APS Kong 江碧珊
CW Lam 林青雲
AOK Chan 陳安琪
SF Yiu 姚少峰
SC Tiu 張秀祥

CASE REPORT

Resistance to thyroid hormone in a Chinese family with R429Q mutation in the thyroid hormone receptor beta gene

一個華裔家庭因甲狀腺素 β 受體基因出現R429Q突變導致甲狀腺素排斥的病例

The combination of elevated serum levels of free thyroid hormones with non-suppressed thyroid-stimulating hormone suggests the differential diagnoses of resistance to thyroid hormone or thyroid-stimulating hormone–secreting pituitary tumour. Clinical differentiation of these two conditions can be difficult, because patients with thyroid hormone resistance may exhibit various combinations of hypermetabolic and hypometabolic features, and laboratory results have limited sensitivity and specificity. We report a case of resistance to thyroid hormone in a Chinese family that illustrates this difficulty. The diagnosis could only be confirmed by the identification of a known disease-causing mutation in the thyroid hormone receptor beta gene in peripheral leukocytes. Availability of genetic tests will identify more cases in the future and improve our understanding of this condition.

游離甲狀腺素和非抑制性促甲狀腺素的血清水平上升，可推斷病人患有甲狀腺素排斥或促甲狀腺素分泌腦下垂體瘤。要對兩者進行臨床區別並不容易，因為對甲狀腺素排斥的病人可能會顯現各種新陳代謝過快和過慢的特徵，而化驗測試亦不夠敏感和具體。本文報告一個華裔家庭患有甲狀腺素排斥並難於斷症的病例，結果在確定病人於外周白血球發現甲狀腺素β受體基因出現因病引起的突變後才可診斷病症。基因測試將有助診斷更多同類病例，同時加深醫學界對這種病例的了解。

Queen Elizabeth Hospital, 30 Gascoigne Road, Hong Kong:
Department of Medicine
APS Kong, MRCP, FHKAM (Medicine)
SF Yiu, FRCP, FHKAM (Medicine)
SC Tiu, MD, FRCP
Department of Chemical Pathology
AOK Chan, BMedSc, MB, ChB
Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong
CW Lam, PhD, MB, ChB

Correspondence to: Dr SC Tiu
(e-mail: tscz01@ha.org.hk)

Introduction

Thyroid hormone secretion is stimulated by thyroid-stimulating hormone (TSH), secretion of which is controlled by thyroid hormones by negative feedback. The unusual combination of elevated serum levels of free thyroid hormones with non-suppressed TSH suggests the diagnosis of resistance to thyroid hormone (RTH) or a TSH-secreting pituitary tumour (TSHoma). We report a case of a Chinese family in whom the diagnosis of RTH was confirmed by a codon 429 mutation in the thyroid hormone receptor beta (THRβ) gene, and discuss the differentiation of these two conditions and the treatment of RTH.

Case report

A 39-year-old Chinese man presented in 1990 with palpitations. He had a diffuse goitre (twice of the normal size) but no ocular signs or other

Table 1. Biochemical findings* of the index patient and his family members in 2003

	TSH (mIU/L)	FT$_3$ (pmol/L)	TT$_3$ (nmol/L)	FT$_4$ (pmol/L)	TT$_4$ (nmol/L)	T-Chol (mmol/L)	SHBG (nmol/L)	CK (IU/L)	Ferritin (pmol/L)
Index patient[†]	←	— (See text and Table 2) —			→	5.6	39.4	107	650
Father	1.87	4.4	1.26	16.0	133	5.4	50.9	98	1010
Mother	1.36	2.8	1.05	19.7	110	7.2	85.2	130	590
Elder sister	3.78	4.6	1.30	15.5	119	5.4	86.4	88	31
Elder brother	1.54	4.4	1.27	15.7	111	7.0	23.4	132	1286
Son[†]	3.39	10.7	3.16	32.6	246	5.6	57.9	183	97
Reference range	0.27-4.20	3.0-6.2	0.70-2.10	12.0-22.0	62-154	<5.2	13.0-71.0	62-328	69-920

