0.08
3	EPO Age Sex eGFR Height Adjusted TKV Liver Volume Phosphate Calcium PTH 1,25D Hemoglobin	-0.00 0.04 -0.13 -0.08 0.13 0.19 0.06 0.03 -0.13 -0.01 0.09	0.99 0.76 0.42 0.59 0.34 0.14 0.64 0.84 0.32 0.95 0.60	1.36 1.59 2.21 1.54 1.42 1.25 1.14 1.33 1.25 1.40 2.16	0.04

EPO: erythropoietin, eGFR: estimated glomerular filtration rate, TKV: total kidney volume, PTH: parathyroid hormone, 1,25D: 1,25-dihydroxyvitamin D.

Supplementary Table S3: Multiple linear regression modeling, dependent variable: C-terminal (total) FGF23, excluding outlying point (n=77)

Model	Independent Variables	Standardized Coefficient	P-value	Variance Inflation Factor	Adjusted R^2
1	**EPO**	**0.27**	**0.016**	1.00	0.06
2	**EPO** Age Sex eGFR Height Adjusted TKV Liver Volume	**0.29** 0.05 -0.06 -0.06 0.05 -0.09	**0.017** 0.69 0.66 0.68 0.67 0.46	1.06 1.40 1.28 1.53 1.23 1.15	0.02
3	**EPO** Age Sex eGFR Height Adjusted TKV Liver Volume Phosphate Calcium PTH 1,25D Hemoglobin	**0.29** 0.08 -0.04 0.02 0.14 -0.13 0.18 -0.38 -0.02 -0.25 0.17	**0.014** 0.57 0.80 0.89 0.28 0.27 0.12 0.002 0.87 0.044 0.28	1.25 1.58 2.24 1.58 1.41 1.24 1.14 1.33 1.25 1.40 2.14	0.18

EPO: erythropoietin, eGFR: estimated glomerular filtration rate, TKV: total kidney volume, PTH: parathyroid hormone, 1,25D: 1,25-dihydroxyvitamin D.

