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Authors

Gitomer, Berenice
Pereira, Renata
Salusky, Isidro
et al.

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Mineral bone disease in autosomal dominant polycystic kidney disease

Berenice Gitomer[1], **Renata Pereira**[2], **Isidro B. Salusky**[2], **Jason W. Stoneback**[3], **Tamara Isakova**[4], **Xuan Cai**[4], **Lorien S. Dalrymple**[5], **Norma Ofsthun**[5], **Zhiying You**[1], **Harmut H. Malluche**[6], **Franklin Maddux**[7], **Diana George**[1], **Vicente Torres**[8], **Arlene Chapman**[9], **Theodore I. Steinman**[10], **Myles Wolf**[11], **Michel Chonchol**[1]

[1]Division of Renal Diseases and Hypertension, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado, USA

[2]Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

[3]Department of Orthopedics, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado, USA

[4]Division of Nephrology and Hypertension, Department of Medicine, Center for Translational Metabolism and Health, Institute for Public Health and Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

[5]Fresenius Medical Care North America, Waltham, Massachusetts, USA

[6]Division of Nephrology, Bone and Mineral Metabolism, Department of Medicine, University of Kentucky Chandler Medical Center, Lexington, Kentucky, USA

[7]Fresenius Medical Care AG & Co, KGaA, Bad Homburg, Germany

[8]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA

[9]Section of Nephrology, University of Chicago, Chicago, Illinois, USA

[10]Department of Medicine and Renal Division, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA

[11]Division of Nephrology, Department of Medicine, Duke Clinical Research Institute, Duke University School of Medicine, Durham, North Carolina, USA

Abstract

Correspondence: Michel Chonchol, Division of Renal Diseases and Hypertension, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado, USA. Michel.Chonchol@cuanschutz.edu.

Study approval

All studies and procedures were approved by the Institutional Review Board of the University of Colorado Anschutz Medical Campus.

DISCLOSURE

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Mice with disruption of *Pkd1* in osteoblasts demonstrate reduced bone mineral density, trabecular bone volume and cortical thickness. To date, the bone phenotype in adult patients with autosomal dominant polycystic kidney disease (ADPKD) with stage I and II chronic kidney disease has not been investigated. To examine this, we characterized biochemical markers of mineral metabolism, examined bone turnover and biology, and estimated risk of fracture in patients with ADPKD. Markers of mineral metabolism were measured in 944 patients with ADPKD and other causes of kidney disease. Histomorphometry and immunohistochemistry were compared on bone biopsies from 20 patients with ADPKD with a mean eGFR of 97 ml/ min/1.73m^2 and 17 healthy individuals. Furthermore, adults with end stage kidney disease (ESKD) initiating hemodialysis between 2002–2013 and estimated the risk of bone fracture associated with ADPKD as compared to other etiologies of kidney disease were examined. Intact fibroblast growth factor 23 was higher and total alkaline phosphatase lower in patients with compared to patients without ADPKD with chronic kidney disease. Compared to healthy individuals, patients with ADPKD demonstrated significantly lower osteoid volume/bone volume (0.61 vs. 1.21%) and bone formation rate/bone surface (0.012 vs. 0.026 μm^3/μm^2/day). ESKD due to ADPKD was not associated with a higher risk of fracture as compared to ESKD due to diabetes (age adjusted incidence rate ratio: 0.53 (95% confidence interval 0.31, 0.74) or compared to other etiologies of kidney disease. Thus, individuals with ADPKD have lower alkaline phosphatase, higher circulating intact fibroblast growth factor 23 and decreased bone formation rate. However, ADPKD is not associated with higher rates of bone fracture in ESKD.

Keywords

ADPKD; bone; mineral metabolism

Autosomal dominant polycystic kidney disease (ADPKD) is a common kidney disease affecting ~ 1 in 1000 individuals.[1] Development and continued growth of multiple fluid-filled cysts within the kidneys lead to end-stage kidney disease (ESKD) in 50% of patients by the age of 60.[2] In most cases, disease results from a mutation in either *PKD1*, which encodes polycystin 1, or *PKD2*, which encodes polycystin 2.[3,4]

Polycystins are expressed in multiple tissues and cell types including osteoblasts and osteocytes.[5] In bone, polycystin 1 acts as a mechanosensor-regulating osteoblastic gene transcription and bone cell differentiation.[6,7] Several animal studies have shown that disruption of *Pkd1* expression in bone results in abnormal bone development and morphology, reduced bone mineral density, cortical thickness, and osteopenia.[5,6,8,9] A role of *Pkd2* in bone is supported by a study demonstrating multiple postnatal craniofacial abnormalities in mice with conditional loss of *Pkd2*.[10] Mechanistically, polycystin 1 modulates osteoblast function through regulation of the bone transcription factor runt-related transcription factor 2.[11]

Although multiple animal studies indicate a bone defect associated with loss of either *Pkd1* or *Pkd2* function and some human PKD studies have suggested a link between ADPKD and suppressed bone formation,[12,13] to date, no human bone histomorphometric studies have interrogated a potential bone defect in adult patients with ADPKD with stage I and II

chronic kidney disease (CKD). Bone developmental defects in children with ADPKD have not been identified and children with either *PKD1* or *PKD2* mutations undergo typical growth and attain normal stature. To test the hypothesis that ADPKD is characterized by low bone turnover and to investigate the clinical consequences, we undertook concurrent studies to examine the following objectives: (i) to characterize markers of mineral metabolism in ADPKD versus estimated glomerular filtration rate (eGFR)—matched populations with CKD without ADPKD by using the HALT-PKD and Chronic Renal Insufficiency Cohort (CRIC) studies; (ii) to examine bone turnover and biology in adults with ADPKD with stage I and II CKD recruited at the University of Colorado; and (iii) to estimate the risk of bone fracture in individuals with ESKD due to ADPKD as compared with other etiologies of ESKD by using the Fresenius Medical Care North America cohort.