* TSH denotes thyroid-stimulating hormone, FT$_3$ free triiodothyronine, TT$_3$ total triiodothyronine, FT$_4$ free thyroxine, TT$_4$ total thyroxine, T-Chol total cholesterol, SHBG sex hormone binding globulin, and CK creatine kinase
[†] Both the index patient and his son had increased serum levels of thyroid hormone with non-suppressed TSH

Table 2. Biochemical parameters* of the patient before and after treatment with 3,5,3'-triiodothyroacetic acid (TRIAC)

	Reference range	Without TRIAC (2 Sept 2003)	Treated with TRIAC (0.35 mg)[†]		
			2 times daily (13 Oct 2003)	3 times daily (20 Oct 2003)	4 times daily (27 Oct 2003)
FT$_3$ (pmol/L)	3.0-6.2	6.8	>46.1	>46.1	>46.1
FT$_4$ (pmol/L)	12.0-22.0	36.0	28.2	30.4	27.0
TSH (mIU/L)	0.27-4.20	1.09	0.19	0.07	0.05

* FT$_3$ denotes free triiodothyronine, FT$_4$ free thyroxine, and TSH thyroid-stimulating hormone
[†] FT$_4$ and TSH levels decreased while FT$_3$ became markedly elevated, probably due to cross-reactivity of TRIAC with FT$_3$ assay

symptoms of hyperthyroidism. His pulse rate varied from 70 to 100 beats per minute. Investigations showed an elevated total thyroxine (T$_4$) level (227 nmol/L; reference range [ref], 77-157 nmol/L) and an increased free triiodothyronine (FT$_3$) concentration (10.03 pmol/L; ref, 3.28-8.2 pmol/L). The TSH level was not measured. Thyroid antibodies were negative.

The patient was treated for thyrotoxicosis with propylthiouracil (up to 600 mg per day), but his FT$_3$ levels remained elevated (9.65-12.3 pmol/L). In 1993, his TSH level was found to be 16.7 mIU/L (ref, 0.3-4.0 mIU/L) with a free T$_4$ (FT$_4$) level of 20.7 pmol/L (ref, 8.5-20.7 pmol/L). This pattern of elevated FT$_4$ and non-suppressed TSH levels persisted on repeated testing (FT$_4$, 25.2 pmol/L and TSH, 7.0 mIU/L; FT$_4$, 23.9 pmol/L and TSH, 7.3 mIU/L). Family history was negative. All family members had normal FT$_4$ and TSH levels (Table 1).

In 1994, magnetic resonance imaging (MRI) revealed an enlarged pituitary gland with lobulated outline, heterogeneous signal intensity, and mild suprasellar extension. Other anterior pituitary function tests were normal. A tentative diagnosis of TSHoma

was made and transsphenoidal excision performed. The "tumour" was macerated in the process of removal, histology of the surgical specimen did not show any definite adenoma, and immunohistochemical staining revealed a mixture of cells, compatible with normal pituitary tissue. Postoperatively, his FT$_4$ and TSH levels remained elevated (23 pmol/L and 6.7 mIU/L, respectively). Propylthiouracil was stopped in 1997 in view of the failure to suppress FT$_4$ values to normal and the clinical suspicion of RTH. Subsequent postoperative MRI follow-up for 10 years revealed no residual or recurrent tumour.

After stopping all medication for at least 4 weeks, results of other investigations performed between 1994 and 2003 were as follows: thyroid scan showed diffusely increased perfusion and uptake. Thyrotropin-releasing hormone (TRH) stimulation test showed exaggerated response of TSH to TRH (3.0, 13.2, and 15.2 mIU/L at 0, 15, and 30 minutes, respectively). Administration of exogenous T$_3$ led to partial suppression of basal TSH and TSH response to TRH stimulation, with basal/post-TRH TSH of 0.5/5 mIU/L, 0.07/1.1 mIU/L, and 0.04/0.2 mIU/L after 50, 100, and 200 μg of levo-triiodothyronine (L-T$_3$) daily, respectively. The alpha-subunit was normal (0.27 and

0.07 IU/L; ref, <0.8 IU/L) in 1995 and 2003. The red blood cell (RBC) zinc concentration was also normal at 158 µmol/L and 176 µmol/L of RBC (ref, 155-237 µmol/L of RBC) in 1994 and 1998. Results of other biochemical tests for assessing tissue sensitivity to thyroid hormones are shown in Table 1. Dual energy X-ray absorptiometry in 2003 revealed osteoporosis (T score: L1-L4, -2.8; neck of femur, -2.7). The alkaline phosphatase level was normal.

