BRAF^{V600E} mutation contributes papillary thyroid carcinoma and Hashimoto thyroiditis with resistance to thyroid hormone: A case report and literature review

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Abstract. Resistance to thyroid hormone (RTH) is a rare autosomal hereditary disorder characterized by increased serum thyroid hormone (TH) levels with unsuppressed or increased thyrotropin concentration. It remains unknown whether the coexistence of RTH with papillary thyroid carcinoma (PTC) and Hashimoto thyroiditis (HT) is incidental or whether it possesses a genetic or pathophysiological association. In the present study, a case of RTH with PTC and HT in an 11-year-old Chinese patient was examined and the clinical presentation of RTH with PTC was discussed. In addition, the possible associations between RTH, PTC and HT were determined. HT was confirmed in the patient using an autoimmune assay and thyroid ultrasound. RTH was diagnosed on the basis of clinical manifestations, laboratory information and gene analysis, and PTC was diagnosed according to histological results. Results of BRAF^{V600E} mutation analysis were positive. A literature review of 14 cases of RTH with PTC was included for comparison. The present case report indicates an association of RTH with PTC and HT coexistence in the patient. Close follow-up, histological evaluation and BRAF^{V600E} mutation detection should be performed in each RTH case with HT, since a persistent increase in TSH may be a risk factor for the development of thyroid neoplasm.

Introduction

Resistance to thyroid hormone (RTH) is a rare autosomal dominant or recessive hereditary disorder resulting from decreased responsiveness of the pituitary and/or peripheral target tissues to thyroid hormone (TH) (1). Thyroid function of the RTH is characterized by unsuppressed (normal or slightly increased) thyroid-stimulating hormone (TSH) levels, despite increased serum free thyroxine (FT_4) and free tri-iodothyronine (FT_3) levels. The first case of RTH was identified in 1967 by Refetoff *et al* (2) who described the clinical features of the disorder, including deaf-mutism and goiter. The association between RTH and mutation of the hormone-binding domain in the TH receptor β gene (THRB) was revealed in 1989 by Sakurai *et al* (3). In total, ~85% of RTH cases result from a number of mutations of the THRB gene (4), located on chromosome 3, with the remaining ~15% of cases arising due to defects on alternative genes, including the TH receptor α gene (THRA) located on chromosome 17, and genes involved in the transport and metabolism of TH (4). RTH exhibits variable clinical presentations; however, the most common clinical feature is goiter with a euthyroid state. On occasion, patients with RTH may suffer from either hyperthyroidism or hypothyroidism. RTH is diagnosed on the basis of clinical findings and laboratory results, and a definite diagnosis relies on the identification of associated gene mutations.

The association between RTH and autoimmune thyroid diseases (AITDs) remains a matter of debate. A previous study (5) demonstrated that RTH is free of autoantibodies against thyroglobulin (anti-TgAb) and thyroid peroxidase (anti-TpoAb), whereas Barkoff *et al* (6) hypothesized that patients with RTH possess an increased risk of developing AITDs including Hashimoto thyroiditis (HT). On the other hand, HT has been associated with papillary thyroid carcinoma (PTC) and may constitute a risk factor for this type of cancer (7). There are a number of studies of adult patients with PTC and HT; however, there are a limited number of studies demonstrating the coexistence of these two diseases in pediatric patients (8,9). In addition, there are a number of case studies that describe RTH with PTC (10-19), which identify the possible association between these diseases. In the present case report, a pediatric patient with newly diagnosed RTH and coexisting PTC and HT is discussed. Additionally, a literature review of PTC in RTH subjects (Table I) is provided.

Case report

A female Chinese patient, aged 11 years, was examined following clinical presentation of mild thyroid enlargement.

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Thyroid ultrasonography (US) revealed marked heterogeneity of the parenchyma, 1 nodule (8x7 mm) with clear margins and no blood flow signal in the right lobe. Fine needle assay (FNA) of the thyroid nodule revealed no malignancy. The patient was diagnosed with hyperthyroidism following a thyroid function assessment in Heze Municipal Hospital (Shandong, China); however, the patient exhibited a poor response to initial methimazole (MMI) treatment (10 mg, once daily) so the dose was increased to 10 mg twice daily. Baseline thyroid function information and during MMI therapy are presented in Table II.

