

Case report**Familial isolated primary hyperparathyroidism due to HRPT2 mutation**

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ABSTRACT

Primary hyperparathyroidism is a common endocrine disorder that is mostly caused by solitary tumors within the parathyroid glands. Characterized by early debut and higher frequency of multiple parathyroid masses, familial forms of primary hyperparathyroidism are caused by the already known mutations of: *menin* (MEN1 syndrome), *RET* proto-oncogene (MEN2 syndrome), *HRPT2*-parafibromin (hyperparathyroidism-jaw tumor syndrome), calcium sensing receptor gene (familial hypocalciuric hypercalcemia). A specific mutation in FIHP has not been identified in the majority of affected families. Recent studies revealed *menin*, *HRPT2* and calcium-sensing receptor mutations in patients with FIHP. Whether FIHP is a variant or an early stage of MEN1 syndrome or hyperparathyroidism-jaw tumor syndrome is yet to be established. We present three siblings with familial isolated hyperparathyroidism due to solitary parathyroid adenoma and favorable evolution post-parathyroidectomy. Genetic tests revealed *HRPT2* mutation.

Key words: Familial isolated hyperparathyroidism, HPT-JT, *HRPT2*

INTRODUCTION

Familial isolated hyperparathyroidism (FIHP) is a rare hereditary disorder characterized by hypercalcemia, high parathyroid hormone (PTH) level and isolated parathyroid tumors. FIHP was suggested as being a syndrome clinically and genetically distinct

from multiple endocrine neoplasia (MEN), familial hypocalciuric hypercalcemia and hyperparathyroidism-jaw tumor syndrome.

CASE REPORTS

We present below a family with isolated HPT. Affected members are the father and the two sons (Figure 1).

Case 1

The younger son (23 years) was the first to be diagnosed. He complained of progressive bone pain

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Received 17-03-2013, Accepted 10-06-2013

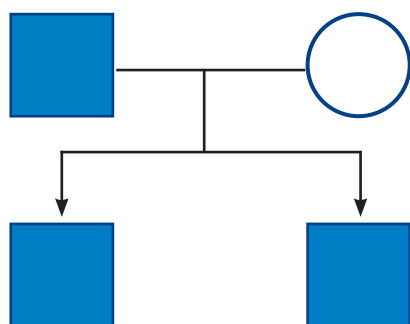


Figure 1. Pedigree.

and marked reduction of muscle force.

Laboratory data (Table 1) revealed hypercalcemia, hypophosphatemia and inappropriately high parathyroid hormone, suggesting primary hyperparathyroidism. There was no evidence of other endocrine neoplasia.

Cervical ultrasound showed a nodule in the lower posterior right thyroid lobe (Figure 2).

Technetium scintigraphy showed increased uptake in the lower pole of the right thyroid lobe. CT scan showed a 28/20mm heterogenous nodule on the right thyroid lobe, confirming it as a potential parathyroid adenoma. We also found osteitis fibrosa cystica i.e. multiple osteolytic bone lesions with intact periosteum located on the clavicle, sternum, bilateral humerus, scapulas, ribs and pelvic bones. The largest (67/34mm)

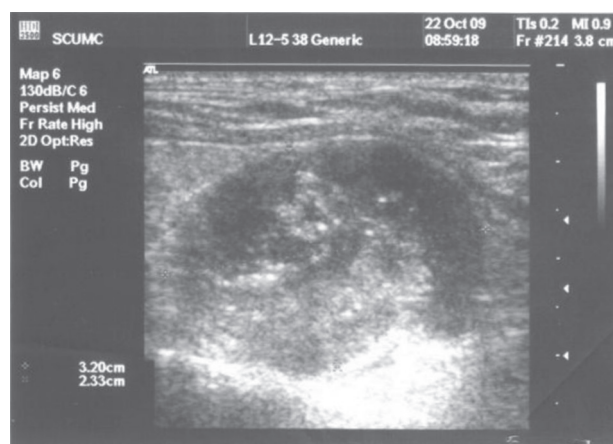


Figure 2. Cervical ultrasound: 26/23mm nodule in right thyroid lobe, located posteriorly, with mixed structure and intense vascularization.

lesion occupied almost entire cortex at the level of the left anterior sacral wing.

Preoperative management consisted of intravenous infusion of 1.5 liters of saline solution 0.9%, 90mg of Pamidronate and 100UI of Calcitonin 3 times a day for 3 days. Right inferior parathyroidectomy was performed and the histopathological report confirmed the presence of a parathyroid adenoma with oxyphil cells, old hemorrhagic areas, areas of cholesteatoma and impregnation with calcareous matter and capsular sclerosis. Unexpectedly, postoperative calcemia and PTH level remained elevated (10-11mg/dl, re-

Table 1.