In 2003, the diagnosis of RTH was confirmed by genomic analysis of the THRβ gene. The patient was heterozygous for R429Q, with CGG to CAG missense mutation at codon 429 on exon 10. Screening of his asymptomatic 7-year-old son revealed the same mutation. Thyroid function tests showed inappropriate TSH secretion (Table 1).

After confirmation of the diagnosis of RTH, the patient was prescribed 3,5,3'-triiodothyroacetic acid (TRIAC) 0.35 mg twice per day and then increased to 4 times per day. His palpitations and insomnia improved with twice daily dosing of TRIAC, but dose escalation produced no further improvement. Pulse rate during TRIAC treatment ranged from 70 to 80 beats per minute. Free T_4 and TSH levels decreased, but FT_3 became significantly elevated (Table 2). Echocardiography showed that the calculated cardiac output decreased from 4.90 to 3.84 L/min after TRIAC therapy, primarily due to a decline in heart rate from 100 to 75 beats per minute. Stroke volume, ejection fraction, and diastolic function remained unchanged.

Serum FT_3 was measured by a two-step chemiluminescent microparticle immunoassay (Axsym; Abbott Laboratories, Abbott Park, US). Serum FT_4 was measured by a competitive immunoassay (ADVIA Centaur; Bayer, Abbott Park, US) and serum TSH by a two-site sandwich immunoassay (ADVIA Centaur). The inter- and intra-assay coefficient of variation were less than 10%. Leukocyte DNA was extracted from blood samples using standard methods. The THRβ gene was amplified using primers and conditions developed by Adams et al,[1] and the polymerase chain reaction product was then sequenced by an automatic DNA sequencer (3100 Genetic Analyzer, Applied Biosystems, Foster City, US).

Discussion

Resistance to thyroid hormone is a syndrome of impaired tissue responsiveness to thyroid hormones. Different body tissues may exhibit variable degrees of resistance to the action of thyroid hormones, depending on the number of THRβ they contain. Since the first description of RTH in 1967,[2] more than 600 cases have been reported.[3] Most patients have been identified on the basis of high or inappropriately normal serum TSH in association with elevated free thyroid hormone levels or by family screening.

Clinical differentiation of RTH from TSHoma can be difficult. A positive family history is suggestive of RTH, which is a dominantly inherited condition. Nevertheless, a negative family history does not exclude the diagnosis, as illustrated by our case. Before his son was born, all his family members had normal thyroid function tests. Resistance to thyroid hormone in our patient was probably due to a de novo mutation, which occurs in 16% of RTH cases.[3] Clinical features of hyperthyroidism may appear in both conditions. Some patients with generalised resistance to thyroid hormone (GRTH) exhibit a combination of hyperthyroid and hypothyroid features, or present with predominantly hypothyroid symptoms. Patients with TSHoma may have impaired visual fields or features of hypopituitarism. The utility of biochemical tests in differentiating TSHoma from RTH is also limited. Alpha-subunit level is elevated in only 66% of patients with TSHoma, and alpha-subunit to TSH molar ratio is elevated in only 80%.[4] Thyroid-stimulating hormone–secreting pituitary tumour may co-secrete growth hormone (16%), prolactin (11%), and gonadotrophins (1.4%), but 71% secrete TSH alone.[4] The TSH response to TRH is typically absent or blunted in 87% of patients with TSHoma[4] and intact or exaggerated in subjects with RTH, but the response of some patients with TSHoma may mimic that of RTH patients. This may be due to somatic mutations in the THRβ gene in the tumour cells.[5] Suppression of TSH by exogenous L-T_3 is impaired in both conditions. The presence of a pituitary adenoma on radiological imaging does not necessarily exclude the diagnosis of RTH, because up to 11.3% of adenomas are incidentalomas.[6]