When first seen at The General Hospital of Jinan Military Command (Shandong, China) on 31 August 2015, the patient's height and weight were 156 cm and 45 kg, respectively, with a pulse rate of 106 beats/min, blood pressure of 100/75 mmHg and a basal metabolic rate of 20%. On observation, the patient's thyroid gland was asymmetrically enlarged with palpable nodules on the two lobes (the largest was located in the left lobe, diameter ~1 cm). The patient was without exophthalmos or myxedematous skin lesions and hepatic function, renal function, sex hormone levels, parathyroid hormone and prolactin concentrations were all within the normal range. Psychological assessment by Raven's Standard Progressive Matrices revealed an intelligence quotient score of 68, an intelligence percentile ranked in the lower 5%, which indicated mild mental retardation.

The laboratory results of thyroid function and autoimmune assays were as follows: FT_3, 14.74 (normal range, 3.8-6.0 pmol/l); FT_4, 44.86 (range, 7.86-14.1 pmol/l); TSH, 3.30 (range, 0.34-5.6 μIU/ml); anti-TgAb, 16.10 (range, 0-4.0); anti-TpoAb, 477.40 (range, 0-9.0 IU/ml). Thyroid US evaluation revealed diffused enlargement with a heterogeneous echotexture and multiple nodules in the two lobes. The dominant nodules were 5x4 and 14x6 mm in size in the left lobe and 15x5, 15x6, 5x5 and 6x5 mm in the right lobe, with micro-calcifications and unclear margins (Fig. 1). FNA identified atypical results of undetermined significance and suggested possible malignancy. ^{99}Tc scintigraphy demonstrated diffused enlargement of the thyroid gland with increased uptake (Fig. 2) and an electrocardiogram revealed sinus tachycardia. A magnetic resonance imaging scan of the pituitary gland excluded the presence of a pituitary tumor or pituitary enlargement and X-ray of the left wrist demonstrated normal bone age. The patient was prescribed with a β-blocker (metoprolol succinate, 23.75 mg, once daily) to control the tachycardia and after 2 weeks achieved the target heart rate (between 70 and 80 beats/min). Subsequently, the patient was hospitalized with a diagnosis of thyroid cancer and suspected RTH. On 18 September 2015, the patient underwent total thyroidectomy and lymph node dissection of the left side of the neck. Biopsy results revealed multifocal PTCs (T1aN1bM0, using the tumor-node-metastasis staging) with 1 papillary focus (1.0x0.7 cm) in the left thyroid lobe, a micro-papillary carcinoma focus (0.6x0.5 cm) with HT and nodular goiter in and around the right lobe, and HT with nodular goiter in the isthmus. Lymph node metastases to the right and left trachea and the esophagus were confirmed by histology results. $BRAF^{V600E}$ mutation analysis demonstrated positivity according to immunohistopathological results (Fig. 3).