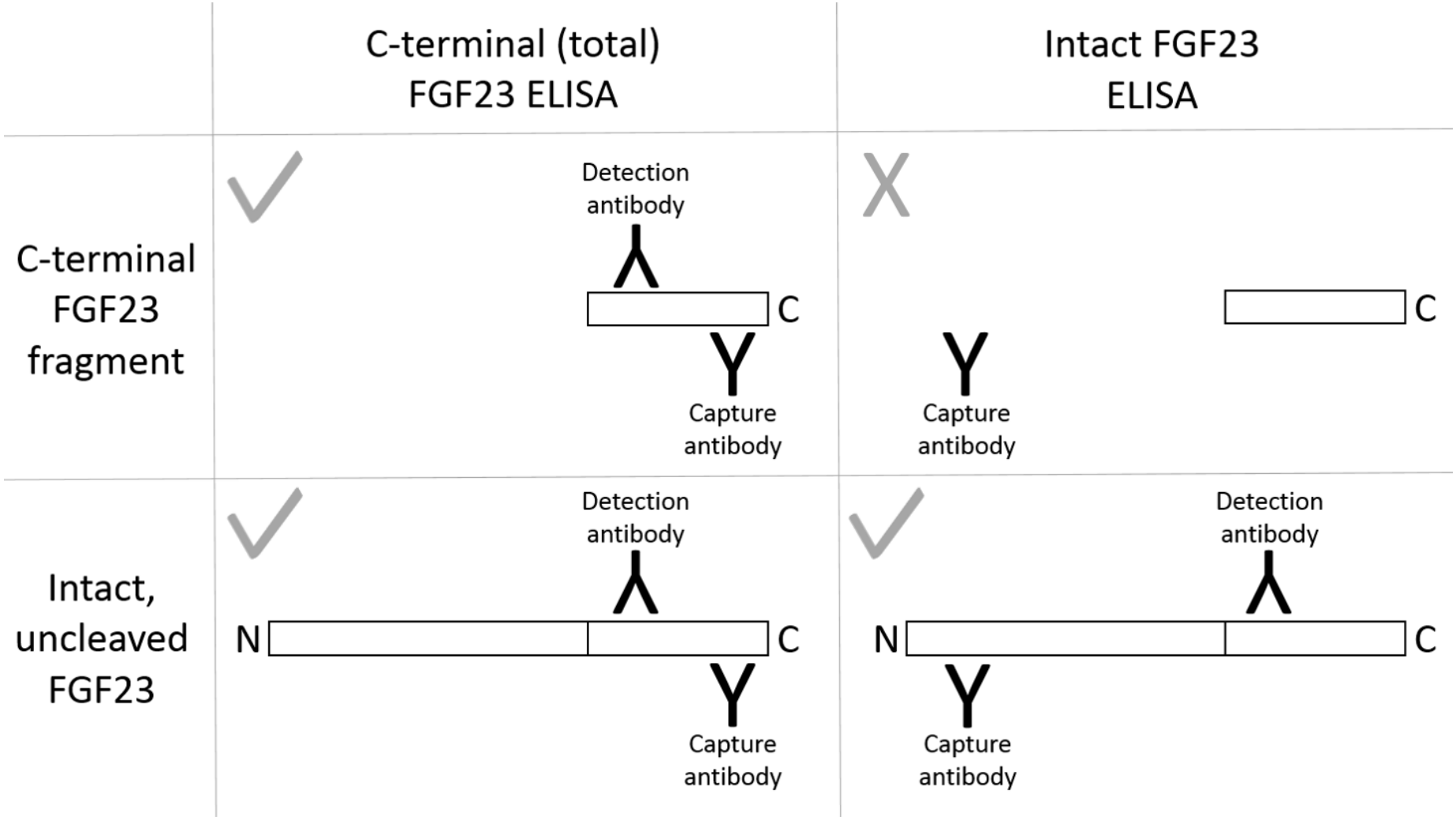
Supplementary Table S4: Multiple linear regression modeling, dependent variable: intact FGF23, excluding outlying point (n=77)

Model	Independent Variables	Standardized Coefficient	P-value	Variance Inflation Factor	Adjusted R^2
1	EPO	-0.03	0.79	1.00	0.00
2	EPO Age Sex eGFR Height Adjusted TKV Liver Volume	0.01 -0.01 -0.22 -0.05 0.11 0.04	0.96 0.96 0.09 0.73 0.37 0.72	1.07 1.39 1.26 1.50 1.23 1.16	0.01
3	EPO Age Sex eGFR Height Adjusted TKV Liver Volume Phosphate Calcium PTH 1,25D Hemoglobin	0.01 0.03 -0.07 -0.06 0.10 0.02 0.11 0.10 -0.13 -0.15 0.09	0.93 0.84 0.67 0.69 0.48 0.85 0.39 0.47 0.32 0.28 0.61	1.36 1.60 2.20 1.54 1.42 1.26 1.14 1.33 1.26 1.43 2.16	0.01

EPO: erythropoietin, eGFR: estimated glomerular filtration rate, TKV: total kidney volume, PTH: parathyroid hormone, 1,25D: 1,25-dihydroxyvitamin D.

