

HRPT2-related Familial Isolated Hyperparathyroidism: Could Molecular Studies Direct the Surgical Approach?

ABSTRACT

It is still debatable which is the best management to familial forms of hyperparathyroidism. Conservative, minimally invasive or aggressive surgical approaches have been proposed from different groups around the world. Our objective was to study the gene mutation, expression of *HRPT2* and the clinical outcome after 32 years of follow-up in one Brazilian kindred with familial isolated hyperparathyroidism (FIHP). Clinical and biochemical data, direct sequencing of the *HRPT2* gene, analysis of parafibromin expression using RT-PCR, and immunohistochemistry were done. A nonsense mutation was found in exon 1 (c.96G>A)(p.Trp32X) in all affected members studied. Using RT-PCR, mRNA transcription was altered with complete absence of both transcripts in tumor tissue. Immunohistochemical analysis of tumors showed loss of parafibromin immunoreactivity. In this kindred there was a high prevalence of recurrence (75%), or persistence after less than subtotal parathyroidectomy that led us to consider a more aggressive surgical approach should be discussed among the affected family members, once surgical criteria was met. We concluded that it is necessary to individualize the surgical approach for HRPT2-related hyperparathyroidism until we can gather a better phenotype-genotype correlation in larger series, to best define their treatment. (**Arq Bras Endocrinol Metab** 2008; 52/8:1211-1220)

Keywords: *HRPT2*; Parafibromin; Parathyroid; Jaw tumor; FIHP; Surgery

RESUMO

Formas Familiares de Hiperparatireoidismo Relacionadas a Mutações no Gene *HRPT2*: Estudos Moleculares Poderiam Direcionar Procedimentos Cirúrgicos?

A melhor conduta nas formas familiares de hiperparatireoidismo relacionadas a mutações no gene *HRPT2* ainda é controversa. Cirurgias conservadoras, minimamente invasivas ou mais agressivas já foram propostas por diferentes grupos. Objetivamos estudar a seqüência e a expressão do gene *HRPT2*, além do desfecho clínico, após seguimento de até 32 anos de uma família brasileira com hiperparatireoidismo familiar isolado (FIHP). Utilizamos dados clínicos e bioquímicos, seqüenciamento direto do *HRPT2* além de análise da expressão da parafibromina através da RT-PCR e imunohistoquímica. Foi identificada mutação *nonsense* no éxon 1 (c.96G>A)(p.Trp32X) em todos os membros afetados que foram estudados. A análise do mRNA transcrito, através da RT-PCR, demonstrou ausência do transcrito no tecido tumoral. A imunohistoquímica também evidenciou ausência da parafibromina. Nessa família houve alta (75%) prevalência de recorrência ou persistência da doença após paratireoidectomia parcial o que nos levou a considerar fundamental discutir uma abordagem cirúrgica mais agressiva com os outros familiares portadores da mutação caso critérios de indicação cirúrgica sejam atingidos. Dessa maneira, até que estudos mais amplos estabeleçam uma correlação genótipo-fenótipo no hiperparatireoidismo familiar relacionado a mutações no *HRPT2*, a abordagem cirúrgica deverá ser individualizada. (**Arq Bras Endocrinol Metab** 2008; 52/8:1211-1220)

Palavras-chave: *HRPT2*; Parafibromina; Paratireóide; FIHP; Cirurgia

clinical case report

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INTRODUCTION

Familial hyperparathyroidism (HPT) encompasses a clinically and genetically heterogeneous group of disorders. These heritable forms are responsible for approximately 10% of primary HPT. They are usually seen in the context of syndromes, such as multiple endocrine neoplasia types 1 and 2 (MEN 1, MIM #131100 and MEN 2, MIM #171400, respectively), hyperparathyroidism-jaw tumor syndrome (HPT-JT, MIM #145001), familial benign hypocalciuric hypercalcemia (FHH, MIM #145980) and familial isolated hyperparathyroidism (FIHP, #145000) (1).

FIHP is a rare autosomal dominant condition, characterized by the occurrence of familial hyperparathyroidism in the absence of other associated lesions or endocrinopathies, with a clinical and genetically heterogeneous presentation (2-6). Most FIHP families do not have identifiable mutations, although some patients have been found to harbor germline mutations in MEN1, CASR, HRPT2 and, more recently, studies have also linked a FIHP locus to chromosome 2p14-p13.3 (HRPT3) (2,3).