RESULTS

Markers of mineral metabolism in HALT-PKD and CRIC studies

We compared baseline serum calcium, phosphorus, intact parathyroid hormone (iPTH), total alkaline phosphatase, and intact fibroblast growth factor 23 (iFGF23) levels in 472 patients with ADPKD who participated in the HALT-PKD study with those in 472 eGFR-matched participants without ADPKD in the CRIC study. At baseline, iFGF23 was significantly higher and total alkaline phosphatase significantly lower in patients with ADPKD than in patients without ADPKD with CKD resulting from other causes (Table 1). In unadjusted evaluations depicted in Figure 1a, there was a linear inverse association between higher baseline iFGF23 and lower baseline eGFR in the HALT-PKD ($r = -0.48$; $P < 0.0001$) and CRIC ($r = 0.21$; $P < 0.0001$) cohorts. I n addition, Figure 1b shows a linear inverse association between higher baseline alkaline phosphatase and lower baseline eGFR in patients without ADPKD with CKD ($r = -0.10$; $P = 0.02$) and in patients with ADPKD ($r = -0.12$; $P = 0.009$). Figure 1c shows a similar pattern for the relationship between serum iPTH and eGFR in both participants with ADPKD ($r = -0.40$; $P < 0.0001$) and those without ADPKD ($r = -0.36$; $P < 0.0001$).

Patients with ADPKD have a reduced bone turnover rate

Demographic and biochemical characteristics of the 20 patients with ADPKD and 9 healthy controls who underwent bone biopsies at the University of Colorado between 2013 and 2016 for purpose of this study are presented in Table 2. Of note, of the 9 healthy controls with biochemical characteristics, only 5 bone biopsies were available for histomorphometric analysis based on 2 available intact cortices. An additional 12 healthy control bone biopsies were obtained from the University of Kentucky and examined by the same observer (RP). To discriminate the effects of the PKD genetic mutation from the known effects of uremia on bone, we evaluated only patients with ADPKD with stage I and II CKD (eGFR, 97 ± 29 ml/min per 1.73 m^2). The bone histomorphometric values obtained from 20 patients with ADPKD were compared to bone biopsies from 17 healthy controls. The age range and sex of the study population with ADPKD were similar to healthy controls (Table 3). An analysis of structural parameters showed that there was a difference between groups in trabecular number (1.97 ± 0.40 mm^2 vs. 1.76 ± 0.18 mm^2; $P = 0.04$) and trabecular separation (387.55 ± 88.53 μm vs. 444.50 ± 70.99 μm; $P = 0.04$), but there was no difference in trabecular

thickness or in bone volume. Patients with ADPKD had significantly lower remodeling parameters including osteoid volume (osteoid volume/bone volume: 0.61% ± 0.34% vs. 1.21% ± 0.81%; $P = 0.01$ and OV/TV: 0.14% ± 0.08% vs. 0.30% ± 0.24%; $P = 0.02$) and osteoid surface (osteoid surface/bone surface: 5.50% ± 3.87% vs. 10.01% ± 6.30%; $P = 0.015$). With regard to dynamic remodeling parameters, significantly lower values were observed for labeled surface (labeled surface/bone surface: 2.17% ± 0.99% vs. 4.88% ± 3.35%; $P = 0.02$), double labeled surface (double labeled surface/bone surface: 1.81% ± 1.06% vs. 4.88% ± 3.35%; $P = 0.02$), mineralizing surface (mineralizing surface/bone surface: 2.17% ± 1.00% vs. 4.99% ± 3.39%; $P = 0.02$), and bone formation rate (bone formation rate/bone surface: 0.012 ± 0.005 $\mu m^3/\mu m^2/d$ vs. 0.026 ± 0.018 $\mu m^3/\mu m^2/d$; $P = 0.03$). Mineral apposition rate, adjusted apposition rate, and mineralization lag time did not differ between patients with ADPKD and healthy controls. Overall, bone data are indicative of decreased bone formation in patients with ADPKD, consistent with the pattern seen in adynamic bone disease.

Osteocyte abnormalities are present in patients with ADPKD as compared with healthy controls

We compared bone expression of the osteocyte proteins FGF23, dentin matrix acidic phosphoprotein1 (DMP1), and sclerostin in bone from 20 patients with ADPKD with that obtained from 9 healthy controls by quantitation of immunohistochemical staining. This analysis included all bone biopsies performed at the University of Colorado, including 4 samples that were inadequate for histomorphometry. We corrected all measurements by bone area. Expression of FGF23 (FGF23/bone area: 6.93 ± 6.57 vs. control: 3.44 ± 2.35; $P < 0.05$) and expression of DMP1 (DMP1/bone area: 0.86 ± 0.41 vs. control: 0.61 ± 0.23; $P < 0.05$) were significantly increased in bone from patients with ADPKD compared with controls (Figure 2). Bone expression of sclerostin was not significantly different in bone from patients with ADPKD compared with healthy controls (sclerostin/ bone area: 0.10 ± 0.08 vs. control: 0.11 ± 0.08; $P > 0.1$) (Figure 2).