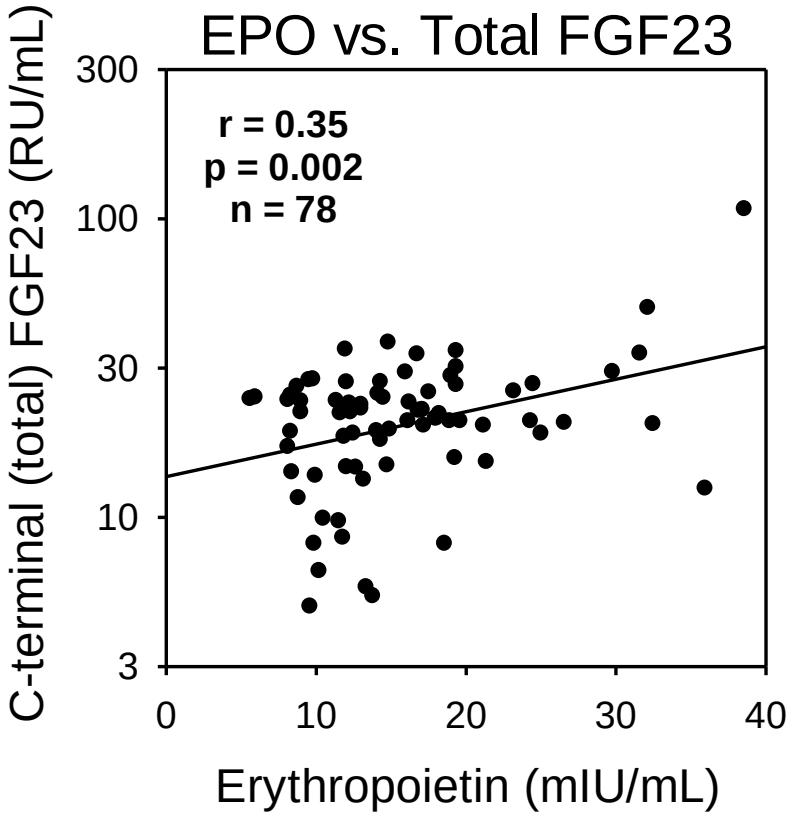
<u>Supplementary Figure S1</u>: The C-terminal (total) FGF23 and intact FGF23 enzyme-linked immunosorbent assays (ELISA).

Whereas the C-terminal (total) fibroblast growth factor 23 (FGF23) ELISA detects both the full-length, intact protein and its C-terminal proteolytic fragments, the intact FGF23 assay detects only the full-length form of the hormone.



<u>Supplementary Figure S2</u>: Associations between erythropoietin and log-transformed FGF23 in the ADPKD HALT-PKD Study A cohort. In patients with autosomal dominant polycystic kidney disease (ADPKD), serum erythropoietin (EPO) levels positively correlated with log-transformed circulating C-terminal (total) fibroblast growth factor 23 (FGF23) concentrations (**Figure S2a**), but not with log-transformed circulating intact FGF23 concentrations (**Figure S2b**).