In patients with FIHP, genetic screening for MEN1 and CASR is most likely to identify mutations in those with young age at onset and multigland parathyroid involvement, whereas HRPT2 mutations should be considered in those families with parathyroid carcinoma, cystic parathyroid tumors, or jaw tumors - present in up to 30% of the affected patients (4-7).

The majority of FIHP with MEN 1 mutations presented with mild hypercalcemia and multiglandular disease or parathyroid hyperplasia, similar to what is usually seen in MEN1 (8-13). On the other hand, in FIHP families where HRPT2 gene mutations have been detected, the clinical presentation is more severe and in all cases the histopathological diagnosis was parathyroid carcinoma or adenoma, frequently associated with atypical or cystic features similar to those seen in HPT-JT syndrome (13-15). These observations suggest that FIHP could be subdivided in at least two major genetic subsets: a MEN1-related FIHP, characterized by milder forms of hyperparathyroidism, typically presenting as multiglandular disease; and HRPT-related variety, characterized by an aggressive disease, parathyroid adenomas or carcinomas and HRPT2 gene mutations that could be regarded as a variant of HPT-JT syndrome.

In view of the atypical behavior of adenomas, and the high prevalence of parathyroid carcinomas in families with syndromes associated with HRPT2 mutations in contrast to the extremely rare occurrence of sporadic parathyroid malignancy, the HRPT2 gene has been investigated in parathyroid carcinoma. Inactivating somatic mutations of HRPT2 were found in the majority of sporadic parathyroid cancers and even germline mutations were detected in some patients with apparently sporadic tumors (16,17). On the other hand, intragenic HRPT2 somatic mutations were detected only in 0.8 to 1.8% of sporadic adenomas, depending on the inclusion criteria used, supporting the view that HRPT2 inactivation is not an important contributor to parathyroid tumorigenesis (18).

The HRPT2 gene is located on chromosome 1q31.2 and consists of 17 exons that span 1.3 Mb of genomic DNA (6) encoding a protein named parafibromin, with 531 amino acids. Recently, parafibromin was identified as a component of the human Paf1 (peroxisome assembly factor-1) complex which is an accessory factor to the RNA polymerase II suggesting, as has been shown for its yeast homologue (Cdc 73), which it may have a role in transcription regulation (19). Overexpression of parafibromin in mammalian cell lines inhibits cell proliferation and blocks expression of Cyclin D1, which has been implicated in parathyroid neoplasia (20). Tan et al. (21) demonstrated loss of parafibromin nuclear immunoreactivity in 96% of parathyroid carcinomas and in eight of nine adenomas associated to HPT-JT syndrome. Furthermore, normal expression of parafibromin has been demonstrated in the majority of the sporadic parathyroid adenomas so far studied (20-22).

The management of familial hyperparathyroidism differs among the specific syndromes and is generally complex due to the underlying causative genetic abnormality that can predispose patients to persistent and recurrent hyperparathyroidism. Therefore it is of utmost importance to identify these syndromes before the surgical procedure is planned. Besides a detailed family history and search of the well-known co-morbidities present in those syndromes, molecular analyses might also influence the therapeutic decision.

We here describe a four generation family with HRPT2 gene mutation and immunohistochemical analyses with the longest ever described follow-up, i.e., 32 years, and debate its surgical outcome.

SUBJECTS AND METHODS

The present study was approved by the Felício Rocho Hospital Ethics Committee and all participating patients and family members signed a written informed consent. Most of the patients, except for II:10, have been followed by the Endocrinology Center, at least once a year. Hyperparathyroidism diagnosis was established by the detection of hypercalcemia associated with elevated serum PTH levels (23). Multiple endocrine neoplasia type 1 was excluded by detecting normal levels of serum glucose, insulin, calcitonin, gastrin, IGF1, and prolactin as well as sequencing all coding regions of the MEN1 gene. The family's pedigree is shown in Figure 1. Peripheral blood samples for DNA extraction were obtained from individuals II:1, II:10, II:12, II:14, II:16, III:4, III:6, III:7, III:8, III:20 and III:21.

