A distinct bone phenotype in ADPKD patients with end-stage renal disease

see commentary on page 261

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Autosomal dominant polycystic kidney disease (ADPKD) is among the most common hereditary nephropathies. Low bone turnover osteopenia has been reported in mice with conditional deletion of the PKD1 and PKD2 genes in osteoblasts, and preliminary clinical data also suggest suppressed bone turnover in patients with ADPKD. The present study compared the bone phenotype between patients with end stage renal disease (ESRD) due to ADPKD and controls with ESRD due to other causes. Laboratory parameters of bone mineral metabolism (fibroblast growth factor 23 and sclerostin), bone turnover markers (bone alkaline phosphatase, tartrate-resistant acid phosphatase 5b) and bone mineral density (BMD, by dual energy x-ray absorptiometry, DXA) were assessed in 518 patients with ESRD, including 99 with ADPKD. Bone histomorphometry data were available in 71 patients, including 10 with ADPKD. Circulating levels of bone alkaline phosphatase were significantly lower in patients with ADPKD (17.4 vs 22.6 ng/mL), as were histomorphometric parameters of bone formation. Associations between ADPKD and parameters of bone formation persisted after adjustment for classical determinants including parathyroid hormone, age, and sex. BMD was higher in skeletal sites rich in cortical bone in patients with ADPKD compared to non-ADPKD patients (Z-score midshaft radius -0.04 vs -0.14; femoral neck -0.72 vs -1.02). Circulating sclerostin levels were significantly higher in ADPKD patients (2.20 vs 1.84 ng/L). In conclusion, patients with ESRD due to ADPKD present a distinct bone and mineral phenotype, characterized by suppressed bone turnover, better preserved cortical BMD, and high sclerostin levels.

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Autosomal-dominant polycystic kidney disease (ADPKD) is an inherited disorder that commonly results in renal failure in humans; ADPKD accounts for 7% to 10% of patients with end-stage renal disease (ESRD).[1,2] More than 85% of patients with ADPKD have mutations in *PKD1*, *PKD2*, or both.[1,3] *PKD1* encodes polycystin (PC)1, which functions as a G protein–coupled receptor.[4] *PKD2* encodes PC2, which is a receptor-activated calcium channel.[1,5] PC1 interacts with PC2 to form heterodimers to colocalize in the primary cilia through interactions between the C-terminus of PC2 and kinesin family member 3A (*KIF3A*). The primary cilium is a solitary, immotile microtubule-based extension present on nearly every mammalian cell. This organelle has established mechanosensory roles in several contexts including kidney, liver, and the embryonic node.[6,7] It is postulated that the primary cilium plays a key role in normal physiologic functions of renal epithelia and that defects in ciliary function may contribute to the pathogenesis of ADPKD.[8] Recent research has implicated the primary cilium as a mechanosensor in bone as well.[9–11] Primary cilia not only play a role in embryonal skeletogenesis but also in postnatal/adult bone homeostasis. Osteocytes, that is, the most numerous bone cells, express the PC1/PC2 complex and exhibit a dendritic structure with extensive connectivity throughout the mineralized matrix of bone. The precise molecular mechanisms whereby osteocytes respond to and convert mechanical stimuli to biochemical signals remain elusive.

Because mechanical loading is the primary functional determinant of bone mass and architecture and a dysfunctional ciliary PC1/PC2 complex may disturb mechanosensation and transduction, it may be hypothesized that ADPKD may associate with a specific bone phenotype. Several lines of experimental and clinical evidence support this hypothesis. Heterozygous *PKD1* mutant mice have a decreased bone mineral density, trabecular bone volume, and cortical thickness. These mice also have downregulated gene expression of the osteoblastic markers Runx2, osterix, and osteocalcin, as well as an increase of the osteoprotegerin to receptor activator of nuclear factor–κB ligand ratio.[12] Along with this finding, Gitomer *et al.*[13] observed dramatically decreased bone formation in a small bone biopsy study including 5 patients with ADPKD who had preserved renal function. Gitomer *et al.*[14]

Table 1 | Demographics and parameters of mineral metabolism in ESRD patients with and without ADPKD