Bone density is low in patients with ADPKD and normal to moderate renal function

Seventeen (9 women and 8 men) patients with ADPKD with bone biopsies also underwent a DEXA scan for determination of bone mineral density. Women had a mean age of 29 ± 12 years, eGFR 101 ± 24 ml/min per 1.73 m^2, and body mass index 26 ± 2 kg/m^2; and men had a mean age of 36 ± 10 years, eGFR 90 ± 37 ml/min per 1.73 ± m^2, and body mass index 26 ± 6 kg/m^2. Bone density was assessed at the lumbar L1 through L4 region, femoral neck, and distal one-third radius. Bone density varied between subjects and did not show any consistent pattern (Supplementary Figure S1).

Fracture incidence in patients with ADPKD with ESKD receiving hemodialysis compared with other etiologies of kidney disease

To assess the clinical consequences on the skeleton of the low bone turnover state, we investigated the risk of first fracture-related hospitalization in patients with ESKD requiring hemodialysis owing to ADPKD as compared with other non-ADPKD etiologies of kidney disease, including diabetes, hypertension, and glomerulonephritis. Baseline characteristics are depicted in Supplementary Table S2. Most patients in this cohort (87%) had been on

dialysis for <5 years before the first fracture requiring hospitalization. In age, sex, race, and time on dialysis before the first fracture-adjusted analyses, patients with ADPKD requiring hemodialysis did not have a higher risk of fracture (Table 4). In fact, the adjusted incident rate ratio of fracture in patients with ADPKD with ESKD was lower than that in patients with diabetes (incident rate ratio, 0.53; 95% confidence interval, 0.31–0.74; $P < 0.0001$).

DISCUSSION

In this study, we present mineral metabolism features that differentiate patients with ADPKD from other causes of CKD and describe a low turnover bone defect in a cohort of patients with ADPKD. In addition, we demonstrate that patients with ADPKD with ESKD do not have a higher rate of fractures compared to other common etiologies of kidney disease.

Circulating FGF23 levels are higher in patients with ADPKD across the spectrum of kidney function than in those with non—ADPKD-related CKD and comparable kidney function.[15,16] Comparison of serum levels of iFGF23 in subjects in the HALT-PKD clinical trial with those in eGFR-matched participants without ADPKD in the CRIC study confirmed this observation. These observations are indicative of a potential dysregulation of osteocyte function in ADPKD. Similarly, evidence for a bone abnormality in ADPKD is further supported by lower levels of circulating alkaline phosphatase observed across a wide range of kidney function in participants in the HALT-PKD study as compared with kidney function —matched subjects in the CRIC study.

Several factors indicate the potential for a bone defect in patients with ADPKD. The gene mutation in most cases of ADPKD results in a defect in the polycystin proteins 1 or 2, both of which are expressed in osteoblasts and osteocytes, cells that also have a primary cilium.[5] In contrast to animal studies with *Pkd1* or *Pkd2* mutations, histomorphometric analysis of both static and dynamic parameters of bone turnover revealed significantly decreased bone formation rate, despite normal bone volume in ADPKD bone samples compared with those in healthy controls. Overall, abnormalities in these key histomorphometric parameters suggest that the bone pattern observed in patients with ADPKD is consistent with adynamic bone disorder. Immunohistochemical staining confirmed increased expression of FGF23 in ADPKD bone samples compared with that of healthy controls, which supports possible dysregulation of osteocytes in ADPKD. Mineral apposition rate and osteoid volume or thickness did not differ between patients with ADPKD and healthy controls.

The observation that patients with ADPKD with ESKD receiving hemodialysis do not demonstrate a higher risk of fracture compared with patients on hemodialysis with non-ADPKD etiologies of kidney disease is surprising given the occurrence of low bone turnover observed in early stages of ADPKD. Such observations are in contrast to the increased risk of fractures in patients on dialysis with adynamic bone disease with other comorbidities such as diabetes. Although the reason for this is unknown at present, it is possible that protective differences in bone hardness or elasticity characterize ADPKD bone. Additional studies will be necessary to address this question.

Evenepoel *et al.* described a distinct bone phenotype of suppressed bone turnover, preserved cortical bone mineral density, and elevated circulating sclerostin levels in patients with ADPKD with ESKD at the time of kidney transplantation.[12] However, in patients with advanced kidney disease, it becomes more difficult to discriminate the effects of gene mutation from the confounding effects of azotemia, changes in CKD—mineral bone disorder, and therapy with vitamin D analogues and calcimimetics. In addition, another important limitation of this study was that only static bone parameters were studied as bone biopsies at the time of transplantation were performed without previous double tetracycline labeling. Therefore, presence of this bone phenotype early in the course of kidney disease and before the presence of significant kidney function decline in patients with ADPKD indicates that the bone lesion is linked to the primary genetic defect. More importantly, these bone alterations that appear to be present in patients with ADPKD at different stages of kidney disease do not appear to increase the risk of fractures in patients with ADPKD once they initiate kidney replacement therapy.