Fresh samples of parathyroid adenomas were obtained during surgical removal of the tumor, for RNA and DNA extraction from III:6 and III:7 in this kindred. In each case, a portion of the tumor was resected, immediately snap frozen and stored at -80°C . For immunohistochemistry, sections were obtained from paraffin-embedded tissue blocks. DNA was extracted as previously described (24).

CASE REPORT

This is a four generation family, in which nine members were diagnosed with isolated hyperparathyroidism and

underwent parathyroidectomy (Figure 1). No other endocrinopathies were reported in any affected individual after 32 years of follow-up since the diagnosis of the index case. Maxillary and/or jaw tumors were ruled out in all patients by clinical examination and plain X-ray of the jaws. Lack of consanguineous marriages and pedigree analysis (Figure 1) suggested autosomal dominant mode of inheritance. Biochemical and molecular screening for MEN1 were negative. Clinical and biochemical details of affected members of this family are shown in Table 1.

The proband (II:12), 32 years of age, was referred in 1976 because of a maxillary lesion diagnosed as brown tumor. She complained of generalized bone pain, polyuria, polydipsia and weight loss (13 kg in one year). After biochemical diagnosis of primary hyperparathyroidism, she underwent surgical removal of a single 1-cm left inferior parathyroid adenoma. She became normocalcemic, needing calcium supplements for about 5 months. The "brown tumor" disappeared and she became asymptomatic until 1981, when she returned with recurrent hypercalcemia and raised parathyroid hormone levels. Once again she underwent cervical surgery, with removal of two more parathyroid adenomas (0.5 and 1.2 cm). Her serum calcium normalized, calcium supplements were needed for a short period of time and her bone mass density improved dramatically. She remained asymptomatic with no laboratory abnormalities for 24 years, when a knee lesion was detected during an orthopedic investigation

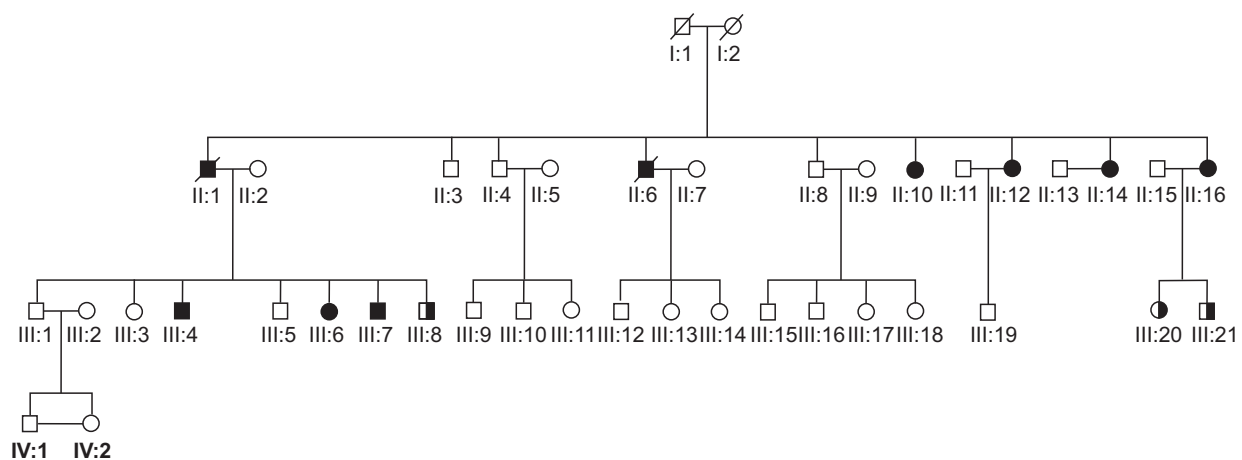


Figure 1. Pedigree indicating the proband (arrow). Half-filled symbols (III:8, III:20 and III:21) denote the presence of heterozygous mutation in patients aged 26, 19 and 17 without any clinical or biochemical disease to date.

Table 1. Clinical and biochemical details of affected members.