Demographics and laboratory parameters	ADPKD (n = 99)	Non-ADPKD (n = 419)	P value
Age, yr	56.9 ± 8.8	54.2 ± 13.5	0.5
Male sex, %	49.5	63.3	0.02
BMI, kg/m^2	24.7 ± 4.0	24.9 ± 4.3	0.8
Dialysis vintage (M)	31.8 (17.0–42.3)	31.8 (18.6–50.9)	0.1
Renal diagnosis, %			<0.0001
Diabetic nephropathy	0	10.7	
Glomerulonephritis/vasculitis	0	31.5	
Interstitial nephritis	0	10.0	
Hypertensive/large vessel disease	0	4.3	
Cystic/hereditary/congenital diseases	100	5.5	
Miscellaneous	0	8.4	
Etiology unknown or missing	0	25.6	
Diabetes mellitus, %	5.1	21.2	0.0002
CVD, %	27.3	42.3	0.005
PTX, %	7.1	14.6	0.05
Fracture, %	6.1	5.7	0.9
Calcium, mg/dl	9.2 ± 0.6	9.2 ± 0.8	0.7
Phosphate, mg/dl	4.7 ± 1.5	4.4 ± 1.4	0.02
Magnesium, mg/dl	2.3 ± 0.3	2.3 ± 0.4	0.2
biPTH, ng/l	133.8 (69.1–220.9)	121.7 (66.4–236.6)	0.9
25(OH)D_3, µg/l	37.7 (25.1–49.2)	35.5 (23.6–48.5)	0.3
1.25$(OH)_2D_3$, ng/l	27.3 (20.2–34.1)	26.7 (17.9–37.5)	1
FGF23, ng/l	3323 (1083–9548)	2040 (606–7573)	0.04
Sclerostin, ng/l	2.20 (1.68–3.16)	1.84 (1.28–2.57)	0.001
OPG, pmol/l	9.97 (8.0–12.3)	10.2 (7.3–14.0)	0.7
sRANKL, pmol/l	0.075 (0.063–0.14)	0.097 (0.063–0.17)	0.01
sRANKL/OPG	0.009 (0.006–0.016)	0.010 (0.005–0.021)	0.2
C-reactive protein, mg/l	3.60 (1.50–8.30)	3.30 (1.30–7.50)	0.6
IL-6, pg/ml	1.71 (0.87–2.77)	1.35 (0.63–2.37)	<0.05
tAP, × UNL	0.72 (0.53–0.95)	0.80 (0.62–1.09)	0.03
BsAP, ng/ml	17.4 (13.2–27.0)	22.6 (16.1–35.5)	<0.0001
PINP, µg/l	77.9 (49.8–111.1)	83.6 (53.7–143.1)	0.1
TRAP5b, U/l	4.65 (3.13–6.57)	5.46 (3.84–7.59)	0.006

ADPKD, autosomal-dominant polycystic kidney disease; BMI, body mass index; biPTH, biointact parathyroid hormone; BsAP, bone-specific alkaline phosphatase; ESRD, end-stage renal disease; FGF23, fibroblast growth factor 23; IL-6, interleukin-6; OPG, osteoprotegerin; P1NP, procollagen type I N propeptide; PTX, parathyroidectomy; sRANKL, soluble receptor activator of nuclear factor–κB ligand; TRAP5b, tartrate-resistant acid phosphatase 5b.
Data are presented as mean ±SD or median (interquartile range).

also reported a lower areal bone mineral density (aBMD) in patients with early stage ADPKD compared with healthy control subjects.

The present observational study aimed to confirm and extend these findings. Laboratory parameters of bone metabolism and turnover, bone mineral density (BMD), and bone histomorphometry were investigated in a large cohort of patients with ESRD who were being referred for kidney transplantation.

RESULTS

Demographics

Five hundred eighteen patients with ESRD, all kidney transplant candidates, were enrolled in the present study. ADPKD was the primary renal disease in 99 patients, corresponding to a prevalence of 19%. Table 1 compares demographics between patients with and without ADPKD. Females were more prevalent among patients with ADPKD. Furthermore, patients with ADPKD had fewer diagnoses of diabetes and cardiovascular morbidity and a borderline significant lower history of parathyroidectomy. Fractures were equally prevalent in patients with and without ADPKD.

Bone turnover markers and laboratory parameters of mineral metabolism

Bone-specific alkaline phosphatase (BsAP) and tartrate-resistant acid phosphatase 5b (TRAP5b) levels were significantly lower in patients with ADPKD than in patients without ADPKD (Table 1; Figure 1). Bone turnover markers strongly correlated with each other ($\rho \geq 0.5$, all $P < 0.0001$, Supplementary Table S1). Importantly, in multivariable regression analyses, ADPKD was identified as determinant of circulating BsAP and TRAP5b levels, independent of age, sex, diabetes, parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and sclerostin. Serum phosphate, sclerostin, and FGF23 levels were significantly higher in patients with ADPKD compared with patients without ADPKD (Table 1). In multivariable regression analysis, age, sex, dialysis vintage, PTH, FGF23, and calcitriol, as well as diagnosis of ADPKD, were identified as independent determinants of circulating sclerostin levels, explaining 24% of its variability. Determinants of FGF23 were quite different. Only calcium, phosphate, and calcitriol were retained in the final model, altogether explaining 44% of the variability of FGF23 (Table 2).