DNA was isolated from peripheral blood leukocytes by QIAamp Blood DNA Mini kit (Qiagen, Inc., Valencia, CA, USA) and all 10 exons of the THRB gene were amplified using the polymerase chain reaction (PCR) and analyzed by automated fluorescence-based sequencing. The primers of exon 1 to 10 of the THRB gene were designed by Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) and the sequence information is listed in Table III. The PCR cycling condtions were as follows: Pre-denaturation at 95˚C for 5 min, followed by 40 cycles of denaturation at 94˚C for 30 sec, annealing at 66-65˚C for 30 sec and extending at 72˚C for 30 sec, Direct sequencing of the PCR products were performed by ABI 3500 sequencer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and compared with reference sequences (NM_000461.4 and NG_009159.1; https://www.ncbi.nlm.nih.gov/nuccore/NM_000461.4 and https://www.ncbi.nlm.nih.gov/nuccore/218156319, respectively). The results revealed a frameshift mutation in exon 10 of THRB (Leu^{454}fs; c.1358dupC, Fig. 4), which has been identified in RTH (20). Diagnosis of RTH, HT and PTC was confirmed on the basis of laboratory and clinical results. The thyroid function of the patient 1-week post-surgery revealed slightly increased FT_3 (6.84 pmol/l), increased FT_4 (26.84 pmol/l) and increased TSH (33.99 μIU/ml) levels following treatment with levothyroxine (L-T_4, 50 μg/day). The patient was subsequently prescribed with 100 μg/day L-T_4 and 1 month later thyroid function assays revealed almost normal FT_3 levels (5.23 pmol/l), slightly increased FT_4 (14.99 pmol/l) and increased TSH (>100 μIU/ml) levels. The levels of anti-TgAb and anti-TpoAb decreased markedly in comparison with pre-surgery amounts (6.9 and 173.3%, respectively). The concentration of thyroglobulin was 0.46 (range, 1.6-59.9 ng/ml) and the patient exhibited no signs of hyperthyroidism. Due to the unavailability of tri-iodothyronine (T_3), the L-T_4 dosage was increased to 125 μg/day and combined with TH tablets (50 mg/day), derived from pig thyroid gland (containing FT_3 and FT_4), and bromocriptine (5 mg/day). After 1 month, thyroid function assessment revealed slightly increased FT_3 and FT_4 (6.23 and 14.47 pmol/l, respectively) and increased TSH (46 μIU/ml) levels. ^{131}I radio remnant ablation therapy was administered at an oral dose of 104 mCi $Na^{131}I$. Subsequently, 4 days after radiotherapy, the patient underwent a whole-body scan which detected a highly radioactive region around the thyroid, but no other abnormal foci of uptake in the rest of the body were identified (Fig. 5). Subsequently, L-T_4 suppression therapy was continued and gradually increased up to 150 μg/day together with 3.75 mg/day bromocriptine and 50 mg/day TH tablets. The latest thyroid function tests, 6 months post-surgery, revealed that FT_4 and TSH levels were slightly increased with normal FT_3 levels (15.97 pmol/l, 6.80 μIU/ml and 5.79 pmol/l, respectively) and the patient exhibited no signs of thyrotoxicosis. Thyroid US demonstrated no signs of disease persistence or relapse, and serum thyroglobulin and carcinoembryonic antigen levels were within the normal range. The patient remains under follow-up. All examined relatives (mother, father and younger brother) exhibited normal thyroid function and morphological features of thyroid US revealed normal results. The patient's relatives were unwilling to undergo testing for the THRB mutation.

All protocols followed were in accordance with the national ethical standards previously approved by Local Ethical Review

Table I. Literature review of papillary carcinoma with resistance to thyroid hormone.

Author, year	Sex/ age	Order of diagnosis	TSH levels	Gene analysis	Histology of DTC	Diameter, mm	HT	Therapy	Follow up for DTC, year/result	(Refs.)
Taniyama *et al* (2001)	F/46	1. TMNG; 2. DTC; 3. RTH	Unsuppressed	Amino acid substitution at codon 429 (R429Q) of THRB	Follicular variant of papillary carcinoma	5	-	1. ATD (MMI); 2. subtotal thyroidectomy	NR	(10)
Siristatidis *et al* (2004)	F/26	1. PTC; 2. TSHoma; 3. RTH	Increased	NR	Papillary carcinoma	NR	NR	Total thyroidectomy and L-T_4	0.75/remission	(11)
Kim *et al* (2010)	F/38	1. TRH; 2. PTC	Unsuppressed	Amino acid substitution at codon 310 (M310T) in exon 9 of THRB	Papillary carcinoma	4 (multifocal)	-	1. Total thyroidectomy; 2. L-T_4	NR	(12)
Paragliola *et al* (2011)	M/48	1. RTH; 2. MNG; 3. PTC	Increased	no mutation identified in THRB	Papillary carcinoma	24	-	1. Total thyroidectomy; 2. L-T_4	9.5/remission	(13)
Paragliola *et al* (2011)	M/63	1. MNG; 2. RTH; 3. PTC	Increased	Missense mutation at codon 453 (P453T) in exon 10 of THRB	Papillary carcinoma	6	-	1. Total thyroidectomy; 2. L-T_4	5/remission	(13)
Sugita *et al* (2012)	F/26	1. PTC; 2. RTH	Unsuppressed	Mutation at codon 447 (P447L) of THRB	Papillary carcinoma	NR	-	1. ATD (MMI); 2. L-T_4 and T_3	8/remission	(14)
Ramos-Prol *et al* (2013)	F/9	1. ADHD; 2. AITD and RTH; 3. PTC	Increased	Missense mutation at codon 243 (R243W) of THRB	Papillary carcinoma	24 (multifocal)	+	1. ATD (first cabimazole, then propylthiouracil) and βB; 2. TRIAC; 3. total thyroidectomy; 4. L-T_4 and TRIAC	3/remission	(15)
Unluturk *et al* (2013)	F/29	1. Hyper- thyroidism; 2. PTC; 3. RTH	Unsuppressed	Missense mutation at codon 334 (T334C) of THRB	Papillary carcinoma	8	-	1. ATD; 2. subtotal thyroidectomy; 3. completion thyroidectomy and radioiodine; 4. L-T_4 and bromocriptine; 5. L-T_4 and βB	21/remission	(16)

Table I. Continued.

