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Osteosclerosis in two brothers with autosomal dominant pseudohypoparathyroidism type 1b: bone histomorphometric analysis

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Abstract

Objective—Pseudohypoparathyroidism (PHP) is a heterogeneous disorder characterized by hypocalcemia and hyperphosphatemia resulting from selective renal resistance to parathyroid hormone (PTH). One autosomal dominant form of PHP type 1b (PHP-Ib) is most frequently caused by a maternally inherited 3-kb deletion within *STX16*, the gene encoding syntaxin 16. To date, increased bone mineral density (BMD) has been described only in PHP type 1a, and there is a lack of detailed information on bone histomorphometry in PHP-Ib. The objective of this report was to present trans-iliac static and dynamic histomorphometry in two brothers with the 3-kb deletion in the *STX16* region and elevated BMD.

Design—Observational study of two brothers (age 18.0 and 22.7 years) with the 3-kb *STX16* deletion and increased BMD.

Results—The brothers had elevated PTH (146 pg/ml (15.6 pmol/l) and 102 pg/ml (10.9 pmol/l); normal: 10–64 pg/ml (1.1–6.8 pmol/l)) and striking osteosclerosis (lumbar spine areal BMD *Z*-scores: +5.4 and +4.9). Bone histomorphometry showed marked elevations in cortical width for both brothers (241 and 209% of the mean result expected for age), with elevations in the bone formation rate on the endocortical (119 and 260% of the healthy mean) and trabecular (220 and 190% of mean) surfaces.

Conclusion—Our findings suggest that PTH in this PHP-Ib genotype can increase cortical thickness due to its anabolic effect on endocortical bone, and underscore the heterogeneity in the skeletal phenotype among patients with PHP-Ib.

Introduction

The α-subunit of the stimulatory G-protein (Gsα) is an intracellular signaling protein essential for the actions of many hormones, including parathyroid hormone (PTH). Although

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Gsα is biallelically expressed in most tissues, it has predominantly maternal expression in renal proximal tubules. Pseudohypoparathyroidism (PHP) is characterized by proximal tubular resistance to PTH due to impaired Gsα expression and results in hypocalcemia and hyperphosphatemia. The two major forms of PHP are PHP type 1a (PHP-Ia) and PHP type 1b (PHP-Ib). PHP-Ia is associated with a distinctive phenotype termed Albright's hereditary osteodystrophy (AHO), which includes short stature, obesity, round faces, brachydactyly, and subcutaneous ossifications. It is caused by maternally inherited inactivating mutations in the *GNAS* gene on chromosome 20q that encodes Gsα (1). Patients with PHP-Ib usually do not have features of AHO; renal resistance to PTH is the main feature of the condition, occurring with sporadic or autosomal dominant (AD) inheritance (2). Most patients with AD-PHP-Ib show loss of methylation at *GNAS* exon A/B involved in Gsα transcription regulation, which is associated with a heterozygous, maternally inherited 3-or 4.4-kb deletion within syntaxin 16 (*STX16*). As a result of biallelic A/B transcription, patients with AD-PHP-Ib are predicted to have impaired maternal Gsα expression in proximal renal tubules (1). PHP-Ib is heterogeneous at the clinical level; for example, the severity of hypocalcemia can vary greatly, and as many as 40% of individuals with maternally inherited *STX16* deletions are asymptomatic (2).

While the renal proximal tubules are resistant to PTH in PHP-Ib, Gsα is derived in osteoblasts from both parental alleles, giving rise to PTH responsiveness at the bone tissue level. Observations of the skeletal phenotype in PHP have been diverse. Some reports have described accelerated subperiosteal resorption and osteitis fibrosa cystica on skeletal radiographs (3–5). However, osteosclerosis has also been described in non-specified PHP (5–7), and more recently, elevated total body bone mineral density (BMD) as measured by dual energy X-ray absorptiometry (DXA) was reported in PHP-Ia (8). Given these disparate findings by indirect assessment methods, bone histomorphometry can precisely define the effect of PHP on bone metabolism, but these studies are lacking. Therefore, the purpose of this report was to present trans-iliac static and dynamic histomorphometry in two brothers with the 3-kb deletion in the *STX16* region and elevated BMD. In doing so, we provide insight into the mechanism of increased BMD observed in these cases.

