

[ CASE REPORT ]

## Congenital Primary Hypothyroidism with the Homozygous Nonsense Mutation P.K1374\* in the Thyroglobulin Gene and a Normal-sized Thyroid Gland on Levothyroxine Replacement

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### Abstract:

Thyroglobulin (*TG*) gene mutations cause thyroid dyshormonogenesis, which is typically associated with a congenital goiter. We herein report the case of a 64-year-old man with congenital primary hypothyroidism who had a normal-sized thyroid gland on levothyroxine replacement. He had short stature (−3.1 standard deviations) and mild intellectual impairment. Thyroid autoantibodies were all negative, and the serum *TG* levels were undetectable. Eventually, he was found to have the novel homozygous nonsense mutation p.K1374\* in the *TG* gene. The possibility of *TG* mutation should be considered for patients with congenital primary hypothyroidism and a very low serum *TG* level, regardless of the thyroid size.

**Key words:** thyroglobulin, gene mutation, congenital hypothyroidism, goiter

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### Introduction

Mutations in the thyroglobulin (*TG*) gene cause defective thyroid hormone synthesis, which usually results in congenital primary hypothyroidism (1-3). Patients with *TG* gene mutations typically develop a large goiter (dyshormonogenetic goiter), and a very low level of serum *TG* is a useful diagnostic marker for the presence of a *TG* mutation in patients with congenital primary hypothyroidism.

We herein report an adult case of childhood-diagnosed primary hypothyroidism associated with a novel homozygous nonsense mutation (p.K1374\*) in *TG*. In contrast to typical cases of dyshormonogenetic goiter, his thyroid gland was not enlarged on adequate levothyroxine (LT4) replacement.

### Methods

Serum levels of free triiodothyronine (T3), free thyroxine (T4), and thyrotropin (TSH) were measured using commer-

cial immunoassays (Roche Diagnostics, Tokyo, Japan). Serum anti-thyroid peroxidase (TPO) antibody, anti-*TG* antibody, and *TG* were measured using commercial electrochemiluminescence immunoassays (ECLusys<sup>®</sup> Anti-TPO, ECLusys<sup>®</sup> Anti-Tg, and ECLusys<sup>®</sup> Tg; Roche Diagnostics). Serum TSH receptor (TSHR) antibody was determined by a first-generation assay (TRAb Cosmic III; Cosmic Corporation, Tokyo, Japan). The thyroid volume was estimated by ultrasound, and we adopted the reference range of the thyroid volume (4.8-17.8 mL) reported by Suzuki et al. (4). The *TSHR* gene was analyzed for all coding sequences (exons 1-10) (5). A sequencing analysis of the *TG* gene in exons 1-48 was performed as previously reported (1). Genetic testing of the *TSHR* and *TG* genes was performed with the patient's consent.