Case	Sex	Age (y) at Diagnose	Serum Calcium (8.5-10.5mg/dL)	PTH (10-65pg/ml)	Parathyroid Histology ^c	Associated Lesions	Recurrence Persistence ^d
II:1	M	65	11.9 mg/dL	664 pg/mL	Adenoma (1)	Bilateral renal cysts	Yes (5y)
II:6	M	52	11.9 mg/dL	525 pg/mL	Adenoma (1)	Bilateral renal cysts	Yes (persistence)
II:10	F	34	11.7 mg/dL	1500 pg/mL	Atypical adenomas (2)	Uterine leiomyomata	Yes (11y)
II:12	F	32	12.4 mg/dL	1600 pg/mL	Cystic adenomas (2)	Uterine leiomyomata	Yes (5 and 30y)
II:14	F	38	13.0 mg/dL	1.1mg/dL NV(<1mg/dL)	Cystic adenomas (2)	Renal tumor and uterine leiomyomata	?
II:16	F	37	11.9 mg/dL	1,100 pg/mL	Adenomas (2) PTX (3 ½)	Renal tumor and uterine leiomyomata	No
III:4	M	27	1.93 mmol/L ^b	354 ^a pg/mL	Adenoma (1)		Yes (1y)
III:7	M	26	11.9 mg/dL	132 ^a pg/mL	Adenomas (2)		Yes (persistence)
III:6	F	25	11.9 mg/dL	73 ^a pg/mL	Adenomas (2)	Uterine leiomyomata	No
III:20 ^e	F	19	1.19 mmol/L ^b	22 ^a pg/ml			
III:8 ^e	M	26	1.26 mmol/L ^b				
III:21 ^e	M	17	1.24 mmol/L ^b	28 ^a pg/ml			
IV:II ^{e,f}	F	19	1.30 mmol/L ^b	49 ^a pg/ml			

II:12, propositus; M: male; F: female; PTX (3 ½): parathyroidectomy of 3 and ½ glands; ^a: PTH normal value: 7-53pg/ml; ^b: Ionized calcium normal value: 1.17-1.32mmol/L; ^c: Histology of the first surgery and number of glands excised in parenthesis; ^d: Time to recurrence/persistence (in years), after the first surgery; ^e: Mutation carrier without biochemical alterations; ^f: Neck ultra-sound showed a 0.8cm x 0.3cm right inferior nodule, compatible with parathyroid gland.

(Figure 2). The patient declined another bone biopsy. Biochemical diagnosis of recurrent hyperparathyroidism was again established. Recently, another adenoma has been localized by ultrasound, after continuous imaging investigations three years after the recurrence diagnosis was made. She is now 65 years old and was recently (for the third time), referred to another surgical procedure.

Surgical outcomes

Except for one patient (II:16) who had three and a half parathyroid glands removed, each of the eight other affected siblings had only one or two adenomas excised at their first surgical procedure (Table 1). Seven of these patients have been followed by our group. This follow-up ranges from 32 months to 32 years, and six

out of seven had recurrence/persistence of their disease during this period (Table 1). Patient III:6 was surgically treated in December 2005, when two adenomas were excised and to date, 32 months later, have not had any disease recurrence.

Mutational screening of the HRPT2 gene

DNA isolated from the tumors and peripheral blood was used to amplify the 17 exons of the HRPT2 gene, using specific intronic primer pairs as previously described (25). PCR reactions were performed in a final volume of 25 µl containing 100-300 ng of template DNA, 10 pmol of each primer, 1.5 to 4 mM MgCl₂, 1.5 mM of dNTPs, 2.5 µl of 10x PCR buffer, and 1.25 U Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA) in a Mastercycler gradient thermocycler

(Eppendorf AG, Hamburg). Amplifications were carried out as follows: an initial denaturation for 8 min at 95 °C followed by 35 cycles of 94 °C for 40 s, 55-59 °C for 40 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. All products were analyzed by electrophoresis on 6.5% polyacrylamide gel followed by silver staining, purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Piscataway, NJ) and sequenced on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). In addition, the amplified PCR products from exon 1 were digested with *Hae*III restriction enzyme as recommended by the manufacturer (New England Biolabs, Ipswich, MA). This enzyme has a restriction site in exon 1 that after digestion results in fragments of 255, 156 and 57 bp. The products obtained by diges-

tion were electrophoresed on 6.5% polyacrylamide gel followed by silver stain.

