Sclerostin—A Debutant on the Autosomal Dominant Polycystic Kidney Disease Scene?

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disease originating from a mutation in genes encoding polycystin 1 and 2. Recent evidence suggests that these polycystins mediate mechanosensation not only in the primary cilia of kidney cells but also in bone cells. The Wnt/β-catenin signaling pathway plays a central role in mechanotransduction in osteocytes. Mechanical unloading causes the upregulation of the Wnt inhibitor sclerostin. We tested the hypothesis that ADPKD associates with higher circulating sclerostin levels.

Methods: In this observational, cross-sectional study, circulating levels of sclerostin and other laboratory parameters of mineral and bone disease, including intact parathyroid hormone (PTH), calcium, phosphate, magnesium, 25(OH) D-vitamin, 1,25 (OH)2 D-vitamin, and bone specific alkaline phosphatase (BALP) were assessed in 100 patients with end-stage renal disease recruited from an ongoing longitudinal cohort study in Stockholm, Sweden.

Results: Patients with ADPKD had higher sclerostin levels and lower BALP levels as compared to patients with other primary renal disease. In multivariate analysis, ADPKD associated with circulating sclerostin levels, independent of the established determinants including age, gender, body mass index, diabetes, phosphate, PTH, and 1,25 (OH)2 D-vitamin.

Discussion: Circulating sclerostin levels are increased in ADPKD, possibly reflecting impaired mechanosensation. The clinical relevance of this finding, especially with regard to bone health, remains to be investigated. Our finding draws attention to the etiology of kidney disease as an important, yet neglected, confounder of the association between renal failure and mineral and bone disease.

Kidney Int Rep (2017) 2, 481–485; http://dx.doi.org/10.1016/j.ekir.2017.01.001
KEYWORDS: autosomal dominant polycystic kidney disease; bone mechanosensors; kidney disease–mineral and bone disorder; polycystin; sclerostin

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disorder and 1 of the leading causes of end-stage renal disease (ESRD). ADPKD originates from a mutation in PKD1 or PKD2 genes encoding polycystins and transmembrane proteins ubiquitously expressed in human tissues.1 Mounting experimental evidence suggests that polycystin-1 (PC1) and polycystin-2 (PC2) co-localize in the primary cilia of epithelial cells, where their role could be to promote mechanosensation and fluid-flow sensation.2 Interestingly, the PC1/PC2 complex is expressed in various other cells besides renal tubular epithelial cells, including osteocytes. Osteocytes exhibit a dendritic morphology with extensive connectivity throughout the mineralized matrix of bone. It is thought that this system forms the bone mechanosensor, acting as the orchestrator of osteoblast and osteoclast activity in response to mechanical stimuli.3 Although the role played by polycystins in osteocytes is not yet fully elucidated, it is tempting to speculate that, as in the renal tubular epithelium, it is related to mechanosensation. The precise molecular mechanisms whereby osteocytes respond to and convert mechanical stimuli to biochemical signals remain largely elusive. Evidence points to sclerostin as a central player in the process of mechanotransduction.4 Sclerostin acts as an inhibitor of Wnt/β-catenin signaling pathway by binding to a receptor.
from the family of low-density lipoprotein receptors. Besides mechanical loading, several hormones and clinical conditions have been documented to affect the expression of sclerostin in bone. For example, sclerostin is stimulated by calcitonin and calcitriol, whereas parathyroid hormone (PTH) and estrogens downregulate its expression. Circulating sclerostin levels increase with aging, and high levels are observed in patients with diabetes and inflammation. Circulating sclerostin levels increase along the progression of chronic kidney disease (CKD) to reach levels in ESRD that are several-fold higher than in healthy controls. Levels rapidly decrease following renal transplantation. Some investigators have reported sclerostin expression in calcifying vessels and aortic valves. Thus, sclerostin is considered to be an important player in chronic kidney disease—mineral and bone disorder and is thought to be involved in the bone—vascular axis.

Intrigued by the above-mentioned considerations, we studied levels of sclerostin in patients with ESRD due to ADPKD in comparison to other primary renal diseases. Our working hypothesis was that impaired bone mechanosensing, related to a dysfunctional PC1/PC2 complex, may lead to increased circulating sclerostin levels in ADPKD.