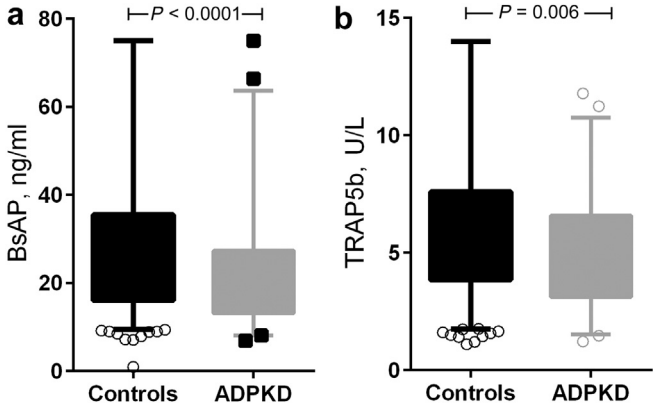

Figure 1 | Levels of bone-specific alkaline phosphatase (BsAP) (a) and tartrate-resistant acid phosphatase 5b (TRAP5b) (b) in patients with end-stage renal disease due to autosomal-dominant polycystic kidney disease (ADPKD) versus control subjects without ADPKD.

ADPKD and bone histomorphometry

Bone biopsies were performed in 90 patients at the time of transplantation and yielded bone specimens of sufficient quality to perform quantitative bone histomorphometry in 71 patients (ADPKD, $n = 10$; non-ADPKD, $n = 61$; Table 3). Inadequate samples were equally distributed between the 2 groups. Bone volume did not differ between patients with and without ADPKD. Mineralization tended to be higher in patients with ADPKD. Static parameters of bone turnover were lower in patients with ADPKD. However, statistically significant differences were reached for bone formation (osteoblast perimeter/tissue perimeter) only.

ADPKD and aBMD

Table 4 presents aBMD in patients with and without ADPKD. Median Z scores, expressing the SD relative to age- and sex-matched control subjects, were below zero across all skeletal sites examined in both groups, confirming that ESRD is a state of low bone mineral density. Z scores were higher in patients with ADPKD, with significance reached both at the mid-shaft radius and femoral neck. Of note, results were not meaningfully affected by the exclusion of patients who had undergone parathyroidectomy.

DISCUSSION

The main finding of the present cross-sectional observational study is that patients with ADPKD who have ESRD show a distinct bone phenotype characterized by suppressed bone turnover and preserved aBMD.

The gold standard for quantifying bone turnover is bone histomorphometry. Bone histomorphometry provides information not only on bone turnover but also on bone volume and mineralization. In the present study, static bone histomorphometric data were available in 71 patients and showed a trend of decreased bone turnover and increased mineralization in patients with ADPKD. Probably because of limited power, significance was reached for osteoblast perimeter/tissue perimeter ($P = 0.04$), that is, a marker of bone formation, only. These data confirm data from a pilot bone biopsy study in patients with early stage ADPKD.[13] They also align with radiologic data from more than 4 decades ago showing suppressed bone erosion in patients with ADPKD who were treated with hemodialysis compared with patients without ADPKD who were treated with hemodialysis.[15] Obtaining a

Table 2 | Factors associated with sclerostin and FGF23: univariate and multivariable regression analyses[a] using Ln sclerostin and Ln FGF23 as the dependent variable[b]

	Sclerostin						FGF23					
	Univariate			Multivariable			Univariate			Multivariable		
	β	*P*	R^2	β	*P*	R^2	β	*P*	R^2	β	*P*	R^2
Demographics—kidney disease												
Age (per yr)	0.01	<0.0001	0.06	0.01	<0.0001		−0.02	0.009	0.01			
Sex (female 0; male 1)	0.1	0.03	0.007	0.1	0.02		0.3	0.03	0.008			
Dialysis vintage (per month)	0.004	<0.0001	0.03	0.003	0.0002		0.001	0.7	0			
ADPKD (no 0; yes 1)	0.19	0.001	0.02	0.2	0.001		0.4	<0.05	0.006			
Mineral metabolism												
Phosphate (per mg/dl)	0.04	0.03	0.008				0.70	<0.0001	0.34	0.73	<0.0001	
Calcium (per mg/dl)	−0.02	0.6	0				0.58	<0.0001	0.07	0.72	<0.0001	
Ln PTH (per ng/l)	−0.12	<0.0001	0.1	−0.13	<0.0001		0.02	0.7	0			
Ln FGF23 (per ng/l)	0.04	0.002	0.02	0.03	0.01		–	–	–			
Ln sclerostin	–	–	–	–	–		0.44	0.002	0.02			
$1.25(OH)_2D$	−0.005	0.0007	0.02	−0.004	0.002		−0.02	0.003	0.02	−0.01	0.02	
Inflammation	–	–										
Ln IL-6	0.05	0.03	0.009				0.1	0.1	0.004			
Overall model						0.24						0.44

ADPKD, autosomal-dominant polycystic kidney disease; FGF23, fibroblast growth factor 23; IL-6, interleukin-6; Ln, natural logarithmic; PTH, parathyroid hormone.
Parameters studied were age, sex, diabetes, ADPKD, dialysis vintage, Ln PTH, Ln FGF23, and Ln sclerostin. Only parameters univariately associated at $P \leq 0.2$ are mentioned in the table.
[a]Generalized linear model.
[b]Because collinearity, only bone alkaline phosphatase was included in the multivariable model. Findings were similar for procollagen type I N propeptide and tartrate-resistant acid phosphatase 5b (data not shown).

