SUSPECTED NON-LRP5 MUTATION ASSOCIATED WITH HIGH BONE MASS UNALTERED BY CONCURRENT SYMPTOMATIC PRIMARY HYPERPARATHYROIDISM OF LONG DURATION

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Abstract

Background. Unexplained high bone mass (HBM) (Bone Mineral Density-BMD Z-score at the lumbar spine or hip of $\geq +3.2$ SD, or a combined spine and hip Z score ≥ 4 SD) after routine bone densitometry occurs with a prevalence of approximately 2 out of 1.000 and is currently believed to be a mild form of skeletal dysplasia (1).

Results. We present the case of a patient with unexplained HBM (Z-scores at L3, L1-L4, total hip and radius total were +3, +2.7, +2 and +1.8, respectively) and concurrent symptomatic primary hyperparathyroidism (total serum calcium 11.9 mg/dL, serum Parathyroid Hormone - PTH 189.3 pg/mL) of long duration. There were no significant BMD changes at any skeletal site after the surgical cure of hyperparathyroidism. Testing for LRP (low density lipoprotein receptor-related proteins) 5 gene mutations was negative.

Conclusions. We presented an unusual case of the association of a HBM with primary hyperparathyroidism with resistance to the catabolic action of PTH. In spite of the negative result of LRP5 testing we do believe that a mutation of a gene involved in the Wnt pathway in bone is responsible.

Key words: Primary hyperparathyroidism, High bone mass, LRP-5.

INTRODUCTION

Few cases of high bone mass (HBM) are explained by LRP5 “gain of function” mutations and other known loci (1, 2). The Wnt signalling cascade has more recently emerged as a key pathway mediating bone homeostasis. Wnt proteins interact with the surface receptors low density lipoprotein receptor-related proteins 5 or 6 (LRP5, LRP6) and a family of co-receptors. Mutations in LRP5 can lead to quite different skeletal effects in humans, ranging from severely low bone mass to a HBM phenotype. HBM due to mutations in LRP5 is supposed to be caused by

augmented bone formation, although bone formation markers were not uniformly increased in all studies. There are virtually no human data on the behaviour of unexplained HBM in concurrent disorders of high bone remodelling.

CASE REPORT

The patient, a 45 years old premenopausal woman, was admitted to our department 3 years ago for the evaluation of resistant hypertension (HTA) and of a hypercalcemia observed recently. The family history was remarkable for HTA in both parents. Her past history was remarkable for having surgery 16 years ago for persistent milk teeth. She was suffering from high blood pressure for 5 years and during the last year more than three medications were needed to control her blood pressure. She has been suffering of nephrolithiasis for almost 20 years, with lithotripsy at least once a year. The physical examination was unremarkable, with the exception of high blood pressure (160/110 mmHg). Her body mass index (BMI) was normal (23.4 kg/m²) and she had regular menses. Initial blood tests (Table 1) confirmed primary hyperparathyroidism and also showed vitamin D deficiency, low bone turnover, mild haematological abnormalities and subclinical hypothyroidism. All others were normal, including serum cortisol, aldosterone, renin, metanephrines and renal function. Small bilateral kidney stones were seen by ultrasound. Neck ultrasonography showed a hypoechoic mass along the posterior aspect of the right lobe of the thyroid, 4/ 1.1 cm and a 5 g right inferior parathyroid adenoma was removed through minimally invasive surgery, which was curative (next day: Ca 8.8 mg/dL, PTH 11.39 pg/mL). Daily oral therapy with calcium (1.8 g) and vitamin D (1.000 U) was started as well 25 µg L- thyroxine. One month postsurgery and

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Table 1. Baseline and follow-up blood chemistries

	Normal range	Baseline preop	1 month postop	6 months postop	1 yr postop	2 yrs postop	3 yrs postop
Serum Calcium	8.4-10.4 mg/dL	11.9	9.6	9.4	9.7	9.2	
Serum Phosphate	2.5-4.5 mg/dL	2.20	2.64	2.76	2.77	2.58	
Serum PTH	15-65 pg/mL	189.30	26.29	43.11	44.61	30.92	
Serum CTX	0.137-0.573 ng/mL	0.190	0.056	0.06	0.055	0.06	0.121
Serum OC	11-43 ng/mL	12.71	11.67	7.74	8.65	6.44	11.52
Serum 25OHD	30-100 ng/mL	13.90	19.30	18.43	15.5	13.15	
Serum AP	38-105 U/L	59	55	59	53	60	
Serum TSH	0.4-4.5mUI/L	5.88	7.58	6.76	7.68	2.76	
Serum FT4	9-19 pmol/L	12.57	12.57	11.45	11.33	10.8	
ATPO	<35UI/L	351.7	-	-	-	-	
WBC	4-10 x10 ³ /μL	12.47	-	11.54	11.34	10.35	
Neutrophiles	2-4.5x10 ³ /μL	9.45	-	8.87	9.51	8.45	
Hemoglobin	12-15.5g/dL	11.5	12.1	11.8	12.1	13.4	
Platelets	150-400x10 ⁹ /L	594	556	607	524	409	