Loss of polycystin 1 has been shown to result in defective skeletogenesis in mice,[8] whereas conditional loss of *Pkd2* has been associated with multiple craniofacial abnormalities.[10] However, patients with ADPKD generally do not undergo abnormal growth and they typically attain normal stature. Conditional disruption of *Pkd1* in murine osteoblasts results in impaired osteoblast differentiation, osteopenia, reduced bone mineral density, trabecular bone volume, cortical thickness, and reduced mineral apposition rate in mice.[5,9] An osteoblast defect may explain the reduced bone formation rate and osteoid volume observed in the present study. However, the overall bone defect in mice differs from the bone abnormalities observed in patients with ADPKD in the present study, where neither reduction in bone volume nor abnormal mineralization was noted. Indication that polycystin 1 may function as a mechanosensor in bone derives from animal and cell studies demonstrating that conditional deletion of *Pkd1* in osteocytes resulted in disrupted mechanosensing in bone.[6] However, although reduced osteocyte expression of DMP1 was observed in the former animal study, in ADPKD patient bone biopsy DMP1 expression was significantly increased based on immunohistochemical staining. Cell studies have shown that polycystin 1 modulates osteoblastic gene transcription and bone cell differentiation via the calcineurin/NFAT signaling pathway.[7]However, additional studies directed toward the analysis of aberrant signaling pathways in ADPKD and bone will be necessary to further elucidate the bone defect in human patients.

In the present study, we observed upregulated expression of DMP1 and FGF23 in ADPKD bone compared with healthy control bone samples on the basis of immunohistochemical staining. Increased expression of sclerostin with respect to its known inhibitory effect on Wnt/β-catenin[17–19] has been associated with decreased osteoblastic activity. However, in the present study, bone expression of sclerostin did not differ in ADPKD from normal bone. This suggests an alternate explanation for reduced bone formation in ADPKD. Abnormalities in expression of osteocyte proteins have been observed in non-ADPKD CKD.[20,21] Increased bone expression of FGF23 and DMP1 has been reported throughout the spectrum of CKD.[21] Hence, our immunohistochemical findings are more in line with what has been reported in other causes of CKD.

There are limitations associated with the present study. All bone analyses were cross-sectional; thus, how bone changes with progression of disease is unknown and circulating sclerostin levels were not measured. In addition, the limited number of bone biopsies available for patients with ADPKD and its comparison with healthy controls may preclude detection of more subtle changes in bone parameters due to variability in the measures. The observed lower risk of bone fracture in the cohort of patients with ESKD and ADPKD as compared with those with ESKD and diabetic kidney disease may relate to fewer comorbidities among patients with ADPKD and other factors associated with the risk of fracture (e.g., frailty). Thus, we cannot rule out the possibility that the difference in fracture rate between patients with ESKD with diabetic kidney disease and those with ADPKD results from complexity intrinsic to diabetes. The ascertainment of fractures from the Fresenius Medical Care North America clinical data system may result in underascertainment of fractures or misclassification of fracture types; however, it is less likely that there was differential misclassification based on the etiology of kidney disease. Finally, an increased cortical thickness has previously been reported in patients with ESKD with ADPKD and may offer an alternate explanation of the decreased fracture rate observed in patients with ADPKD compared with those with other etiologies of kidney disease.

In conclusion, renal osteodystrophy associated with CKD is usually evident in patients with advanced kidney disease. However, in the present study, we observed evidence of a bone defect in patients with ADPKD with stage I and II CKD. Unlike in patients with other causes of CKD, the nature of the bone defect in patients with ADPKD was uniform, consistent with the genetic origin of disease. The described bone defect in adult patients with ADPKD with stage I and II CKD is consistent with adynamic bone disorder and appears to be present before kidney function decline. Despite the presence of the bone defect in the early stages of disease, patients with ADPKD with ESKD receiving hemodialysis do not have an increased risk of fracture when compared with patients with other etiologies of ESKD. Future studies with focus on the longitudinal changes in ADPKD bone and associated aberrant regulatory pathways will be necessary to fully understand the bone lesion in ADPKD.

METHODS

Study populations

HALT-PKD and CRIC studies.—We compared serum calcium, phosphorus, iPTH, total alkaline phosphatase, and iFGF23 in baseline samples from 472 participants in the HALT-PKD study and 472 eGFR-matched participants in the CRIC study by using the Chronic Kidney Disease—Epidemiology Collaboration equation.[22] The HALT-PKD study was a prospective randomized trial that tested the effect of the dual blockade of the renin-angiotensin-aldosterone system using lisinopril plus telmisartan as compared with lisinopril monotherapy on the progression of ADPKD.[23–25] The study included 2 parallel substudies of hypertensive adults with different levels of kidney function. Study A enrolled 558 patients aged 15 to 49 years with an eGFR of >60 ml/min per 1.73 m^2, and study B enrolled 486 patients aged 18 to 64 years with an eGFR of 25 to 59 ml/min per 1.73 m^2. Study A also evaluated the effect of the level of blood pressure control on outcome measures. The primary

outcome of study A was annual percent change in height-corrected total kidney volume; and of study B, time to doubling of baseline serum creatinine, ESKD (initiation of dialysis or transplantation), or death. The CRIC study is a multicenter prospective cohort study of risk factors for cardiovascular disease and progression of CKD.[26] Adult patients aged 21 to 74 years with an eGFR of 20 to 70 ml/min per 1.73 m^2 at the screening visit were enrolled. Patients with ADPKD were excluded.

Biochemistry assays.—We measured iFGF23 in the HALT-PKD and CRIC studies by using a 2-site enzyme-linked immunosorbent assay for iFGF23 (Kainos Laboratories, Inc., Tokyo, Japan).[27] The intra-and interassay coefficient of variations (CVs) are 3.8% and 3.0%, respectively, for this assay. We assayed serum iPTH 1 to 84 with a 2-site immunoassay with a Beckman UniCel Dxl analyzer. The detection range is 1 to 3500 pg/ml, with the inter- and intra-assay CVs being <5% at 37 pg/ml. We measured serum calcium, phosphorus, and total alkaline phosphatase with standard multianalyte autoanalyzers. All serum samples were separated and stored at the National Institute of Diabetes and Digestive and Kidney Diseases repository at −80°C until assays were performed. All samples were analyzed at the same time, and we performed all assays at the University of Washington clinical laboratory.