Table 3 | Key demographics, laboratory parameters, and bone histomorphometry data in ESRD patients with and without ADPKD

	ADPKD (n = 10)	Non-ADPKD (n = 61)	P value
Demographics			
Age, yr	59.2 ± 10.7	54.4 ± 13.1	0.4
BMI, kg/m^2	28.2 ± 7.6	25.5 ± 4.2	0.4
Laboratory parameters			
Calcium, mg/dl	9.5 ± 0.5	9.3 ± 0.7	0.7
Phosphate, mg/dl	5.3 ± 1.1	4.4 ± 1.4	<0.05
biPTH, ng/l	197.3 (112.0–210.8)	204.4 (99.0–315.9)	0.5
FGF23, ng/l	5231 (1544–15913)	1159 (427–5245)	0.02
Sclerostin, ng/l	1.90 (1.68–2.97)	1.58 (1.07–2.28)	<0.05
BsAP, ng/ml	17.4 (14.2–22.1)	20.4 (15.3–35.5)	0.2
PINP, µg/l	83.0 (63.7–89.1)	80.0 (53.0 –131.2)	0.6
TRAP5b, U/l	4.34 (3.26–6.43)	5.80 (4.34–7.86)	0.2
Bone histomorphometry			
B.Ar/T.Ar, %	19.1 (14.4–23.1)	21.8 (17.5–26.5)	0.2
O.Ar/B.Ar, %	1.13 (0.85–1.60)	2.05 (1.11–3.14)	0.08
O.Pm/B.Pm, %	11.2 (8.40–19.3)	20.1 (11.5–25.4)	0.06
O.Wi, µm	6.72 (6.14–8.37)	7.41 (6.41–9.41)	0.3
Ob.Pm/O.Pm, %	0.00 (0.00–6.86)	9.56 (0.00–19.9)	0.08
Ob.Pm/T.Pm, %	0.00 (0.00–1.27)	1.61 (0.00–4.04)	0.04
E.Pm/B.Pm, %	4.10 (2.00–5.32)	4.23 (2.69–7.45)	0.3
Oc.Pm/E.Pm, %	10.8 (0.00–21.4)	16.6 (0.00–22.6)	0.6
Oc.Pm/T.Pm, %	0.31 (0.00–1.09)	1.61 (0.00–4.04)	0.4
Tb.th, µm	135.5 (109.2–165.4)	145.4 (126.4–168.0)	0.3
Tb.N, mm^{-1}	1.97 (1.65–2.27)	1.70 (1.47–2.00)	0.3
Tb.Sp, µm	481.1 (365.4–515.1)	372.9 (292.7–456.2)	0.2

ADPKD, autosomal-dominant polycystic kidney disease; B.Ar, bone area; biPTH, biointact parathyroid hormone; BMI, body mass index; B.Pm, bone perimeter; BsAP, bone-specific alkaline phosphatase; E.Pm, eroded perimeter; ESRD, end-stage renal disease; FGF23, fibroblast growth factor 23; M, mineralization; O.Ar, osteoid area; Ob.Pm, osteoblast perimeter; Oc.PM, osteoclast perimeter; O.Pm, osteoid perimeter; O.Wi: osteoid width; PINP, procollagen type I N propeptide; T, turnover; T.Ar, tissue area; Tb.N, trabecular number; Tb.Sp, trabecular spacing; Tb.th, trabecular thickness; Tb.Wi, trabecular width; TRAP5b, tartrate-resistant acid phosphatase 5b; Tt.Pm, total perimeter; V, volume.
Data are presented as mean ± SD or median (interquartile range).

bone biopsy specimen is invasive and requires the necessary skills; furthermore, analyzing bone biopsy specimens is expensive and necessitates specific histopathologic expertise that is not widely available. Therefore, a bone biopsy is not feasible in all patients all of the time.[16] Noninvasive imaging techniques (including isotope techniques)[17] and bone turnover markers have been suggested as a surrogate of or adjuvant to bone biopsy to assess bone turnover. In the present study, we assessed bone turnover by measuring circulating levels of BsAP, trimeric N-terminal propeptide of type I collagen (PINP), and TRAP5b, because these analytes are stable and undergo little degradation, are not cleared by the kidneys, exert little circadian rhythm, and are not affected by food intake. BsAP (–30%) and TRAP5b (–17%) were significantly lower in patients with ADPKD, whereas PINP (–7%) was only nominally lower in patients with ADPKD. The apparent discrepancy between BsAP and PINP remains to be explained but could be related to limitations inherent to the biomarker. Thus far, BsAP, PINP, and TRAP5b are not used routinely in clinical practice. In the absence of frank liver dysfunction, the total alkaline phosphatase level may be a valid alternative. In agreement with previous cohort studies in patients with early[18] and advanced[19] stage renal disease, we observed suppressed total alkaline phosphatase levels in patients with ADPKD. Of interest, a low total alkaline phosphatase level recently has been shown to be independently associated with a higher height-corrected total kidney volume in patients with early ADPKD[20] and thus might qualify as a biomarker of disease severity.