MATERIALS AND METHODS

Methods

Participants in the ongoing cohort study at the Department of Renal Medicine, Karolinska University Hospital, Stockholm, Sweden, were included in this cross-sectional study. Inclusion criteria comprised age ≥18 years, ESRD, qualification to undergo living donor renal transplantation (LD-RTx), and consent to participate. The ADPKD group consisted of patients fulfilling clinical criteria for the diagnosis (i.e., medical history and/or radiological imaging). The non-ADPKD group consisted of patients with other primary diagnoses underlying ESRD. We chose to include a LD-RTx cohort to reduce the heterogeneity of the investigated cohorts besides the difference in primary kidney disease. The elective procedure of LD-RTx involves, among other things, strict control of active infection, inflammation, neoplastic processes, and cardiovascular disease.

Blood was sampled immediately prior to RTx. Analyses of circulating intact parathyroid hormone (PTH), creatinine, albumin, calcium, phosphate, magnesium, 1,25 (OH)2 D-vitamin, and 25(OH) D-vitamin were performed with validated routine methods at the accredited Clinical Laboratory of Karolinska University Hospital, Stockholm, Sweden. Sclerostin and bone alkaline phosphatase (BALP) were analyzed with enzyme-linked immunosorbent assays from R&D System (Abingdon, UK) and Immunodiagnostic Systems (Bolden, UK), respectively. The limit of detection of the sclerostin assay is 1.74 pg/ml (range, 0.370–3.80 pg/ml). Total alkaline phosphatase (ALP) activity was assessed using a commercial reagent kit (Thermo Fisher Scientific Oy, Vantaa, Finland).

The study adhered to the principles of the Declaration of Helsinki and was approved by the regional committee of ethics in Stockholm. All participants provided informed consent.

Statistical Analyses

Data are expressed as median (range of 10th–90th percentile) or percentage. Statistical significance was set at the level of $P < 0.05$. Comparisons between groups were assessed with the nonparametric Kruskal–Wallis test for continuous variables and $\chi^2$ test for nominal variables. Spearman rank correlation analysis was used to determine associations between continuous and ordinal parameters. Determinants of circulating sclerostin levels were investigated by multiple linear regression analysis in a model including the presence of ADPKD versus other CKD etiology, 1-standard deviation (1-SD) higher age, gender, diabetes, 1-SD higher body mass index (BMI), 1-SD higher phosphate, 1-SD higher PTH, 1-SD higher calcium, and 1-SD higher dialysis vintage. Statistical analyses were performed using SAS statistical software version 9.4 (SAS Campus Drive, Cary, NC).

RESULTS

A total of 100 adult ESRD patients who were qualified to undergo LD-RTx were included in this study. The median age was 46 years; 61% were male and 39% female. Of the 100 participants, 19% had ADPKD, 38% glomerulonephritis, 7% diabetes mellitus (DM), and 36% were diagnosed with other primary kidney diseases (tubulointerstitial nephritis, Alport’s syndrome, amyloidosis, Wilms tumor, atypical hemolytic uremic syndrome (aHUS), antiphospholipid syndrome, nephronophthisis, multicystic dysplasia, myeloma kidney, renal cell cancer, and unknown).

Demographic and clinical characteristics of the patients are shown in Table 1. ADPKD patients were older than patients with other primary renal diseases and had shorter dialysis vintage. All other clinical and demographic parameters were comparable among groups. Mineral and bone metabolism biomarkers are summarized in Table 2. ADPKD patients were characterized by higher concentrations of sclerostin, calcium, and 25(OH) D-vitamin and lower concentrations of BALP. Circulating PTH levels did not differ between ADPKD and non-ADPKD patients. Results of univariate analysis of sclerostin versus other variables in the whole cohort are displayed in Table 3, showing...
associations with age, PTH, creatinine, body mass index, and calcium, which may have been expected. In multivariate regression analysis, ADPKD was identified as a determinant of circulating sclerostin levels, independent of age, serum phosphate, and PTH (Table 4).

DISCUSSION

To the best of our knowledge, we are the first to report that patients with ESRD due to ADPKD have significantly higher circulating sclerostin levels than patients with ESRD due to other primary renal diseases. ADPKD is independently associated with sclerostin levels with an impact similar to that of age or PTH.