Author, year	Sex/ age	Order of diagnosis	TSH levels	Gene analysis	Histology of DTC	Diameter, mm	HT	Therapy	Follow up for DTC, year/result	(Refs.)
Unluturk *et al* (2013)	M/33	1. MNG; 2. PTC; 3. RTH	Unsuppressed	Amino acid substitution at codon 364 (I364F) of TSHR	Papillary carcinoma	12	-	1. Total thyroidectomy and radioiodine; 2. L-T_4	0.75/remission	(16)
Vinagre *et al* (2014)	F/19	1. Hyper-thyroidism; 2. PTC and follicular adenoma; 3. RTH	Unsuppressed	Mutation at codon 320 (R320C) in exon 9 of THRB and $BRAF^{V600E}$ mutation in PTC by gene sequence	Papillary carcinoma	4	-	1. ATD (MMI); 2. total thyroidectomy and radioiodine; 3. L-T_4 and βB	11.00/remission	(17)
Aoyama *et al* (2015)	F/54	1. PTC; 2. RTH	Unsuppressed	Point mutation at codon 453 (P453S) of THRB	Papillary carcinoma	10 (multifocal)	-	1. Total thyroidectomy; 2. L-T_4	2.25/remission	(18)
Karakose *et al* (2015)	F/56	1. MNG; 2. RTH; 3. PTC	Unsuppressed	Missense mutation at codon 234 (A234D) in exon 8 of THRB	Papillary carcinoma	2	-	1. Subtotal thyroidectomy; 2. total thyroidectomy; 3. L-T_4 and T_3	0.33/remission	(19)
Karakose *et al* (2015)	M/33	1. RTH; 2. PTC	Increased	Missense mutation at codon 234 (A234D) in exon 8 of THRB and $BRAF^{V600E}$ mutation negative	Papillary carcinoma	4 (two focus)	-	1. Total thyroidectomy and radioiodine; 2. L-T_4	0.17/remission	(19)
Present case (2015)	F/12	1. Hyper-thyroidism; 2. PTC; 3. RTH	Unsuppressed	Mutation at codon 454 (L454FS) in exon 10 of THRB and $BRAF^{V600E}$ mutation in PTC	Papillary carcinoma	10 (multifocal)	+	1. ATD (MMI); 2. total thyroidectomy and radioiodine; 3. L-T_4, βB and bromocriptine	0.25/remission	

ADHD, attention-deficit hyperactivity disorder; AITD, autoimmune thyroid disease; ATD, anti-thyroid drug; DTC, differentiated thyroid carcinoma; βB, β-blocker; FTC, follicular thyroid carcinoma; follow-up for DTC, the duration of the follow-up after the initial treatment of thyroid cancer/result; F, female; M, male; MMI, methimazole; MNG, multinodular goiter; L-T_4, levothyroxine; PTC, papillary thyroid carcinoma; RTH, resistance to thyroid hormone; TMNG, toxic multinodular goiter; NR, not reported; THRB, thyroid hormone receptor β; TSH, thyrotropin; TSHR, thyrotropin receptor; TSHoma, thyrotropin-secreting adenoma; TRIAC, tri-iodothyroacetic acid, T_3, tri-iodothyronine; -, negative; +, positive; HT, Hashimoto thyroiditis.

Table II. Alterations in thyroid function at the initial visit and during MMI therapy.

Variable	FT_3, ng/ml	FT_4, ng/ml	TSH, μIU/ml
Normal range	1.82-3.86	0.78-1.86	0.38-5.57
Initial	6.56	1.85	10.02
2 months after MMI therapy (5 mg, bid)	8.65	3.84	17.00
6 months after MMI therapy (10 mg, tid)	9.54	4.09	>100
3 months after withdrawal of MMI	6.60	2.84	18.91
1 month after MMI therapy (10 mg, bid)	9.08	4.79	5.22

MMI, methimazole; FT_3, serum-free tri-iodothyronine; FT_4, serum-free thyroxine; TSH, thyroid-stimulating hormone; bid, twice daily; tid, three times daily.