Of interest, these clinical observations perfectly align with data from recent *in vitro* and animal studies using advanced genetic approaches. Xiao and Quarles[10] demonstrated decreased osteoblast-mediated bone formation along with decreased expression of osteoblast-related genes including *Runx2*, Osteocalcin, Osteopontin, *SOST*, and *FGF23* in mice with conditionally and selectively deleted *PKD1* and *PKD2* in osteoblasts or osteocytes. Moreover, these mice showed significant reductions in both serum concentrations and bone mRNA expression of receptor activator of nuclear factor–κB ligand and TRAP, whereas serum PTH and osteoprotegerin did not differ from the wild-type mice. Altogether, these experimental data support the concept that primary cilium/polycystin complex plays an important role in bone mechanosensing. The precise mechanisms involved in translating mechanical signals into (re)modeling response remain unclear. Mounting evidence points to Wnt signaling pathway components, and the antiosteogenic canonical Wnt inhibitor Sost/sclerostin in particular, as important players in regulating the bone's adaptive response to loading. Wnt–β-catenin signaling directly affects both the osteoblast and the osteoclast bone cell lineages and also indirectly affects these cells through cross talk in the bone environment, inducing an overall increase in osteoblastogenesis together with a decrease in osteoclastogenesis.[21] Experimental and clinical evidence demonstrated that bone sclerostin expression and circulating sclerostin levels increased during skeletal mechanical unloading.[21,22] Starting from the premise that a disrupted mechanosensation mimics in some way the condition of unloading, increased bone sclerostin expression and higher circulating sclerostin levels would be anticipated in persons with ADPKD. This was actually observed in the present study and in a previous similar but smaller cohort study.[19] Importantly, ADPKD is associated with higher circulating sclerostin levels, independent of classic determinants, including PTH, age, sex, and inflammation. Remarkably, in aforementioned mice models of conditional deleted polycystins, bone sclerostin mRNA was not increased but suppressed. Residual confounding, assay related limitations, and altered translation all may be hypothesized to contribute to the discrepancy. If increased protein expression is confirmed in persons with ADPKD, additional studies will be required to decipher the molecular pathways involved. Besides being the consequence of impaired mechanosensation, increased sclerostin levels in persons with ADPKD also could be an adaptive counterregulatory response to enhanced canonical Wnt signaling as observed in polycystic kidneys.[23] Recent evidence points to high levels of hypoxia-inducible factor 1–α as the

Table 4 | aBMD in ESRD patients with and without ADPKD

	ADPKD	Non-ADPKD	*P* value
R1/3 ($n = 342$)			
BMD	0.708 (0.647–0.767)	0.683 (0.607–0.754)	0.07
T score	−0.172 (−0.595 to −0.017)	−0.251 (−1.070 to −0.101)	0.03
Z score	−0.04 (−0.15 to 0.61)	−0.14 (−0.37 to −0.00)	<0.0001
NI/osteopenia/osteoporosis, %	79.2/12.5/8.3	73.7/15.6/10.7	0.6
UDR ($n = 342$)			
BMD	0.390 (0.357–0.439)	0.391 (0.328–0.448)	1.0
T score	−1.757 (−2.994 to −0.776)	−2.036 (−2.813 to −1.012)	0.4
Z score	−0.83 (−1.85 to 0.10)	−1.19 (−2.09 to −0.29)	0.1
NI/osteopenia/osteoporosis, %	32.9/31.4/35.7	24.1/41.7/34.2	0.2
LS ($n = 518$)			
BMD	0.902 (0.789–1.037)	0.942 (0.839–1.058)	0.06
T score	−1.880 (−2.87 to −0.450)	−1.467 (−2.407 to −0.433)	0.09
Z score	−0.84 (−2.02 to 0.51)	−0.77 (−1.66 to 0.34)	0.5
NI/osteopenia/osteoporosis, %	34.4/36.5/29.2	39.0/38.5/22.5	0.4
FN ($n = 502$)			
BMD	0.705 (0619–0.758)	0.671 (0.583–0.767)	0.2
T score	−1.591 (−2.203 to −0.991)	−1.828 (−2.450 to −1.079)	0.08
Z score	−0.72 (−1.30 to −0.05)	−1.02 (−1.57 to −0.27)	0.01
NI/osteopenia/osteoporosis, %	26.0/58.3/15.6	22.2/54.2/23.7	0.2
TH ($n = 502$)			
BMD	0.849 (0.748–0.947)	0.855 (0.720–0.917)	0.1
T score	−1.048 (−1.837 to −0.379)	−1.286 (−2.025 to −0.645)	<0.05
Z score	−1.37 (−2.08 to −0.37)	−1.25 (−2.18 to −0.55)	1.0
NI/osteopenia/osteoporosis, %	47.9/43.8/8.3	37.2/51.7/11.1	0.1

aBMD, areal bone mineral density; ADPKD, autosomal-dominant polycystic kidney disease; BMD, bone mineral density; ESRD, end-stage renal disease; FN, femoral neck; LS, lumbar spine; R, radius; R1/3. mid-shaft radius; TH, total hip; UDR, ultradistal radius.
Data are presented as median (interquartile range).

