Clinical and genetic evaluation of Danish patients with pycnodysostosis

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\begin{abstract}

\textbf{Background:} Pycnodysostosis is a rare autosomal recessive osteosclerotic skeletal dysplasia caused by variants in the cathepsin K gene (CTSK). Clinical features include short stature, bone fragility, characteristic facial features and acro-osteolysis of the distal phalanges. Usually, patients suffer from multiple bone fractures. The purpose of this study was to describe the Danish population of pycnodysostosis patients with respect to genotype, phenotype and the prevalence of complications.

We collected medical history, performed clinical examination, collected blood- and urine samples, performed dual-energy x-ray absorptiometry scan (DXA) and high-resolution peripheral quantitative computed tomography scan (HRpQCT) and obtained clinical photos. Information about complications, bone mineral density and bone markers in the blood were collected and analysed.

\textbf{Results:} Ten patients with a median age of 32 years ranging from five to 51 years participated. The pycnodysostosis phenotype varied with respect to the number of bone fractures and degree of complications. DXA and HRpQCT showed high bone mineral density. A tendency of growth hormone treatment escalating growth and increasing final height was seen. A marker of bone resorption measured in blood was within normal range in nine patients and elevated in one patient. A novel pathogenic variant in CSTK causing pycnodysostosis was detected in two related patients. Moreover information about the patients’ own health perception was reported. An example being they rated their mental health to be good despite multiple bone fractures.

\textbf{Conclusion:} This study provides information about genotypes and phenotypes in a Danish pycnodysostosis population. It reports new data about the complications such as bone fractures and it elucidates the levels of bone turnover markers as well as the density of the bones in one of the biggest cohort of pycnodysostosis patients ever published. An individualised approach to treatment in this patient group is necessary as the phenotype including complications varies between patients. Additional studies are needed to further understand genotype-phenotype correlations.

\end{abstract}

1. Introduction

Pycnodysostosis (OMIM 265800) is a rare autosomal recessive osteosclerotic skeletal dysplasia with complete penetrance. Its clinical features, which were first described in 1962, include short stature, bone fragility, delayed closure of the cranial sutures, dental abnormalities and acro-osteolysis of the distal phalanges (Andren et al., 1962; Maroteaux and Lamy, 1962). The short stature can be very disabling both physically and psychologically; moreover patients are troubled by multiple and often complicated bone fractures. The disorder does not affect cognitive functions. Pycnodysostosis can be diagnosed clinically and/or radiologically and confirmed by genetic testing.

The disease gene Cathepsin K (CTSK) is located to chromosome 1q21 (Polymeropoulos et al., 1995; Gelb et al., 1995) and various variants have been reported (Gelb et al., 1996, 1998; Johnson et al., 1996; Hou et al., 1999; Ho et al., 1999; Haagerup et al., 2000; Arman et al., 2014). The \textit{CTSK} gene is a lysosomal cysteine protease that is predominantly expressed in osteoclasts. Patients with pycnodysostosis have a normal number of osteoclasts, but the region of demineralised bone surrounding the individual osteoclasts is increased (Everts et al., 1985). This suggests that the osteoclasts do not sufficiently degrade the organic matrix and the old bone material is not resorbed properly causing the patients to be...
more susceptible to bone fractures (Nishi et al., 1999). Only 197 cases of pycnodysostosis have been presented in the literature, but a founder effect has previously been described in Denmark, Egypt, Brazil and Turkey (Haagerup et al., 2000; Arman et al., 2014; Otaify et al., 2018; Araujo et al., 2016).

As the current knowledge is limited, current treatment is based on limiting symptoms, however, no previous study has reported genotype-phenotype correlations in a population of pycnodysostosis patients including radiology and endocrine evaluation.

The aims of this study are to describe the Danish population of patients with pycnodysostosis including the phenotype and genotype, to examine the prevalence of complications including radiologic and endocrine evaluations and to investigate the parameters of quality of life and social outcome.

2. Methods

2.1. Participants

The study was a national cross-sectional descriptive study. The patients were recruited through the two Centres for Rare Diseases at Aarhus University Hospital and Rigshospitalet, the Danish departments of Clinical Genetics, the Danish Dwarf Association and through the patients’ network. Inclusion criteria were a clinical or genetic diagnosis of pycnodysostosis. There were no exclusion criteria. The inclusion period was from January 2017–August 2017. We knew of 15 patients whereas two of them did not want to participate and three did not get back to us. Ten Caucasian patients from eight unrelated families participated in the study including seven females and three males with non-consanguineous parents. Approval from the Danish Data Registry and the Ethical Committee was obtained as well as informed consent from all participants. All of the results from the study were kept at the approved database REDCap (Research Electronic Data Capture), which is a secure web-based application designed to support data capture for research studies (Harris et al., 2009).

