Hyperfunctioning Thyroid Adenoma Concomitant with Papillary Thyroid Carcinoma, Follicular Thyroid Adenoma and Primary Hyperparathyroidism

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Abstract. A case of 67-year-old woman with hyperthyroidism due to functioning thyroid adenoma is reported. The patient had concomitant follicular thyroid adenoma and primary hyperparathyroidism in addition to functioning adenoma. Histological examination of the excised thyroid tissue revealed occult papillary carcinoma within a functioning adenoma. Genetic analysis of such tumors indicated that functioning adenoma and papillary carcinoma may be etiologically independent. There have been a number of case reports on the coexistence of functioning thyroid adenoma and thyroid cancer or hyperparathyroidism, but none of the studies had examined the etiologic relationship of these lesions on a genetic basis. Furthermore, to our knowledge, this is the first report of the concurrence of four tumors in the neck, functioning thyroid adenoma, papillary thyroid carcinoma, follicular thyroid adenoma and parathyroid adenoma.

Key words: Hyperthyroidism, Hyperparathyroidism, Thyroid tumor, Mutation

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We report a case of concurrent functioning thyroid adenoma and primary hyperparathyroidism which were both excised. Follicular thyroid adenoma was also found and surgically enucleated. Furthermore, preoperatively unrecognized papillary thyroid carcinoma within a functioning adenoma was found by histological examination. Genetic analyses of the tumors was performed to examine their etiologic relationship.

Case Report

A 67-year-old woman was referred to our hospital in October, 1994, due to hyperthyroidism with an enlarging thyroid mass. The patient was diagnosed with hyperthyroidism in 1989 and had been maintained in the euthyroid state with thiamazole. In 1994, rapid enlargement of the thyroid right lobe and deterioration of hyperthyroidism was noted. The patient had neither a family history of thyroid diseases nor a history of irradiation of the neck. Laboratory data on the day after admission are summarized in Table 1 (Pre-op). Thyroid auto-antibodies were negative. The patient was a well nourished woman, weighing 47.8 kg. Her height was 155.3 cm. Blood pressure was 116/76 mmHg, pulse was 84 beats per min and regular. On physical examination, a soft, smooth and diffusely enlarged right lobe of the thyroid gland (6 x 4 cm) was palpated. The left lobe was mildly enlarged (3 x 2 cm). There was neither exophthalmos nor lid lag. Examination of the chest and abdomen were unremarkable. On the ultrasonography, diffuse enlargement of the right lobe with heterogeneous echogenicity was seen (Fig. 1A). In the left lobe, a well capsulated low echoic mass (1.2 x 1.2 x 2.0 cm) was observed (Fig. 1B). A CT scan of the neck revealed enlargement of the right lobe with irregular enhancement (Fig. 1C). Scintigraphy indicated intense uptake of $^{123}$I in the right lobe. No uptake to the left lobe was evident (Fig. 1D). On laboratory examination, hypercalcemia, albeit mild grade, was noted. Although hypercalcemia is often observed in hyperthyroidism, her PTH level was unexpectedly high (Table 1).

Surgery was performed on January 30, 1995. Right thyroid lobectomy (6.5 x 4.0 x 3.5 cm) and enucleation of the well capsulated nodule in the left lobe (2.0 x 1.5 x 1.0 cm) was performed. Histopathological examination of the functional adenoma in the right lobe revealed that tumors consisted of poorly stained small follicles which were surrounded by cuboidal or columnar epithels. Well differentiated papillary carcinoma (0.7 x 0.7 x 0.7 cm) was also found in the right lobe. Hyalination, calcification and psammoma bodies were observed in the stroma, and invasion of carcinoma cells to adjacent lymph ducts was seen. An enlarged right-upper parathyroid gland (1.2 x 0.8 x 0.6 cm) was identified and excised. Resected gland consisted of homogenously proliferated chief cells. Lobular structure and fat cells were not seen.

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ND: not done.
The other three parathyroid glands could not be identified during the operation.

After the operation, her thyroid and parathyroid function returned to normal. Laboratory data obtained 9 months after the operation are shown in Table 1 (Post-op.). During the follow-up period until July, 1997, she has not shown any clinical signs of hyperthyroidism or hyperparathyroidism.