a



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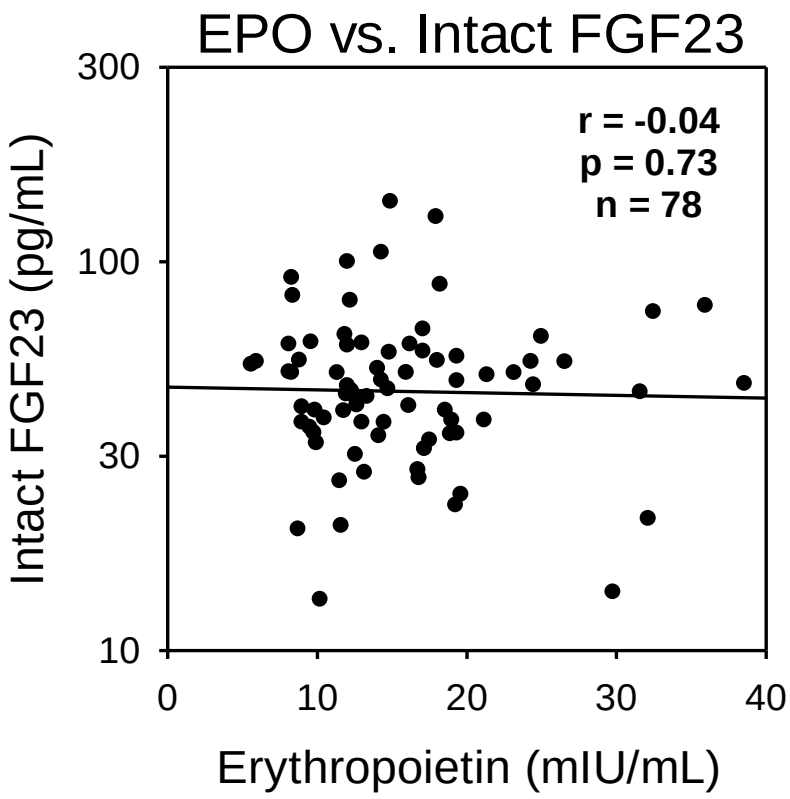
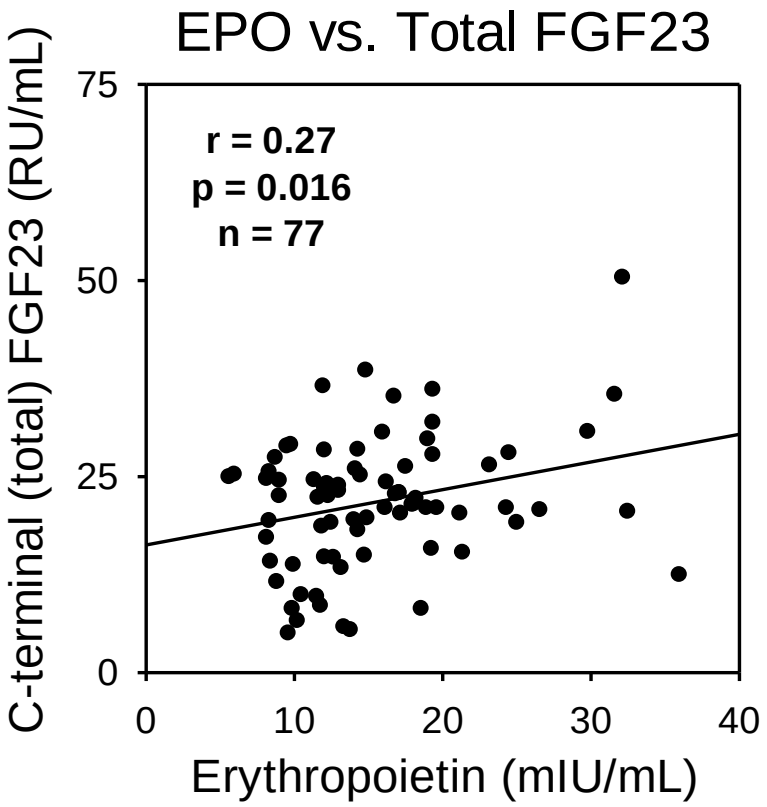


<u>Supplementary Figure S3</u>: Associations between erythropoietin and FGF23 in the ADPKD HALT-PKD Study A cohort, excluding outlying points. In patients with autosomal dominant polycystic kidney disease (ADPKD), serum erythropoietin (EPO) levels positively correlated with circulating C-terminal (total) fibroblast growth factor 23 (FGF23) concentrations (**Figure S3a**), but not with circulating intact FGF23 concentrations (**Figure S3b**).

