

Brief Report

DOI: 10.5582/irdr.2012.v1.1.27

Identification of a germline mutation in the *HRPT2* gene in a Chinese family with parathyroid carcinomas**Mei Zhang¹, Qin Li¹, Lili Zhang¹, Rongzhan Fu¹, Yulong Wang¹, Shouhua Chen¹, Kai Yuan¹, He Gu^{1,*}, Yazhou Cui^{2,*}**¹Department of Thyroid & Breast Surgery, Qianfoshan Hospital Affiliated with Shandong University, Ji'nan, Shandong, China;²Shandong Academy of Medical Sciences, Shandong Medical Biotechnological Center, Ministry of Health Key Laboratory for Biotech Drugs, Ji'nan, Shandong, China.**Summary**

This study reported a family with primary hyperparathyroidism due to parathyroid carcinoma and investigated the pathological and genetic features of family members. Three members of the family had clinical manifestation of primary hyperparathyroidism and tumors in the neck. All three patients underwent parathyroidectomy, thyroidectomy and level-VI neck dissection and were definitively diagnosed based on pathology. The index case was a patient that was found to have parathyroid carcinoma on the right side and parathyroid adenoma on the left side. The other two patients had local tumor recurrence and metastasis to distant organs. A germline mutation in the *HRPT2* gene (Arg91Pro) was identified in all of the patients in this family. Study of the literature indicated that this is the first report of familial parathyroid carcinomas with an *HRPT2* gene missense mutation. Results also indicated that *HRPT2* may play an important role in the development of parathyroid carcinoma.

Keywords: Familial, parathyroid tumor, hyperparathyroidism, *HRPT2*

1. Introduction

Parathyroid carcinoma (PTC) is a rare malignant tumor of the endocrine system and accounts for fewer than 1-3% of all cases of primary hyperparathyroidism (1,2). Primary hyperparathyroidism usually occurs sporadically but may be inherited as an autosomal dominant trait. However, few previous reports have described familial parathyroid carcinoma. The current study reported a family with three parathyroid carcinoma patients, and a germline mutation in the *HRPT2* gene was identified in these family members.

2. Materials and Methods**2.1. Clinical data**

The index case, a 30-year-old man, complained of pain in the lower extremities for 2 years and headache, fatigue, nausea and vomiting for 1 month when seen on November 14, 2008. A computed tomography (CT) scan revealed two tumors with distinct margins (17 × 17 × 25 mm and 15 × 14 × 25 mm) in the posterior and lower portions of both lobes of the thyroid. No significant increase in shadows from neck lymph node shadows on either side was noted (Figure 1A). An emission CT scan revealed nodules in the right lower lobe that had radioactive distribution equal to the surrounding thyroid tissue, and the lower pole of the left lobe had limited radiation uptake (Figure 1B). The serum levels of parathyroid hormone (PTH), alkaline phosphatase (ALP), calcium, phosphorus, and potassium were 1,367 pg/mL, 392 U/L, 4.03 mmol/L, 0.56 mmol/L, and 3.43 mmol/L, respectively. The patient in the index case underwent parathyroidectomy, thyroidectomy, and level-VI neck dissection. Postoperative pathology confirmed parathyroid carcinoma on the right side (Figure 2A) and

*Address correspondence to:

Dr. Yazhou Cui, Shandong Academy of Medical Sciences, Shandong Medical Biotechnological Center, Ministry of Health Key Laboratory for Biotech Drugs, Ji'nan, Shandong, China.

E-mail: cuiyazhou@yahoo.com.cn

Dr. He Gu, Department of Thyroid & Breast Surgery, Qianfoshan Hospital affiliated with Shandong University, Ji'nan, Shandong, China.

E-mail: meizhang0925@yahoo.com.cn

parathyroid adenoma on the left side, and no lymph node metastasis was found.

