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

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CONTRIBUTIONS

P.E. 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, E.C. performed part of the biochemical assays.

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DISCLOSURES

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ABSTRACT

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 gender. 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.

BACKGROUND

Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder that commonly results in renal failure in humans; ADPKD accounts for 7-10% of patient with end stage renal disease (ESRD) (1;2). More than 85% of ADPKD patients have mutations in PKD1 and/or PKD2(1;3). PKD1 encodes polycystin (PC)1, which functions as a G protein coupled receptor(4). PKD2 encodes PC2 that is a receptor-activated calcium channel(1;5). PC1 interacts with PC2 to form heterodimers to co-localize 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 mechano-sensory 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 mechano-sensor in bone as well (9-11). Primary cilia not only play a role in embryonal skeletogenesis but also in postnatal/adult bone homeostasis. Osteocytes, i.e. the most numerous bone cells, express the PC1/PC2 complex and exhibit a dendritic morphology 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.

As 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 supports 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 kappa-B ligand (OPG/RANKL) ratio (12). Along this finding, Gitomer *et al.* (ASN 2014 [SA-OR094]) observed dramatically decreased bone formation in a small bone biopsy study including five ADPKD patients with preserved renal function. The same authors (ASN 2016 [SA-PO61]) also reported a lower aBMD in patients with early stage ADPKD as compared to healthy controls .

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

RESULTS

Demographics

Five hundred eighteen (518) patients with ESRD, all renal 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 ADPKD with non-ADPKD counterparts. Females were more prevalent among ADPKD patients. Furthermore, ADPKD patients were characterized by less diabetes and cardiovascular morbidity and a borderline significant lower history of parathyroidectomy. Fractures were equally prevalent in ADPKD and non-ADPKD patients.

Bone turnover markers and laboratory parameters of mineral metabolism

Bone specific alkaline phosphatase (BsAP) and TRAP5B levels were significantly lower in ADPKD patients than in non-ADPKD counterparts (***Table 1, Figure 1***). Bone turnover markers strongly correlated with each other (rho ≥0.5, all p<0.0001, ***supplementary Table 1***). Importantly, in multivariable regression analyses, ADPKD was identified as determinant of circulating BsAP and TRAP5B levels, independent of age, gender, diabetes, PTH, FGF23 and sclerostin. Serum phosphate, sclerostin and FGF23 were significantly higher in ADPKD vs non-ADPKD patients (***Table 1***). In multivariable regression analysis, age, gender, 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***).

ADPKD and bone histomorphometry

Bone biopsies were performed in 90 patients at the time of transplantation and yielded bone specimen 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 two groups. Bone volume did not differ between ADPKD and non-ADPKD patients. Mineralization tended to be higher in

ADPKD patients. Static parameters of bone turnover were lower in ADPKD patients. However, statistically significant differences was reached for bone formation (Ob.Pm/T.Pm) only.

ADPKD and areal bone mineral density

Table 4 presents aBMD in ADPKD and non-ADPKD patients. Median Z-scores, expressing the standard deviation relative to age- and gender-matched controls, 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 ADPKD patients, with significances reached both at the mid-shaft radius and femoral neck. Of note, results were not meaningfully affected by the exclusion of parathyroidectomized patients.