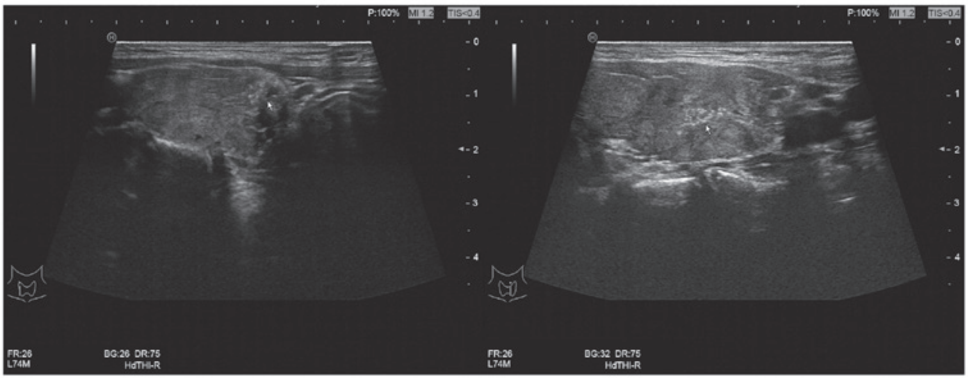

Figure 1. Thyroid ultrasonography scan. Thyroid ultrasonography scan demonstrated decreased echo nodule with sand and gravel-like calcification in the right lobe (arrow) and decreased echo nodule with dense calcification in the left lobe (arrow).

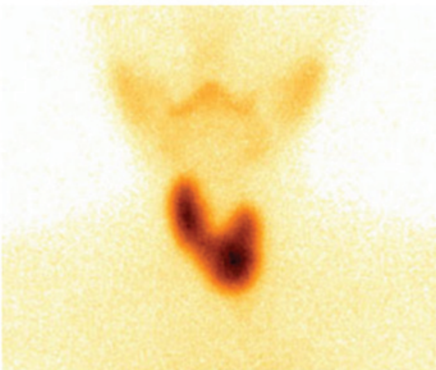

Figure 2. Emission computed tomography of the thyroid gland.

Committees. The present case report was approved by the Regional Ethical Review Board of General Hospital of Jinan Military Command, China. Informed consent was obtained from the patient's parents.

Discussion

RTH is the most common type of decreased sensitivity to TH with an incidence of ~1/40,000 live births (21). RTH is caused by mutations of a number of genes, including THRB, THRA and others involved with TH transport and metabolism (4). Clinical manifestations of RTH are heterogeneous (22); the most common signs include goiter and sinus tachycardia, as identified in the present case report. Distinct mutations of THRB have been studied in >3,000 individuals and ~1,000 families (23). In the present case report, a frameshift mutation in exon 10 of THRB (Leu^{454}fs; c.1358dupC) was identified by gene sequencing. Among all reviewed cases, including the present case study, only 1 revealed a mutation in the TSH receptor (16) and all remaining cases exhibited gene mutations within the three clusters rich in CpG (24-26) between residues 310 and 353, 429 and 461 and 232 and 282 (clusters 1, 2 and 3, respectively) within the ligand-binding domain of THRB.

THRB is the cellular homolog of the transcriptionally inactive oncogene v-erbA, which may have an influence on the development of cancer. In thyroidectomized tissues of PTC, THRB1 mutations were identified in 93.8% of cases and no mutations were detected in healthy euthyroid controls (27). In animal studies, the association of THRB and thyroid carcinoma has been demonstrated (28,29) and Kim *et al* (12) proposed that the THRB mutation itself may also exert oncogenic effects. Additionally, patients with PTC in RTH were all relatively young, ranging between 9 and 63 years (mean, 35.1 years). Thus, thyroid US evaluation and FNA may be performed in the follow-up of patients with RTH in order to determine carcinogenesis.