The father and sister of the patient in index case underwent surgery at other hospitals and were confirmed to have parathyroid carcinoma by a pathological examination. Furthermore, both had distant bone metastasis, and the sister had already died of multiple bone metastases.

2.2. Immunohistochemical staining

Immunohistochemistry was performed on the tissues of the parathyroid carcinoma and the adenoma from the index case. Immunohistochemical staining was performed with commercially available monoclonal antibodies against human Syn, CgA, Calcitonin, TG, TTF-1 and Ki-67 as described previously (2).

2.3. *HRPT2* gene mutation analysis

Peripheral blood was obtained from all family

members (3 patients and 3 asymptomatic relatives) with their informed consent to identify germline *HRPT2* mutations. This was approved by the local ethics committee. Extracted DNA was amplified in 15 segments (from 226 to 599 bp) to allow mutation screening of the 17 translated *HRPT2* exons and their exon-intron boundaries. The primers used for PCR amplification are described in supplemental data. A 20- μ L mixture was prepared for each reaction and included 1 \times HotStarTaq buffer, 2.0 mM Mg²⁺, 0.2 mM dNTP, 0.2 μ M of each primer, 1 U HotStarTaq polymerase (Qiagen Inc. CA, USA), and 1 μ L template DNA. The cycling program was 95°C for 15 min; 11 cycles of 94°C for 15 sec, 62°C-0.5°C per cycle for 40 sec, 72°C for 1 min; 24 cycles of 94°C for 15 sec, 56°C for 30 sec, 72°C for 1 min; 72°C for 2 min. PCR products were purified using SAP and Exo I and then sequenced on an ABI 3130XL sequencing system (Applied Biosystems, CA, USA).

3. Results and Discussion

Mutations of the *HRPT2* gene have recently been implicated in the development of parathyroid carcinoma (3-5). Familial isolated hyperparathyroidism with the *HRPT2* mutation may represent a risk factor for developing parathyroid carcinoma. However, familial

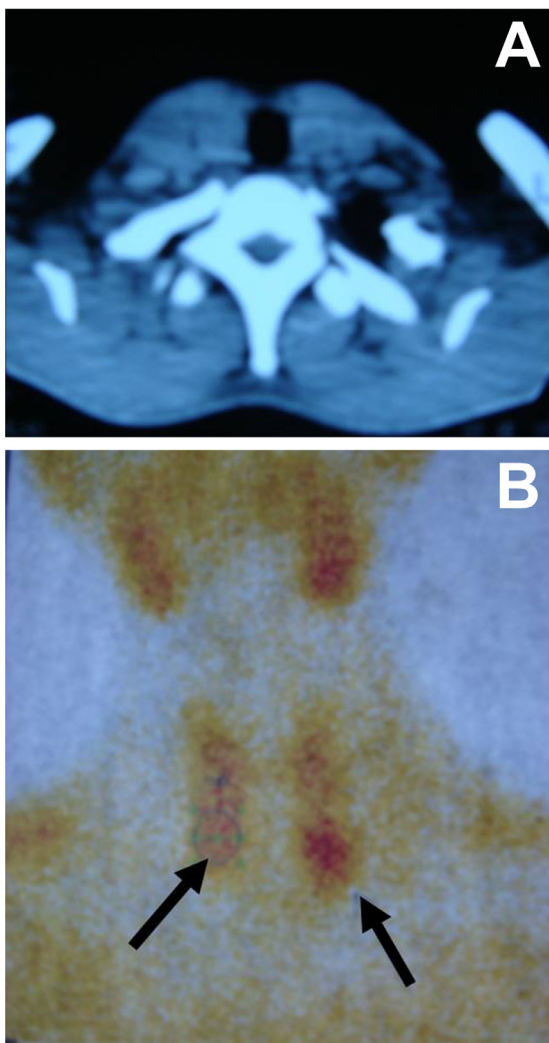


Figure 1. Images of the parathyroid tumors in the index case. (A), Computed tomography. (B) Emission computed tomography.