Clinical report

Proband 1 presented at 11 years of age with a 1-year history of increasing leg pain with exercise. He was born to non-consanguineous French–Canadian parents, and his maternal uncle had been diagnosed with PHP at 13 years of age after a hypocalcemic seizure. At presentation, proband 1 measured 148.4 cm (75th percentile), weighed 44.8 kg (90th percentile), and had dental enamel hypoplasia, but no clinical features of AHO or tetany. The serum ionized calcium was low, whereas serum levels of phosphate, alkaline phosphatase activity (ALP) and PTH were elevated (Table 1). Serum creatinine, magnesium, TSH, and free thyroxine were normal. Based on these features, he was diagnosed with PHP-Ib and treated with calcitriol and elemental calcium.

When proband 1 was diagnosed with PHP-Ib, his older brother (proband 2) was examined for similar features and was found to be normocalcemic. However, a second assessment a few months later at 15.9 years of age revealed a decreased ionized calcium and elevated levels of serum phosphate, ALP, and PTH (Table 1), in the presence of normal serum creatinine and magnesium levels, consistent with a diagnosis of PHP-Ib (see also Jü ppner *et al.* (9) for the family pedigree where the probands were D-III/31 and D-III/32 respectively). He had no symptoms related to the hypocalcemia. He was clinically euthyroid, though thyroid function tests were not performed. Given the elevated PTH, he was treated with calcitriol and calcium supplementation. Neither boy reported a history of fractures nor dietary intakes of calcium that exceeded the dietary reference intake for age (10).

Skeletal radiography, bone densitometry, trans-iliac histomorphometry, and genetic testing were carried out in both patients to further characterize their clinical and genetic features.

Materials and methods

The study was approved by the ethics committee at the Children's Hospital of Eastern Ontario. Height, weight, and pubertal status are reported at the time of the detailed skeletal evaluation. Pubertal status was evaluated according to the method of Marshall & Tanner (11). Height was measured with a Harpenden stadiometer and weight with a digital weight scale. Height and weight *Z*-scores were calculated using reference data provided by the National Center for Health Statistics (12).

Serum concentrations of calcium, phosphate, magnesium, creatinine, and ALP were measured using standard methods. Serum PTH was quantified by IRMA (N-tact*; Incstar Corp., Stillwater, MN, USA), and serum 25-hydroxyvitamin D was measured by RIA (Osteo SP; Incstar Corp.). Genetic testing had been detailed in a prior publication (9).

Radiographs were performed using standard methods. Lumbar spine (vertebrae L2 to L4) and total body areal BMD (aBMD) were measured by DXA in the anterior–posterior direction (Lunar Prodigy; General Electric, Madison, WI, USA). Results were transformed to age- and sex-specific *Z*-scores using reference data supplied by the manufacturer.

Trans-iliac bone biopsies were performed at a site 2 cm posterior to the anterior superior iliac spine on days 4 or 5 after dual labeling with demeclocycline (15–20 mg/kg per day taken orally for 2 days, and repeated for 2 more days after a 10-day free interval). Biopsy preparation and histomorphometric analyses were performed as described previously (13, 14). Measurements were carried out using a digitizing table with OSTEOMEASURE software (Osteometrics, Inc., Atlanta, GA, USA). Results were compared to reference data of healthy age-matched controls established in our laboratory, and expressed as percentages of the average value (13, 14).

Results

For proband 1, height was 170.4 cm (*Z*-score −0.8) and weight was 70.4 kg (*Z*-score +0.3) at 18 years of age. Radiographs around the time of diagnosis showed thick diaphyseal cortices without medullary sclerosis. aBMD was markedly elevated both at the spine (1.8 g/cm^2 (age- and gender-matched *Z*-score +5.4)) and for the total body (1.6 g/cm^2 (*Z*-score +4.8)). Proband 2, aged 22 years, measured 177 cm (*Z*-score 0) and weighed 66 kg (*Z*-score −0.4). He also had an increased spine aBMD (1.8 g/cm^2 (*Z*-score +4.9)) and total body BMD ((1.5 g/cm^2 (*Z*-score +3.9)). Both brothers were Tanner stage V for pubertal development at the time of BMD testing.

Results of trans-iliac histomorphometry are presented in Table 2. The outer size of the biopsy sample (core width) was above average for proband 1 and average for proband 2. Both brothers had extremely thick cortices (Fig. 1). Cortical thickness was ~7 and 6 standard deviation score respectively above the mean value of the reference range. The amount of trabecular bone was also somewhat elevated due to increased trabecular thickness, whereas the number of trabeculae was close to the age-appropriate average value.