a



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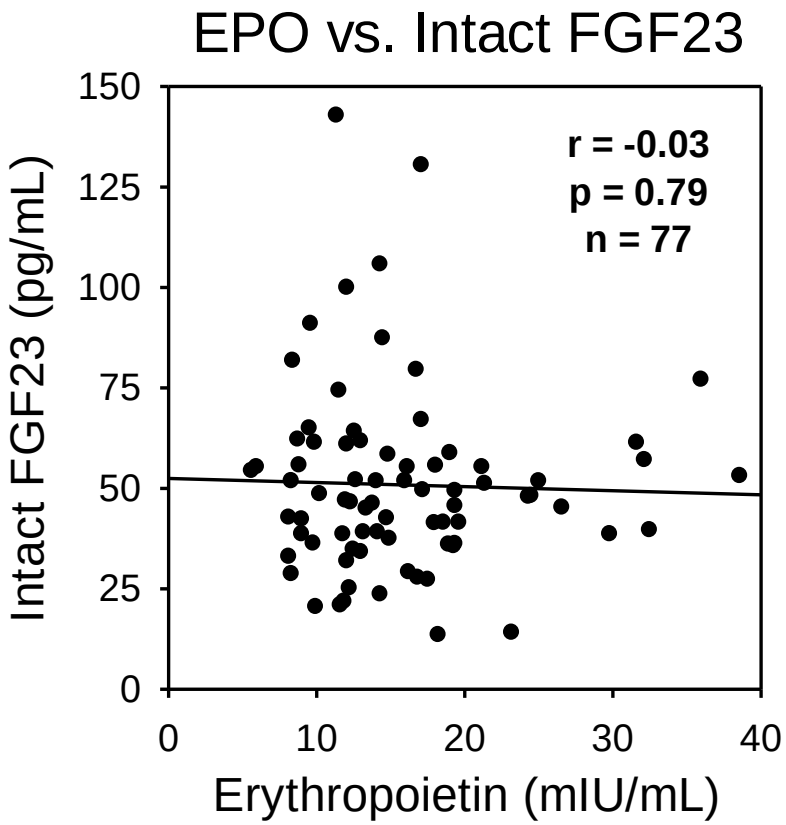
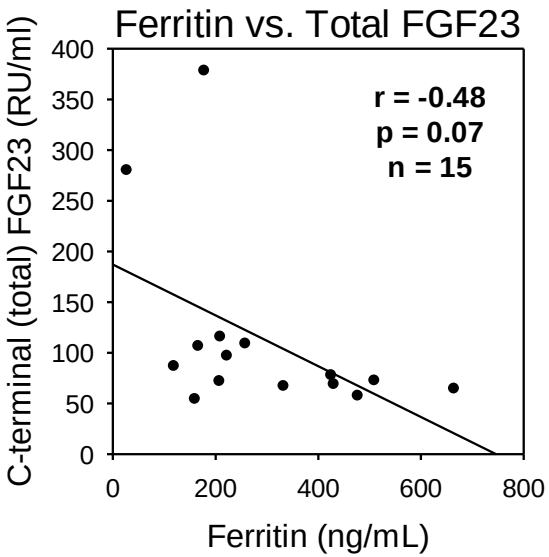


<u>Supplementary Figure S4</u>: Associations between ferritin and FGF23 in the ADPKD bone biopsy cohort. In patients with autosomal dominant polycystic kidney disease (ADPKD), serum ferritin levels tended to inversely correlate with circulating C-terminal (total) fibroblast growth factor 23 (FGF23) concentrations (**Figure S4a**), but not with circulating intact FGF23 concentrations (**Figure S4b**) or with bone FGF23 levels (**Figure S4c**).

a



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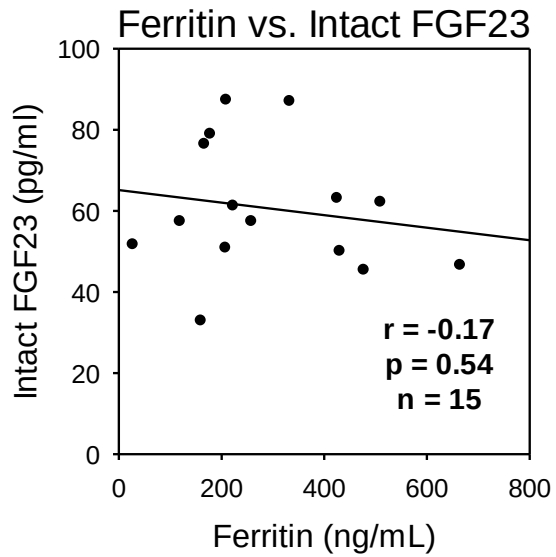