Bone turnover and biology study.—A total of 20 patients with ADPKD with a mean eGFR of 97 ± 29 ml/min per 1.73 m^2 and 9 healthy controls were admitted to the outpatient surgical unit at the University of Colorado Hospital after undergoing a double tetracycline labeling procedure.[28] We obtained bone biopsies containing both cortices 0.5 cm in width by 1 to 2 cm long from the anterior iliac crest. In all participants undergoing bone biopsy at the University of Colorado, we measured serum calcium, phosphorus, 25-hydroxyvitamin D, 1,25-dihidroxyvitamin D, iPTH, total and bone alkaline phosphatase, iFGF23, urinary creatinine, and urinary phosphorus at the University of Washington clinical laboratory. In the bone biopsy cohort, we also measured plasma C-terminal FGF23 by enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA).[29] We measured bone-specific alkaline phosphatase by enzyme-linked immunosorbent assay (Quidel, San Diego, CA). The intra- and interassay CVs are 2.2% and 10.3%, respectively, for this assay. We also assayed C-terminal telopeptides of type 1 collagen by enzyme-linked immunosorbent assay (Immunodiagnostic Systems, Gaithersburg, MD). The intra- and interassay CVs are 7.7% and 8.6%, respectively, for this assay. Of note, 25-hydroxyvitamin D and 1,25-dihidroxyvitamin D levels were analyzed using immunoaffinity extraction and liquid chromatography—tandem mass spectrometry. The intra- and interassay CVs are 10% and 15%, respectively, for 1,25-dihidroxyvitamin D, and 3.2% and 6.2%, respectively, for 25-hydroxyvitamin D.

Bone histomorphometry.—Only bone biopsies containing both intact cortices were used for bone histomorphometry. Bone biopsies were fixed in 70% alcohol, cleared with xylene, and embedded in methyl methacrylate. We examined static histomorphometric parameters in undecalcified 5 μm sections treated with toluidine blue stain, and we assessed tetracycline labeling in unstained 10 μm sections. Primary bone histomorphometric parameters were evaluated in trabecular bone using the OsteoMetrics system (OsteoMetrics, Decatur, GA). We calculated all derived indices by using the standard formulas.[14] We used the standard

turnover, mineralization, and volume classification for renal osteodystrophy to characterize pathology.[30] Of note, only 5 bone samples of the 9 bone biopsies obtained from healthy controls at the University of Colorado were used for histomorphometric analyses. We obtained 12 additional bone biopsies from healthy controls at the University of Kentucky (HHM), which were also analyzed for histomorphometry. All bone biopsy histomorphometric analyses were performed at the University of California, Los Angeles, by the same observer (RP).

Immunohistochemistry and quantification of bone protein expression.—We performed in ADPKD (n = 20) and healthy control (n = 9) bone biopsy immunohistochemical staining and quantitation of bone FGF23, sclerostin, and DMP1 as previously described[20,21] by using the following primary human directed antibodies: FGF23 (Immunotopics, Inc, San Clemente, CA), monoclonal anti-sclerostin (R&D Systems, Minneapolis, MN), monoclonal anti-osteopontin (Santa Cruz Biotechnology Inc., Santa Cruz, CA), and monoclonal anti-DMP1 (Santa Cruz Biotechnology Inc). Immunoreactivity for FGF23 was quantified by counting the number of osteocytes expressing FGF23 in one 5 μm section of trabecular bone and normalizing this number by bone area. The Ariol scanning system was used to correct for artifacts and nonspecific staining in quantitation of immunoreactivity for DMP1 and sclerostin, and data were also normalized by total bone area.[21] All slides were scanned at 20× magnification with a red filter and digitized (Applied Imaging Inc., San Jose, CA). Analyzed fields were manually selected to avoid areas with tissue damage occurring during immunostaining.[31–34] Staining was expressed as pixels per square millimeter. Immunohistochemistry results and quantification of bone protein expression in ADPKD were compared with double tetracycline—labeled iliac crest bone biopsy specimens from 9 healthy control participants.

Bone mineral density.—All scans were completed using the array beam mode. Standardized positioning and use of QDR software were based on the manufacturer's recommended protocol. Scans were read centrally and monitored for quality control at the University of Colorado Hospital reading center by using Hologic software version 7.10. The CV for lumbar spine, hip, and distal radius was <0.75%.

ESKD cohort.—Adults (age, 18–100 years) treated for ≥30 days without a history of kidney transplantation initiating in-center hemodialysis between January 1, 2002, and December 31, 2013, in Fresenius Medical Care North America outpatient hemodialysis facilities were included. The cohort was restricted to individuals with ESKD due to ADPKD, diabetes, hypertension, or glomerulonephritis. Data were collected from the Fresenius Medical Care North America clinical data warehouse, which collects information on demographic characteristics, cause of ESKD, dialysis treatment information, laboratory results, medications, and clinical outcomes.

Baseline data.—Demographic and clinical information included age, sex, race, time on dialysis before the first fracture, and cause of kidney disease based on *International Classification of Diseases, Ninth Revision, Clinical Modification* codes.