Histomorphometric parameters of bone metabolism for trabecular, intracortical, endocortical and periosteal surfaces are shown in Table 3. There was no evidence of a mineralization defect, as osteoid thickness was within normal limits on all surfaces. Bone formation rate on trabecular surfaces was above average, and was also elevated on the endocortical surface,

especially for proband 2. Trabecular bone formation rate was extremely elevated as demonstrated by the elevation in mineralizing surface/bone surface (data not shown).

Biochemistry obtained on the same day as the biopsy included elevated PTH and normal 25-hydroxyvitamin D levels (Table 1). When the pattern of serum PTH secretion was reviewed, it was found that proband 1's PTH was normal (12 pg/ml (1.3 pmol/l)) 1 year before his bone biopsy, and proband 2 also had a normal PTH value (37 pg/ml (4.0 pmol/l)) 3 years preceding his biopsy. Taken together, these findings provided evidence for fluctuating serum PTH levels in both patients. Magnesium and creatinine were normal. Genetic testing had previously revealed (for both patients) a 3-kb *STX16* deletion upstream of *GNAS* on chromosome 20q13.3 (9, 15). Their mother and a maternal cousin had the 3-kb deletion when previously investigated (by maternal inheritance for their mother and paternal inheritance for their maternal cousin) (9). The mother's lumbar spine aBMD *T*-score was below average (−0.5) while her PTH was normal at 63 pg/ml (6.7 pmol/l); N: 15–65 pg/ml (1.6–6.9 pmol/l), and their cousin had a mildly increased spine aBMD *Z*-score (+1.0) at age 11 years with a concomitant normal PTH of 49 pg/ml (5.2 pmol/l); N: 11–68 pg/ml (1.2–7.3 pmol/l). Neither the mother nor cousin required treatment with calcitriol to maintain eucalcemia.

Discussion

We present two brothers with PHP-Ib caused by a 3-kb deletion within *STX16* associated with marked osteosclerosis due to thickened cortices. Although elevated BMD in PHP-Ia (8) and osteosclerosis in genetically undefined PHP have been previously described (5–7), this is the first report linking a mutation known to cause AD-PHP-Ib with marked increases in DXA-derived BMD parameters and elevations in cortical width on trans-iliac histomorphometry. The increase in cortical width could have resulted from skeletal resistance to PTH had bone resorption been impaired, since it has recently been demonstrated that a predominance of maternally inherited Gsα can exist in PHP-Ib skeletal tissue (16). However, the patients' core width values combined with increased cortical width implicate elevated endosteal bone formation, which was confirmed on dynamic histomorphometry. Dynamic histomorphometric analysis showed elevated endosteal bone formation that was particularly evident in proband 2 (endocortical bone formation rate 260% of the mean). The best dynamic parameter for measuring the effect of hyperparathyroidism on bone turnover is bone formation rate per bone surface (17). Our data show that the bone formation rates per intracortical, endocortical, and trabecular bone surfaces were increased in both probands. A high bone formation rate does not necessarily lead to a net gain of bone if the remodeling balance is zero or negative (18), and the index case did have an elevated eroded intracortical surface. However, bone resorption is the least informative aspect of histomorphometric analysis since it is quantified only with static parameters (17), and the augmented cortical width and trabecular thickness in our cases suggest that the high bone formation rates do, in fact, indicate net bone gain.

Our findings contrast with other reports describing catabolic effects of PTH on bone biopsy in children with PHP, particularly cortical bone (3, 4, 19). However, it is important to take the age dependency of histomorphometric parameters into account (18), and the only case study on histomorphometric data in PHP by Duquesnoy *et al.* (19) was published before established reference data for pediatric quantitative histomorphometry were available. Furthermore, there is a paucity of information on dynamic parameters such as bone formation rate per bone surface in the study by Duquesnoy *et al.* (19). In our report, the elevated PTH in the brothers concurrent with their bone histomorphometric results demonstrates that PTH can have a positive effect on cortical bone formation, consistent with

studies showing that subcutaneous PTH administration increases the cortical thickness of the ilium when used for osteoporosis treatment in men and women (20, 21).

Our report highlights the heterogeneity in the skeletal phenotype among patients with PHP-Ib. While there are other reports of osteosclerosis on skeletal radiographs (5–7) suggesting increased bone formation as in our patients, there are also reports of accelerated subperiosteal resorption, osteitis fibrosa cystica, and elevated bone turnover markers in PHP (3–5), specifically PHP-Ib (22). DXA-derived BMD has been reported to be normal in a patient with PHP-Ib (22), whereas another case report of patients with PHP-Ib caused by a 3-kb deletion in *STX16* showed mild osteopenia (23).