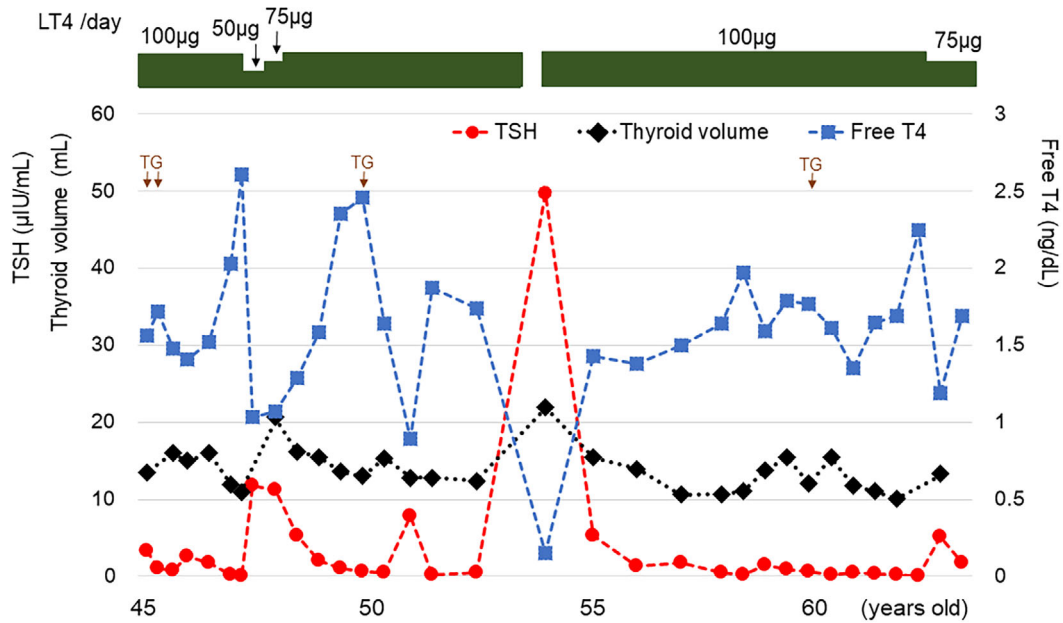
### Case Report

A 45-year-old man visited our clinic to continue thyroid hormone replacement therapy of LT4 at a dose of 100 µg/

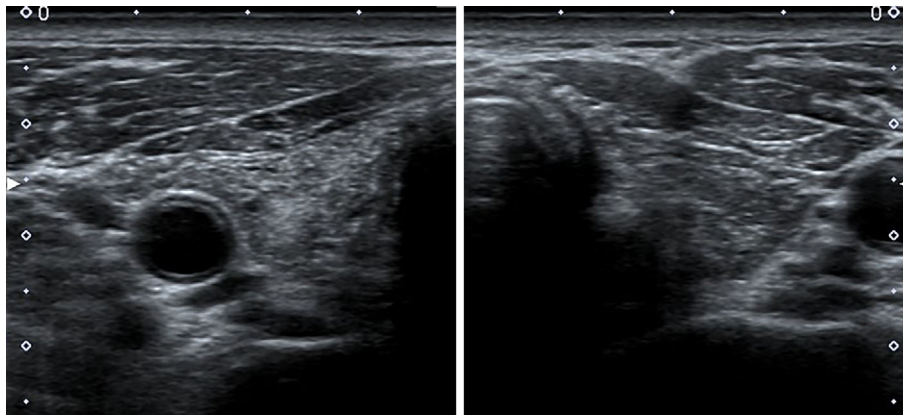
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**Figure 1.** Course of the thyroid function and thyroid volume. TG: measurement of serum thyroglobulin level (each measured value, <0.10 ng/mL).

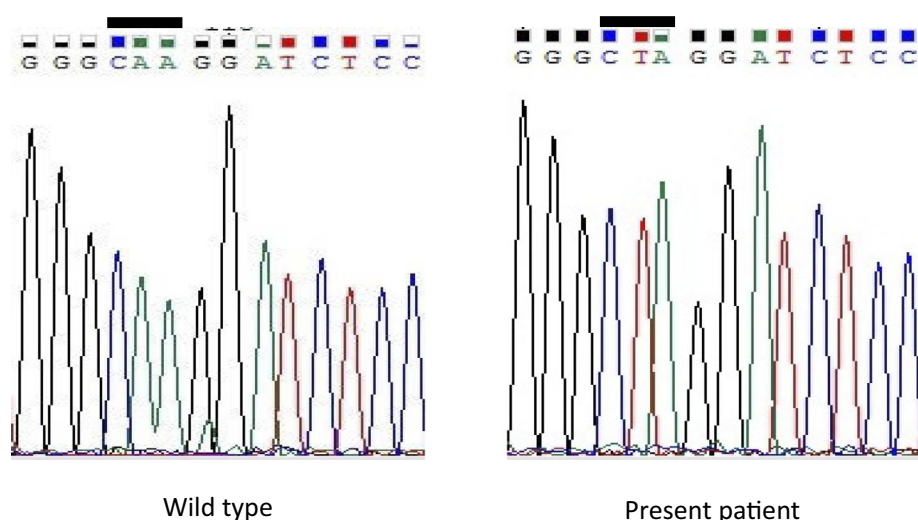


**Figure 2.** Ultrasonogram of the thyroid (transverse section). The thyroid size is normal. The echogenicity of the thyroid parenchyma is increased diffusely, showing heterogeneous echogenicity. Granular hyperechoic lesions are scattered throughout the thyroid gland.