Outcomes.—The outcome of interest was the first fracture requiring hospitalization after the initiation of hemodialysis (recorded as a discharge diagnosis posthospitalization). The cohort was followed until the time of first fracture-related hospitalization or until the time of kidney transplantation, change in dialysis modality, discharge from Fresenius Medical Care, death, or end of study on December 31, 2014. We identified fractures using *International Classification of Diseases, Ninth Revision, Clinical Modification* codes (Supplementary Table S1). We included the following types of fractures: (i) vertebral; (ii) femur/pelvis/hip; (iii) tibia, fibula, patella, and ankle (lower leg); (iv) ribs and sternum; (v) humerus, scapula, and clavicle (shoulder and upper arm); and (vi) forearm (radius and ulna) and wrist. Unspecified fractures and fractures involving hands and feet were excluded.

Statistical analysis

We calculated the mean and SD for continuous variables and the count and proportion for categorical variables. We used the Fisher exact test for between-group comparisons for categorical variables and the 2-sample *t* test for continuous variables with the assumption of normality. We used nonparametric tests for nonnormally distributed variables. Pearson correlations were used to investigate the correlation of iFGF-23, alkaline phosphatase, and iPTH with eGFR in the HALT-PKD and CRIC cohorts. Differences in histomorphometric parameters between patients with ADPKD and healthy controls were performed using a *t* test for continuous variables and a chi-square test for categorical variables. Fracture rates were calculated according to the etiology of kidney disease (diabetic kidney disease, ADPKD, hypertension, and glomerulonephritis). Fracture incidence rates were adjusted for age, sex, race, and time on dialysis before the first fracture and adjusted incidence rate ratios were estimated. We performed all analyses by using SAS 9.4 or higher (SAS Institute, Cary, NC).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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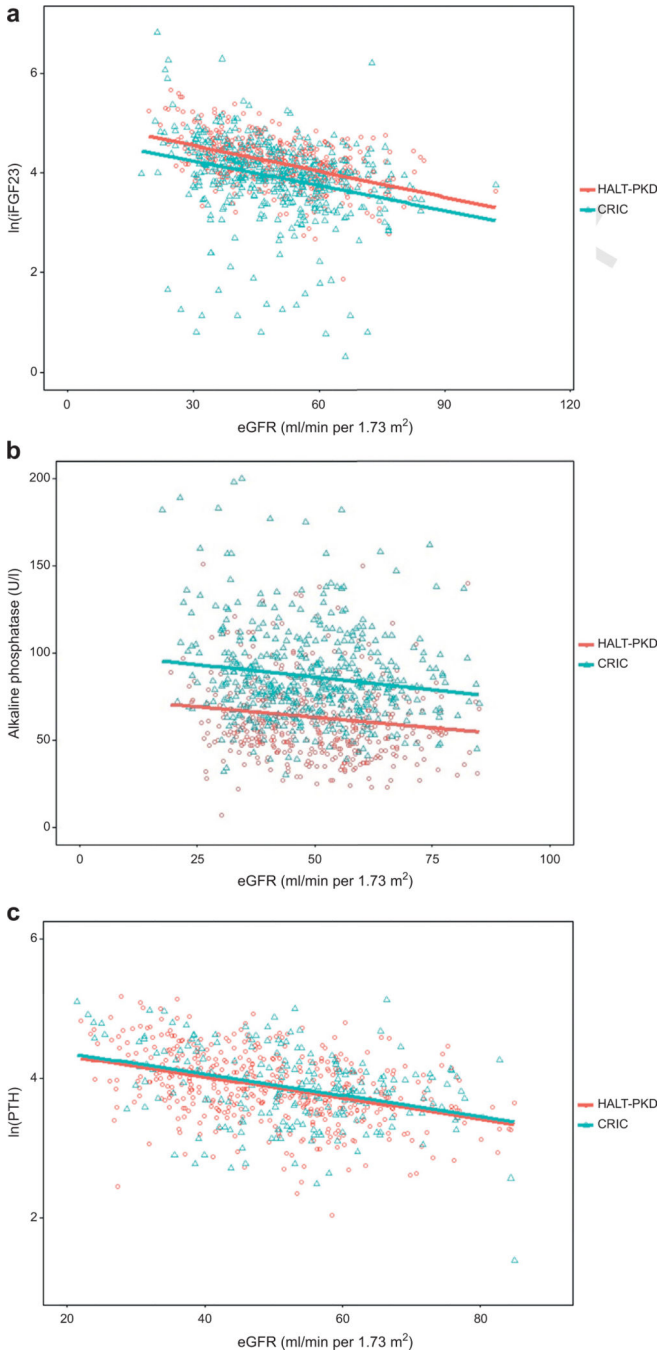

Figure 1. (a) Intact fibroblast growth factor-23 (iFGF23) increase is greater in patients with autosomal dominant polycystic kidney disease (ADPKD).

Unadjusted associations of iFGF23 with estimated glomerular filtration rate (eGFR) decline in the HALT-PKD and Chronic Renal Insufficiency Cohort (CRIC) cohorts. (**b**) Alkaline phosphatase is lower in patients with ADPKD. Unadjusted associations of alkaline phosphatase with eGFR decline in the HALT-PKD and CRIC cohorts. (**c**) Intact parathyroid hormone (PTH) in patients with ADPKD and non- ADPKD chronic kidney disease.

Unadjusted associations of intact PTH with eGFR decline in the HALT-PKD and CRIC cohorts.

