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Non-Truncated Amino-Terminal PTH Overproduction in Two Patients with Parathyroid Carcinoma: A Possible Link to HRPT2 Gene Inactivation

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Summary

Objective—Some patients with parathyroid carcinoma present with an over-production of non-truncated amino-terminal (NT-N) PTH, a post-transcriptionally modified form of PTH(1-84). This is usually picked up on an elevated Whole (W) PTH (3rd generation) / Total (T) (2nd generation) PTH assay ratio (N > 0.8).

Patients and Design—Two parathyroid cancer patients with several episodes of hypercalcemia and multiple surgeries are described. In both, W-PTH, T-PTH and circulating PTH molecular forms separated by HPLC were measured with the same assays. qPCR was used to study HRPT2 gene mutation.

Results—The first patient had total calcium of 3.8 and 3.22 mmol/L before the 4th and 5th surgeries, and 3rd/2nd generation PTH ratios of 2.95 and 3.6, respectively. After the 4th surgery, the ratio remained normal for one year and increased progressively to 3.6 over 15 months. This preceded hypercalcemia by 6 months. The ratio became normal after the 5th surgery. HPLC analysis disclosed an over-expression of NT-N PTH to 82.2% (N < 10%) relative to hPTH(1-84) before the 5th surgery. A deletion of all the tested exons of the HRPT2 gene was identified. In the second patient, W-PTH/T-PTH ratio was 0.89 when serum calcium was 3.3 mmol/L. NT-N PTH was also over-expressed at 51.9%. An inactivating mutation of the HRPT2 gene was also identified.

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Conclusions—This may suggest that a progressive rise in 3rd/2nd generation ratio may have possible clinical utility to monitor parathyroid cancer recurrence. A possible association between NT-N PTH overproduction and HRPT2 gene inactivation is also suggested.

Keywords

Hypercalcemia; hyperparathyroidism; parathyroid carcinoma; PTH assays; HRPT2 gene

Introduction

Non-truncated amino-terminal (NT-N) parathyroid hormone (PTH) is a new molecular form of human (h) PTH (1-84) which has been discovered through the combined use of a third generation PTH assay with a 1-7 epitope and more efficient HPLC gradients to separate circulating PTH molecular forms.[1] It normally represents less than 10% of 3rd generation PTH results in normal individuals and up to 15% in patients with advanced renal failure.[1] NT-N PTH is poorly reactive in most second generation PTH assays with a 13-20 epitope in relation to a probable post-translational phosphorylation of serine 17.[1,2] In rare patients, NT-N PTH can be overexpressed with third generation PTH values being higher than second generation PTH values resulting in a ratio higher than 0.8.[3-9] An elevated 3rd generation/2nd generation PTH ratio has been described so far in 34 patients with primary hyperparathyroidism (PHPT),[3-6,9] including 26 patients with parathyroid cancer[4,6] mostly with metastatic disease.[9] Two cases have been found in renal failure patients with either tertiary[7] or secondary hyperparathyroidism.[8] The exact cause of this abnormality is unknown. Using high pressure liquid chromatography (HPLC) to separate circulating PTH molecular forms and the Whole (W) 3rd generation PTH assay to reveal them, we demonstrated the overexpression of NT-N PTH in one patient with osteitis fibrosa cystica,[3] in about half a group of patients with parathyroid cancer[4] and in one patient with tertiary hyperparathyroidism.[7] After successful surgery, this ratio returns to normal.[3-8] Its evolution in relation to an hypercalcemic recurrence in parathyroid cancer has not been described.

We report two patients with parathyroid cancer who had several episodes of hypercalcemic recurrence followed by surgeries, including evolution of the W-PTH (3rd generation)/Total (T)-PTH (2nd generation PTH assay) ratio before and after the 4th and 5th surgeries in one case. Both patients also had an inactivating mutation of the HRPT2 gene suggesting that NT-N PTH overproduction could be linked to HRPT2 gene inactivation.