It is tempting to speculate that the higher circulating sclerostin levels in ADPKD patients reflect dysfunction of osteocytic polycystins, which translates into attenuated mechanosensation. Because osteocytes react in response to mechanical loading by downregulation of sclerostin, cells that are deprived of the signal from mechanosensors or from the intracellular communication net may secrete more sclerostin. Alternatively, increased circulating sclerostin levels may be a consequence of impaired binding of sclerostin to low-density lipoprotein receptors, similar to PTH resistance causing increased PTH levels. Mutations in genes encoding low-density lipoprotein receptors are thought to be

Table 2. Parameters of mineral metabolism in 100 ESRD patients undergoing living donor kidney transplantation grouped according to primary kidney disease (i.e., autosomal polycystic kidney disease, versus other primary kidney diseases)

|                  | ADPKD (n = 19) | CGN (n = 38) | DM (n = 7) | Other (n = 36) | P value |
|------------------|----------------|--------------|------------|----------------|---------|
| Sclerostin (pg/ml) | 614 (335–1167) | 378 (182–702) | 564 (373–711) | 406 (254–823) | 0.001   |
| PTH (ng/l)       | 184 (82–902)   | 280 (158–970) | 319 (49–560) | 302 (90–567) | 0.14    |
| Calcium (mmol/l) | 2.4 (2.2–2.6)  | 2.2 (2.0–2.5) | 2.3 (1.5–2.5) | 2.3 (2.2–2.7) | 0.001   |
| Phosphate (mmol/l) | 1.6 (1.2–2.4) | 1.7 (1.0–2.3) | 1.4 (0.9–1.6) | 1.7 (0.9–2.5) | 0.29    |
| Magnesium (mmol/l) | 0.90 (0.77–1.10) | 0.84 (0.65–1.03) | 0.87 (0.71–1.24) | 0.84 (0.74–1.00) | 0.41 |
| 25 (OH) D-vitamin (nmol/l) | 48 (20–92) | 39 (23–60) | 45 (28–102) | 30 (17–47) | 0.003   |
| 1,25 (OH)2 D-vitamin (ng/l) | 17 (10–26) | 16 (10–42) | 16 (5–39) | 19 (9–36) | 0.48    |
| Alkaline phosphatase (μU) | 63 (38–93) | 56 (37–135) | 76 (47–92) | 70 (34–123) | 0.18    |
| Bone alkaline phosphatase (μg/l) | 11.2 (7.8–25.7) | 15.6 (9.3–46.7) | 23.2 (9.6–34.0) | 21.2 (6.6–53.3) | 0.022   |

Significant differences are indicated in bold.
ADPKD, autosomal dominant polycystic kidney disease; CGN, glomerulonephritis; DM, diabetes mellitus; ESRD, end-stage renal disease; PTH, intact parathyroid hormone.
involved in cystogenesis in polycystic liver disease and are believed to be responsible for the occurrence of sporadic ADPKD. Finally, increased sclerostin levels could be an adaptive response to enhanced canonical Wnt signaling caused by ciliopathy.

A key question ensuing from our observation relates to the clinical relevance of increased circulating sclerostin levels in ADPKD. Studies investigating the association between circulating sclerostin levels and mortality have yielded heterogeneous results. Although both analytical and statistical issues and case mix may explain the apparent controversy, our study findings indicate that primary renal disease should be considered as an additional confounder. Further studies exploring the relationship between ADPKD, circulating sclerostin levels, and outcome are required. Irrespective of the mechanism by which the concentration of circulating sclerostin increases in ADPKD, it may have a broad array of clinical consequences for vascular calcification and bone disease.

Table 3. Correlation matrix (Spearman rank) showing correlations between sclerostin and other variables in 100 ESRD patients undergoing living donor kidney transplantation

| Variable                  | Sclerostin (pg/ml) | P value  |
|---------------------------|-------------------|----------|
| PTH (ng/l)                | −0.315            | 0.0018   |
| Age (yr)                  | 0.303             | 0.0087   |
| BMI (kg/m²)               | 0.212             | 0.0347   |
| Diabetes comorbidity      | 0.181             | 0.0715   |
| Cardiovascular disease    | 0.197             | 0.05     |
| Calcium (mmol/l)          | 0.236             | 0.0179   |
| Phosphate (mmol/l)        | 0.089             | 0.379    |
| Creatinine (μmol/l)       | 0.225             | 0.0247   |
| Albumin (g/l)             | 0.047             | 0.641    |
| hs-CRP (mg/l)             | 0.060             | 0.512    |
| 1,25 (OH)_2 D vitamin (ng/l) | −0.096          | 0.368    |

Significant differences are indicated in bold.