day (Fig. 1). He had begun taking LT4 at seven years of age when he had first been diagnosed with primary hypothyroidism. There was no consanguineous marriage or thyroid disorder in his family history. He had mild intellectual impairment without hearing problems. He was 153 cm tall (−3.1 standard deviations) and weighed 65 kg. His blood pressure was 138/96 mmHg, and his pulse was regular at 85 beats/min. The thyroid gland was not palpable. The heart, lungs, and abdomen were unremarkable on a physical examination. Under treatment with 100 µg/day LT4, the serum levels of free T3, free T4, and TSH were 3.68 pg/mL (reference range, 2.20-4.30 pg/mL), 1.56 ng/dL (0.90-1.80 ng/dL), and 3.31 µIU/mL (0.220-3.30 µIU/mL), respectively. Serum antibodies against TPO, TG, and TSHR were 10.3 IU/mL (reference range, <16 IU/mL), 14.7 IU/mL (<28 IU/mL), and <0.1% (<10.0%), respectively. The serum TG level was <0.10 ng/mL (reference range, <25.0 ng/mL). Ultrasonography re-

vealed a normal-sized thyroid with diffusely heterogeneous echogenicity (estimated thyroid volume, 13 mL) (Fig. 2).

The patient continued LT4 replacement therapy. Nine months after the reduction of the LT4 dosage to 50-75 µg/day at 48 years of age, the serum levels of free T4 and TSH were 1.07 ng/dL and 11.24 µIU/mL, respectively. At that time, the thyroid volume slightly increased to 21 mL. Two months after the discontinuation of LT4 against medical advice at 54 years of age, the patient's serum levels of free T3 and free T4 were markedly reduced to 0.66 pg/mL and 0.15 ng/dL, respectively, whereas the serum TSH level had increased to 49.77 µIU/mL. The thyroid was slightly enlarged (estimated volume, 22 mL). The patient is currently still being treated with 75 µg/day LT4; at 64 years of age, the estimated thyroid volume was 13 mL, and the serum levels of free T3, free T4, and TSH were 3.10 pg/mL, 1.69 ng/dL, and 1.700 µIU/mL, respectively. He does not have any



**Figure 3.** Sequencing chromatograms of the thyroglobulin (*TG*) gene. A homozygous adenine to thymine transition at position 4177 was identified in the present patient (bar).

nodular lesions in the thyroid gland according to ultrasonographic follow-up.

The patient had childhood-diagnosed primary hypothyroidism, and serum thyroid autoantibodies were all negative. His thyroid gland was not enlarged on adequate LT4 replacement. Since a germline *TSHR* mutation prevents the growth of the thyroid due to resistance to TSH, we first suspected the presence of a *TSHR* mutation and therefore performed genetic testing of the *TSHR* gene. However, no *TSHR* mutation was identified. Despite performing 4 different time measurements, the serum TG levels were all undetectable (simultaneous serum TSH levels 3.31, 0.99, 0.06, and 0.583  $\mu$ IU/mL) without anti-TG antibodies. Therefore, genetic testing of the *TG* gene was performed next. A homozygous A to T transition (c.4177A>T) was identified, which resulted in a nonsense mutation at codon 1374 (p.K1374\*) (Fig. 3). This finding led to a diagnosis of primary hypothyroidism associated with a homozygous *TG* mutation at p.K1374\*.