Parameter	Case 1	Case 2	Case 3	Normal range
Calcium (mg/dl)	13.89	14.5	16.65	8.5 – 10.3
	18.86	15.44	15.2	
	15.7		14.3	
Phosphate (mg/dl)	1.45	2.3	2.64	2.5 – 4.5
	1.85	1.9	2.4	
	2.42			
PTH (pg/ml)	1600	643	1172	15 - 65
Parathyroid tumor	right lower adenoma	left lower adenoma	right lower adenoma	
Complications	nitrogen retention osteitis fibrosa cystica	nitrogen retention nephrolithiasis	osteitis fibrosa cystica nephrolithiasis	

spectively, 100pg/ml) but spontaneously normalized after 3 months.

Case 2

The second patient was the 55-year old father, who had a history of high blood pressure (170/100mmHg) and calcium oxalate nephrolithiasis. His blood tests revealed primary hyperparathyroidism (Table 1). Thyroid ultrasound showed a 40/30mm transonic nodule with floating echoes inside the lower left thyroid lobe and another 8mm nodule in the lower posterior right lobe. Technetium scintigraphy showed increased uptake in the lower pole of the left thyroid lobe (Figure 3).

Cervico-mediastinal CT scan showed in the prevascular space, in intimate contact with the left thyroid lobe, a 35/33mm nodule with mixed structure well defined by a thin and iodophilic capsule.

Left inferior parathyroidectomy and right hemithyroidectomy were performed. The histopathological report confirmed the presence of a parathyroid adenoma with oxyphil cells, sclerous capsule and septa and areas of cholesteatoma. Postoperative evolution was similar to that of Case 1 with persistent hypercalcemia and elevated PTH, spontaneously normalized after 3 months. Thyroid replacement therapy with 50µg LT4/day was prescribed.

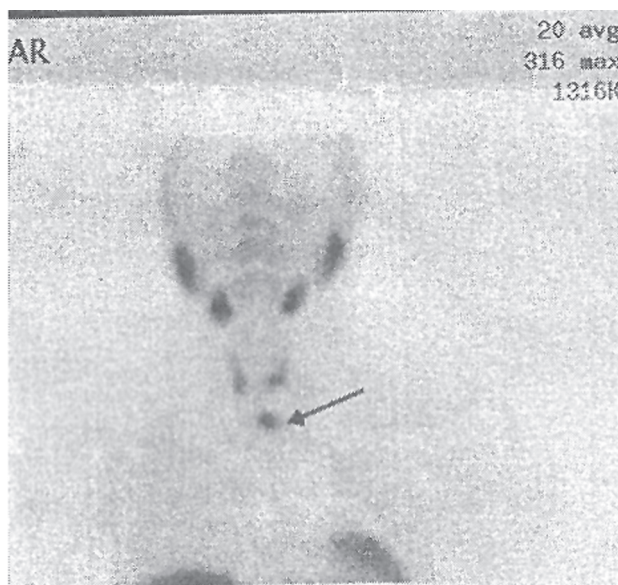


Figure 3. Technetium scintigraphy: increased uptake in lower pole of left thyroid lobe.

Case 3

The last member of the affected family is the eldest son (31 years). He has a history of repeated incidence of renal colic and diffuse bone pain. Laboratory data revealed primary hyperparathyroidism (Table 1).

Cervical ultrasound revealed: two nodules in the middle and lower part of the right thyroid lobe (Figure 4).

Technetium scintigraphy showed increased uptake in the lower right thyroid lobe. CT scan indicated the same 2 nodules as suspected parathyroid lesions: superior = 26/23/30mm and inferior = 17/18/14mm.

Renal ultrasound revealed the presence of a 14mm stone in the right kidney.

DXA scan indicated loss of cortical bone:

- L1-L4: BMD = 1.037; T = -1.5; Z = -1.3
- femoral neck: BMD = 0.757; T = -2.4; Z = -2.3
- radius: BMD = 0.434; T = -4.6; Z = -4.6

Evidence of decreased BMD in the distal radius is often used as an early indicator in hyperparathyroidism. Moreover, tomography and scintigraphy showed osteolytic lesions of the left humeral head and left proximal tibia.

Right inferior parathyroidectomy and right hemithyroidectomy were performed (the second lesion was a thyroid nodule). Parathyroid adenoma



Figure 4. Thyroid ultrasound: in right thyroid lobe a 20/30mm mixed nodule and a 7/18mm hypodense nodule, both intensely vascularized.

with chief cells was confirmed by the histopathological report and the second lesion proved to be a thyroid nodule. After surgery, calcemia and PTH level normalized. Thyroid replacement therapy with 50µg LT4/day was prescribed.