These conflicting descriptions of the bone density phenotype in patients with PHP suggest differential effects of PTH on bone metabolism. One possibility to explain these findings may relate to the magnitude of PTH elevation. Markedly high PTH values that are persistently elevated can cause accelerated skeletal resorption and osteitis fibrosa cystica, but a chronically mild increase in PTH secretion has been associated with an increased net bone formation in humans (24), as observed in our patients with extremely elevated spine and total body BMD. In contrast, Laspa *et al.* (23) reported that patients with PHP-Ib caused by the 3-kb deletion had DXA-derived spine BMD indices that were −0.9 to −1.35 standard deviation scores below the mean. The most striking difference between our patients and those of Laspa *et al.* (23) was the magnitude of the PTH elevation (PTH was 9- and 6-fold higher than the upper limit of normal compared to 2- and 1.6-fold higher in our patients), suggesting that the higher PTH levels in the Laspa study (23) may have exerted a catabolic effect on bone. Along the same lines, the mother and maternal cousin in our report had normal PTH levels that were associated with relatively normal spine aBMD *Z*-scores of −0.5 and +1 respectively. Taken together, these observations further support that the magnitude of the PTH elevation may modulate the skeletal phenotype.

Besides the degree of PTH elevation, the duration of the PTH increase may also impact bone tissue responsiveness. Intermittent PTH administration can induce large increases in cortical thickness and spine BMD in postmenopausal women (20, 21, 25). Additionally, PTH stimulates endosteal and periosteal bone formation when intermittently released in growing mice and adult humans (26, 27), resulting in both increased bone modeling and remodeling. Specifically, Lindsay *et al.* (27) reported increased bone formation on the endocortical surface in postmenopausal women, consistent with our cases. The increase in bone formation in this report was largely due to an increase in osteoblast number. PTH appears to increase osteoblast numbers by exerting pro-differentiating and prosurvival effects on pre-osteoblasts in periosteal bone (26), resulting in an increased cortical diameter similar to our cases. It is possible that periosteal bone formation was also elevated while the boys were growing, though we were not able to fully evaluate this aspect since linear growth was complete at the time of the biopsy, and periosteal bone growth is minimal following cessation of longitudinal growth (28, 29).

In addition to increased cortical bone, our patients had elevated trabecular thickness. Animal models show that daily PTH injections attenuate the rate of osteoblast apoptosis in trabecular bone, which has a higher rate of apoptosis, resulting in increased trabecular thickness (30). While our main findings of increased cortical width and elevations in bone formation on cortical and trabecular surfaces support an anabolic effect of PTH at the bone tissue level, they are based on only two individuals. Therefore, we cannot exclude the possibility that other genetic factors may have contributed to the marked osteosclerosis. For example, activating LRP5 mutations can cause osteosclerosis (30); however, in our patients, there were no physical features (such as torus palatinus) to support further exploration of this or other specific diagnoses. Alternatively, acquired factors such as hepatitis C, heavy

metals, and fluoride exposure can influence BMD (6), but these possibilities are also unlikely in our probands based on their history and physical examinations. On the other hand, a recent publication by Long *et al.* (8) provides further support that high bone mass may be an integral component of the PHP phenotype in some patients. This group showed that total body BMD measurements were significantly greater than normal in 20 children and adults with PHP-Ia; for example, the highest total body BMD *Z*-score was +4.8 with a PTH value of 253 pg/ml (27.8 pmol/l). Further study of BMD as well as bone histomorphometry in relation to PTH among patients with the 3-kb deletion in *STX16* will be helpful in dissecting the relative contribution of elevations in PTH to the osteosclerotic phenotype.

In conclusion, we present novel findings in two patients with PHP-Ib harboring the 3-kb *STX16* deletion, including quantitative static and dynamic histomorphometry on trans-iliac specimens. Our bone histomorphometry results suggest that PTH has had an anabolic effect as opposed to a catabolic effect on bone in these cases. In particular, we have shown in these patients that mild elevations in PTH appear to be beneficial for cortical bone structure, although we cannot eliminate the possibility that alterations in other genes may have contributed to the observed skeletal phenotype. Overall, the histomorphometric data provide important insight into the pathogenesis of the bone phenotype at the tissue level in these brothers.