**Materials and Methods**

**DNA preparation**

Genomic DNA was prepared from tumor tissues obtained at surgery or embedded in paraffin by proteinase K digestion and phenol/chloroform extraction [14]. A blood sample was obtained from the patient with her informed consent and high-molecular-weight DNA was isolated from 0.5 ml of whole blood with a DNA Extractor WB Kit (Wako Pure Chemicals, Osaka, Japan) and used...
for polymerase chain reaction (PCR).

**Direct sequencing of Gsa, Gia, N-RAS and H-RAS genes**

Exons 8 and 9 of Gsa, exons 5 and 6 of Gia, exons containing codons 12, 13 and 61 of N- and H-RAS genes in excised tissues and peripheral leukocytes were amplified by PCR. The oligonucleotide sequence for each PCR was determined according to the method reported in a previous publication [15]. Each PCR cycle consisted of denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C, and extension for 2 min at 72 °C. Amplified fragments were purified with a Sephaglas™ BandPrep Kit (Pharmacia, Uppsala, Sweden), and directly sequenced using Applied Biosystems mode 373A automated sequencer with a Taq DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

**Loss of heterozygosity (LOH) of chromosome 11**

Two polymorphic microsatellite markers, (CA) (GA) repeat in the PYGM locus and D11S913, were used for LOH analysis of the MEN1 locus on the chromosome 11 [16, 17]. MEN1 gene was recently identified in the region bounded by PYGM on the centromeric side and newly developed polymorphic marker D11S4936 on the telomeric side [18]. The latter is centromeric to D11S913 and thus closer to the MEN1 gene than D11S913. The following oligonucleotides were used for PCR:

**For PCR, forward oligonucleotide primers were end-labeled with [γ-32P]ATP (ICN, Costa Mesa, CA, USA) and T4 polynucleotide kinase (Takara Biomedicals, Kyoto, Japan), and approximately 50,000 cpm of 32P-labeled primer was included in the PCR mixture. PCR protocol was identical to that described above. Amplified fragments were separated by gel electrophoresis in 5% polyacrylamide gels containing 8M urea. Gels were dried in a vacuum and exposed to FUJI New RX X-ray film for 24 h.**

**Results**

A heterozygous gsp mutation at codon 201 of Gsa (CGT to CAT), which resulted in the encoded amino acid substitution from arginine to histidine, was found in the functioning thyroid adenoma (Fig. 2). This mutation was not found in other tumor or leukocyte DNA. No samples harbored mutations in Gia and critical codons of N-RAS and H-RAS genes (data not shown).

As shown in Fig. 3, the patient was heterozygous for two polymorphic markers, PYGM and D11S913.

![Fig. 2. gsp Mutation in the functioning thyroid adenoma. Nucleotide sequences of exon 8 of Gsa gene. DNA was obtained from (A) leukocyte, (B) papillary thyroid carcinoma, and (C) functioning thyroid adenoma. Note heterozygous CGT (arginine) to CAT (histidine) substitution at codon 201 of functioning thyroid adenoma.](image-url)
All DNA samples from thyroid and parathyroid tissues had patterns identical to that for leukocyte DNA, indicating there were no large deletions in the MEN1 gene on chromosome 11 in any tumor.