2.2. Methods

The clinical and paraclinical part of the project comprised one visit at the Osteoporosis Clinic, Aarhus University Hospital. The visit included obtaining medical history, clinical examination, blood- and urine sampling, dual-energy x-ray absorptiometry scan (DXA), high-resolution peripheral quantitative computed tomography scan (HRpQCT) and clinical photos. The participants were systematically interviewed with focus on symptoms and complications from pycnodysostosis. The clinical examination was performed including anthropometric measurements and neurological examination.

2.2.1. Biochemistry

Following an overnight fast (8 h), blood samples were collected by venous puncture between 8 a.m. and 9 a.m. and approximately 50 ml were drawn. Serum and plasma were stored at −80 °C until analysis. A urine sample of approximately 20 ml was also collected and stored at −20 °C. Bone turnover markers as well as other parameters presented in additional file 1 were analysed in all patients. Plasma collagen type 1 cross linked C-telopeptide (p-CTX) is a marker of bone resorption. Serum alkaline phosphatase bone specific (S-BAP), p-osteocalcin and plasma type 1 procollagen N-terminal propeptide (p-P1NP) are markers of bone formation. P-CTX, p-osteocalcin and p-P1NP were measured using COBAS 8000 and s-BAP was measured using ISYS. Measuring in-securities was p-CTX ±10% (95% confidence interval (CI)), s-BAP ±20% (95% CI), p-osteocalcin ±6% (95% CI) and p-P1NP ±7.4% (95% CI).

2.2.2. DXA

Areal bone mineral density (aBMD) was measured using DXA (Hologic, Europe, Vilvorde, Belgium) at the lumbar spine (L1-L4), left hip, right forearm, and total body. The coefficient of variation was 1.0% at all sites.

T-scores were calculated for the adult participants as the difference between the measured BMD and mean for young adults at the lumbar spine, femoral neck and the right arm and expressed in standard deviations (SDs). Z-scores were also calculated for every participant as the difference between the measured BMD and the age- and gender-specific mean value of the population expressed in SDs. The DXA measurements were analysed by trained technicians.

2.2.3. HRpQCT

HRpQCT system (Xtreme CT; Scanco Medical, AG, Basserdorf, Switzerland) was used to investigate bone geometry, volumetric BMD (vBMD), microarchitecture and to estimate bone strength at the right distal radius and right distal tibia in the patients. In case of a previous fracture at the site, the left extremity was used. Image acquisition and analysis were performed according to the manufacturer’s standard protocol for in vivo patient scanning. A reference line was placed manually on a 2D scan at the radius and tibia endplate. A 3D-representation of 9.02 mm of the distal radius and 22.5 mm of the distal tibia were obtained. The operator immediately examined the scans for any motion artefacts and the scan was repeated if any artefacts were present.

Geometrical areas, vBMD, and trabecular number (TbN) were assessed directly, whereas other parameters were derived (Boutroy et al., 2005). Trabecular bone volume fraction (BV/TV) was derived from vBMD in the trabecular department according to conventions made by the manufacturer. Trabecular thickness (TbTh) and trabecular separation (TbSp) were derived from BV/TV and TbN using standard histomorphometry methods (TbTh = BV/TV/TbN and TbSp = (1-BV/TV)/TbN). Cortical thickness (CtTh) was extracted from an automatic segmentation algorithm, which could determine the endosteal and periosteal edges of the cortex by dual threshold input (Buie et al., 2007; Laib et al., 1998). The mechanical properties of the radius and Tibia corresponding to the estimated bone strength were estimated using a micro-finite element (FE) analysis solver provided by the manufacturer. An estimate of bone failure load (kN) was calculated as described in the study by Pistoia et al. on the assumption that bone failure occurs if >2% of the elements are strained beyond 0.7% (Pistoia et al., 2004). Failure load in combination with bone stiffness (kN/mm) were used as an index for bone strength as described (Macneil and Boyd, 2008).