To date, most cases of RTH have been due to mutations in the THRβ gene, and a genomic search for THRβ mutations, if successful, may help differentiate between a TSHoma and RTH, as illustrated by our case. However, RTH without a structural THRβ defect occurs in approximately 10% of the cases.[7] It has been postulated that a cofactor interacting with THR may be responsible for the manifestation of RTH.[7] Subjects with the same mutation may exhibit different phenotypes, suggesting modulation of thyroid hormone actions by other factors.[8]

An intriguing question is whether our patient has concomitant TSHoma and RTH. An example of this type of co-morbidity is a patient with Cushing's disease and generalised glucocorticoid resistance due to a germline mutation of the glucocorticoid receptor.[9] Nevertheless, among more than 600 RTH patients reported in the literature with mutations in the THRβ gene, none developed a TSHoma.[1,10,11] A patient with a TSH-secreting microadenoma and pituitary resistance to thyroid hormone (PRTH) has been reported, but no genetic analysis was performed.[12] In our patient, while the diagnosis of a concomitant TSHoma remained possible, it was not definitively substantiated by operative or immunohisto-chemical findings. Alternative explanations for his MRI findings are pituitary incidentalomas or pituitary hyperplasia. Pituitary enlargement compatible with hyperplasia has been reported in a patient with RTH, but only following inadvertent radioactive iodine therapy: regression occurred after supraphysiological doses of thyroxine replacement.[13] In our case, there was no prior history of ablative therapy, and his FT_3 levels remained elevated despite treatment with propylthiouracil.

In the English medical literature, only one plausi-ble case of RTH has been reported among Chinese. The diagnosis was based on compatible thyroid func-tion test results, normal alpha-subunit levels, and non-suppression of TSH by exogenous T_3. Family history was negative, and no mutations in THRβ or thyroid hormone receptor alpha genes could be identified.[7] The low incidence of RTH in Chinese may be due to underreporting or ethnic differences. The Chinese RTH family we report here is heterozygous for R429Q, with a CGG to CAG missense mutation at codon 429 of the THRβ, leading to replacement of arginine by glutamine. This mutation has been reported to impair co-repressor release in the TSH gene.[14]

Resistance to thyroid hormone has been subclassified into two phenotypes: GRTH and PRTH. Patients with GRTH are typically euthyroid or hypothyroid, whereas patients with PRTH are usually hypermetabolic. Symptoms of thyroid dysfunction are however relatively non-specific, and there is no good tissue marker for thyroid hormone actions. Differentiation into GRTH and PRTH can thus be difficult. Previous studies have failed to demonstrate an association between clinical features and the nature or location of a receptor mutation.[1] More recent studies suggest that specific mutations may be related to the PRTH phenotype.[15,16] An example is R429Q mutation. Of three patients with R429Q

mutation reported in the literature, two had PRTH[1] and one had GRTH.[17] Our index patient had palpitations, anxiety, osteoporosis, and increased cardiac output—features which are compatible with PRTH.

Treatment of RTH should be based on assessment of tissue sensitivity to thyroid hormones. In most cases of GRTH, tissue RTH appears to be adequately compensated for by the increase in endogenous thyroid hormones, and no treatment is required. For GRTH patients who have features of hypothyroidism, or who cannot compensate by increasing thyroid hormone secretion because of prior erroneous diagno-sis and ablative therapy, supraphysiological doses of thyroid hormones are recommended. Patients with PRTH who exhibit hyperthyroid features at tissue levels generally require treatment to reduce the elevated thyroid hormone levels. 3,5,3'-Triiodothyroacetic acid, a physiological metabolite of T_3, can reduce TSH and endogenous thyroid hormone levels and alleviate symptoms.[3] However, the efficacy of TRIAC is variable,[18] and its effect on heart rate is often minimal, probably because the decrease in thyroid hormone levels is offset by the intrinsic thyromimetic effect of the drug. 3,5,3'-Triiodothyroacetic acid also cross-reacts in T_3 assays. Glucocorticoids and dopaminergic drugs can suppress TSH secretion, but their side-effects and difficulty in administration preclude long-term use.[3] When treatment with TRIAC is unsuccessful, thyroid ablative therapy followed by replacement with physiological doses of thyroid hor-mone may be required. In our patient, low doses of TRIAC ameliorated his symptom of palpitations and reduced his cardiac output. Assessment of osteoporo-sis has not been possible because of the short duration of treatment.