DISCUSSION

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

The gold standard for quantifying bone turnover is bone histomorphometry. Bone histomorphometry not only provides information 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 ADPKD. Probably due to limited power, significance was reached for Ob.Pm/T.Pm (p=0.04), i.e. a marker of bone formation, only. These data confirm data from a pilot bone biopsy study in patients with early stage ADPKD (Gitomer et al. ASN Renal week 2014 [SA-OR094]). They furthermore align with radiological data from more than four decades ago, showing suppressed bone erosion in ADPKD patients treated with hemodialysis as compared to non-ADPKD hemodialysis patients (13). Taking a bone biopsy is invasive and requires the necessary skills whilst its analysis is expensive and necessitates specific histopathological expertise which is not widely available. Therefore, a bone biopsy is not feasible in all patients all of the time (14). Noninvasive imaging techniques (including isotope techniques) (15) and bone turnover markers (BTM) have been suggested as 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 P1NP, 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 ADPKD patients, while P1NP (-7%) was only nominally lower in ADPKD. The apparent discrepancy between BsAP and P1NP remains to be explained, but could be related to limitations inherent to the biomarker. So far, BsAP, P1NP, and TRAP5bare not routinely used in clinical practice. In the absence of frank liver dysfunction, total alkaline phosphatase (tAP) may be a valid alternative. In agreement with previous cohort studies in patients with early (16) and advanced (17) stage renal disease we observed suppressed tAP in ADPKD patients. Of interest, low tAP has recently been shown to be independently associated with a higher height-corrected total kidney volume in patients with early ADPKD (Gitomer *et al.* ASN 2016 [SA-PO957]) and thus might qualify as a biomarker of disease severity.

Of interest, these clinical observations do perfectly align with data from recent in vitro and animal studies using advanced genetic approaches. Quarles *et al.* 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 (10). These mice moreover showed significant reductions in both serum concentrations and bone mRNA expression of RANKL and TRAP, while serum PTH and OPG did not differ from the wild type mice. Altogether, these experimental data support the concept of primary cilium/polycystin complex playing an important role in bone mechano-sensing. The precise mechanisms involved in translating mechanical signals into (re)modelling response remain unclear. Mounting evidence points to Wnt signalling pathway components, and the anti-osteogenic 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 crosstalk in the bone environment, inducing an overall increase in osteoblastogenesis together with a decrease in osteoclastogenesis (18). Experimental and clinical evidence demonstrated that bone sclerostin expression and circulating sclerostin levels increased during skeletal mechanical unloading (18;19). Starting from the premise that a disrupted mechano-sensation mimics in some way the condition of unloading, increased bone sclerostin expression and higher circulating sclerostin levels would be anticipated in ADPKD. This was actually observed in the present study and in a previous similar but smaller cohort study (17). Importantly, ADPKD associated with higher circulating sclerostin levels, independent of classical determinants including PTH, age, gender and inflammation. Remarkably, in abovementioned mice models of conditional deleted polycystins, bone sclerostin mRNA was not increased, but suppressed. Residual confounding, assay related limitations, and altered translation may all be hypothesized to contribute to the discrepancy. If increased protein expression is confirmed in ADPKD, additional studies will be required to decipher the molecular pathways involved. Besides being the consequence of impaired mechano-sensation, increased sclerostin levels in ADPKD could also be an adaptive counterregulatory response to enhanced canonical Wnt signaling as observed in polycystic kidneys (20). Recent evidence points to high levels of hypoxia-inducible factor 1-alpha as the culprit of increased osteocytic sclerostin expression and secretion in ADPKD (21;22).

A body of experimental and clinical evidence indicates that sclerostin does not only suppress bone formation (23-25) but also influences serum concentrations of hormones that regulate mineral accretion, including calcitriol and FGF23 (26). 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 (26).

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 as compared to healthy controls has been reported (Gitomer *et al.* ASN 2016 [SA-PO61]). To the contrary, in the present study we observed a better preserved aBMD in ADPKD patients as compared to non-ADPKD patients. Most probably, the different stage of kidney disease explains this controversy (***supplementary figure 2***). In the setting of advanced CKD, ADPKD-related suppression of bone remodeling may limit hyperparathyroidism-mediated bone loss. Bone remodeling activity affects bone volume and degree of mineralization, 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 for a cortical or trabecular bone structural unit to be resorbed before the completion of its secondary mineralization increases. This leads –at the tissue level- to a greater proportion of younger and submaximally mineralized bone (27). In early stage CKD, 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.