Patient and Methods

Human studies consent

All patient studies were performed with informed consent under clinical protocols approved by the Centre Hospitalier Universitaire Larrey (patient 1) or National Institute of Diabetes and Digestive and Kidney Diseases (patient 2) Institutional Review Boards.

Parathyroid carcinoma patients

The first patient, a 62-year-old female, has been described up to her 4th episode of hypercalcemia.[6] At the ages of 33 and 43 years, she presented left and right renal stones, respectively. She had hypercalcemia, hypercalciuria, low serum phosphate level and increased PTH concentration suggestive of PHPT. A right inferior parathyroid tumor was excised during surgery. Pathology concluded that it was an adenoma with oxyphil cells. At the ages of 49 and 54 years, she had 2 episodes of PHPT relapse. Each time, a nodule embedded in the right sternocleidomastoid muscle was excised. During the 1st surgery, right thyroid lobectomy was also performed. Histological examination was identical each time

and showed compact parathyroid tissue, large cells with an oxyphil cytoplasm, large nucleolated nuclei and rare mitoses. At the age of 60 years, she manifested another PHPT relapse and underwent another surgery. The histological results were similar to those of previous surgeries, and an irregular fibrous capsule limited the lesion with dense fibrous trabeculae and vascular emboli beyond the capsule. The proliferation index was 5% (KI-67). Taking into account the recurrent episodes of PHPT and the histological data, a diagnosis of parathyroid carcinoma was made. Two years later, she presented a 5th episode of PHPT recurrence (Table 1). Echography, MIBI scintigraphy and MRI confirmed the presence of a 2-cm nodule in the inferior left parathyroid area. Another surgery was performed with ablation of the nodule and left thyroid lobectomy. The histological features were identical to those after previous surgeries, and muscular invasion was noted. Because of local invasiveness, anterior neck irradiation was decided after surgery.

The second patient has also been partially published[10] and has had a total of nine cervical or mediastinal operations for recurrent parathyroid carcinoma. He presented at age 34 with elevated calcium values, up to 4.45 mmol/L, discovered on screening because of a strong family history of PHPT. A 2.5 cm left inferior parathyroid tumor was excised that was composed primarily of chief cells partially divided by strands of fibrous connective tissue and was described as an adenoma. The serum calcium remained elevated post-operatively but did finally normalize after 5 days. The patient developed recurrent PHPT one year later and underwent cervical re-exploration at which time left superior parathyroid and left inferior parathyroid tissue was excised. Five years later the patient presented with lethargy and fatigue and a diagnosis of recurrent PHPT was made, at which time cervical re-exploration failed to find any abnormalities. He was referred to another institution where cervical re-exploration revealed recurrent parathyroid tumor encasing the left recurrent laryngeal nerve. The parathyroid tumor was excised with sacrifice of the nerve. The tissue diagnosis was parathyroid carcinoma. Six years later the patient developed recurrent PHPT and underwent cervical re-exploration of removal of a 2 cm parathyroid carcinoma tumor mass adherent to the left jugular vein. Six years later the patient again developed recurrent PHPT and underwent limited upper sternotomy with removal of parathyroid tumor mass adherent to the innominate vein. His seventh operation two years later was directed at a separate parathyroid tumor mass in the right mid-neck. His eighth operation involved an extensive dissection clearing cervical tissue down to the vertebral fascia, trachea, and carotid artery and removal of parathyroid tumor mass appearing to invade the medial portion of the right carotid artery. His ninth operation, four years later for recurrent PHPT, involved cervical re-exploration and thoracotomy with removal of parathyroid tumor and stripping of serosal tissues from the vagus nerve, innominate artery, the thoracic portion of the left carotid artery, the junction of the left subclavian vein and left internal jugular vein and the brachiocephalic vein. Because of residual hypercalcemia, he was treated with Cinacalcet HCl to try to improve his total calcium concentration. But this therapy had no effect on his total calcium concentration.

Laboratory test

Blood samples were collected after an overnight fast. Calcium, phosphate, total protein and creatinine were measured by standard chemistry methods. PTH was quantified in serum with 3rd generation W-PTH and 2nd generation T-PTH assays from Scantibodies Laboratory Inc. (Santee, CA, USA). The detection limits were 1 and 1.23 pg/ml, and normal ranges were 5-39 and 14-66 pg/ml for the W-PTH and T-PTH assays, respectively.