The reference values used as controls are from Hansen et al. where they examined 164 healthy Danish citizens and established reference ranges for men and women in the age group 20–35 years (Hansen et al., 2014). The controls are therefore only gender-matched with the participants in this study and some of the participants fall into the age group of 20–35 years but the controls and patients are not matched in other ways. This should be kept in mind when comparing the results. Hansen et al. used the HRpQCT instrument as in this study and the results are therefore comparable.

2.2.4. Questionnaire

Before the visit, the participants had completed a questionnaire regarding their own physical and mental health perception. The questionnaire was designed to this project and adjusted from the standardised SF-36 Health Survey Questionnaire (Ware and Gandek, 1990).

3. Results

3.1. Phenotype and genotype

3.1.1. Phenotype

The ten participants had a median age of 32 years ranging from five to 51 years. The patients had the typical phenotypic characteristics of pycnodysostosis such as short stature and characteristic facial features. See Table 1 and Fig. 1. BMI was normal in eight patients; two patients
when calculating the median Z-score from the four scan areas (additional file 2). As seen in Table 1 there is no association between the median Z-score and amount of bone fractures. Moreover the patient with the normal BMD had suffered ten fractures.

4. Genotype

Five different variants in the ten participants were found and the variant c.547 A > C (NM_000396.3) is described for the first time in this study (ClinVar accession number SCV001426414). The genotypes are presented in Tables 1 and 2.

4.1. Analyses

4.1.1. Blood analyses

P-CTx was elevated and p-P1NP was at the upper limit in the seven-year-old boy compared to children in other studies (Herrmann et al., 2005; Bayer, 2014). Plasma parathyroid hormone (P-PTH) and serum insulin-like growth factor binding protein 3 (s-IGFBP-3) were also marginally low in two patients but glycosylated haemoglobin (Hb1Ac) was normal in both the patients.

4.1.3. HRpQCT

Five of the adult patients had very large cortical bone areas of the radius compared to the reference values. Three patients also had larger cortical area of the tibia. The six adult patients all had elevated levels of total BMD of both the radius and tibia. The trabecular BMD was elevated compared to the cortical BMD. Cortical BMD was not higher in the pycnodysostosis patients compared to controls. On the other hand, the expected increased cortical thickness and cortical bone area was confirmed in the patients. Trabecular BMD was higher but the trabecular bone area was not. Further verification of the high trabecular BMD in the patients was the higher levels of BV/TV and TrB as well as reduced TrSn. Thickness of the trabeculae was also higher Stiffness and estimated failure load, which combined gave an estimate of bone strength, were both higher in most of the adult patients. The two parameters were however lower than the reference values in the tibia bone of the two adult male patients. All HRpQCT results are presented in additional file 3. Table 4 presents median values of the results from the HRpQCT. Only the adults were included as the reference group was 20–35 years old.

4.1.4. Bone fractures

The patients included in this study suffered from bone fractures in various degrees (Table 1). Characteristically the fractures were mostly low impact fractures such as a fracture of the tailbone due to sitting down on a chair. Another example was a fracture of the femur due to the impact of a volleyball. Low impact fractures of the femur and the spine were not uncommon in this group. Prolonged recovery time was reported from all the patients. One example was a patient wearing a cast for a broken hand for approximately a year. Two participants had shin protectors whereas one wore them permanently as the risk of bone fractures was very high just from walking. Age of first bone fracture was within the first year of life for one patient, otherwise in early childhood in the rest of the cohort except for one patient being 22 years old.

5. Growth hormone treatment

The two children were not treated with GH as they followed their own growth chart at the time. Six of the eight adult patients had been treated with GH and the period of treatment varied from one to eleven years. Five of the six patients treated reported the treatment to have been beneficial on their growth and final height. The patients not treated...
expressed a wish to have been treated. Fig. 2 shows the eight adult patients divided into two groups of either being treated with GH or not being treated compared to the height of the patients. The mean height of the two patients not treated is slightly lower than the group of patients treated with GH albeit the difference is not significant.

6. Health perception

Generally the ten patients rated their physical health as “well” (Fig. 3a). The reason for not saying “really well” was typically because of multiple bone fractures, short height and intermittent respiratory problems. Six patients rated their mental health to be “really well”. The main reason for the rest of the patients not saying “really well” was the limitations due to the bone fractures.