Genetic tests

The HRPT2 and menin genes were studied by amplification of exon and intron regions by PCR and direct sequencing of amplicons. The results were expressed according to the nomenclature of the Human Genome Variation Society.

17 exons were studied for HRPT2 gene and the same mutation was found in all three cases. Nucleotide variation was:

Exon (s) 6
 Nucleotide nomenclature c.505C>T
 Proteic nomenclature p. Gln169X

The pathological presence of a stop-codon leads to the synthesis of a truncated protein.

Using Multiplex Ligation-dependent Probe Amplification, the presence of HRPT2 gene deletion or duplication was disproven.

9 exons were studied for MEN1. No nucleotide variations, gene deletion or duplication were found.

DISCUSSION

Compared to sporadic primary hyperparathyroidism, familial hypercalcemia has a lower prevalence, a younger age at diagnosis and an equal ratio between affected men and women. The most frequent histopathological lesion is solitary parathyroid adenoma in sporadic primary hyperparathyroidism and hyperplasia or multiple parathyroid adenomas in familial hypercalcemia (Table 2).

Causes of familial hypercalcemia include hereditary

forms of primary hyperparathyroidism and impaired cellular response to extracellular calcium fluctuations.

There are three syndromes associated with primary hyperparathyroidism:

- MEN 1 and 2A;
- hyperparathyroidism-jaw tumor syndrome (HPT-JT);
- familial isolated hyperparathyroidism (FIHP).
- Impaired cellular response to extracellular calcium fluctuations is responsible for:
 - familial benign hypocalciuric hypercalcemia;
 - severe neonatal hypercalcemia.

MEN1 syndrome has an autosomal dominant inheritance with 100% genetic penetrance by age of 50.^{2,3} In most cases, primary HPT is the first clinical manifestation.⁴ Associated tumors are located in the endocrine pancreas, pituitary, adrenal cortex or carcinoid tumors.

The etiology of MEN1 syndrome is menin inactivation. The *MEN1* tumor suppressor gene was cloned from 11q13 by positional cloning.^{5,6} Its product, menin, has been found to bind specifically to JunD, whereas disruption of this binding activity by *MEN1* mutations leads to inhibition of JunD-activated transcription.⁷

Over 100 germline mutations of this tumor suppressor gene have been reported (exons and introns: missense, nonsense, frameshift, ARNm splicing defects). The type of mutation is not associated with a specific phenotype but is present in all affected family members. Genetic testing is required to confirm the clinical diagnosis.²

MEN2A syndrome has an autosomal dominant inheritance with 95% genetic penetrance. Only 25% of these patients have primary hyperparathyroidism.⁴ Associated tumors are: medullary thyroid carcinoma and feocromocitoma. Etiology of this syndrome is activating mutations of the RET proto-oncogene (cz

Table 2.

	Sporadic primary hyperparathyroidism	Familial hypercalcemia
Prevalence	21 cases/100,000 inhabitants ¹	7 cases/100,000 inhabitants
Age at diagnosis	≈50 years	<50 years
Women/Men	3/1	1/1
Most frequent histopathological lesion	Solitary parathyroid adenoma	Hyperplasia or multiple parathyroid adenomas

10q11.2)⁸ which encodes a tyrosine kinases receptor. When codon 634 is affected incidence of HPT is maximum.⁹ RET proto-oncogene mutations have never been documented in patients with familial isolated hyperparathyroidism.

HPT-JT syndrome is an autosomal dominant disorder consisting of parathyroid tumors, ossified jaw fibromas and renal lesions (Wilms tumor, hamartoma, polycystic kidney disease).¹⁰ In the parathyroid, potential histopathological lesions are: solitary solid or cystic adenoma, multiple adenomas or carcinoma.^{11,12}

HPT-JT syndrome has an autosomal dominant inheritance with 70% genetic penetrance (smaller if the mutation carrier is a woman).¹³

Etiology of this syndrome is inactivating mutations of the HRPT2 (CDC73) gene which encodes a tumor suppressor named parafibromin.¹⁴⁻¹⁶ Because of the 15-20% of PT carcinoma linked to parafibromin inactivation, HRPT2 is considered to be a marker of parathyroid cancer.