Typically, anti-thyroid antibodies are negative in RTH which eliminates autoimmunity in the etiology of this disorder (30). HT is a common type of AITD worldwide, whereas RTH is a rare condition and so the coexistence of these two diseases is considered to be incidental (5). In the last decade, the coexistence of RTH and AITD has become increasingly prevalent in

Table III. Primer sequences of exon 1 to 10 of TH receptor β gene.

Exon	Primer	Sequence	Size of product (bp)
1	Forward	5'-GCTGCGGCCGCCTCTCTTCGC-3'	420
	Reverse	5'-GCCTCCGGGTTCTTGCGACGC-3'	
2	Forward	5'-GAGTTTGAGGTTCACATTGAA-3'	541
	Reverse	5'-AATACCTATAGAGTTCAACCT-3'	
3	Forward	5'-ATTGCTAGCATAGGCATTGGC-3'	525
	Reverse	5'-ATATATTTCAGTTAAGTACAGC-3'	
4	Forward	5'-AAATTATCACAGATATATGACG-3'	418
	Reverse	5'-GTGAGGATGCATCTTATATGAG-3'	
5	Forward	5'-ACAACTTGCCTTCCAAAAGTGT-3'	492
	Reverse	5'-GAAAAGCGACGCGCTAGTAAAG-3'	
6	Forward	5'-GTGGGCCTATGTTAAGTCTAT-3'	370
	Reverse	5'-TTGAATTTAACTTAACATTGC-3'	
7	Forward	5'-AAGTGTGCCCAGTGTGAGCCAG-3'	458
	Reverse	5'-TATCAGTAAAATGAGGCAATAAC-3'	
8	Forward	5'-GATAAATAAAGCTCCCTTCAAC-3'	384
	Reverse	5'-TAAATACAGAAAGTGGGAATC-3'	
9	Forward	5'-CTTTGAGTATGAAATGGTTG-3'	502
	Reverse	5'-TTAGCGCTAGAGAAGCAAAAG-3'	
10	Forward	5'-TGGAGCACCAGAGTTCACC-3'	469
	Reverse	5'-ACAAATGCAGCTAGCTAGAT-3'	

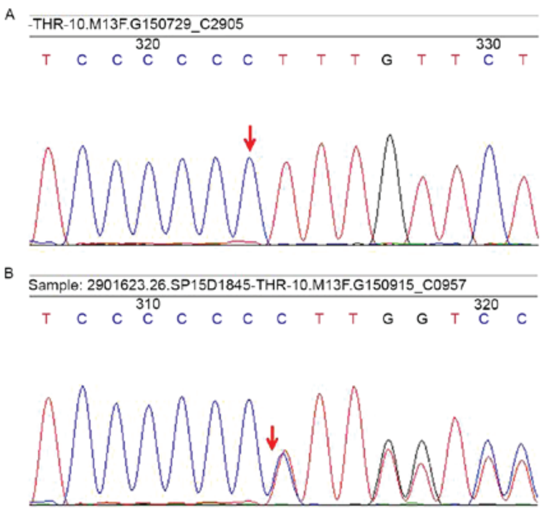

Figure 3. Partial sequencing results of the PCR product of the tenth exon of THRB. (A) No mutation was detected in the control sample; (B) a heterozygous mutation was identified in the patient's sample at c.1358 (arrow).

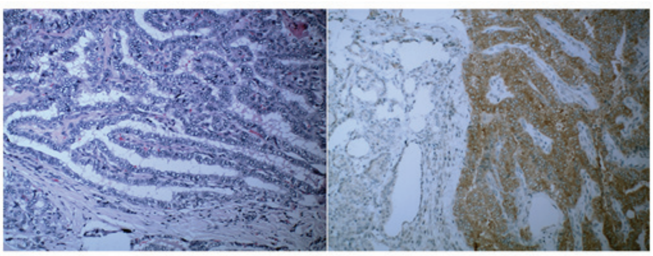

Figure 4. Pathology and immunohistochemistry of BRAF^{V600E} mutated thyroid gland resected. Thyroid pathology indicated papillary carcinoma (magnification, x200; hematoxylin and eosin stain, left) and immunohistochemical staining revealed positive BRAF^{V600E} mutation (dark brown) of the resected thyroid tissue (right).