Additionally, stress and depression with the latter being associated with the bone fractures. One patient was to some degree inhibited in daily life because of an inherited heart condition with arrhythmia, which gave her palpitations and shortness of breath. Three of the female patients reported frequent intestinal problems such as pain and constipation.

7. Quality of life and social parameters

All the patients were either in employment or in education. Six of the eight adult patients had partners and five of them had children or were pregnant at the time of the study. Most of the patients had not experienced bullying or isolation during their time in school and had always been surrounded by friends. Two patients had however experienced
buckling and one of them to such a degree that the patient developed social anxiety. Multiple fractures also resulted in staying at home for long periods of time and home schooling for this patient. Some patients explained difficulties finding partners due to self-consciousness regarding their physical appearance. Many of the patients described people staring and pointing at them when in public as very bothersome. Regarding their physical appearance, many of the patients described social anxiety. Multiple fractures also resulted in staying at home for long periods of time and home schooling for this patient. Some patients explained difficulties finding partners due to self-consciousness regarding their physical appearance.

8. Discussion

With this study a big cohort of pycnodysostosis patients is presented as well as important findings about pycnodysostosis and the associated complications. We present thorough descriptions of the patients’ bone phenotype based on DXA, HRPQCT, and biochemical markers of bone turnover and a tendency of GH treatment having a favourable effect on final height. Moreover data on the quality of life for pycnodysostosis patients is published for the first time and their quality of life was reported as high. Based on the number of patients with pycnodysostosis in Denmark found in this study the prevalence in Denmark was 2–3/1,000,000, which was within the internationally reported prevalence of 1–9/1,000,000 (orpha.net). Four of the patients (case 03, 06, 07 and 10) in this study also participated in the previous Danish study where a founder effect was discovered (Haagerup et al., 2000). In this study 50% of the participants had a homozygous genotype suggesting a probable distant consanguinity.

A connection between homozygosity for the c. 926 T > C variant and a tendency of suffering more than ten bone fractures during a lifetime were investigated. There was no significant difference but numbers were small. Comparing our study to a recent study from Egypt we could report a higher rate of pathological bone fractures (Otify et al., 2018). Although a patient in the Egyptian study similar in age to our youngest patient had suffered from more fractures than our youngest patients. The genotypes in the Egyptian study were all different from the genotypes in our study and all the Egyptian patients had consanguineous parents.

As seen in Table 3 the results of the bone markers in the children were significantly higher than in the adult patients. Children have an increased bone turnover due to growing and this may have been an explanation for their elevated levels. The eldest sibling of the children had considerably higher bone turnover markers than his sister and because children do not grow at a steady rate he may have been at a more active growing period than his sister at the time of the examination.

Reference ranges for p-CTX have been reported in a population of healthy European children (Herrmann et al., 2005). The five-year-old girl’s result of p-CTX was within range of the reference values in girls her age in the study whereas the seven-year-old boy’s result was elevated compared to the reference values in boys his age. That was surprising but we saw that p-CTX was all within normal range in the adult patients albeit in the lower range. As p-CTX is a marker of bone resorption we would expect p-CTX to be low in patients with pycnodysostosis and there was no obvious explanation for the elevated level in the seven-year-old boy. P-P1NP was also in the lower range in the adult patients. Reference ranges for p-P1NP have been reported in a population of healthy Central European children (Bayer, 2014). Both of the children in our study were within normal range when comparing to the other study, however the seven-year-old boy’s result was close to the upper limit in the reference interval. The boy being in a more active growing period may explain this. Precaution comparing the results is necessary as P1NP was measured in serum in the other study and the growing period may explain this. Precaution comparing the results is necessary as P1NP was measured in serum in the other study and the growing period may explain this.

Another study found normal levels of CTX and BAP in a patient from China equivalent to results in this study (Huang et al., 2015). One study examining three siblings found normal ranges of osteocalcin but elevated levels of S-BAP in two of the three pycnodysostosis patients (Schilling et al., 2007). However the latter two children were 12 and 13 years old and therefore not comparable in age to the two youngest patients in this study. To summarise, the findings in our study may indicate that biochemical markers of bone turnover reflect the inhibited degradation of bone in pycnodysostosis patients, as both CTX and P1NP were in the lower range in all the adult patients. The results of the GH analyses were difficult to interpret due to a single measurement, as there is great circadian variability and intra-individual variation. The seven-year-old had significantly lower GH than his sister as well as elevated values of IGFBP-3 and PTH. His serum insulin-like growth factor-1 (IGF-1) result was however within normal range, which is an indicator of sufficient GH secretion. PTH is an effective stimulus for osteoblastic IGF-1 production and the high PTH level may have contributed to his high level of IGF-1 albeit within normal range. PTH also varies intra-individually from day to day and we...
The highest median Z-score was 6.3 SDs above the normal vitamin D as three of the patients were examined in the beginning of March after a Danish winter. The two adult patients with low values of vitamin D had values below the lower level of the reference interval. No apparent explanation could be found.