Parafibromin is a protein localized and functionally linked to the nuclear, nucleolar as well as cytoplasmic compartments.¹⁷ Parafibromin is considered a tumor suppressor protein based on its ability to induce apoptosis, down-regulate cyclin D1 levels, inhibit G1 to S phase transition as well as to regulate gene expression of various growth factors.¹⁸⁻²⁰ In addition, parafibromin exhibits histone modulating properties, such as recruiting histone methyltransferases and processing histone mRNAs.²¹⁻²³

Familial benign hypocalciuric hypercalcemia is an autosomal dominant disorder consisting of right-shifted calcium set-point. Over 150 inactivating mutations of the calcium sensing receptor gene (cz 3q13.3-q21) have been reported.²⁴ The heterozygous form associates moderate non-progressive hypercalcemia with hypocalciuria and elevated PTH. The homozygous form, also named severe neonatal hypercalcemia, associates severe hypercalcemia, symptomatic at birth, with very high levels of PTH.²⁵

Familial isolated primary hyperparathyroidism is an autosomal dominant disorder with reduced genetic penetrance. There are two histopathological entities: hyperplasia/multiple parathyroid adenoma and solitary parathyroid adenoma/potential carcinoma.¹³

FIHP is a genetically heterogenous disease. No specific genetic anomaly has been reported.¹⁶ Instead, specific mutations of other forms of familial hypercalcemia have been described.⁹ That is why FIHP could be considered a mild form with incomplete penetrance or an early stage of the above syndromes.

FIHP with HRPT2 mutations and solitary parathyroid adenoma with a cystic component is considered a variant of HPT-JT syndrome.^{26,27} It is associated with increased risk for parathyroid carcinoma.^{28,29}

When menin mutation is present, we can find multiglandular involvement but a more favorable clinical progression than in MEN1 syndrome.³⁰ Two separate regions of menin have been shown to separately bind JunD, and at the C-terminus two nuclear localization signals have been identified.^{7,13} The two reported FIHP-associated *MEN1* mutations fall outside all these regions, suggesting a functional basis for this milder variant of MEN1.^{13,30}

Analysis of the previously published MEN1 mutations in FIHP reveals that 41% of these are missense mutations and 37% are frameshift or nonsense mutations.^{31,33} This contrasts significantly with the situation in patients with MEN1, in whom more than 80% of the germline mutations are nonsense or frameshift and less than 20% are missense mutations.^{32,33}

Five families with isolated HPT and calcium sensing receptor mutations have been reported. Especially mutations in the 3q region in homozygous form can lead to the development of a parathyroid adenoma.^{3,24,34}

An important fact that one must keep in mind is that different mutations of the same gene are responsible for different syndromes (Table 3).

Regarding the pattern of tumor growth, two main

Table 3.

HRPT2 mutations	HPT-JT syndrome
	FIHP
Menin mutations	MEN1 syndrome
	FIHP
Calcium sensing receptor mutations	FBHH/severe neonatal hypercalcemia
	FIHP

types of pathological growth of parathyroid cells have been postulated,³ apparently depending on mutation type.

Most of the sporadic parathyroid adenomas have stopped their proliferation at the time of surgery. This could be explained by a right-shift in set-point for PTH secretion, which is a stimulus for cell growth because of continuous stimulation by the relative hypocalcemia. This clone of cells continues to grow until the secretion of PTH is enough to raise calcemia to a level that matches the new set-point, and then the growth is stopped. Mutations of CaR have been incriminated for the increase in set-point of PTH secretion seen in primary HPT, but it has been difficult to establish the role of CaR in the tumorigenesis of sporadic parathyroid tumors.¹³

The second pattern of parathyroid growth is considered to be the result of a mutation in a cell cycle gene. In this situation, tumors grow exponentially. Possible genetic events involved are: somatic deletions and amplifications of specific chromosomal regions harboring putative tumor suppressor genes and oncogenes. Overexpression or rearrangement of PTH-cyclinD1/PRAD1 are commonly seen in parathyroid tumorigenesis.³⁵

CONCLUSION

Familial hypercalcemia is a heterogeneous group of disorders. Like other forms of hypercalcemia, it is often asymptomatic, and calcium determination in routine testing is vital for early diagnosis. Complex laboratory investigations confirm the diagnosis and curative treatment is surgical. Genetic testing has an important role in patient evaluation, especially in situations where it is necessary to assess the risk of developing parathyroid carcinoma. Testing of blood relatives requires careful monitoring of those with confirmed mutations.

ACKNOWLEDGMENTS:

We acknowledge the Oncogenetics Laboratory of Groupe Hospitalier Cochin, Paris – Pr. E. Cluser, Dr. M. O. North, Dr. N. Hamzaoui for their very good work with the genetic analysis.

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