culprit of increased osteocytic sclerostin expression and secretion in persons with ADPKD.[24,25]

A body of experimental and clinical evidence indicates that sclerostin not only suppresses bone formation[26–28] but also influences serum concentrations of hormones that regulate mineral accretion, including calcitriol and FGF23.[29] In this regard, the observation of an independent negative association between sclerostin and calcitriol and positive association between sclerostin and FGF23 aligns with findings in *SOST* knockout mice.[29]

Mice with conditionally and selectively deleted *PKD1* and *PKD2* in osteoblasts or osteocytes showed a reduced BMD, trabecular bone volume, and cortical thickness.[12] Also, in patients with early stage ADPKD, a lower aBMD compared with healthy control subjects has been reported.[14] To the contrary, in the present study we observed a better preserved aBMD in patients with ADPKD compared with patients without ADPKD. The different stage of kidney disease probably explains this controversy (Figure 2). In the setting of advanced chronic kidney disease, ADPKD-related suppression of bone remodeling may limit hyperparathyroidism-mediated bone loss. Bone remodeling activity affects bone volume and degree of mineralization, which are both important determinants of aBMD. As a consequence of an imbalance between resorption and formation at the individual bone remodeling units, high bone turnover causes accelerated bone (volume) loss. Moreover, when bone turnover is high or increased, the probability increases that a cortical or trabecular bone structural unit will be resorbed before completion of its secondary mineralization, which leads at the tissue level to a greater proportion of younger and submaximally mineralized bone.[30] In early stage chronic kidney disease, conversely, a low bone volume resulting from an imbalance between bone resorption and bone formation may be speculated to negate the impact of any pivotal benefit related to the suppression of bone turnover.

A key question is whether aforementioned alterations affect bone strength and fracture risk in patients. The present cohort study was not powered to answer this question. Clinical fractures were as prevalent in patients with ADPKD as in those without ADPKD. Notably, in a recent large population study in kidney transplant recipients, ADPKD was observed to confer an increased fracture risk, similar to diabetic nephropathy.[31] In patients undergoing dialysis, on the other hand, the incident fracture rate was shown to vary according to the cause of kidney disease: patients with ADPKD had the lowest rate and patients with diabetes had the highest rate.[32] Future epidemiologic studies should account for ADPKD as a potential confounder.

Besides increased circulating sclerostin levels, we also observed increased FGF23 levels in patients with ADPKD compared with patients without APKD. It remains to be defined whether these increased FGF23 levels result from increased skeletal or extraskeletal production. In regression analysis, the association between ADPKD and FGF23 disappeared after adjustment for serum phosphate. Serum phosphate levels were significantly higher in patients with ADPKD, even after adjustment for age, sex, and residual renal function. Previous observations in patients with early stage ADPKD support the hypothesis that the higher serum phosphate levels in persons with ADPKD might be a