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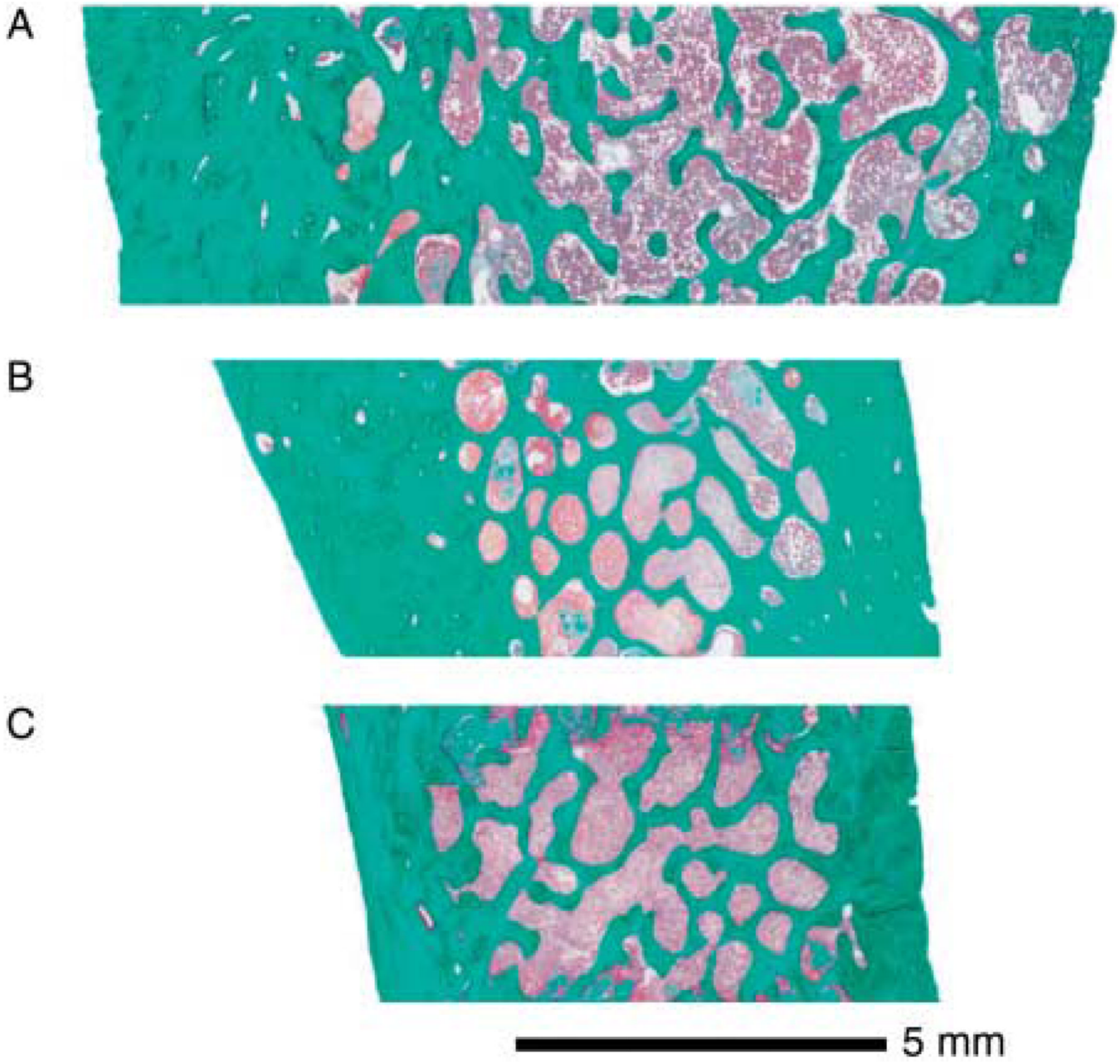

Figure 1.
(A) Iliac bone sample from proband 1. Cortical width is 2429 μm. (B) Sample from proband 2. Cortical width is 2106 μm. Note the difference in their cortical width when compared to the control (mean value based on the healthy average = 1006 μm) (C).

Table 1

Biochemistry.