Table 4. Predictors of 1-SD of sclerostin level based on output from multivariate linear regression analysis

| Variable                  | β       | P value  |
|---------------------------|---------|----------|
| 1-SD of age (yr)          | 0.29    | 0.007    |
| Gender, male versus female| 0.27    | 0.12     |
| ADPKD versus no ADPKD     | 0.51    | 0.03     |
| Diabetes versus no diabetes| −0.05  | 0.82     |
| 1-SD of BMI, kg/m²        | 0.03    | 0.73     |
| 1-SD of 1,25 (OH)_2 D vitamin (ng/l) | −0.07 | 0.42 |
| 1-SD of phosphate, mmol/l | 0.21    | 0.03     |
| 1-SD of PTH, ng/l         | −0.29   | 0.002    |
| 1-SD of intact calcium, mmol/l | 0.06 | 0.51 |
| 1-SD of dialysis vintage, yr | 0.02   | 0.79     |

Significant differences are indicated in bold.

In particular, it underscores the importance of taking primary renal disease into account when analyzing the association between circulating sclerostin levels and hard outcomes.

In animal models, loss of PKD1 in osteocytes has been associated with reduced bone mineral density (BMD), total bone volume, cortical thickness, and impaired bone formation rate. Patients with ADPKD, however, do not have clinically obvious skeletal abnormalities. Plausibly, kidney disease—mineral and bone disorder may mask a specific skeletal phenotype associated with heterozygous inactivation of PKD1 in bone. In addition, a “second hit” affecting a second allele and overlapping heterozygous mutation in ADPKD may not occur in bone, and, if it did, an insufficient fraction of osteoblasts might be affected to cause clinically detectable skeletal abnormalities.

It is well established that the Wnt/β-catenin signaling pathway also plays a prominent role in vascular pathobiology. Notwithstanding a matter of ongoing controversy, several lines of experimental and clinical evidence indicate that sclerostin may attenuate the progression of vascular calcification. Although it is premature to conclude that ADPKD patients are, to some extent, protected against vascular calcification by high sclerostin exposure, it should be noted that already in 1974 it was observed that ESRD patients with ADPKD showed less vascular calcification than their counterparts with other primary renal disease.

Some strengths and limitations of our study need consideration. An important strength is that recruited patients were carefully phenotyped. We acknowledge that conclusions based on this cohort of selected relatively young ESRD patients eligible for RTx may not necessarily apply to the typical older dialysis patient. On the other hand, as the investigated ESRD patients are likely to have had less comorbidity than an unselected population of ESRD patients, the interference of confounders is probably lower than in frail and sedentary dialysis populations. The apparent limitation of the study is that the observation is confined solely to ESRD patients. Certainly, confirming this finding also in a cohort of ADPKD patients with intact kidney function and normal glomerular filtration rate would importantly strengthen our hypothesis.

In summary, for what is, to the best of our knowledge, the first time, we report that ADPKD patients exhibit higher sclerostin levels than patients with other primary kidney diseases. We speculate that this could be linked to a generalized defective mechanosensation in ADPKD. Additional studies are required to confirm our observation in an independent cohort, and to investigate whether higher sclerostin levels in ADPKD translate into a different bone and vascular phenotype.
and contribute to the survival benefit of ADPKD in comparison to ESRD of other etiology.

**DISCLOSURE**

BL is affiliated with Baxter Healthcare. All the other authors declared no competing interests.

**ACKNOWLEDGMENT**

We thank the patients who participated in the study. We are grateful to all those who carried out the extensive clinical and laboratory work at the Renal Lab at Karolinska University Hospital (Ann-Christin Bragfors Helin, Björn Anderstam, Monica Eriksson) in the current study. Swedish Medical Research Council (VR) and Njurfonden supported Peter Stenvinkel’s research. Baxter Novum is a result of a grant from Baxter Healthcare to Karolinska Institutet.

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