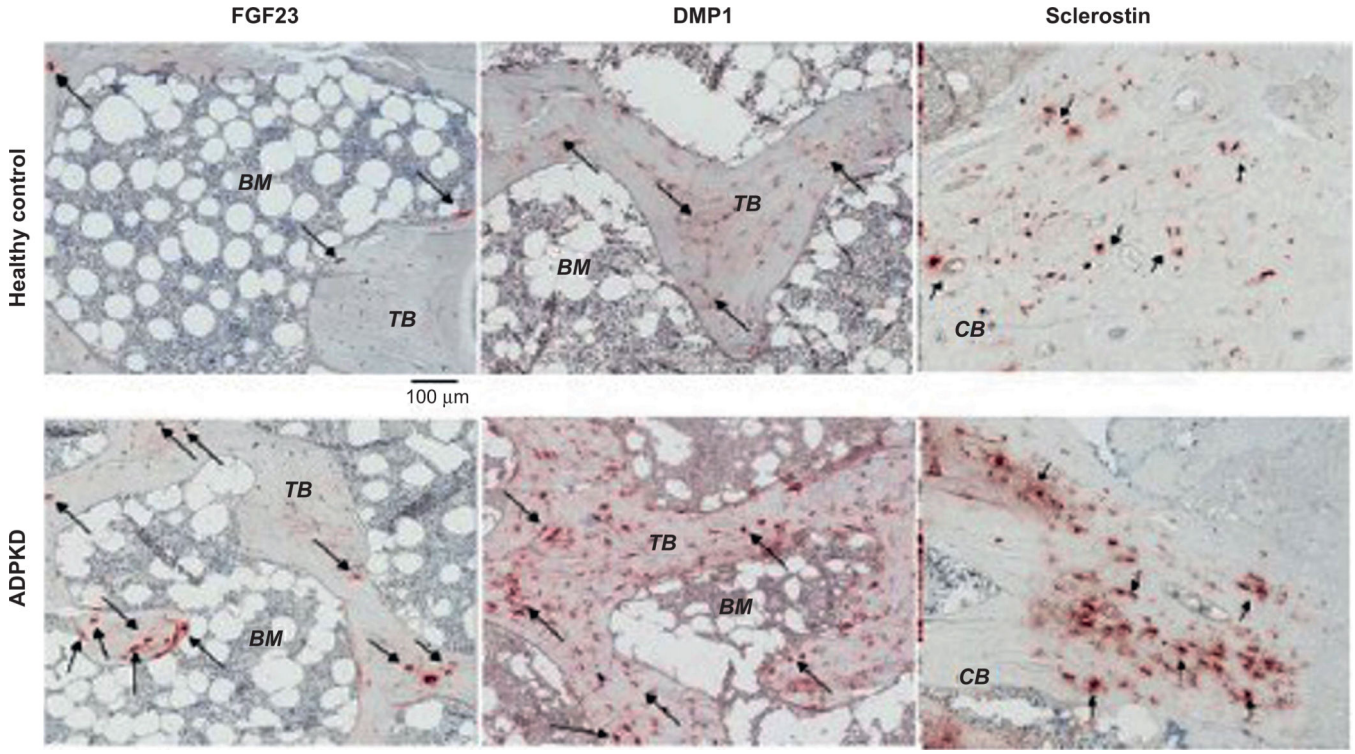

Figure 2. Immunohistochemical trabecular bone expression of fibroblast growth factor 23, dentin matrix acidic phosphoprotein1 (DMP1), and sclerostin in early autosomal dominant polycystic kidney disease (ADPKD).

BM, bone marrow, CB, cortical bone; TB, trabecular bone.

Table 1 |

Demographic and biochemical characteristics of subjects with ADPKD from the HALT-PKD cohort and subjects with non-ADPKD chronic kidney disease from the CRIC cohort

Characteristic	CRIC (*n* = 472)	HALT-PKD (*n* = 472)	*P*
Age, yr	58 ± 11	48 ± 9	<0.0001
Female	42	52	0.01
African American	0.2	3.0	0.001
Body mass index, kg/m^2	31 ± 8	28 ± 5	<0.0001
Systolic blood pressure, mm Hg	122 ± 20	129 ± 15	<0.0001
Hypertension	82	100	<0.0001
Diabetes at baseline	42	0	<0.0001
Kidney function			
Creatinine, mg/dl	1.5 ± 0.5	1.5 ± 0.4	0.69
eGFR (CKD-EPI equation),[14] ml/min per 1.73 m^2	49 ± 14	50 ± 13	0.62
Laboratory results (serum)			
Calcium, mg/dl	9.3 ± 0.5	9.4 ± 0.7	0.23
Phosphate, mg/dl	3.5 ± 0.5	3.4 ± 0.5	0.36
iPTH, pg/ml	47.0 (33–68)	47 (36–67)	0.90
iFGF23, pg/ml	54 (38–80)	69 (50–91)	<0.0001
Total alkaline phosphatase, IU/l	87 ± 29	63 ± 28	<0.0001

ADPKD, autosomal dominant polycystic kidney disease; CKD-EPI, Chronic Kidney Disease–Epidemiology Collaboration; CRIC, Chronic Renal Insufficiency Cohort; eGFR, estimated glomerular filtration rate; HALT-PKD, XXXX; iFGF23, intact fibroblast growth factor 23; iPTH, intact parathyroid hormone.

Data are expressed as mean ± SD, median (interquartile range), or percentage.