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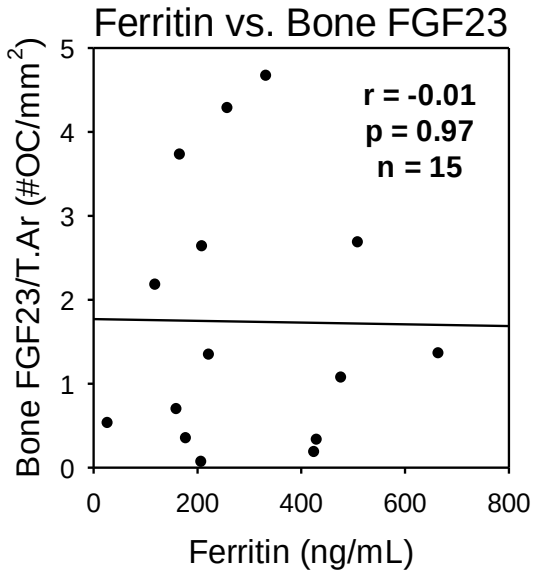
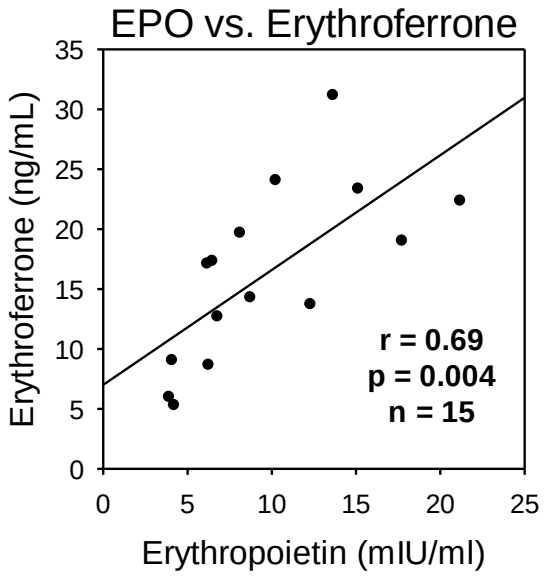


<u>Supplementary Figure S5</u>: Associations among iron-related parameters in ADPKD subjects. In patients with autosomal dominant polycystic kidney disease (ADPKD), serum erythropoietin (EPO) levels positively correlated with erythroferrone (ERFE) (**Figure S5a**), but ERFE did not correlate with hepcidin (**Figure S5b**). Serum ferritin positively correlated with hepcidin (**Figure S5c**).

a



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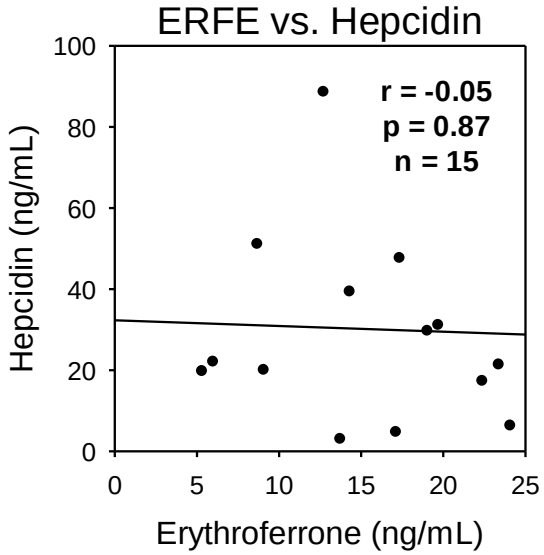

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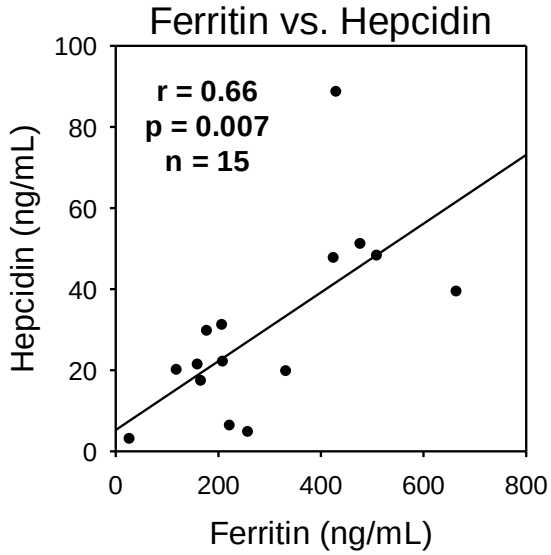

Methods:

ADPKD HALT-PKD Study A Cohort

This cohort included 78 ADPKD subjects from the HALT-PKD Study A.[S22] The University of Colorado Anschutz institutional review board and the HALT-PKD Study Executive Steering Committee approved this ancillary study. Study subjects were at least 20 years old, with eGFR of at least 60 mL/min/1.73m^2. Demographic and anthropomorphic data were obtained from medical records. Kidney and liver volumes were calculated using magnetic resonance imaging data.