## Discussion

The present patient had already been taking LT4 at the time of his first visit to our clinic, but he discontinued the treatment at 54 years of age, which led to the onset of apparent primary hypothyroidism within 2 months of the treatment discontinuation. He had been diagnosed with primary hypothyroidism at the age of 7, although the actual onset timing of his hypothyroidism is unknown. Primary newborn screening for congenital hypothyroidism has been adopted by most countries around the world, although mass screening for congenital hypothyroidism was not initiated in Japan until 1979, so the present patient did not undergo mass screening for congenital hypothyroidism at birth. However, he was suspected of having congenital primary hypothyroidism because of the lack of evidence of thyroidal autoimmunity, his short stature, and his intellectual impairment.

Regarding the etiology of hypothyroidism in the present patient, undetectable serum TG levels functioned as a clue for detecting the novel homozygous *TG* mutation. In general, a very low or undetectable serum TG level in patients without anti-TG antibodies suggests thyroid dysgenesis, resistance to TSH, or a *TG* mutation (6). The serum TG levels were all undetectable without anti-TG antibodies in the present patient. Unfortunately, the TSH-stimulated serum TG level during LT4 withdrawal could not be determined.

Thyroid dysmorphogenesis due to a *TG* mutation, which is one of the causes of congenital primary hypothyroidism, has an estimated prevalence of approximately 1:67,000 in Japan (2) and 1:100,000 in China (6). Although most known *TG* pathogenic variants typically cause congenital primary hypothyroidism, the actual clinical presentation varies from euthyroid to mild or severe hypothyroidism (1-3, 7). In the presence of a sufficient iodine supply, some patients with *TG* mutations can compensate for the impaired morphogenesis and generate sufficient thyroid hormones (8). To date, a total of 117 deleterious mutations have been identified in the human *TG* gene (9). *TG* mutations are inherited in an autosomal recessive manner, and affected individuals are either homozygous or compound heterozygous. The nonsense mutation p.K1374\* identified in the *TG* gene of the present patient is novel. The TG protein is composed of four structural and functional regions: N-terminal regions (regions I, II, and III) and a C-terminal region (ACHE-like domain) (10). Lysine 1374 is located in the hinge region (exon 20) between regions I and II.

Most *TG* mutations are associated with characteristic goiter (dysmorphogenetic goiter), which is very large with an elastic and soft consistency (2, 3). The goitrous phenotype can be attributed to long-term TSH stimulation and the accumulation of misfolded proteins in the endoplasmic reticulum of the thyrocytes (1, 11). The thyroid gland of the present patient was not enlarged under adequate LT4 replacement therapy during the follow-up period. There was slight

**Table. Previously Reported Cases of Homozygous or Compound Heterozygous *TG* Mutations without an Enlarged Thyroid.**

Case No.	Gene No.	Ethnic	Sex	<i>TG</i> mutation	Diagnosis of hypothyroidism		Last evaluation on LT4		[Reference]
					Age (year)	Thyroid size	Age (year)	Thyroid size	
1	1	Taiwanese	F	c.1348delT	neonatal	NA	3	no goiter	[12]
2	2	Taiwanese	F	p.Q1765*/ g.IVS3+2T>G	neonatal	NA	6	no goiter	
3	3	Taiwanese	F	p.R432*/c.1348delT	neonatal	no goiter	NA	NA	
4	4	Taiwanese	M	g.IVS3+2T>G/ c.6047delA	neonatal	no goiter	NA	NA	
5	5	French	F	c.3788-3789insT/ g.IVS19+3_+4delAT	neonatal	enlarged right lobe normal left lobe	3	NA	[13]
6	6	Turkish	F	p.Q630*	neonatal	upper limit of normal	NA	NA	[14]
[6]		Turkish	M	p.Q630*	neonatal	enlarged thyroid	NA	NA	
7	7	Turkish	F	p.W637*	14 y	hypoplasia	NA	NA	
8		Turkish	F	p.W637*	15 y	hypoplasia	NA	NA	
9	8	Chinese	F	c.274+2T>G	neonatal	normal	7.3	normal	[6]
10		Chinese	M	c.274+2T>G	neonatal	normal	5	normal	
11		Chinese	M	c.274+2T>G	neonatal	NA	3.7	normal	
12		Chinese	M	c.274+2T>G	neonatal	enlarged thyroid	1.5	normal	
13	9	Chinese	F	c.6391delTTGT	neonatal	normal	2.5	normal	
14	10	Chinese	F	c.6782+1delG	neonatal	enlarged thyroid	5	normal	
15		Chinese	F	c.6782+1delG	neonatal	normal	2.5	normal	
16	11	Chinese	M	p.P1012L	neonatal	normal	5.5	normal	
17	12	Chinese	M	p.T1111R/p.R2585W	neonatal	NA	2.7	normal	
18	13	Chinese	M	p.C1051*/p.P1012L	neonatal	normal	2.0	normal	
19	14	Japanese	M	p.K1374*	7 y	NA	64	normal	present case