Key question is whether abovementioned 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 the ADPKD patients as compared to non-ADPKD patients. Notably, in a recent large population study in renal transplant recipients, ADPKD was observed to confer an increased fracture risk, similar to diabetic nephropathy (28). In dialysis patients, differently, incident fracture rate was shown to vary across etiology of kidney disease: patients with ADPKD had the lowest rate and patients with diabetes had the highest rate (Gitomer et al. ASN 2017 [TH PO784]). Future epidemiological studies should account for ADPKD as a potential confounder.

Besides increased circulating sclerostin levels, we also observed increased FGF23 levels in ADPKD patients as compared to non-APKD patients. It remains to be defined whether these increased FGF23 levels result from increased skeletal or extra-skeletal production. In regression analysis, the association between ADPKD and FGF23 disappeared after adjustment for serum phosphate. Serum phosphate levels were

significantly higher in ADPKD patients, even after adjustment for age, gender and residual renal function. Previous observations in early stage ADPKD patients support the hypothesis that the higher serum phosphate levels in ADPKD might be a reflection of Klotho deficiency, thus implying FGF23 resistance (29). Additional research is needed to clarify this issue.

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

MATERIAL AND METHODS

Design and Study population

This 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 (age > 18 years) with ESRD referred for single kidney transplantation at the University Hospital Leuven, Belgium between April 23, 2006 and Dec 21, 2013 were eligible for inclusion in this cross-sectional observational study (n=950) (***supplementary figure 1***). Only patients with available DXA scan within two weeks following transplantation were included in the present analysis (n=518). Baseline demographics, laboratory parameters of mineral metabolism and areal bone mineral density data in the overall cohort have been discussed previously (30). The present study focuses on differences between ADPKD (n=99) and non-ADPKD (n= 419) patients 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, co-morbidities 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, non-fasted). Samples were stored for <2 h at 5°C until centrifugation. Upon arrival at the laboratory, the blood

samples were centrifuged at 3000 rpm for 10 min, aliquoted, and either processed immediately or stored at –80°C until analysis. 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) parathyroid hormone (PTH) were determined by immunoradiometric assays, as described elsewhere (31-33). 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 (TECO medical, Sissach, Switzerland: Reference range (RR): 450±150, 510±140 and 590±130 pg/mL in men, pre and postmenopausal women, respectively), biointact fibroblast growth factor 23 (FGF23) (Kainos Laboratories, Inc., Tokyo, Japan; . RR: 8-78 pg/mL), Osteoprotegerin (OPG, Biomedica, Vienna, Austria. p50 of a healthy population: 2.7 pmol/L), soluble receptor activator of nuclear factor kappa-B ligand (sRANKL, Biomedica, Vienna, Austria. p50 of a healthy population: 0.14 pmol/L) were measured according to the manufacturers' instructions. Interleukin 6 (IL-6) was measured on a MESO QuickPlex SQ120 multiplex imager (Meso Scale Discovery, Rockville, Maryland, USA) using an electrochemiluminescence multiplex immunoassay (Human Proinflammatory I- 4plex, MSD) according to the manufacturer's instructions. Bone specific alkaline phosphatase (BsAP; RR: 7.9-25.5 µg/L in men; 6.1-22.2 and 7.1-23.9 µg/L in pre- and postmenopausal women, respectively), trimeric ("intact") N-terminal propeptide of type I collagen (P1NP; RR: 12.8-71.9 µg/L in men, 13.7-71.1 and <82.6 µg/L in premenopausal and postmenopausal women, respectively) and tartrate-resistant acid phosphatase isoform 5b (TRAP5b; RR: 1.4-6.1 U/L in men; 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 since large inter-method variation has been observed in CKD patients (34). All the coefficients of variation of the assays used in this study were <10%.