No general reference ranges for S-osteocalcin, P-1NP and P-CTX in the children were available. Reference ranges:

- S-BAP (µg/l) >150
- S-Osteocalcin (µg/l) 99
- P-1NP (µg/l) 563
- P-CTX (ng/ml) 1.14
- S-PTH (pmol/l) 2.7
- S-25-OH-Vitamin D (nmol/l) 56.3
- S-GH (ng/ml) 7.041
- S-IGF-1 (ng/ml) 156
- S-IGFBP-3 (ng/ml) 3833

Table 3

<table>
<thead>
<tr>
<th>Blood analyses</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
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<th>08</th>
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<tr>
<td></td>
<td>Female</td>
<td>5 y</td>
<td>Male</td>
<td>7 y</td>
<td>Female</td>
<td>24 y</td>
<td>Female</td>
<td>27 y</td>
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<td>&gt;150</td>
<td>23.3</td>
<td>6.5</td>
<td>15.2</td>
<td>9.1</td>
<td>25.8</td>
<td>13.5</td>
<td>14.7</td>
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<td>9</td>
<td>22</td>
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<td>20</td>
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<tr>
<td>P-1NP (µg/l)</td>
<td>563</td>
<td>1109</td>
<td>80</td>
<td>47</td>
<td>43</td>
<td>32</td>
<td>73</td>
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<td>0.39</td>
<td>0.13</td>
<td>0.15</td>
<td>0.17</td>
<td>0.24</td>
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<td>S-PTH (pmol/l)</td>
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<td>6.3</td>
<td>5.4</td>
<td>3.4</td>
<td>4.7</td>
<td>5.9</td>
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<td>S-25-OH-Vitamin D (nmol/l)</td>
<td>56.3</td>
<td>55.1</td>
<td>70.1</td>
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<td>115</td>
<td>113</td>
<td>37</td>
<td>40.2</td>
<td>116</td>
<td>108</td>
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<td>7.041</td>
<td>0.239</td>
<td>1.092</td>
<td>0.095</td>
<td>2.551</td>
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<td>0.054</td>
<td>0.060</td>
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<td>3831</td>
<td>3490</td>
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</table>

Saw great variation in the PTH results of the other nine patients although within normal range. No patients had suffered from any bone fractures within a year before the examination. The median time period since the most recent bone fracture at the time of the examination was five years. No general reference ranges for S-osteocalcin, P-1NP and P-CTX in the children were available. Reference ranges:

- S-BAP: 1-10 y 1-10 y 130
- S-Osteocalcin (µg/l) 99
- P-1NP (µg/l) 563
- P-CTX (ng/ml) 1.14
- S-PTH (pmol/l) 2.7
- S-25-OH-Vitamin D (nmol/l) 56.3
- S-GH (ng/ml) 7.041
- S-IGF-1 (ng/ml) 156
- S-IGFBP-3 (ng/ml) 3833

For the first time data on HRpQCT analyses of eight pycnodysostosis patients were presented with this study (Table 4 and additional file 3). The high values of cortical bone area are in agreement with what to be expected in pycnodysostosis patients. The elevated levels of total BMD of both the radius and tibia confirm osteosclerosis, which is associated with pycnodysostosis. Trabecular BMD was high as expected due to thickening of the trabeculae. The stiffness and estimated failure load analyses suggested the patients’ bones are stronger than the reference values from the general population. In agreement with previous studies we confirm the fragile bones of persons with pycnodysostosis because of the clinical observation of multiple bone fractures. The estimated failure load based on HRpQCT does not take bone composition into account and the accumulated amount of old bone material that has not been remodelled because of the lack of cathepsin K likely affects bone strength. Bone strength can therefore only be estimated reliably based on HRpQCT if the bone material properties are normal.