Cases 6 and [6], and Cases 7 and 8 are siblings.

NA: not available

temporary enlargement of the thyroid gland after the dosage reduction and discontinuation of LT4, indicating some effect of LT4 treatment on the thyroid gland. However, *TG* mutation-associated goiters are often remarkably large and display gradual, continuous growth (11). In the present patient, not only long-term LT4 replacement therapy but also other factors may have prevented the progressive enlargement of the thyroid.

Several *TG* mutations associated with congenital primary hypothyroidism and normal-sized or hypoplastic thyroid have been reported (6, 12-14) (Table). Despite involving the same *TG* mutations, the phenotype of the goiter differs among cases (14). The cause of such phenotypic differences in the goiter remains unclear. The majority of cases with normal-sized or hypoplastic thyroid were found to have congenital primary hypothyroidism in the neonatal period. Although patients with a *TG* synthesis defect generally present with a congenital goiter or goiter appearing shortly after birth, the absence of an obvious goiter may be partly ascribed to the early detection and treatment of congenital hypothyroidism by neonatal screening in those reported cases (15).

Mutations in *TPO*, *SLC26A4/PDS*, and *DUOX2* are major causes of dyshormonogenetic goiter. Recently, normal-sized

or hypoplastic thyroid gland in congenital primary hypothyroidism associated with mutations of *TPO*, *SLC26A4/PDS*, or *DUOX2* has been reported (16-18). Since those *TPO* or *SLC26A4/PDS* gene mutations are found not only in goitrous patients but also in non-goitrous patients (16, 17), a second additional genetic or epigenetic hit in the thyroid tissue is assumed to contribute to the small (or at least not obviously enlarged) thyroid (16). The present patient might have had some genetic or epigenetic factors that inhibited the development of a typical goitrous phenotype.

*Tg* mutations have also been described in animals, such as Afrikaner cattle, Dutch goats (mixed Saanen and dwarf goats), *cog/cog* mice, *WIC-rdw* rats, and Wistar Hannover GALAS rats (9). All animal models of congenital primary hypothyroidism due to a *Tg* mutation, except for *WIC-rdw* rats, develop goitrogenesis. *WIC-rdw* rats, by contrast, present with a hypoplastic thyroid gland despite elevated circulating TSH and reduced serum thyroid hormone levels, suggesting that the mutant proteins in the *WIC-rdw* rat may be cytotoxic for thyroid growth and proliferation (19, 20). The influence of the novel p.K1374\* mutation on thyrocytes remains to be elucidated.

Many adult patients with *TG* mutation present with nodular thyroid hyperplasia (3). A high incidence of thyroid can-



cer is also a characteristic of long-standing goiters with *TG* mutations, and *TG* mutations are associated with the development of thyroid cancer (21). At present, our patient has no nodular lesions in the thyroid gland according to ultrasonographic follow-up.

Molecular genetic *TG* studies are usually performed for goitrous patients with very low serum TG levels. However, in patients with congenital primary hypothyroidism and a very low serum TG level, *TG* mutations should be considered, regardless of the thyroid size and lack of a typical goitrous phenotype.

**The authors state that they have no Conflict of Interest (COI).**

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