PTH – Parathyroid hormone, CTX – C-telopeptide, OC-Osteocalcin, 25OHD -25 hydroxy-vitamin D, AP-Alkaline Phosphatase, TSH – Thyrotropin, FT4 – Free Thyroxine, ATPO- Anti Thyroid-Peroxidase Antibodies, WBC – White Blood Cells

Figure 1. Three years follow-up of bone turnover markers (pre-and postoperatively).

follow-up blood chemistries are presented in Table 1. Blood pressure was 130/90 mmHg with lower doses of 3 medications. She was followed-up for 3 years and her turnover markers are presented in Figure 1.

BMD studies

Dual-energy X-ray absorptiometry (DXA) (GE Lunar iDXA) was used to measure BMD in the spine, hip and forearm. The coefficients of variation were 1.2% for the lumbar spine and total hip measurements (LSC 3.3%), 1.5% for femoral neck (LSC 4.2%) and 1.3 % for the forearm (LSC 3.6%). BMD by DXA revealed a generalized high bone mass (Z-scores at L3, L1-L4, total hip and radius total were +3, +2.7, +2 and +1.8 SD, respectively). BMD changes at all measured sites (LS, TH, RT) were non-significant compared to calculated LSC of our DXA instrument (Table 2). As known causes of osteosclerosis/ osteopetrosis were excluded, their specific mutations were not searched for.

Genetic testing

Genomic DNA was extracted from venous blood using standard methods. PCR was performed using Taq DNA polymerase (ThermoFisher Scientific) and primers were designed in order to only amplify exons 2-4 of the LRP5 gene (reference sequence: NM_002335). Primer sequences are available from authors upon request. Sanger sequencing was performed according to standard procedures using the BigDye Terminator v3.1 Cycle Sequencing kit (Life Technologies, USA). Sequencing reactions were resolved on 3730 ABI (Applied Biosystems) and the results were analyzed using SeqScape (Applied Biosystems). The sequencing did not lead to the identification of any abnormal genetic variants of LRP5 gene.

DISCUSSION

We presented the case of a premenopausal woman with primary hyperparathyroidism (PHPT) and concurrent high bone mass. Her PHPT was of long duration, almost 20 years based on her history of frequent lithotripsy for kidney stones. Unexpectedly, BMD by DXA revealed a generalized high bone mass (Z-scores at L3, L1-L4, total hip and radius total were +3.3, +2, +2 and +1.8 SD, respectively) fulfilling the definition of the recently defined unexplained HBM (1, 3, 4). There were no degenerative changes at the spine and no other sclerosing disorders. Recently, several features were found associated with HBM: (a) BMI >30 kg/m², (b) broad frame, (c) sinking when swimming, (d) mandible enlargement on examination and (e) extra

Table 2. Baseline spine, hip and radius BMD and Z-scores and changes at 1 and 3 years postoperatively

	Baseline Preop		% Increase postop vs baseline	
	BMD	Z-score	1 yr	3 yrs
L1-L4	1.510	2.7	3.10%	2.50%
Total Hip	1.201	2	0.50%	3.70%
Neck	1.044	0.7	1.20%	-0.20%
Radius 33%	0.869	-0.1	-2.60%	0.80%

bone identifiable on clinical examination (1). Our patient has a normal BMI, normal frame and mandible. Remarkably, she had oral surgery for bilateral persistent milk teeth when 31 year old, one of the features of unexplained HBM phenotype. Quite recently it has been acknowledged that mutations in known HBM loci may be asymptomatic (2). Our patient had persistent moderate increase in platelet counts which resolved 2 years post-surgery; increased counts *vs* control have been also observed by others in patients with LRP5 - HBM (5).