Reverse transcription-PCR analysis

Total RNA was extracted from parathyroid tumor cells with Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's recommendations and treated with DNase (Invitrogen Life Technologies, Carlsbad, CA). First-strand cDNA was prepared from 1 µg of total RNA treated with DNase using the Superscript first strand synthesis system (Invitrogen Life Technologies, Carlsbad, CA). After reverse transcription, the cDNA was used as a template for PCR amplification of the full length transcript of the human *HRPT2* cDNA, using three primer pairs: exon 1 to 7, forward primer 5'-GCGACAAGAGAAGAAGGAG-3' (5' UTR region) and reverse primer 5'-TCCACTGACATAGCTTCAGACAA-3', exon 5 to 12, forward primer 5'-GTCAAACGAGCTGCAGATGA-3' and reverse primer 5'-CTTCTGATTTGGGGGAGGTC-3', exon 10 to 17, forward primer 5'-TTGACACTATGGGAACCTACCA-3' and reverse primer 5'-CCTTGAAGCACA AAGCATCA-3' (3' UTR region). The amplifications were performed in a volume of 25 µl containing 10 pmol of each primer, 2.5 mM MgCl₂, 1.5 mM of dNTPs, 10x PCR buffer, and 1.25 unit of Taq DNA Polymerase, Recombinant (Invitrogen Life Technologies, Carlsbad, CA). Amplifications were carried out in a Mastercycler gradient thermocycler (Eppendorf AG, Hamburg) as follows: an initial denaturation for 5 min at 95°C followed by 35 cycles of 94°C for 40 s, 59°C for 40 s, 72°C for 1 min, and a final extension for 5 min at 72°C. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified as control for cDNA quality. The amplified products were submitted to electrophoresis on a 6.5% polyacrylamide gel, thereafter following the same protocol described above for *HRPT2* mutational analyses.

Parafibromin immunohistochemistry

Tissue sections from the lesions were immunostained with parafibromin antiserum. Briefly, 4 µm paraffin-embedded sections were dewaxed in xylene and hydrated with graded ethanol. Endogenous peroxidase activity was blocked with 3% H₂O₂ in water for 10 min. Heat-induced epitope retrieval was performed with 10 mM citrate buffer pH 6.0 for 30 min in a steamer at 96 °C; avidin D solution for 15 minutes (Vector Labs, Burlingame, CA); biotin solution for 15 minutes (Vec-

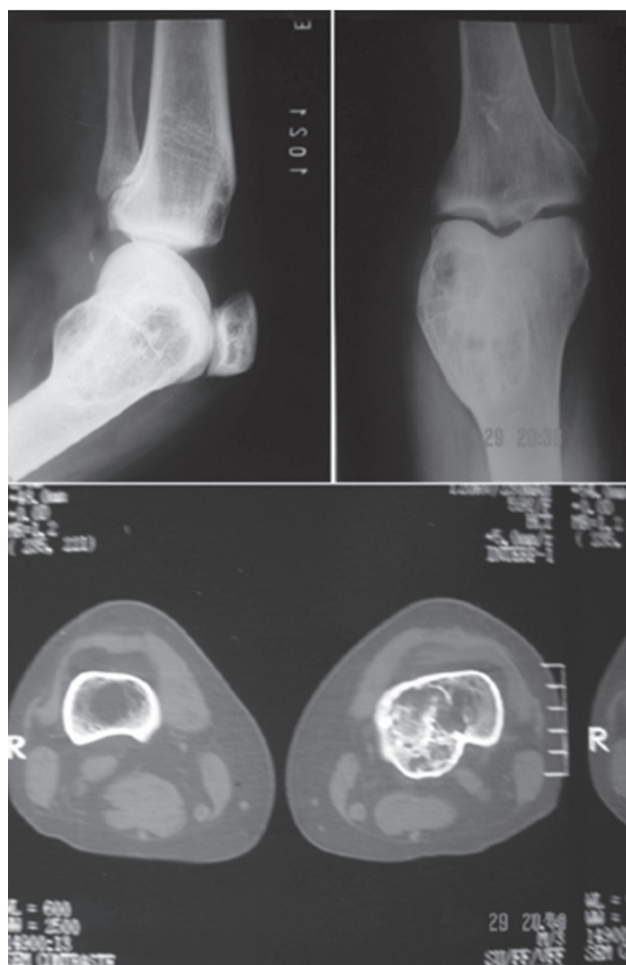


Figure 2. Salient radiographic signs of the propositi showing a large lytic lesion in the distal right femur and multiple knee lesions of varying sizes.