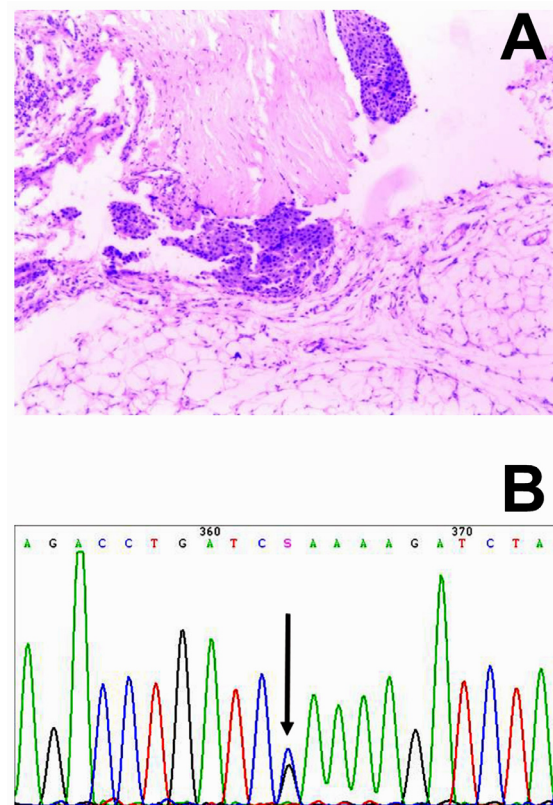


Figure 2. Pathological images of the parathyroid carcinoma in the index case (A, HE staining, Original magnification 100 \times), and a *HRPT2* gene missense mutation at exon 3 (Arg91Pro, B).

parathyroid with primary hyperparathyroidism has rarely been reported.

The current study is the first to report a family with 3 cases of parathyroid carcinoma with the *HRPT2* gene mutation. A 3-generation pedigree of this family revealed that 3 members had parathyroid carcinoma with a *HRPT2* gene missense mutation at exon 3 (Arg91Pro) (Figure 2B), and asymptomatic relatives were negative for the Arg91Pro germline mutation. Furthermore, the index case had both benign and malignant parathyroid tumors, a finding that has been reported in previous studies (6). These findings suggest that the Arg91Pro mutation of *HEPT2* gene might be required for multistage development of parathyroid carcinoma. The molecular characteristics of benign and malignant tumors were also compared based on immunohistochemistry. Immunohistochemistry results for the parathyroid carcinoma tissues (right side) were follows: Syn (weak positive), CgA (weak positive), Calcitonin (-), TG (-), TTF-1 (-), and Ki-67 (positive cells 5-10%). Immunohistochemistry results for the left parathyroid adenoma were as follows: Syn (weak positive), CgA (weak positive), Calcitonin (-), TG (-), TTF-1 (-), and Ki-67 (positive cells < 2%). Molecular characteristics of the parathyroid adenoma and carcinoma were similar, indicating that the parathyroid carcinoma may have originated from the adenoma, which was induced by the *HEPT2* gene mutation at Arg91Pro.

In a previous study, Cetani *et al.* (7) identified the Arg91Pro mutation in patients with early onset of primary hyperparathyroidism (PHPT), and they suggested this *HRPT2* mutation may be ascribed to modification of parafibromin conformation and/or stability. The Arg91Pro mutation is also associated with different *HRPT2* somatic alterations in parathyroid tumors, which is consistent with the "two hit" concept of biallelic inactivation of classical tumor suppressor genes.

In conclusion, this report is the first to identify the pedigree of parathyroid carcinomas with the *HRPT2* gene missense mutation. Together with previous findings, the current results suggest that the Arg91Pro mutation of the *HRPT2* gene might be associated with early onset of PHPT and a high risk of developing a parathyroid carcinoma. Results also suggest that *HRPT2* may serve as an important suppressor gene in the development of parathyroid carcinoma.