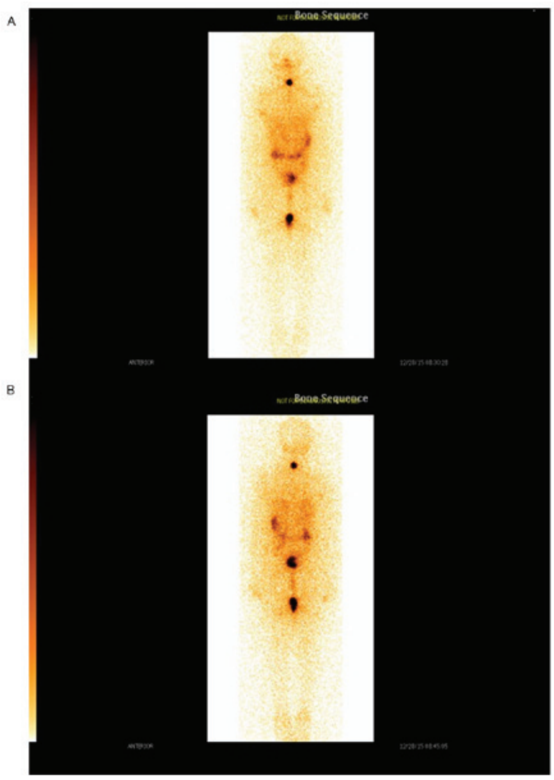

Figure 5. Whole body scan following ^{131}I radio remnant ablation therapy. (A) Anterior; (B) posterior. A highly radioactive region was detected close to the thyroid in the neck and no abnormal foci of uptake were observed in other parts of the body.

single patients (31,32) and also in families with RTH (33,34). Since the TSH level is unsuppressed or abnormally increased, in comparison with serum TH concentrations, a number of studies hypothesized that chronic stimulation of TSH in RTH may activate the intra-thyroidal lymphocytes, leading to thyroid damage and autoimmune thyroiditis including HT (35). More recently, Barkoff *et al* (6) revealed that patients with RTH exhibit an increased risk of AITD, compared with unaffected relatives, due to the THRB gene mutation which suggests that the coexistence of HT and RTH may not be accidental. Furthermore, the presence of HT and the resulting thyroid failure, caused by destructive antibodies, may decrease serum TH levels, thereby masking the cardinal features of RTH and leading to misdiagnosis (33). Since HT is more common in females than males (7), the ideal approach may be to test for thyroid antibodies in females suspected with RTH with close follow-ups in patients with HT and RTH.

HT is commonly observed following histological examination of thyroidectomy specimens. The association between HT and PTC was first proposed by Dailey *et al* (36) in 1955. Subsequently, the clinical association of the two diseases has been extensively debated with a number of studies confirming a relatively high incidence of PTC in HT (37,38); however, other studies have identified contradictory results (39). Recently, Koibuchi *et al* (40) investigated three cases of children with PTC and HT; however, the underlying molecular mechanism of the association between HT and PTC remains unknown, with one study suggesting that increased reactive oxygen species levels may contribute to the development of PTC in HT (41). The $BRAF^{V600E}$ mutation, identified in between 29 and 83% of PTC cases (42) and considered an early or initiating event in PTC, is typically in papillary microcarcinoma and minute incidental cases (43). An identical $BRAF^{V600E}$ mutation has been identified in solid cell nests in the thyroid and adjacent PTC (44), indicating that HT and PTC may be initiated by similar stem cell remnants, and may be etiologically related (7). In studied cases of RTH with PTC, $BRAF^{V600E}$ mutation testing was carried out in two studies (17,19) in the histological section and only one positive mutation was found (17). To the best of our knowledge, the present case report is the second patient to exhibit $BRAF^{V600E}$ mutation in PTC with RTH. Since the $BRAF^{V600E}$ mutation is rare in children and adolescents with PTCs (45), and TSH suppression therapy not always effective, using $BRAF^{V600E}$ mutation tests in cases with PTC and RTH was hypothesized in the present study, in order to identify the patients at risk of metastases and patients with poor prognosis.