The eldest participant had less elevated cortical area and trabecular- and cortical thickness than the five other adult patients. These findings may be due to her age and the postmenopausal change in the porosity of the bones. The scan results from the 31-year-old participant were comparable with scan results from another female with pycnodysostosis close to her age regarding BMD, TbN, TbSp, trabecular and cortical thickness (Chavassieux et al., 2008).

Six of the eight adult patients had been treated with GH and they were treated for different periods of time. There was a slightly higher mean height in patients treated with GH indicating that there may have been an actual effect of the GH treatment (Fig. 2). Four of the six patients treated were taller than 150 cm which was tall compared to the mean height of 130–150 cm (Xue et al., 2011). Interestingly almost all the patients expressed satisfaction with their GH treatment regarding their final height and patients not treated wished to have been treated. The difference of the mean height was only a couple of centimetres though and it was evaluated from the results of eight patients. GH treatment is rather invasive, as it involves injection of the hormone under the skin every day often for a long period of time. When a patient is willing to go through this treatment a belief in the effect is presumed. It has also been shown when a syringe is part of the treatment the patients expect a greater therapeutic benefit (Benedetti et al., 2011). Placebo may therefore be an important factor in the patients’ perception of the effect. GH has however proven effective in previous studies in pycnodysostosis.
Table 4

<table>
<thead>
<tr>
<th>Radius</th>
<th>vBMD</th>
<th>Cortical vBMD</th>
<th>Trabecular vBMD</th>
<th>Cortical thickness</th>
<th>Cortical areal</th>
<th>Trabecular areal</th>
<th>Trabecular BV/TV</th>
<th>TbN</th>
<th>TbSp</th>
<th>Failure load</th>
<th>Stiffness</th>
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<tr>
<td>Median</td>
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<td>931.0 mgHA/cm³</td>
<td>35.0 mgHA/cm³</td>
<td>1.4 mm</td>
<td>89.0 mm²</td>
<td>144.9 mm²</td>
<td>29.9 %</td>
<td>2.40 ± 0.30 mm</td>
<td>6.5 kN</td>
<td>140.6 kN/mm</td>
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<tr>
<td>Reference</td>
<td>342 ± 72 mgHA/cm³</td>
<td>898 ± 49 mgHA/cm³</td>
<td>160 ± 36 mgHA/cm³</td>
<td>0.94 ± 0.20 mm</td>
<td>57 ± 11 mm²</td>
<td>194 ± 46 mm²</td>
<td>13.3 ± 3 %</td>
<td>1.93 ± 0.26 mm</td>
<td>0.448 ± (0.41–0.50) mm</td>
<td>3.99 ± 0.73 kN</td>
<td>79 ± 15 kN/mm</td>
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<table>
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<tr>
<th>Radius</th>
<th>vBMD</th>
<th>Cortical vBMD</th>
<th>Trabecular vBMD</th>
<th>Cortical thickness</th>
<th>Cortical areal</th>
<th>Trabecular areal</th>
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<td>950.8 mgHA/cm³</td>
<td>318.6 mgHA/cm³</td>
<td>1.5 mm</td>
<td>113.4 mm²</td>
<td>204.8 mm²</td>
<td>26.6 %</td>
<td>2.08 ± 0.36 mm</td>
<td>7.3 kN</td>
<td>161.3 kN/mm</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>354 ± 53 mgHA/cm³</td>
<td>873 ± 42 mgHA/cm³</td>
<td>199 ± 33 mgHA/cm³</td>
<td>1.02 ± 0.17 mm</td>
<td>75 ± 12 mm²</td>
<td>278 ± 62 mm²</td>
<td>16.5 ± 2.8 %</td>
<td>2.06 ± 0.22 mm</td>
<td>0.406 ± (0.37–0.45) mm</td>
<td>5.92 ± 0.82 kN</td>
<td>118 ± 17 kN/mm</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Tibia</th>
<th>vBMD</th>
<th>Cortical vBMD</th>
<th>Trabecular vBMD</th>
<th>Cortical thickness</th>
<th>Cortical areal</th>
<th>Trabecular areal</th>
<th>Trabecular BV/TV</th>
<th>TbN</th>
<th>TbSp</th>
<th>Failure load</th>
<th>Stiffness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>446.1 mgHA/cm³</td>
<td>950.3 mgHA/cm³</td>
<td>307.9 mgHA/cm³</td>
<td>1.6 mm</td>
<td>157.9 mm²</td>
<td>464.4 mm²</td>
<td>25.7 %</td>
<td>2.38 ± 0.31 mm</td>
<td>14.4 kN</td>
<td>305.3 kN/mm</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>323 ± 54 mgHA/cm³</td>
<td>902 ± 33 mgHA/cm³</td>
<td>183 ± 34 mgHA/cm³</td>
<td>1.26 ± 0.24 mm</td>
<td>125 ± 22 mm²</td>
<td>545 ± 115 mm²</td>
<td>15.3 ± 2.8 %</td>
<td>1.99 ± 0.31 mm</td>
<td>0.427 ± (0.38–0.48) mm</td>
<td>10.9 ± 1.72 kN</td>
<td>218 ± 35 kN/mm</td>
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</tbody>
</table>