Bone densitometry

Areal bone mineral density (aBMD) measurements were performed within 2 weeks after transplantation by DXA using a Hologic Discovery® densitometer (DXA, Hologic Inc, Marlborough, MA Hologic QDR-4500A) at the lumbar spine (L1 through L4, n=518), total hip (TH, n=502), and femoral neck (FN, n=502). In a subset of patients aBMD (n=342) was also assessed at the radius of the non-dominant arm, both midshaft (R1/3) and ultradistal (UDR). 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 (standard deviation [SD] relative to 20-30 year-old white U.S. women according to the NHANES reference), and as Z-score (SD relative to age-

and gender-matched controls). Osteopenia was defined as a T-score between -1 and -2.4 and osteoporosis 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), at a site 2 cm posterior and 2 cm inferior to the anterior iliac spine. Since the timing of deceased donor kidney transplantation is unpredictable, bone biopsies at the time of transplantation were performed without prior double tetracycline labelling. The method for quantitative histomorphometry of bone has been described elsewhere (35). 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 two dimensions using nomenclature established by the American Society for Bone and Mineral Research (36). Bone analysis was performed in the Laboratory of Pathophysiology of the University of Antwerp, Belgium, using a semi-automatic image analysis program (AxioVision v 4.51, Zeiss, 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.

As the absence of tetracycline labelling 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 tetracyclin labelled patients(37).

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's t-test for parametric data and the Mann–Whitney U-test for

nonparametric data. Categorical data were compared between groups using chi-square test. Simple and multivariable linear regression analyses were used to identify independent determinants of circulating sclerostin and FGF23 levels and bone turnover markers. Non-parametric distributed analytes were ln-transformed to achieve normality for the regression analyses. The SAS version 9.4 (SAS Institute, Cary, NC) software program was used for the statistical analysis. Two-sided $p < 0.05$ was considered statistically significant.

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FIGURE LEGENDS:

FIGURE 1: Levels of TRAP5B and BsAP in patients with ESRD due to ADPKD vs non-ADPKD controls

FIGURE 2: Working model linking 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 mechano-sensation and increased sclerostin expression; in advanced stage disease, ADPKD mitigates hyperparathyroidism related bone mineral density loss by suppressing bone turnover. T: turnover; M: mineralization; V: volume. CKD: chronic Kidney Disease

TABLES

Table 1: demographics and parameters of mineral metabolism in in ADPKD vs non-ADPKD patients with ESRD

	ADPKD (n=99)	Non-ADPKD (n=419)	p-value
Age (yrs)	56.9 ± 8.8	54.2 ± 13.5	0.5
Gender M (%)	49.5	63.3	0.02
BMI (kg/m²)	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/heriditary/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, x 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

Abbreviations:: BMI: body mass index; biPTH: biointact PTH, FGF23: fibroblast growth factor 23; OPG: osteoprotegerin; sRANKL: soluble Receptor activator of nuclear factor kappa-B ligand; BsAP: bone specific alkaline phosphatase; P1NP: procollagen type I N propeptide, TRAP5b: tartrate-resistant acid phosphatase 5b; IL-6: interleukin 6; PTX: parathyroidectomy

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Table 2: Factors associated with sclerostin and FGF23: univariate and multivariable regression analyses[1] using ln sclerostin and ln FGF23 as the dependent variable

	Sclerostin						FGF23				
	Univariate			Multivariable			Univariate			Multivariable	
	β	p	R^2	β	p	R^2	β	p	R^2	β	p
Demographics-kidney disease											
Age (per yr)	0.01	<0.0001	0.06	0.01	<0.0001		-0.02	0.009	0.01		
Gender (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
LnPTH (per ng/L)	-0.12	<0.0001	0.1	-0.13	<0.0001		0.02	0.7	0		
LnFGF23 (per ng/L)	0.04	0.002	0.02	0.03	0.01		-	-	-		
LnSclerostin	-	-	-	-	-		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	-	-									
LnIL-6	0.05	0.03	0.009				0.1	0.1	0.004		
Overall model						0.24					