tor Labs); and primary monoclonal antibody to parafibrin (targeting the peptide RRPDRKDLLGYLNC) (26) at 10 µg/mL for 18 h at 4 °C. This was followed by incubation with the labeled streptavidin-biotin (LSAB) Kit (DakoCytomation California Inc, Carpinteria, CA). Peroxidase activity was developed with DAB (Sigma, St Louis, MI) with timed monitoring using a positive control sample. The sections were then counterstained with hematoxylin, dehydrated and mounted. Two experienced independent pathologists examined multiple fields and scored tissue sections for the extent of nuclear staining regardless of staining intensity. A primary parathyroid hyperplasia and one sporadic parathyroid adenoma were used as positive controls. Data not shown.

RESULTS

The entire coding sequence of the *HRPT2* gene was amplified and sequenced from tumor DNA obtained from patients III:4, III:6 and III:7 from this kindred. Genomic DNA was also screened in patients II:10, II:12, II:14, III:4, III:6, III:7, III:8, III:20 and III:21 to determine the somatic or germline origin of the mutation.

In this kindred a G to A transversion in exon 1 (c.96G>A) was identified in germline and tumor tissue from the proband (II:12), leading to a stop codon (W32X), demonstrating loss of heterozygosity in the tumor tissue (data not shown).

The same mutation was found in heterozygous state in the germline DNA from patients II:10, II:14, III:4, III:6 and III:7. Germline DNA was also available from individuals III:8 (age 26), III:20 (age 19) and III:21 (age 17) who were found to carry the mutation without any clinical or biochemical symptoms of the disease. They have been closely followed with calcium and PTH screening twice a year.

RNA extracted from the parathyroid adenomas underwent RT-PCR amplification to assess the presence of aberrant transcripts, originating from the mutated gene. In the adenoma from patient III:4, who carried the W32X mutation, no *HRPT2* transcript was detected (data not shown). At another parathyroid adenoma, from a patient with HPT-JT syndrome used for comparison, we only found the normal transcript. Sporadic adenomas used as controls showed normal *HRPT2* transcript after RT-PCR.

Parathyroid adenomas showed diffuse loss of parafibrin immunostaining, compared with normal pa-

rathyroid or sporadic parathyroid adenoma (data not shown).

DISCUSSION

While surgical management of sporadic hyperparathyroidism is currently well established, management of familial forms requires a careful approach. Sporadic hyperparathyroidism is typically caused by a single adenoma and excision of the affected gland is the treatment of choice, as recurrence is rarely seen (27,28).

On the other hand, surgical procedure in familial cases is still controversial in the literature, especially concerning *HRPT2*-related FIHP (29-32). To allow a proper management of the parathyroid tumors, it is essential that we better comprehend the natural history of familial syndromes associated with hyperparathyroidism.

Although in the majority of FIHP cases no genetic alterations can be identified, it is important to distinguish *MEN1* from *HRPT2* associated FIHP, whenever possible.

FIHP associated with germline mutations of the *MEN1* gene is thought to render the parathyroids susceptible to develop a tumor after a somatic mutation (the second hit). It has been proposed that the initial surgical procedure in patients with *MEN1* syndrome or FIHP who have *MEN1* mutations is either subtotal parathyroidectomy, leaving a remnant of approximately 20-30 mg of one of the glands or total parathyroidectomy with heterotopic autotransplantation of resected parathyroid tissue. Previous studies of the parathyroid histology in *MEN1* report, simultaneous and asymmetric tumors involving 3-4 parathyroid glands (33), and less than subtotal parathyroidectomy is associated with an unacceptably high frequency of persistent and recurrent hyperparathyroidism in patients with mutations of the *MEN1* gene (32).

In contrast with *MEN1*, parathyroid disease in *HRPT2*-related FIHP is yet to be defined. Some authors suggested that therapeutic approach is the resection of all grossly enlarged parathyroid glands, unless parathyroid cancer is suspected during the surgical procedure (31,32). On the contrary, it has also been suggested that, given the high rate of the disease's persistence or recurrence, the surgical re-interventions morbidity and the associated risk of developing parathyroid carcinoma, a more aggressive surgical interven-

tion with ipsilateral hemithyroidectomy and total parathyroidectomy may be warranted.(29,34,35).