Before surgery (active PHPT) her bone turnover was rather low, based on serum markers: CTX 0.19 ng/mL (0.137-0.573) was 6 times lower than mean CTX of our PHPT patients (1.174 ng/mL, n=178) and OC 12.71 ng/mL (11-43), almost 5 times lower than mean OC of our PHPT patients (61.33 ng/mL, n=178). Measured bone markers behaved differently after surgery (Fig. 1), as expected after parathyroidectomy (PTX). Serum CTX decreased significantly as early as one month (0.056 ng/mL) and stayed for 2 years, and became slightly lower than normal after 3 years (0.121 ng/mL). Serum OC decreased after 6 months (7.74 ng/mL) stayed for 2 years and was low normal (11.52 ng/mL) after 3 years; serum P1NP was normal 3 years after surgery, 24.42 ng/mL (15-74). Instead, 3 years BMD changes at all measured sites (LS, TH, RT) were non-significant compared to calculated LSC of our DXA instrument (Table 2). More specifically there was no significant BMD increase 1 year post-surgery, at any skeletal site. Successful PTX is associated with increases in BMD that are the greatest and most rapid at the lumbar spine and hip, followed later by increases in the distal 1/3 radius (6). At the third distal radius, specific site involved in primary hyperparathyroidism, before surgery BMD Z-score was -0.1 SD and also did not change significantly 3 year post-surgery. Typically in PHPT, bone loss is the greatest at the forearm (33% radius), a skeletal site that is almost totally comprised of cortical bone, and least at the lumbar spine, a skeletal site with a large component of trabecular bone (7).

We thought it is reasonable to assume that her bone mass and turnover at 3 years post-surgery

are her normal values. Therefore, our interpretation of the case is unexplained HBM concurrent with PHPT and resistant to the catabolic action of high PTH of long duration. She has generalized high bone mass, as has been recently quoted in unexplained HBM, persistent milk teeth into adulthood, no other skeletal abnormality. Her markers of bone turnover were constantly low, lower for CTX than OC, which is in contrast with other observations: in the normal range but low compared to controls (5), and those showing increased bone formation (8). Our data do not suggest an increased rate of bone formation as both OC and PINP were slightly below the lower normal range or in the lower half 3 years after surgery. The occurrence of PHPT increased bone turnover markers, CTX and OC, 3x and 1.5 respectively, but much less than the mean concentrations of our PHPT patients (5-6 x less). Therefore, the low bone turnover associated with HBM could be stimulated by a high remodelling disorder as PHPT. Interestingly BMDs at all sites, including third distal radius which has a normal Z-score, were unchanged after the curative surgery. This is in agreement with the relatively low turnover before surgery, and suggests that her skeleton was protected from the catabolic action of PTH. Interestingly enough she reported accidental trauma unfollowed by fracture suggesting increased bone strength; there is a report suggesting such cases are resistant to fractures despite exposure to heavy trauma such as road traffic accident (9). The patient's only child, a 21 years female, had an attenuated HBM: Z-score at the lumbar spine and hip were 2.3 and 1.4 SD, respectively and normal bone markers: CTX 0.44 ng/mL, OC 22.31 ng/mL.

As the patient had generalized high bone mass and apparently resistant to the catabolic action of PTH we were looking for a mutation of the LRP5 gene. Less severe phenotypes (Z-scores +2.4 to +6.2 SD), like our patient, have been described in unexplained HBM (2). It has been also shown quite recently that the Lrp5 HBM alleles offer significant protection from the catabolic effects of disuse and estrogen deficiency in mice (10). Previous studies have identified some common loci associated with HBM. In the largest and

recent population study on HBM five novel mutations were identified in exons 2-4 of the LRP5 gene (2). The same authors identified 13 different mutations in LRP5 causing HBM and all of them locate in these 3 exons. In order to genetically investigate the HBM phenotype, we sequenced exons 2-4 of LRP5 in the index patient and her daughter. The sequencing did not lead to the identification of any abnormal genetic variants. Mutations in LRP5 only explain less than 3% of HBM cases and this could explain our negative findings. Furthermore, other genes such as SOST and LRP4 are known to associate with HBM. For this reason, the sequencing of other HBM genes could help to find a genetic diagnosis. However, the genetic background of HBM is to a great extent unknown and many genes underlying the phenotype still remain to be identified.

In conclusion, we presented an unusual case of the association of a HBM with primary hyperparathyroidism with resistance to the catabolic action of PTH. In spite of the negative result of Lrp5 testing we do believe that a mutation of a gene involved in the Wnt pathway in bone might be responsible.

Conflict of interest

The authors declare that there is no conflict of interest. Written informed consent was obtained from the patient after a complete and clear explanation of the nature and purpose of the study.

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