Parameters studied: age, gender, diabetes, ADPKD, dialysis vintage, LnPTH, Ln FGF23, LnSclerostin. Only parameters univariately associated at p≤0.2 are mentioned in the table.
* because collinearity, only BAP was included in the multivariable model. Findings were similar for P1NP and TRAP5b (data not shown)
[1] Generalized linear model

Table 3: Key demographics, laboratory parameters and bone histomorphometry data in ADPKD vs non-ADPKD patients with ESRD

	ADPKD (n=10)	Non-ADPKD (n=61)	p-value
Demographics			
Age (yr)	59.2 10.7	54.4 13.1	0.4
BMI (kg/m²)	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	135.5 [109.2-165.4]	145.4 [126.4-168.0]	0.3
TB.N	1.97 [1.65-2.27]	1.70 [1.47-2.00]	0.3
Tb.Sp	481.1 [365.4-515.1]	372.9 [292.7-456.2]	0.2

Abbreviations: B.Ar: bone area; T.Ar: tissue area; O.Ar: Osteoid area; O.Pm: osteoid perimeter; B.Pm: bone perimeter; O.Wi: osteoid width; Ob.Pm: osteoblast perimeter; E.Pm: eroded perimeter; Oc.PM: osteoclast perimeter; Tt.Pm: total perimeter: Tb.Wi: trabecular width; TB.N: trabecular number; T: turnover; M: mineralization; V: volume

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Table 4 : aBMD in ADPKD vs non-ADPKD patients with ESRD

		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
	Nl/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
	Nl/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
	Nl/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
	Nl/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
	Nl/osteopenia/osteoporosis	47.9/43.8/8.3	37.2/51.7/11.1	0.1

Abbreviations: BMD: bone mineral density; R: radius; R1/3: midshaft radius; UDR: ultradistal radius; LS: lumbar spine; FN: femoral neck; TH: total hip

LEGENDS FOR THE SUPP: LEMENTARY MATERIAL

SUPPLEMENTARY TABLE : Pearson correlation matrix of bone turnover markers (all $p<0.0001$).

SUPPLEMENTARY FIGURE 1: patient disposition

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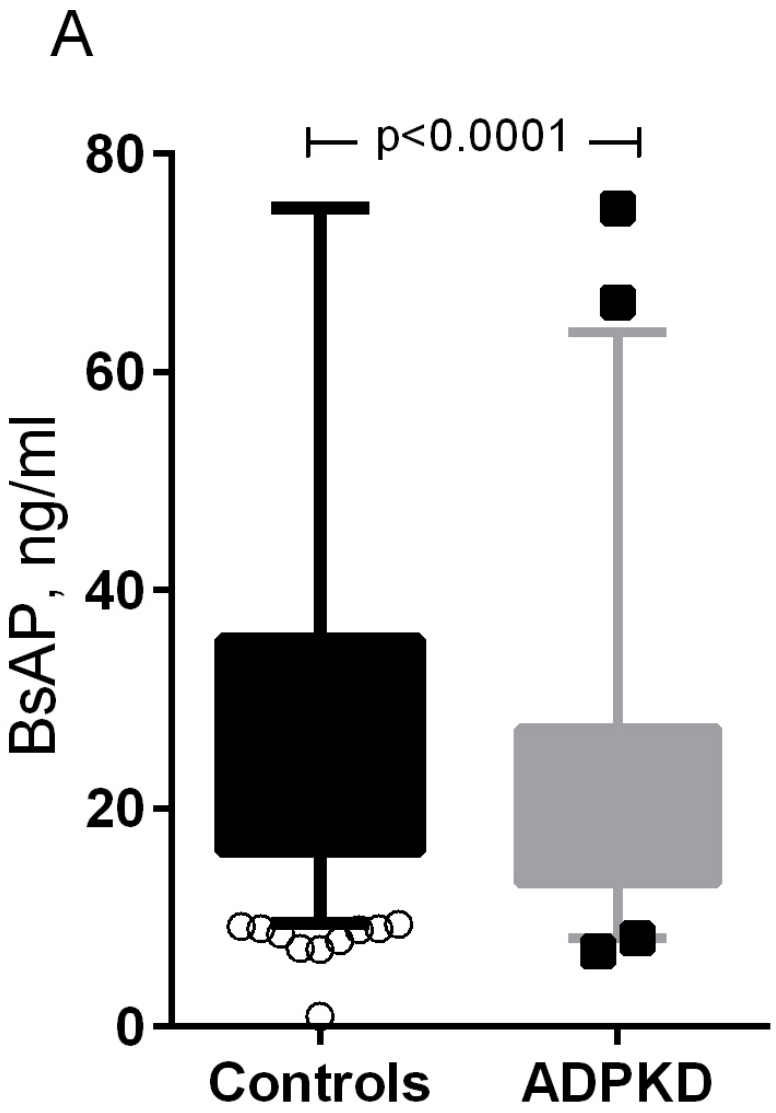