Table 2 |

Demographic and biochemical characteristics of ADPKD and healthy controls with a bone biopsy

Characteristic	ADPKD (*n* = 20)	Healthy controls (*n* = 9)	*P*
Age, yr	31 ± 11	28 ± 9	0.37
Male	11 (55)	3 (33)	0.01
Body mass index, kg/m^2	26 ± 4	24 ± 4	0.35
eGFR, ml/min per 1.73 m^2	97 ± 29	96 ± 16	0.90
Serum calcium, mg/dl	9.3 ± 0.3	9.6 ± 0.3	0.04
Serum phosphate, mg/dl	4.0 ± 0.5	3.6 ± 0.4	0.04
Serum total alkaline phosphatase, IU/l	55 ± 14	61 ± 17	0.33
Bone-specific alkaline phosphatase, µg/l	11.0 ± 4	14 ± 4	0.12
iPTH, pg/ml	31 ± 14	38 ± 14	0.20
25-Hydroxyvitamin D, ng/ml	30 ± 9	35 ± 11	0.15
1,25-Dihydroxyvitamin D, ng/ml	45 ± 12	65 ± 26	0.05
CTX, pg/ml	0.38 ± 0.22	0.41 ± 0.19	0.68
Intact FGF23, pg/ml	63 (51–85)	44 (40–67)	0.10
C-terminal FGF23, RU/ml	87 (69–116)	76 (72–81)	0.31
Urine phosphorus, mg/dl	68 ± 50	67 ± 28	0.95
Fractional excretion of phosphate, %	12 ± 6	9 ± 2	0.03

ADPKD, autosomal dominant polycystic kidney disease; CTX, serum collagen type 1 cross-linked C-telopeptide; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; iPTH, intact parathyroid hormone.

Data are expressed as mean ± SD, median (interquartile range), or n (%).

All data presented in Table 2 are from participants with ADPKD and healthy controls recruited from and studied at the University of Colorado.

Table 3 |

Comparison of bone static and dynamic histomorphometric indices between ADPKD and healthy controls

Parameter	ADPKD (*n* = 20)	Healthy controls (*n* = 17)	*P*
Demographic characteristics			
Age, yr	31 ± 10	37 ± 13	0.10
Female	47	45	0.68
Structural parameters			
BV/TV, %	26.49 ± 8.10	22.91 ± 5.71	0.14
Tb.N., #mm^2	1.97 ± 0.40	1.76 ± 0.18	0.04
Tb.Th., μm	134.98 ± 34.79	130.20 ± 56.93	0.66
Tb.Sp., μm	387.55 ± 88.53	444.50 ± 70.99	0.04
Remodeling parameters, %			
OV/BV	0.61 ± 0.34	1.21 ± 0.81	0.01
OV/TV	0.14 ± 0.08	0.30 ± 0.24	0.02
OS/BS	5.50 ± 3.87	10.01 ± 6.30	0.015
Ob.S/BS	0.7 ± 0.64	1.19 ± 1.76	0.28
Oc.S/BS	0.31 ± 0.31	0.39 ± 0.50	0.57
ES/BS	1.28 ± 1.36	1.15 ± 0.55	0.76
Dynamic remodeling parameters			
LS/BS, %	2.17 ± 0.99	4.88 ± 3.35	0.02
dLS/BS, %	1.81 ± 1.06	4.46 ± 3.24	0.02
MS/BS, %	2.17 ± 1.00	4.99 ± 3.39	0.02
MAR, μm/d	0.55 ± 0.15	0.53 ± 0.17	0.75
Aj.AR, μm/d	0.31 ± 0.34	1.45 ± 3.69	0.35
BFR/BS, μm^3/μm^2/d	0.012 ± 0.005	0.026 ± 0.018	0.03
BFR/BV, %/d	0.0.17 ± 0.007	0.041 ± 0.028	0.02
BFR/TV, %/d	0.005 ± 0.002	0.010 ± 0.007	0.04
Mlt, d	39.98 ± 27.83	48.75 ± 80.10	0.74

ADPKD, autosomal dominant polycystic kidney disease; Aj.AR, adjusted apposition rate; BFR/BS, bone formation rate/bone surface; BFR/BV, bone formation rate/bone volume; BFR/TV, bone formation rate/total volume; BV/TV, bone volume/total volume; dLS/BS, double labeled surface/bone surface; ES/BS, eroded surface/bone surface; LS/BS, labeled surface/bone surface; MAR, mineral apposition rate; Mlt, mineralization lag time; MS/BS, mineralizing surface/bone surface; Ob.S/BS, osteoblast surface/bone surface; Oc.S/BS, osteoclast surface/bone surface; OS/BS, osteoid surface/bone surface; OV/BV, osteoid volume/bone volume; OV/TV, XXXX; Tb.N, trabecular number; Tb.Sp, trabecular spacing; Tb.Th, trabecular thickness.

Data are expressed as mean ± SD or percentage.

The data from healthy controls are derived from 5 of the bone biopsies obtained at the University of Colorado and 12 bone biopsies from the University of Kentucky. All bone biopsy histomorphometric analyses were performed at the University of California, Los Angeles, by the same observer (RP).

Table 4 |

Incidence rate of fractures by etiology of kidney disease

Etiology of kidney disease	Adjusted incidence rate[a] per 1000 person-years (95% CI)
Diabetes	15.1 (13.9–16.3)
Hypertension	11.0 (10.2–11.9)
Glomerulonephritis	11.1 (9.9–12.4)
ADPKD	8.9 (7.2–10.9)

ADPKD, autosomal dominant polycystic kidney disease; CI, confidence interval.

[a]Adjusted for age, sex, race, and time on dialysis before the first fracture.