		Proband 1		Proband 2	
	Reference range	**Result at diagnosis**	**Result at the time of biopsy**	**Result at diagnosis**	**Result at the time of biopsy**
Ionized calcium (mg/dl; (mmol/l))	4.4–5.2 (1.10–1.30)	2.6 (0.66)	4.2 (1.05)	3.2 (0.81)	3.6 (0.91)
Phosphate (mg/dl; (mmol/l))	3.1–5.3 (1.0–1.7)	8.7 (2.8)	3.7 (1.2)	7.1 (2.3)	2.8 (0.9)
Alkaline phosphatase (IU/l)	58–237	95	63	181	64
PTH (pg/ml; (pmol/l))	10–64 (1.1–6.8)	382 (40.8)	146 (15.6)	217 (23.2)	102 (10.9)
25-hydroxyvitamin D (ng/ml; (nmol/l))	8.0–36.1 (20–90)	ND	39.7 (99)	ND	33.7 (84)

ND, not done.

Table 2

Histomorphometric results in trans-iliac bone samples: structural parameters.

		Proband 1		Proband 2	
	Reference range[a]	**Raw result**	**Percentage of age- and gender-matched mean**	**Raw result**	**Percentage of age- and gender-matched mean**
Core width (μm)	8.2 ± 1.6	13.3	162	8.4	102
Cortical width (μm)	1006 ± 195	2429	241	2106	209
Cortical porosity (%)	6.6 ± 5	7.2	128	10.8	220
Bone volume/tissue volume (%)	27.8 ± 4.5	35.5	128	45.6	164
Trabecular number (/mm)	1.8 ± 0.3	1.8	96	2	108
Trabecular thickness (μm)	153 ± 24	203	132	230	150

[a]Mean ± S.D. for age 17.0–22.9 years in a reference population (Glorieux 2000).

Table 3

Histomorphometric results on trans-iliac bone samples: bone formation and resorption parameters on trabecular, intracortical, endocortical and periosteal surfaces.

		Proband 1		Proband 2	
	Reference range[a]	Raw result	Percentage of age- and gender-matched mean	Raw result	Percentage of age- and gender-matched mean
Trabecular surface					
Osteoid thickness (μm)	6.9 ± 1.2	8.4	122	5.8	84
Osteoid surface/bone surface (%)	17 ± 5	19	117	17	103
Mineralizing surface/bone surface (%)	7.9 ± 2.7	13.5	172	14.8	188
Mineral apposition rate (μm/day)	0.8 ± 0.1	1	131	0.8	104
Bone formation rate/bone surface ($\mu m^3/\mu m^2$ per year)	22 ± 9	48.8	220	42.1	190
Eroded surface/bone surface (%)	18 ± 6	24	132	21	116
Intracortical surface					
Osteoid thickness (μm)	7.7 ± 3.3	9.6	125	6.9	90
Osteoid surface/bone surface (%)	22.5 ± 14	15.5	69	16.5	73
Mineralizing surface/bone surface (%)	14.5 ± 8	14.1	97	14.1	97
Mineral apposition rate (μm/day)	0.8 ± 0.3	0.9	115	1.0	125
Bone formation rate/bone surface ($\mu m^3/\mu m^2$ per year)	41.5 ± 23.5	48.1	116	53.2	128
Eroded surface/bone surface (%)	14 ± 9	21.5	154	16.5	118
Endocortical surface					
Osteoid thickness (μm)	6.3 ± 4.4	5.6	90	6	96
Osteoid surface/bone surface (%)	16 ± 14	15	94	24	150
Mineralizing surface/bone surface (%)	9.5 ± 7.5	11.1	116	18.4	193
Mineral apposition rate (μm/day)	0.5 ± 0.2	0.6	114	0.8	159
Bone formation rate/bone surface ($\mu m^3/\mu m^2$ per year)	20 ± 15.5	23.8	119	52.1	260
Eroded surface/bone surface (%)	14.5 ± 10.5	30.5	210	10	68
Periosteal surface					
Osteoid thickness (μm)	4.1 ± 1.5	3.9	96	3.5	86
Osteoid surface/bone surface (%)	38.5 ± 26	22	58	30.5	79
Mineralizing surface/bone surface (%)	2.9 ± 6.4	0	0	0	0

	Reference range[a]	Proband 1		Proband 2	
		Raw result	Percentage of age- and gender-matched mean	Raw result	Percentage of age- and gender-matched mean
Mineral apposition rate (μm/day)	0.2 ± 0.2	0	0	0	0
Bone formation rate/bone surface ($\mu m^3/\mu m^2$ per year)	4.9 ± 2.0	0	0	0	0
Eroded surface/bone surface (%)	32.5 ± 30.5	52.5	162	17	52

[a]Mean ± S.D. for age 17.0–22.9 years in a reference population (13, 14).