Levels of BsAP (A) and TRAP5B (B) in patients with ESRD due to ADPKD vs non-ADPKD controls

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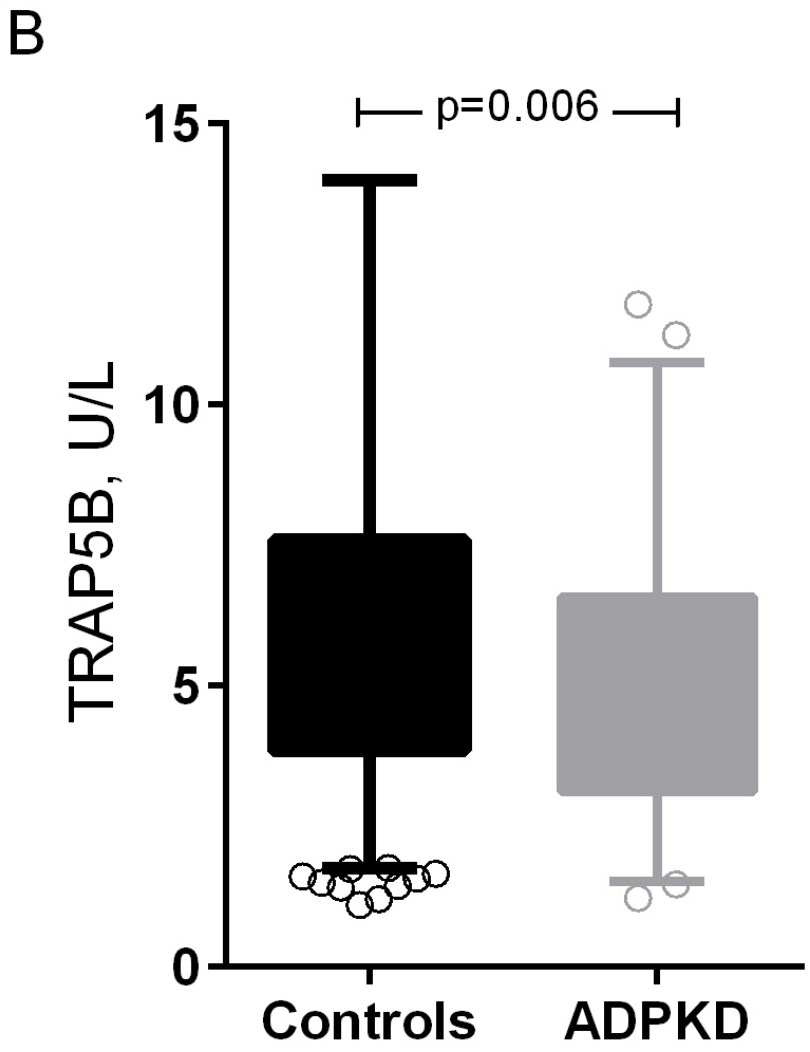