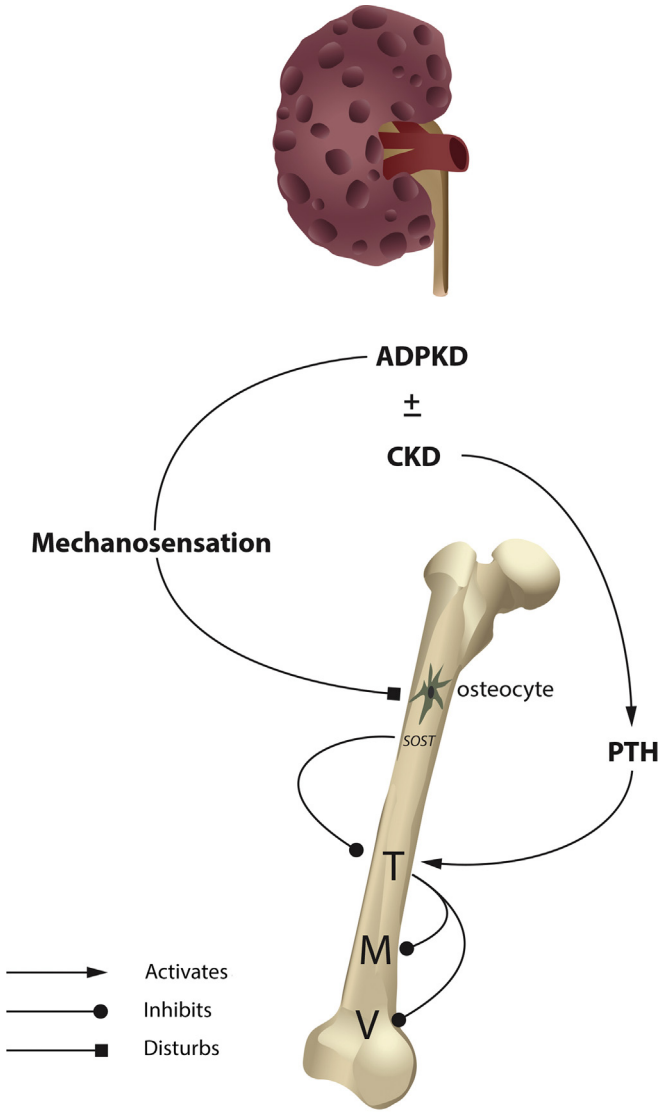

Figure 2 | Working model linking autosomal-dominant polycystic kidney disease (ADPKD) to bone phenotype according to stage of disease. In early stage disease, ADPKD associates with low bone turnover, osteopenia, probably as a consequence of disrupted mechanosensation and increased sclerostin expression. In a person with advanced stage disease, ADPKD mitigates hyperparathyroidism-related bone mineral density loss by suppressing bone turnover. CKD, chronic kidney disease; M, mineralization; PTH, parathyroid hormone; T, turnover; V, volume.

reflection of Klotho deficiency, thus implying FGF23 resistance.[33] Additional research is needed to clarify this issue.

In conclusion, patients with ADPKD and ESRD present a specific bone phenotype characterized by suppressed bone turnover, preserved aBMD, and high sclerostin levels. Clinical implications and therapeutic consequences remain to be defined.

MATERIALS AND METHODS

Design and study population

This study is an ancillary analysis of data collected in the frame of other studies exploring various aspects of bone health in renal transplant candidates before and after engraftment (NCT00547040, NCT01886950).

Adult patients (>18 years) with ESRD who were referred for single kidney transplantation at the University Hospital Leuven, Belgium, between 23 April 2006 and 21 December 2013 were eligible for inclusion in this cross-sectional observational study ($n = 950$; Supplementary Figure S1). Only patients with an available DXA scan within 2 weeks after transplantation were included in the present analysis ($n = 518$). Baseline demographics, laboratory parameters of mineral metabolism, and aBMD data in the overall cohort have been discussed previously (other data [P. Evenepoel *et al.*, unpublished data, 2018]). The present study focuses on differences between patients with ADPKD ($n = 99$) and without ADPKD ($n = 419$) and includes data on bone histomorphometry obtained in a subset of patients (ADPKD, $n = 10$ vs. non-ADPKD, $n = 61$). The study adhered to the principles of the Declaration of Helsinki and was approved by the Ethical Committee of KU Leuven. All patients provided written informed consent.

Clinical data

Relevant demographics, therapy (including details on mineral metabolism therapy), routine biochemistry, comorbidities, and fracture history were extracted from electronic files. Skull and digit fractures were excluded, as well as fractures associated with major trauma (e.g., motor vehicle accidents).

Biochemistry

Blood samples were collected at the time of admission for the renal transplant procedure (random, nonfasted). Samples were stored for <2 hours at 5 °C until centrifugation. Upon arrival at the laboratory, the blood samples were centrifuged at 3000 rpm for 10 minutes, aliquoted, and either processed immediately or stored at –80 °C until analysis. Levels of creatinine, hemoglobin, total calcium, phosphate, magnesium, total alkaline phosphatase, and albumin were measured using standard laboratory techniques. $1,25(OH)_2$VitD (calcitriol), 25(OH)VitD (calcidiol), and full-length (biointact) PTH were determined by immunoradiometric assays, as described elsewhere.[34-36] Total alkaline phosphatase levels were expressed as times upper normal limit to harmonize for the various assays being used for the duration of the study.

Serum sclerostin (TECOmedical, Sissach, Switzerland; reference range [RR] 450 ± 150, 510 ± 140, and 590 ± 130 pg/ml in men, premenopausal women, and postmenopausal women, respectively), biointact FGF23 (Kainos Laboratories, Inc., Tokyo, Japan; RR 8–78 pg/ml), osteoprotegerin (Biomedica, Vienna, Austria; p50 of a healthy population: 2.7 pmol/L), and soluble receptor activator of nuclear factor–κB ligand (Biomedica; p50 of a healthy population: 0.14 pmol/l) were measured according to the manufacturers' instructions. Interleukin-6 was measured on a MESO QuickPlex SQ120 multiplex imager (Meso Scale Discovery, Rockville, MD) using an electrochemiluminescence multiplex immunoassay (Human Proinflammatory Panel I [4-Plex], Meso Scale Discovery) according to the manufacturer's instructions. Bone-specific alkaline phosphatase (BsAP; RR 7.9–25.5 μg/l in men and 6.1–22.2 and 7.1–23.9 μg/l in pre- and postmenopausal women, respectively), trimeric ("intact") PINP (RR 12.8–71.9 μg/l in men and 13.7–71.1 and <82.6 μg/l in premenopausal and postmenopausal women, respectively) and TRAP5b (RR 1.4–6.1 U/l in men and 1.2–4.8 and 1.1–6.9 U/l in pre- and postmenopausal women, respectively) were measured with the IDS iSYS instrument (IDS, Boldon, UK). These cut-offs are obviously method dependent because large intermethod

variation has been observed in patients with chronic kidney disease.[37] All the coefficients of variation of the assays used in this study were <10%.