Most of the already reported good results using minimally invasive parathyroidectomy (MIP) in FIHP, required intraoperative parathyroid hormone (IOPTH) assay combined with accurate and expensive preoperative and intraoperative identification of the parathyroid glands, including those that may exist in ectopic locations (30). However, citations available in PubMed when HRPT2 AND hyperparathyroidism AND surgery are searched, show that the time of follow-up, the scarcity of molecular data and the small number of patients reported in several series are not sufficient to lead to a definitive conclusion (Table 2) (25,29,31,36-42). Furthermore, when multiglandular disease is present, minimally invasive approach, based solely on preoperative imaging studies, may result in treatment failure (43).

In a study of 36 kindreds with familial hyperparathyroidism, it has been noticed that the number of operations per case was higher in the HPT-JT subgroup (2) due to the high prevalence of parathyroid cancer in these families (36%) (2). Nevertheless the authors considered unnecessary the indication for total parathyroidectomy as the initial operation, if compliance with long-term follow-up was likely to happen, given that there were patients who remained normocalcemic up to 29 years after 1 or 2 glands were removed (2). It is important to emphasize that HRPT2 had not been cloned at that time.

In 2007, Iacobone et al. (31) reported 3 kindreds (12 patients) with germline HRPT2 mutations (not depicted). They described a single gland involvement in all cases and a recurrence rate of 25% (3 cases: 2 adenomas and 1 carcinoma) after a disease free interval of 5, 9 and 27 years, respectively. They proposed that limited parathyroidectomy could be an adequate procedure in HRPT2-related hyperparathyroidism, especially when preoperative imaging techniques concordantly localize a single affected gland (60% in their study), allowing focused and minimally invasive parathyroidectomy, with the potential advantage of causing lower risk of hypoparathyroidism and minimal tissue trauma, facilitating reoperations in case of recurrent hyperparathyroidism.

The family herein described has up to 32 years of follow up since its index case had her first surgical procedure performed. Out of nine patients, only one has been followed by another Hospital, but we have been informed that she is asymptomatic and normocalcemic.

Patient II:1 died from metastatic prostate carcinoma and recurrence of hyperparathyroidism after 5 years of follow-up. Patient II:6 died following a myocardial infarction associated with renal failure and persistent hyperparathyroidism. The proposita had now her third recurrence diagnosed after 32 years of follow-up. This long follow-up allowed us to perceive a very high (75%) prevalence of recurrence or persistence of the disease after less than subtotal parathyroidectomy. This percentage could be even higher as one of the patients underwent surgery only 32 months ago (III:6). Taking this family as an example, we would like to emphasize that the follow-up time is of the utmost importance to determine the recurrence rates. Along with our data, PubMed search showed a high frequency (80%) of persistence or recurrence considering the minimal follow-up of 120 months (Table 2). Finally, treatment in patients with FIHP has to be individualized and the options of a conservative approach or a more radical initial surgery, with three and a half parathyroidectomy, should be discussed with the patients.

Another point to be considered is the importance of identifying and reporting all the HRPT2 mutations and/or polymorphisms, so that an attempt to establish a genotype-phenotype correlation be made, as previously done with RET and medullar thyroid carcinoma. Unfortunately, descriptions of the mutations are usually unavailable for further analyses (Table 2).

In the present study, a mutation (c.96G>A) in exon 1 of the HRPT2 gene leading to a premature stop codon (W32X) was detected. While the germline alteration was heterozygous, analysis of tumor DNA showed that the parathyroid adenoma had undergone loss of the wild type allele demonstrating biallelic inactivation, typical of tumor suppressor genes (44). Both RT-PCR and immunohistochemistry support the notion that there is no transcript or protein as a result of this mutation, supporting the pathogenicity of this molecular alteration. Although none of the affected members in this kindred, all with the HRPT2 mutation, had jaw tumors even after a long follow-up period, the clinical features of the patients such as aggressive hyperparathyroidism with recurrent and atypical parathyroid adenomas, sometimes with cystic changes, the presence of bilateral renal cysts (II:1 and II:6) and benign renal tumors (II:14 and II:16), besides the association with uterine lesions, strongly favors the HPT-JT syndrome or simply HRPT2-related FIHP.

Table 2. Surgical, clinical and molecular data from literature review of HRPT2- related hyperparathyroidism.