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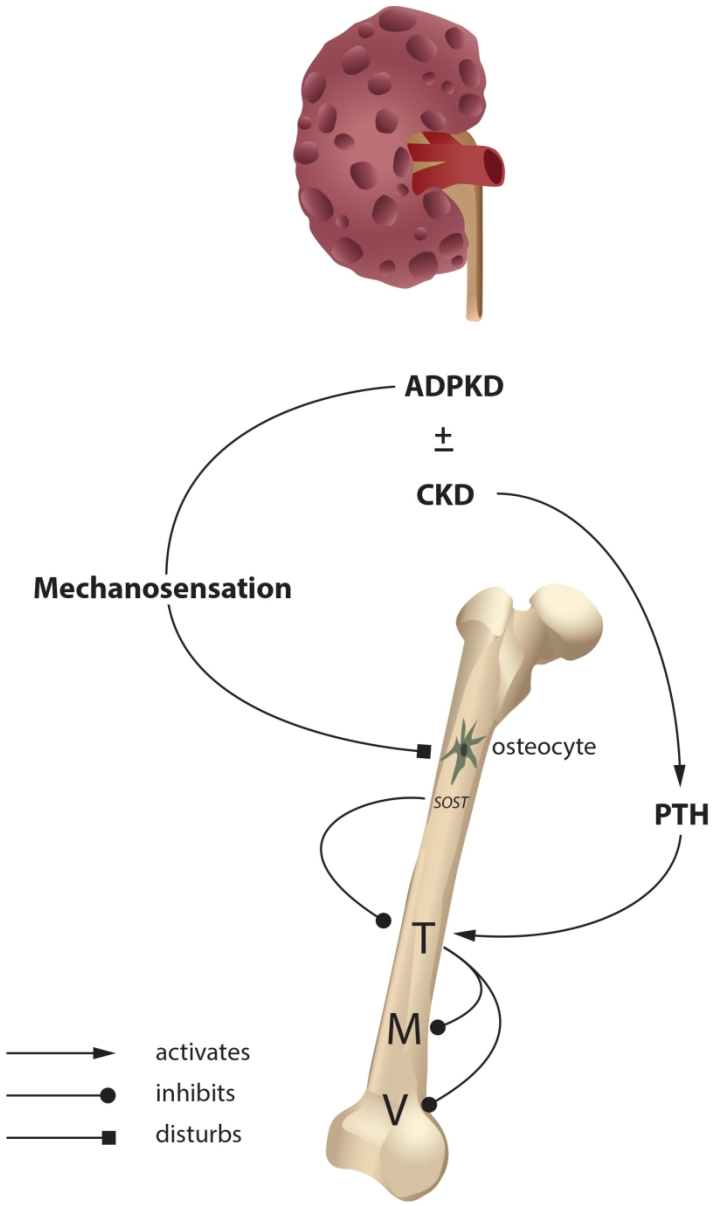