References

1. Fang SH, Lal G. Parathyroid cancer. *Endocr Pract.* 2011; 17:36-43.
2. Sharretts JM, Simonds WF. Clinical and molecular

genetics of parathyroid neoplasms. *Best Pract Res Clin Endocrinol Metab.* 2010; 24:491-502.

3. Kim HK, Oh YL, Kim SH, Lee DY, Kang HC, Lee JI, Jang HW, Hur KY, Kim JH, Min YK, Chung JH, Kim SW. Parafibromin immunohistochemical staining to differentiate parathyroid carcinoma from parathyroid adenoma. *Head Neck.* 2012; 34:201-206.
4. Carlson D. Parathyroid pathology: Hyperparathyroidism and parathyroid tumors. *Arch Pathol Lab Med.* 2010; 134:1639-1644.
5. Okamoto T, Iihara M, Obara T, Tsukada T. Parathyroid carcinoma: Etiology, diagnosis, and treatment. *World J Surg.* 2009; 33:2343-2354.
6. Lee YS, Nam KH, Chung WY, Chang HS, Park CS. Coexistence of parathyroid adenoma and papillary thyroid carcinoma. *J Korean Surg Soc.* 2011; 81:316-320.
7. Cetani F, Pardi E, Ambrogini E, Viacava P, Borsari S, Lemmi M, Cianferotti L, Miccoli P, Pinchera A, Arnold A, Marcocci C. Different somatic alterations of the *HRPT2* gene in a patient with recurrent sporadic primary hyperparathyroidism carrying an *HRPT2* germline mutation. *Endocr Relat Cancer.* 2007; 14:493-499.

(Received December 24, 2011; Revised January 26, 2012; Accepted January 31, 2012)

Supplemental data

PCR Primers

| | |
|------|---------------------------|
| P2F | GGGGCGCAGTCAGGCGTTCTC |
| P2R | CCCGAACACCCGTTTTATCCCATCC |
| P3F | TCATTGTTGTAGCAAGTTGTTTT |
| P3R | ATGAATTATTGGCCAGGCACA |
| P4F | CGCCCAGCCAAGAAAATTA |
| P4R | CCATAAGGGCAAAGACAGTGC |
| P5F | TGCAGAGCTGCTTTAAACTGAA |
| P5R | CCTTGAGCCAATAGGTTTCATCC |
| P6F | TTGACTCTGGTGAAGGCTTGTC |
| P6R | CCAATCCCCACACATGTTCTT |
| P7F | TTGCCATGTAAGTGTTTTTACCAGA |
| P7R | AACAGGAAAACCTGGGCCATTC |
| P8F | GCCTCCCGAATGTAGCAGTTT |
| P8R | TTTAATGTTGGGGAGGGCTTT |
| P9F | TGAGCCATGGTCATGCTACTG |
| P9R | CCCTTAAACATCAGGCCACAC |
| P10F | TGCCCTATGACTGAACTTTTGA |
| P10R | CCACACAGATCAATCAGCACAA |
| P11F | GGTTTTTCCAACAGGAGGGTA |
| P11R | TCGACAGTCTCAAAGAAACATGA |
| P12F | TTTCCTTTTGACTACAGATTGTGGA |
| P12R | GCCTATAGCACAGAAACCGAAA |
| P13F | TTGAAACCAGAAAGGTGGAGGT |
| P13R | CAACGTCATCAACGGCAATAA |
| P14F | AAGGGAACAGAAAGGGCAAAC |
| P14R | TGTTGTGGGATAGGCAATATCA |
| P15F | TTATAATACGGCTTCAGTTGGTGA |
| P15R | TTGCAAAAACACAGGGTTCTC |
| P16F | CGGATGGTTACAAAAGGAAA |
| P16R | CTTGAAGCACAAAGCATCAAA |