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<tr>
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<th>TbSp</th>
<th>Failure load</th>
<th>Stiffness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>412.4 mgHA/cm³</td>
<td>973.9 mgHA/cm³</td>
<td>240.1 mgHA/cm³</td>
<td>1.6 mm</td>
<td>155.9 mm²</td>
<td>496.7 mm²</td>
<td>20.0 %</td>
<td>1.76 ± 0.48 mm</td>
<td>12.5 kN</td>
<td>268.3 kN/mm</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>339 ± 54 mgHA/cm³</td>
<td>876 ± 37 mgHA/cm³</td>
<td>218 ± 30 mgHA/cm³</td>
<td>1.31 ± 0.25 mm</td>
<td>160 ± 32 mm²</td>
<td>737 ± 152 mm²</td>
<td>18.1 ± 2.5 %</td>
<td>2.20 ± 0.26 mm</td>
<td>0.362 ± (0.34–0.42) mm</td>
<td>15.1 ± 1.9 kN</td>
<td>302 ± 40 kN/mm</td>
</tr>
</tbody>
</table>

vBMD, volumetric bone mineral density; BV/TV, trabecular bone volume fraction; TbN, trabecular number; TbSp, trabecular separation.

Results in bold font are not within normal range. The right radius and tibia were scanned in all patients except patient 10 whose left radius was scanned because of most fractures in the right radius. Reference values are from Hansen et al. (Hansen et al., 2014).

patients (Soliman et al., 2001; Karamizadeh et al., 2014; Rothenbuhler et al., 2016; Bizaoui et al., 2019). In two of the studies the pycnodysostosis patients suffered from GH deficiency and in one study hypoplasia of the anterior pituitary was established in the four participating patients. The mechanisms underlying the GH deficiency observed in some pycnodysostosis patients are still unknown. However, it has been hypothesised that the increased bone volume of the sella turcica might increase intrasellar pressure, which then results in hypopituitarism (Arafah et al., 2000). It was not known whether the patients in this study suffered from GH deficiency and hypopituitarism, and the efficacy of GH treatment in pycnodysostosis patients has still to be determined. More data about growth hormone therapy are needed, especially dosage, start and length of treatment to evaluate growth and final height of pycnodysostosis patients.

The level of physical activity in daily life differed greatly between the patients. One patient exercised 5–6 times a week and another patient was formerly part of the bench press national team. A third patient performed free diving and participated in underwater hunting contests. Other patients were less physically active due to physical restrictions of pycnodysostosis. Remarkably most of the patients were coping with pycnodysostosis and especially the fractures very well. The pycnodysostosis patients seemed less limited by fractures than expected, except in the case of larger fractures such as a fracture of the femur where they were physically limited for a long period of time. One patient once suffered a fracture in the foot at school and was still able to walk home. More stories like this give an impression of a higher pain threshold and endurance in this patient group than in the general population.

Unpublished data from Haagerup et al. (2000) showed some of the patients are socially isolated and do not feel as an integrated part of society. Some of the patients had difficulty finding jobs despite proper education and they experienced discrimination due to their appearance. Moreover, suicide and drug addiction was present. All the patients in this study were either employed or studying. Furthermore all the patients had partners and five patients were parents or soon to be parents. Likewise most did not feel that pycnodysostosis had impacted their
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Fig. 2. Height of the eight adult patients divided into two groups of either being treated with GH or not. Mean height of the two groups is shown as a cross symbol.