HPLC analysis

For the first patient, three pools of serum were analyzed on the same HPLC column. The first pool was made up of serum obtained after the 4th surgery, the second serum pool was

obtained before the 5th surgery, and the third serum pool was obtained after the 5th surgery. Serum extraction with Sep-Pak Plus C-18 cartridges (Waters, Milford, MA) and separation of PTH molecular forms on a Waters C18 uBondapak analytical column (300×3.9 mm, inner diameter) eluted with a non-continuous linear gradient of acetonitrile in 1 g/L trifluoroacetic acid have been described in detail elsewhere.[4] For the second patient, a different HPLC column was used and a study of circulating PTH molecular forms was obtained when he was on Cinacalcet HCl to try to reduce his elevated calcium concentration.[4] The slight difference in the elution position of PTH molecular forms in the 2 patients is explained by the used of different columns.

Results

Table 1 illustrates the biochemical course of the first patient before and after her 4th and 5th surgeries. She became slightly hypocalcemic after her 4th surgery, but maintained normal total calcium over the next 18 months. At that time, even if W-PTH and T-PTH levels were normal, her W-PTH/T-PTH ratio rose above 0.8 to 1.19. Over the next 6 months, her total serum calcium rose to 2.60 mmol/L, her W-PTH level to 154.4 pg/ml, her T-PTH remained normal at 43.7 pg/ml, and her W-PTH/T-PTH ratio increased to 3.53. Over the next 6 months, her total calcium climbed to 3.22 mmol/L, her W-PTH to 590 pg/ml, her T-PTH to 163 pg/ml, and her W-PTH/T-PTH ratio remained unchanged at 3.61. After her 5th surgery, she became hypocalcemic and required calcium supplementation and 1alpha-vitamin D therapy. Both PTH levels and the ratio value remained normal over the next 4 months. HPLC analysis of circulating PTH molecular forms after the 4th surgery (Figure 1A) and before and after the 5th surgery (Figure 1B, 1C) disclosed that the W-PTH assay was able to identify peaks of immunoreactivity corresponding to hPTH(1-84) and NT-N PTH, while T-PTH assay peaks corresponded to hPTH(1-84), NT-N PTH and non(1-84) PTH fragments. As expected, the T-PTH assay reacted poorly with NT-N PTH. NT-N PTH corresponded to 23.8, 82.2 and 19.3% of W-PTH after the 4th and before and after the 5th surgery respectively, indicating clear NT-N PTH oversecretion prior to the 5th surgery but also post-surgery compared to normal individuals. Plasma calcitonin and urinary catecholamine levels were normal, excluding multiple endocrine neoplasia (MEN) type 2a. MEN type 1 analysis was normal. All 17 exons of HRPT2 gene were tested on a blood specimen by quantitative polymerase chain reaction (Light Cycler 480; Roche Diagnsotics, 38242 Meylan, Cedex, France), and complete deletion of all exons was identified. The mutation was heterozygous and neither the sister nor the daughter of the patient presented similar mutation.

The second patient had a 3rd generation/2nd generation PTH ratio of 0.89 when studied on Cinacalcet HCl therapy and a calcium concentration of 3.3 mmol/L. The amount of NT-N PTH present on the W-PTH HPLC profile accounted for 51.9% of the total immunoreactivity in this patient. Biochemical testing revealed no evidence of pituitary or enteropancreatic endocrine tumors. Germline mutation testing of the MEN1 and CASR genes were negative. Germline HRPT2 mutation testing revealed a heterozygous 2 bp insertion in exon 7 (679insAG) that predicted addition of 27 amino acid residues of unrelated sequence in the altered reading frame with truncation after codon 229 of parafibromin. The frameshift mutation tracked with a phenotype of surgically-proven primary hyperparathyroidism among first-degree relatives as previously described.[10] Although gene mutational analysis of leukocyte DNA collected at our institution was permitted, analysis of somatic DNA alterations at the HRPT2 locus in parathyroid tumor tissue, all collected during surgeries at outside institutions, was not covered under the protocol for which the patient gave informed consent.