Number of Patients	Surgery	HRPT2 germline mutation	HRPT2 somatic mutation	Recurrence Persistence	Percentage	Time of Follow-up (months)	Pathology Findings	Reference
1	PTX ^a (1 gland)	c.686delGAGT (exon 7)		1	100	60	Adenoma / Carcinoma ^c	29
1	PTX (1 gland)	c.679delAG (exon 7)		1	100	96	Adenoma	29
1	PTX (1 gland)	c.685-688delAGAG (exon 7)		1	100	180	Atypical adenoma	34, 36
2	?	c.685-688delAGAG (exon 7)		?	?	?	Atypical adenoma and carcinoma ^c	34, 36
1	PTX	c.415C>T (exon 5)		1	100	60	Carcinoma	34, 36
1	PTX	c.518-521delTCTC (exon 7)		0	0	24	Carcinoma	34, 36
1	PTX (1 gland)	c.343G>C (exon 4)		1	100	180	Atypical adenoma / Carcinoma ^c	34, 36
1	PTX (1 gland)	R91P (exon 3) CGA>CCA	A2S and Y54X	1	100	204	Adenoma	37
11	limited	? ^d		3	27	113 (average)	Adenoma	31
1	bloc PTX and thyroid lobectomy	? ^d		1	100	29	Carcinoma	31
7	radioguided PTX	? ^d		0	0	32 (average)	Adenoma / Hyperplasia	38
1	PTX (2 glands)	c.140-144del5 (exon 2)	c.61-64del4 (exon 1)	1	100	24	Atypical adenoma	39
1	PTX (1 gland)	c.140-144del5 (exon 2)		1	100	216	Adenoma / Carcinoma ^c	39
1	PTX (1 gland)	c.140-144del5 (exon 2)		0	0	6	Adenoma	39
4	?	518-521del (exon 7)	70-73del, 95-102del, 518-521del	1	25	102	2 adenomas and 2 Atypical adenomas	25
1	PTX (1 gland)	62-66del (exon 1)		1	100	108	Adenoma	25
1	?	39delC (exon 1)		0	0	?	Adenoma	25
2	PTX (1 gland)	IVS2-1G>A (intron 2)	85delG and 13-30del	0	0	24 and 72	Carcinoma	40
3	?	IVS2-1G>A (intron 2)		0	0	50 (average)	1 Adenoma and 2 Atypical adenomas	25
4	less than total	IVS2-1G>C (intron 2)		2	50	120	1 Hyperplasia and 3 Adenomas	41
5	less than total	191T>C (L64P) (exon 2)		1	20	?	Adenoma	41
10	PTX (resection guided by IOPTH)	? ^e		2	20	> 6	Adenoma	42
1	PTX (resection guided by IOPTH)	? ^{de}		0	0	9	Carcinoma	42
3	PTX (resection guided by IOPTH)	? ^e		0	0	< 6	Adenoma	42

^a PTX: parathyroidectomy; ^b IOPTH: intra-operative PTH assay; ^c Carcinoma recurrence after previous adenoma excision; ^d Confirmed HRPT2 mutations without their description; ^e Confirmed familial isolated primary hyperparathyroidism and absence of MEN1, but patients were not evaluated for genetics traits.

It is important to note that HRPT2 mutations and the finding of loss of parafibromin immunostaining are associated with more aggressive forms of hyperparathyroidism and a high risk of parathyroid carcinoma. It has been shown that HRPT2 mutation is an early event that may lead to parathyroid malignancy and, moreover, it has been suggested that intragenic mutation of HRPT2 should be considered as a marker of malignant potential in both familial and sporadic parathyroid tumors (16). Therefore it is essential to be aware of this diagnostic possibility, so that adequate genetic or clinical screening could be undertaken in both patients and relatives, enabling earlier diagnosis and treatment (17). Parafibromin immunostaining, a rather simple diagnostic test, should be widely used to analyze parathyroid tumors especially in the context of aggressive behavior or young age (26). The finding of loss of parafibromin immunoreactivity would further implicate a high likelihood of familial cases and would also help to select more subjects for genetic analysis.

We conclude that it is necessary to gather additional data related to the molecular aspects of HRPT2-related FIHP, and better follow the outcomes in order to find risk factors for recurrence or malignancy. Until this is done it is premature to define the best surgical approach to these patients and this matter must be kept open for further discussions.

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