**Discussion**

Hypercalcemia occurs in up to 23% of patients with hyperthyroidism, although severe and symptomatic hypercalcemia is rare [10]. The mechanism by which thyrotoxicosis induces hypercalcemia has been well documented. Thyroid hormone directly activates osteoclastic bone resorption which predominates over increased bone formation by thyroid hormone [12, 19]. The plasma level of PTH is thus usually suppressed, which leads to hypercalciuria due to decreased tubular reabsorption of calcium. Coexistence of hyperparathyroidism and hyperthyroidism without neck irradiation is uncommon, and the etiologic relationship between the two disorders is controversial. Stoffer et al. found the incidence of coexisting hyperparathyroidism identified during surgery for hyperthyroidism was 1.2%, which was higher than in the general population [20]. Meanwhile, Lever et al. were unable to confirm this, reporting the incidence of coexisting hyperparathyroidism to be only 0.43% [21]. It has been suggested that persistent stimulation of thyroid hormone on adrenergic receptors may lead to hyperparathyroidism [22], which is probably mediated by the induction of β-adrenergic receptors on parathyroid cells by thyroid hormone [23–25]. Along with this hypothesis, Barsotti et al. have reported two patients whose PTH levels returned to normal when they became euthyroid [26]. It has also been suggested that relative decrease in calcitonin in thyrotoxicosis stimulates parathyroid hormone production [11]. Some reports have therefore recommended observation of the serum levels of calcium and PTH until the patient becomes euthyroid due to antithyroid therapy [11, 27]. We were unable to have an observation period since rapid growth of the thyroid nodule in our patient necessitated immediate surgical treatment. It is questionable, however, that an histologically confirmed parathyroid adenoma in our case could have spontaneously disappeared even if the patient had been rendered euthyroid before the operation. Also, if those hypotheses were the case, one might expect parathyroid hyperplasia rather than a solitary adenoma, and parathyroid hyperplasia should be more common in patients with thyrotoxicosis. In the review by Lam et al. of 43 reported cases with well-documented pathologic reports, 32 had solitary parathyroid adenomas, 10 had hyperplasias and one had a parathyroid carcinoma [28].

The etiological relationship between functioning thyroid adenoma and papillary thyroid carcinoma is also unclear. Recent molecular genetics revealed that about 30% of the functioning adenoma has an activating mutation in the gene encoding α-subunit of Gs protein, termed gsp [7], although this mutation is less frequent among Japanese patients [29]. This mutation is also observed in some differentiated thyroid carcinoma [8, 9]. A higher rate of the coexistence of occult carcinoma in functional thyroid autonomy than in Graves' disease has recently been reported by several independent groups [3–5]. These results indicate that the functioning thyroid adenoma and papillary thyroid carcinoma imbedded therein could be etiologically linked in some cases. Meanwhile, other groups

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**Fig. 3.** Lack of LOH in MEN1 locus in the thyroid and parathyroid tumors. LOH analysis of DNA from leukocyte and tumor DNA samples. Lane 1, leukocyte; lane 2, parathyroid adenoma; lane 3, hyperfunctioning thyroid adenoma; lane 4, papillary thyroid carcinoma; lane 5, follicular thyroid adenoma. Amplified fragments are indicated by arrows. Note identical patterns in all DNA samples. The lower bands associated with the “true” bands (arrows) are called the “shadow”, which is frequently seen in the PCR detection of microsatellite repeats. PYGM locus exists at about 50 kb centromeric to the MEN1 gene [18], and D11S913 locus exist at about 7 cM telomeric to the MEN1 gene.
have previously claimed that concomitant malignancy in functioning thyroid nodules documented by radioiodine studies is very rare [30-32], and cytologic findings suspicious for malignancy can be observed in functioning nodules at high frequency, of which the vast majority would be benign [33]. Genetic analysis of our case showed that the two lesions were clonally distinct, although the possibility that Gsa mutation in the functioning thyroid adenoma is a second mutation, occurring after an unidentified first step mutation common to the papillary thyroid carcinoma, cannot be ruled out. It is likely that the papillary thyroid carcinoma had already existed when the functioning thyroid adenoma replaced normal thyroid tissue in the right lobe, because if the papillary carcinoma was generated in the functioning adenoma, it should harbor a gsp mutation as was seen in the functioning adenoma. We therefore reason that normal thyroid tissue in the right lobe was eliminated by the expansion of the functioning adenoma, but the preexisting carcinoma was not.

Sequential mutational events which may underlie the initiation and progression of thyroid neoplasia have been revealed [34-37]. Along with those reports, we examined genetic abnormalities in each tumor in our patient, but we could not find any abnormality except for gsp mutation in the functioning adenoma. The etiologic relationships among thyroid and parathyroid lesions were therefore unclear.

In summary, we report a rare case of concomitant functioning thyroid adenoma, papillary thyroid carcinoma, follicular thyroid adenoma and parathyroid adenoma, which seemed in no way related to each other. Accumulation of a lot of experience on a genetic basis is necessary to elucidate a possible association between hyperthyroidism and hyperparathyroidism, and between functioning thyroid adenoma and thyroid papillary carcinoma, both of which have long been controversial.

References

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COEXISTING THYROID- AND PARATHYROID-TUMORS

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