TSH is a growth factor for the thyroid gland and nodules; however, whether it additionally serves a pathogenic role in thyroid oncogenesis remains unclear. A previous study identified that patients with increased serum TSH concentrations exhibited an increased risk of developing thyroid malignancy (46). Within the normal range of TSH, a value above the mean level for the general population is associated with a markedly increased likelihood of thyroid cancer, compared with TSH values below the mean (47). In a retrospective study based on 637 medical records, Medenica *et al* (48) revealed that patients with increased serum TSH concentrations and/or AITD, exhibited an increased risk of thyroid malignancy. Since RTH is characterized by increased TH concentrations, accompanied by unsuppressed or increased serum TSH levels, whether patients with RTH are at increased risk of thyroid malignancy remains unknown (19). Owing to the low incidence of RTH and the lack of specific symptoms associated with the disorder, RTH is commonly misdiagnosed as hyperthyroidism or Grave's disease. Patients who have been previously misdiagnosed and prescribed anti-thyroid drugs (ATDs) may be at an increased risk of neoplasm formation (30). In the present case report, the patient with RTH was misdiagnosed with hyperthyroidism and administered with MMI for ~8 months, and serum TSH levels were increased to above the detection limit. It is hypothesized that chronic HT and increased TSH stimulation caused by inappropriate therapy may have contributed to the development of PTC in the present case.

A literature review of 14 cases of differentiated thyroid carcinoma DTC with RTH (Table I) revealed that HT coexisted in only a 9-year-old girl from Germany (15). Therefore, to the best of our knowledge, the present case report is the first to reveal PTC and HT with RTH in an Asian adolescent. Lymph node metastases of the right and left trachea and esophagus were confirmed in the present case, indicating a more aggressive thyroid malignancy in this case when compared with the aforementioned 9-year-old female patient. It was hypothesized that increased serum TSH levels in patients with RTH and HT, who possess the $BRAF^{V600E}$ mutation, may be a contributing factor for malignancy considering the relatively young age of the present patient, the rare incidence of the $BRAF^{V600E}$ mutation in children, HT coexistence, increased serum TSH levels prior to and during treatment and metastasis occurrence. TSH stimulation in RTH may be an important growth factor for the thyroid gland and minute neoplastic nodules.

Total thyroidectomy and post-operative L-T_4 suppression therapy is typically administered for the treatment of patients with PTC as this therapy is considered to prevent cancer relapse or progression. However, in a patient with RTH and thyroidectomy, TSH suppression may not be achieved despite increasing doses of L-T_4. In the literature review of 14 cases, 3 patients (14,15,19) were prescribed T_3 or tri-iodothyroacetic acid (TRIAC), in addition to L-T_4 suppression therapy. Owing to the lack of T_3 or TRIAC, the dosage of L-T_4 was increased in combination with TH tablets and the dopaminergic agent bromocriptine. To the best of our knowledge, only the study by Unluturk *et al* (16) demonstrated that bromocriptine in combination with L-T_4 may be used to decrease serum TSH levels in a patient with RTH and PTC. Previous studies have indicated that bromocriptine may inhibit TSH secretion and additionally decrease the enlarged goiter in RTH (49,50). In the present case, the TSH level decreased under combination therapy of L-T_4 and bromocriptine in the patient with RTH and PTC, following surgery. Considering the limited evidence for the use of bromocriptine in RTH, additional studies are required to assess its value as a treatment of RTH.

Tests to identify a BRAF mutation, e.g. DNA sequencing or PCR-based molecular assays, are not routinely applied as they are costly and time-consuming. Recently, an immunohistochemical technique was introduced into clinical practice. The aforementioned technique identifies BRAF mutations using the mouse anti-human $BRAF^{V600E}$ monoclonal antibody VE1 and clinical information suggested a marked consistency between this method and the BRAF mutation assessment in PTC1 and other BRAF mutation-related cancers, including colon cancer (51,52). Therefore, the use of BRAF immunohistochemistry in clinical practice for $BRAF^{V600E}$ mutation detection is considered to be useful due to its effectiveness, simplicity and economical benefit. The limitations of the present study included small sample size (only one patient), unknown lifestyle habits of the patient, as well as a focus on only one region (Asian).

In conclusion, the present case report indicates that RTH with increased or unsuppressed serum TSH concentrations and positive anti-thyroid antibodies, suspect for HT, may be an indication for thyroid carcinoma development. It is hypothesized that, in clinical practice, treatment with ATDs which lead to increased TSH, in addition to HT and RTH, may be avoided. Close follow-up and genomic analysis should be performed in cases with suspected RTH. Additional studies are required to disclose the possible association of HT and PTC in RTH and explore the long-term effects of medication.

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