The entire Study A cohort included over 500 participants, recruited between 2006 and 2009. Serum intact FGF23 was previously measured in this group.[S41] The current 78 study subjects were selected based on the availability of serum samples in which to measure EPO and C-terminal (total) FGF23. All serum samples were drawn at the University of Colorado Anschutz Medical Campus in Denver, at the baseline visit (i.e. before any study-assigned treatment with angiotensin converting enzyme inhibitors or angiotensin receptor blockers). Most of the study subjects did not live in Colorado, so they traveled to Denver the day prior to the study visit.

From the serum samples, we measured C-terminal (total) FGF23, intact FGF23, EPO, creatinine, phosphate, calcium, intact PTH, 25-hydroxyvitamin D (25D), 1,25-dihydroxyvitamin D (1,25D), and 24,25-dihydroxyvitamin D (24,25D). Circulating total and intact FGF23 concentrations were measured via ELISA (Immutopics/Quidel, San Diego and Kainos, Japan, respectively). Serum EPO was measured via ELISA (R&D Systems, Minneapolis). Serum creatinine, phosphate, and calcium were measured by standard auto-analyzers. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Intact PTH was assayed by a two-site immunoassay on a Beckman Unicell Dxl analyzer. Serum 25D was assayed using heptane extraction and liquid-chromatography-tandem mass spectrometry, and 1,25D and 24,25D were assayed by immunoaffinity purification and LC-MS/MS. Hemoglobin was measured by a standard hematology analyzer.

Categorical data are presented as numbers and percentages. Continuous data are presented as means and standard deviations. Pearson product-moment correlation coefficients and linear regression modeling were used to assess associations between serum EPO and FGF23 levels. Multiple linear regression modeling included the unadjusted model, a partially adjusted model (including the covariables of age, sex, eGFR, height adjusted total kidney volume, and liver volume), and a fully adjusted model (including the additional covariables of calcium, phosphate, PTH, 1,25D, and hemoglobin). Sensitivity analyses were performed, repeating the multiple linear regression modeling with log-transformed FGF23 values or outlier exclusion. Statistical analysis was performed using SigmaPlot 12.5 (San Jose, CA). P-values of <0.05 were considered statistically significant.

ADPKD Bone Biopsy Cohort

This cohort study included 15 adult ADPKD subjects (not from the HALT-PKD study) who underwent bone biopsies as part of a research protocol. Subjects were at least 21 years old, with eGFR of at least 60 mL/min/1.73m^2. Thirteen subjects lived in Colorado, one lived in Oklahoma, and one lived in Texas. Subjects were recruited between 2013 and 2017 at the University of Colorado Anschutz Medical Campus and provided written informed consent. The protocol was approved by the Colorado Multiple Institution Review Board. Demographic and anthropomorphic data were obtained from the medical records.

We measured plasma C-terminal (total) FGF23; serum intact FGF23, EPO, creatinine, phosphate, calcium, PTH, 25D, 1,25D, and 24,25D; and whole blood hemoglobin, as described above. In this cohort, we additionally measured serum erythroferrone (ELISA, used as previously described),[S42] hepcidin (ELISA, used as previously described),[S43] ferritin (ELISA; Abcam, Cambridge, UK), and iron (colorimetric assay; Genzyme, Cambridge, MA). Iliac crest bone biopsies were performed, and bone FGF23 immunohistochemical staining was performed using an antibody directed against C-terminal FGF23. The total number of trabecular osteocytes with positive FGF23 staining was counted and normalized by the total tissue area analyzed (mm^2). Pearson product-moment correlation coefficients were used to assess associations between variables.

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