Fig. 3. (a) Graphic presentation of how the ten patients viewed their own physical and mental health. (b) Graphic presentation of how the patients rated pycnodysostosis to have complicated their relationships.

relationships (Fig. 3b). As the patients in our study managed socially better than in Haagerup et al. it may suggest a greater acceptance in society of people who look different and a lesser discrimination based on appearance.

A limitation of this study was the small cohort size and therefore no firm conclusion can be made. Furthermore some of our results were from the patients’ own recollection. On the contrary, the strength of our study is that we present the biggest cohort of pycnodysostosis patients published with this study design.

Pycnodysostosis has been of great interest in regards to the disease osteoporosis. Osteoporosis is characterised by low bone mass and a high risk of fractures and the disorder represents a substantial health problem (Seeman and Delmas, 2006). In this respect the CTSK gene responsible for pycnodysostosis is interesting because the inactivity of the gene increases bone density, which is characteristic for pycnodysostosis. Thus there is a major interest in targeting the enzymatic activity of the CTSK gene to increase bone density in osteoporosis patients. It has been shown that the CTSK inhibitors do not reduce bone formation and may even increase it (Stroup et al., 2009; Cusick et al., 2012; Bone et al., 2015; Cabal et al., 2017; Langdahl et al., 2012). Paradoxically studies show that low doses of cathepsin K inhibitors increase levels of CTx but higher doses decrease the levels as would be expected (Bone et al., 2010; Pirapaharan et al., 2019).

9. Conclusion

This study reports on a well described Danish pycnodysostosis cohort regarding phenotype and genotype and discloses a novel variant. It reports an original overview of the clinical manifestations such as frequency of bone fractures. The study furthermore elucidates the levels of bone turnover markers as well as the density of the bones from DXA and HRpQCT in one of the largest cohorts of pycnodysostosis patients reported. Moreover important information about the patients’ own health perception and associated complications to the disorder is described. From this study we learn that individual treatment in this patient group is necessary since they have various degrees of complications. All patients below the age of 18 years should be enrolled to yearly clinical controls at skeletal dysplasia clinics with expertise in pycnodysostosis. Extra clinical controls should take place if indicated by symptoms. Adult patients may also benefit from yearly controls and the experts may also be able to help and educate doctors at other departments when needed.

Since our results regarding growth hormone therapy shows a possible increase in final height, we recommend doctors to consider growth hormone treatment for their pycnodysostosis patients and for larger RCT studies to be performed.

The patients in our study share the characteristic clinical features of the disorder previously described but we now know more about the disorder regarding the different aspects and complications. Further research with additional pycnodysostosis patients is needed to investigate possible genotype-phenotype correlations.

Ethics approval and consent to participate

Approval from the Danish Data Registry (Project ID 1-16-02-422-16) and the Ethical Committee (Region Midt) (Project ID 1-10-72-286-16) was obtained as well as written informed consent from all the participants and the parents of underage participants.

Consent for publication

Consent for publication of the images of the participants has been obtained from the participants and the parents of underage participants.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Mia Aa. Doherty: Conceptualisation, methodology, investigation, resources, writing – original draft, visualisation, funding acquisition.
Bente L. Langdahl: Conceptualisation, methodology, resources, writing – review and editing.

Glossary

**CTSK:** Cathepsin K

**GH:** Growth hormone

**DXA:** Dual-energy x-ray absorptiometry

**HRpQCT:** High-resolution peripheral quantitative computed tomography

**P-CTx:** Plasma collagen type I cross-linked C-telopeptide

**S-BAP:** Serum alkaline phosphatase bone specific

**P-P1NP:** Plasma type 1 procollagen N-terminal propeptide

**CI:** confidence interval

**aBMD:** Areal bone mineral density

**SD:** Standard deviation

**vBMD:** Volumetric BMD

**TbN:** Trabecular number

**BV/TV:** Trabecular bone volume fraction

**TbTh:** Trabecular thickness

**TbSp:** Trabecular separation

**CtTh:** Cortical thickness

**FE-analysis:** Micro-finite element analysis

**HbA1c:** Glycosylated haemoglobin

**P-PTH:** Plasma parathyroid hormone

**S-IGFBP-3:** Serum insulin-like growth factor binding protein 3

**P1CP:** Type 1 procollagen C-terminal propeptide

**S-IGF-1:** Serum insulin-like growth factor-1

**S-25-OH-vitamin D:** Serum 25-hydroxy vitamin D2 and D3 and D2+D3

**ODN:** Odanacatib