References

1. Adams M, Matthews C, Collingwood TN, Tone Y, Beck-Peccoz P, Chatterjee KK. Genetic analysis of 29 kindreds with generalized and pituitary resistance to thyroid hormone. Identification of thirteen novel mutations in the thyroid hormone receptor beta gene. J Clin Invest 1994; 94:506-15.
2. Refetoff S, DeWind LT, DeGroot LJ. Familial syndrome combining deaf-mutism, stuppled epiphyses, goiter and abnormally high PBI: possible target organ refractoriness to thyroid hormone. J Clin Endocrinol Metab 1967;27:279-94.
3. Refetoff S. Resistance to thyroid hormone. In: Bardin CW. Current therapy in endocrinology and metabolism. 6th ed. St Louis: Mosby; 1997.
4. Shomali ME, Katznelson L. Medical therapy for gonadotroph and thyrotroph tumors. Endocrinol Metab Clin North Am 1999;28:223-40,viii.
5. Ando S, Sarlis NJ, Oldfield EH, Yen PM. Somatic mutation of TRbeta can cause a defect in negative regulation of TSH

in a TSH-secreting pituitary tumor. J Clin Endocrinol Metab 2001;86:5572-6.

6. Molitch ME. Pituitary incidentalomas. Endocrinol Metab Clin North Am 1997;26:725-40.

7. Pohlenz J, Weiss RE, Macchia PE, et al. Five new families with resistance to thyroid hormone not caused by mutations in the thyroid hormone receptor beta gene. J Clin Endocrinol Metab 1999;84:3919-28.

8. Weiss RE, Weinberg M, Refetoff S. Identical mutations in unrelated families with generalized resistance to thyroid hormone occur in cytosine-guanine-rich areas of the thyroid hormone receptor beta gene. Analysis of 15 families. J Clin Invest 1993;91:2408-15.

9. Karl M, Lamberts SW, Koper JW, et al. Cushing's disease preceded by generalized glucocorticoid resistance: clinical consequences of a novel, dominant-negative glucocorticoid receptor mutation. Proc Assoc Am Physicians 1996;108: 296-307.

10. Weiss RE, Refetoff S. Resistance to thyroid hormone. Rev Endocr Metab Disord 2000;1:97-108.

11. Brucker-Davis F, Skarulis MC, Grace MB, et al. Genetic and clinical features of 42 kindreds with resistance to thyroid hormone. The National Institutes of Health Prospective Study. Ann Intern Med 1995;123:572-83.

12. Watanabe K, Kameya T, Yamauchi A, et al. Thyrotropin-

producing microadenoma associated with pituitary resistance to thyroid hormone. J Clin Endocrinol Metab 1993;76: 1025-30.

13. Gurnell M, Rajanayagam O, Barbar I, Jones MK, Chatterjee VK. Reversible pituitary enlargement in the syndrome of resistance to thyroid hormone. Thyroid 1998;8:679-82.

14. Clifton-Bligh RJ, de Zegher F, Wagner RL, et al. A novel TR beta mutation (R383H) in resistance to thyroid hormone syndrome predominantly impairs corepressor release and negative transcriptional regulation. Mol Endocrinol 1998; 12:609-21.

15. Flynn TR, Hollenberg AN, Cohen O, et al. A novel C-terminal domain in the thyroid hormone receptor selectively mediates thyroid hormone inhibition. J Biol Chem 1994;269:32713-6.

16. Kahaly GJ, Matthews CH, Mohr-Kahaly S, Richards CA, Chatterjee VK. Cardiac involvement in thyroid hormone resistance. J Clin Endocrinol Metab 2002;87:204-12.

17. Taniyama M, Ishikawa N, Momotani N, Ito K, Ban Y. Toxic multinodular goitre in a patient with generalized resistance to thyroid hormone who harbours the R429Q mutation in the thyroid hormone receptor beta gene. Clin Endocrinol (Oxf) 2001;54:121-4.

18. Kunitake JM, Hartman N, Henson LC, et al. 3,5,3'-triiodothyroacetic acid therapy for thyroid hormone resistance. J Clin Endocrinol Metab 1989;69:461-6.