Bone densitometry

Measurements of aBMD were performed within 2 weeks after transplantation by DXA using a Hologic Discovery densitometer (Hologic QDR-4500A, Hologic, Marlborough, MA) at the lumbar spine (L1 through L4, $n = 518$), total hip ($n = 502$), and femoral neck ($n = 502$). In a subset of patients, aBMD ($n = 342$) also was assessed at the radius of the nondominant arm, both mid-shaft (R1/3) and ultradistal. All DXA scans were analyzed by a single certified and highly experienced operator. Results were expressed as absolute BMD (g/cm^2), as T score (SD relative to 20- to 30-year-old white U.S. women according to the National Health and Nutrition Examination Survey reference), and as Z score (SD relative to age- and sex-matched control subjects). Osteopenia was defined as a T score between –1 and –2.4 and osteoporosis was defined as a T score of –2.5 and below.

Bone histomorphometry

In a subset of 90 patients, a bone biopsy was performed at the end of the kidney transplant procedure using a needle with an internal diameter of 4.5 mm (Osteobell, Biopsybell, Mirandola, Italy) at a site 2 cm posterior and 2 cm inferior to the anterior iliac spine. Because the timing of deceased donor kidney transplantation is unpredictable, bone biopsies at the time of transplantation were performed without prior double tetracycline labeling. The method for quantitative histomorphometry of bone has been described elsewhere.[38] Briefly, biopsy specimens were fixed in ethanol 70% and subsequently embedded in a methylmethacrylate resin. Undecalcified 5-µm–thick sections were stained by the method of Goldner for quantitative histology to determine static bone parameters. All results are reported as measurements in 2 dimensions using nomenclature established by the American Society for Bone and Mineral Research.[39] Bone analysis was performed in the Laboratory of Pathophysiology of the University of Antwerp, Belgium, using a semiautomatic image analysis program (AxioVision version 4.51, Zeiss, Jena, Germany) running a custom program. Key parameters that were assessed included bone, perimeter of active osteoblasts on osteoid perimeter (Ob.Pm/O.Pm, %), perimeter of active osteoclasts on eroded perimeter (Oc.Pm/E.Pm, %), eroded perimeter on bone perimeter (E.Pm/B.Pm, %), bone area on tissue area (B.Ar/T.Ar, %), osteoid area on bone area (O.Ar/B.Ar, %) and osteoid width (µm). Fibrosis was scored as present or absent. Osteoid seams less than 2 µm in width were not included in primary measurements of osteoid width or area.

Because the absence of tetracycline labeling precluded determination of dynamic parameters, we used the bone area to total tissue area (B.Ar/T.Ar), osteoid area to bone area (O.Ar/B.Ar), and the ratio of osteoblast-covered perimeter to total bone perimeter (Ob.Pm/B.Pm) as surrogate markers for bone volume, mineralization, and turnover, respectively. Diagnostic cut-off values of these surrogate markers were determined after comparison static bone with dynamic bone parameters in bone biopsies of a separate cohort of tetracycline-labeled patients.[40]

Statistics

Results were expressed as mean ± SD or median (interquartile range), as appropriate. Patients were categorized according to primary renal disease (ADPKD vs. non-ADPKD). Differences between groups were evaluated using the unpaired Student *t*-test for parametric data and the Mann–Whitney U test for nonparametric data. Categorical data were compared between groups using χ^2 test. Simple and multivariable linear regression analyses were used to identify independent determinants of circulating sclerostin and FGF23 levels and bone turnover markers. Nonparametric distributed analytes were natural logarithm–transformed to achieve normality for the regression analyses. The SAS version 9.4 software program (SAS Institute, Cary, NC) was used for the statistical analysis. Two-sided $P < 0.05$ was considered statistically significant.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

PE designed the study, collected the data, supervised the biochemical analyses and wrote the first draft of the manuscript. All co-authors contributed to the analysis of the data and writing of the manuscript. In addition, EC performed part of the biochemical assays.

SUPPLEMENTARY MATERIAL

Figure S1. Patient disposition. ADPKD, autosomal dominant polycystic kidney disease; DXA, dual energy x-ray absorptiometry.
Table S1. Pearson correlation matrix of bone turnover markers (all $P < 0.0001$).
Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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