Discussion

An elevated 3rd generation/2nd generation PTH ratio related to NT-N PTH over-production has been reported in rare patients with primary, secondary or tertiary hyperparathyroidism.[3-9] Detection of this abnormality requires a 3rd generation assay with a 1-7 epitope (W-PTH) and a 2nd generation assay with a 13-20 epitope (T-PTH). NT-N PTH, a phosphorylated[1,2,11] PTH(1-84) molecule on serine 17, reacts poorly in 2nd generation PTH assays with a 13-20 epitope but normally in 2nd generation PTH assays with more distal 26-34 epitope.[11] Thus, a higher 3rd generation/2nd generation PTH ratio would not be observed with a 2nd generation PTH assay having a distal epitope[3] (Elecsys PTH assay).

Elevated 3rd generation/2nd generation PTH ratios have been reported mainly in parathyroid cancer patients (4-6, 9).[9] Parathyroidectomy can correct the abnormal ratio by removing the responsible parathyroid tissue but this is difficult to achieve definitively in parathyroid cancer patients.[6] Cinacalcet HCL can cause a significant decrease in total calcium concentration in parathyroid cancer patients but a high 3rd generation/2nd generation PTH ratio has been reported during such treatment, as observed in patient 2.[4] The first patient is the only one described so far in whom an hypercalcemic recurrence was preceded by a progressive increase in the 3rd generation/2nd generation ratio, indicating a possible value of this parathyroid cancer marker in patients who overproduce NT-N PTH. More studies are required to see if this is always the case.

Both patients also presented with an inactivating mutation of HRPT2 gene identified by qPCR.[6,10] Somatic and/or germline HRPT2-inactivating mutations are often found in parathyroid cancer patients[12] and associated with multiple parathyroid neoplasms.[13,14] Inactivating HRPT2 mutations are associated with decreased expression of protein(s) involved in cyclinD1 regulation. It is unknown if NT-N PTH overexpression could be linked, directly or indirectly, to HRPT2 inactivation, but our study suggest that it could be the case. Further studies either in patients with N-PTH over-production or in patients with HTPR2 mutations should permit to see if this association is frequently present.

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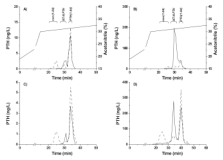

Figure 1.

HPLC profiles of circulating PTH molecular forms, obtained with Whole PTH (—) and total PTH (----) assays, are illustrated after the 4th surgery (A) and before (B) and after 5th surgery (C) for the first patient and during Cinacalcet HCl therapy for the second patient (D). The regions of elution of hPTH(1-84), NT-N PTH and non(1-84) PTH fragments are indicated at the top (A, B). NT-N PTH overexpression is seen in B and D where it represents 82.2% and 51.9% of W-PTH immunoreactivity respectively. The different elution positions observed in A, B, C vs D are related to the use of different HPLC columns.

Table 1

Date	Surgery	Ca_{tot} (mmol/l) Normal range (2.17-2.56)	W-PTH (pg/ml) Normal range (9-34)	T-PTH (pg/ml) Normal range (10-46)	W-PTH/T-PTH ratio (< 0.8)
2006-05	4th	3.8	675	229	2.95
2006-11-03		1.85	29.7	42.1	0.70
2007-02-05		2.18	26.2	39.3	0.66
2007-09		2.25	21.9	33.2	0.66
2007-12		2.35	26.6	34.3	0.72
2008-06		2.33	37.5	31.4	1.19
2008-12		2.60	154.4	43.7	3.53
2009-05-11		2.82	534.3	140.8	3.79
2009-05-25	5th	3.22	590.3	163.4	3.61
2009-07-06		1.63	7.3	12.5	0.58
2009-10-16		2.27	25.2	32.9	0.77

Results (for the first patient) are single values. Arrows indicate the 4th (2006-11-02) and 5th surgeries (2009-07-02).

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