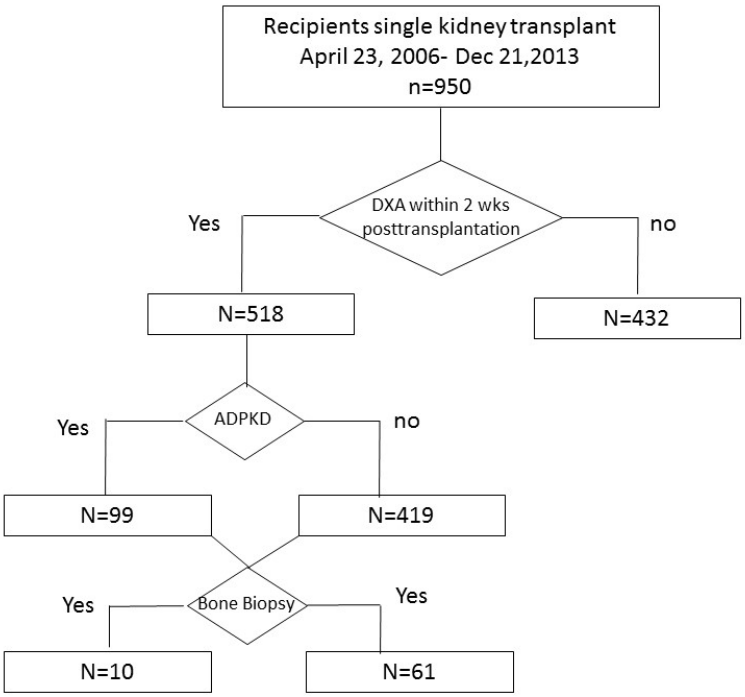
Working model linking ADPKD to bone phenotype according to stage of disease. In early stage disease, ADPKD associates with low bone turnover, osteopenia, probably as a consequence of disrupted mechano-sensation and increased sclerostin expression; in advanced stage disease, ADPKD mitigates hyperparathyroidism related bone mineral density loss by suppressing bone turnover. T: turnover; M: mineralization; V: volume. CKD: chronic Kidney Disease

125x202mm (300 x 300 DPI)

Supplementary Table 1: Pearson correlation matrix of bone turnover markers (all p<0.0001).

	tAP	BsAP	P1NP	TRAP5B
tAP	1.00	0.85	0.56	0.50
BsAP		1.00	0.75	0.64
P1NP			1.00	0.72
TRAP5b				1.00

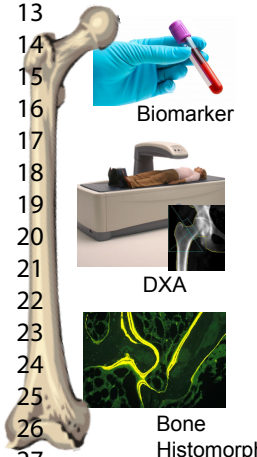
Abbreviations: tAP: total alkaline phosphatase; BsAP: bone specific alkaline phosphatase; P1NP: procollagen type I N propeptide, TRAP5b: tartrate-resistant acid phosphatase 5b



254x190mm (96 x 96 DPI)

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

Bone phenotype in 518 patients with end stage renal disease



Biomarker

Sclerostin	ADPKD (n=99)	>	Non-ADPKD (n=419)
BsAP; Trap5b	ADPKD (n=99)	<	Non-ADPKD (n=419)

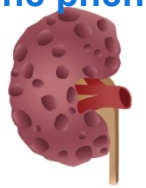
DXA

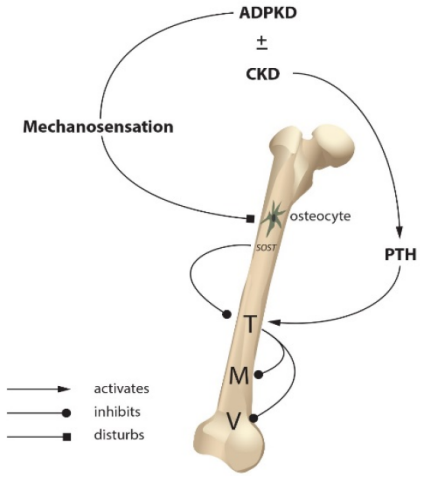
BMD (midshaft Radius, Femoral Neck)	ADPKD (n=96)	>	Non-ADPKD (n=406)

Bone Histomorphometry

Turnover (Ob.Pm/T.Pm)	ADPKD (n=10)	<	Non-ADPKD (n=61)

Working model linking ADPKD to bone phenotype





Conclusion

ADPKD patients with ESRD present a distinct bone and mineral phenotype, characterized by suppressed bone turnover, preserved areal bone mineral density and high sclerostin levels.