



Ultrasonographic Development and Progression of a Thyroid Nodule in a Girl with *TPO*-Mutated Dyshormonogenesis during Levothyroxine Supplementation

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Dyshormonogenesis is caused by genetic defects in thyroid hormone synthesis. The most common form is thyroid peroxidase (TPO) deficiency. Clinically variable degree of hypothyroidism and thyroid gland enlargement depend on the severity of the defect. We report 22-year-old female with congenital hypothyroidism (CH) caused by TPO deficiency. Since goitrous CH was diagnosed at 8-year-old, L-thyroxine has been supplemented. Her goiter size was fluctuated according to the compliance on the medication. After 3.5 years of medication, ultrasonography found solid nodule, which was interpreted as nodular hyperplasia pathologically. The nodule size did not change during recent 10 years except peripheral calcification. Genetic analysis using NGS for CH revealed compound heterozygous variants of c.2757del;p.(Met921Trpfs*53) and c.1580G>T;p.(Trp527Leu) in TPO gene. The first variant inherited from asymptomatic mother is pathogenic frame-shift mutation associated with stop codon, and the second one inherited from her asymptomatic father is predicted as deleterious in bioinformatics software program. From this case, we have observed that nodular change and calcification developed from diffuse enlarged goiter in dyshormonogenetic patient. Early molecular diagnosis of dyshormonogenesis and TSH suppression is important for not developing thyroid nodules in case of childhood euthyroid goiter without thyroid autoantibodies.

Key Words: Congenital hypothyroidism, Thyroid dyshormonogenesis, Thyroid peroxidase, TPO

Introduction

Early hormonal treatment of congenital hypothyroidism (CH) can prevent mental retardation. The frequent etiology of CH is thyroid dysgenesis, which includes agenesis, ectopy, and hypoplasia, followed by defects in thyroid hormone biosynthesis (dyshormonogenesis).¹⁾ Reduced thyroxine synthesis during dyshormonogenesis induces increased thyrotropin (TSH) secretion through reduced negative feedback of

thyroxine. Consequently, affected patients has congenital goiter, otherwise postnatal goiter without L-thyroxine (LT4) supplement.

Even if CH is diagnosed in the newborn period based on the levels of TSH and free T4, etiologic diagnosis is not required before starting LT4 replacement because the management remains the same, irrespective of the cause. At the age of 3 years, reevaluation of CH patients based on hormone levels and thyroid imaging, should be performed after stopping LT4 for 4 to 6 weeks.²⁾ Scintigraphy is useful for the diag-

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nosis of ectopic thyroid. For the diagnosis of TPO deficiency, the perchlorate discharge test is useful,³⁾ but is not commonly used due to the risk of radiation exposure. In addition, a genetic diagnosis of CH is not essential for determining the treatment because treatment is always LT4 replacement. However, molecular diagnosis of these heterogeneous entities could provide prognostic insights and reliable genetic counseling along with more specific targets for molecular therapies.⁴⁾ Panel sequencing based on next-generation sequencing (NGS) is useful in cases of dyshormonogenesis.⁵⁾

In patients with dyshormonogenesis, the thyroid peroxidase (*TPO*) gene is the most commonly mutated.⁶⁾ However, recent studies from China, Thailand, and Korea have reported that dyshormonogenesis is more common than dysgenesis, and dual oxidase2 (*DUOX2*) is the most commonly mutated gene.⁷⁻⁹⁾ Multinodular goiters have also been reported to exhibit dyshormonogenesis.¹⁰⁾ Herein, we report the

sequential changes in the ultrasonographic appearance of a solitary thyroid nodule during LT4 supplementation in a patient who was genetically confirmed to have a *TPO* mutation using NGS.

Case Report

An 8.2-year-old girl visited our clinic because of thyroid enlargement. In her medical history, neck swelling was noted during the newborn period, but further investigation was not performed because of a normal newborn screening test. Upon examination, her vital signs were nonspecific. Thyroid function test was normal as follows: free T4 0.91 ng/dL (normal, 0.7–1.48), TSH 4.68 uIU/mL (normal, 0.35–4.94) (Table 1). Thyroid autoantibodies were not detected. ^{99m}Tc scan showed a diffusely enlarged thyroid gland with increased uptake, and ultrasonography (US) showed diffuse thyroid enlargement with heterogeneous echogenicity (Fig. 1). LT4 was administered as

Table 1. Laboratory findings and clinical course

Age (yr)	8.2	11.0	11.6	13.6	14.4	18.1	22.1
Free T4	0.91	1.15	0.65	1.36	0.9	0.83	0.97
TSH	4.68	1.17	8.68	0.72	8.1	2.87	2.35
Tg							202.3
Goiter	Moderate	Mild	Moderate	Mild	Severe	Mild	Mild
LT4	(+)	(-)	(+)	(-)	(+)	(+)	(+)
US	DE	Decreased	DE with SN			DE with SN	DE with calcified SN
Course			FNAB				NGS

Normal levels: free T4 (0.7–1.48 ng/dL), TSH (0.35–4.94 uIU/mL), thyroglobulin (0.2–70 ng/mL).

DE: diffuse enlargement, FNAB: fine needle aspiration biopsy, LT4: Levothyroxine, NGS: next-generation sequencing, SN: solid nodule, Tg: thyroglobulin, US: ultrasonography

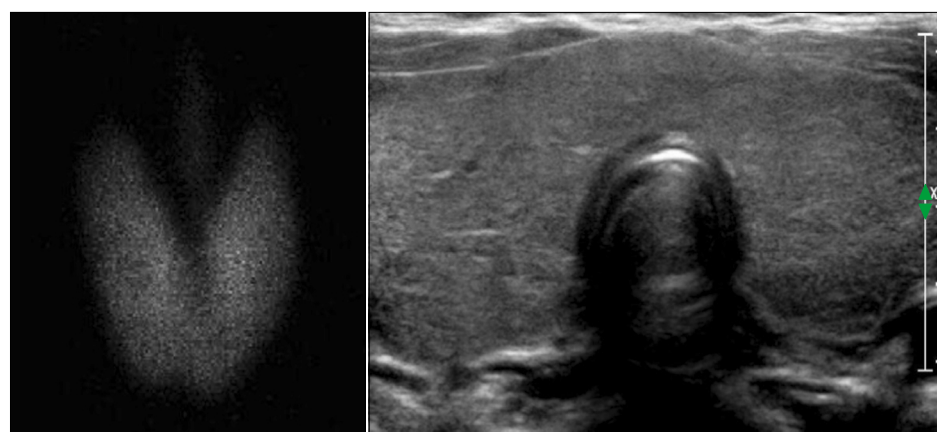


Fig. 1. Initial thyroid examination at 8.2 years of age, shows diffuse enlargement with increased uptake in the ^{99m}Tc scan (left) and diffuse thyroid enlargement with heterogeneous echogenicity in ultrasonography (right).

dyshormonogenetic CH was suspected. The goiter decreased in size; however, it did not disappear completely. At 11.5 years of age, the goiter size increased again and thyroid function became mildly hypothyroid after discontinuation of LT4 for 6 months (Table 1). US showed diffuse enlargement of the thyroid gland with heterogeneously decreased echogenicity, as well as an isoechoic solid nodule of size 1.8×1.7×1.6 cm with a peripheral halo in the inferior aspect of the right thyroid. The nodule was reported to be a benign follicular lesion with nodular hyperplasia on fine-needle aspiration biopsy (FNAB) (Fig. 2). After resuming LT4, the goiter size decreased again, and the euthyroid state was maintained until the patient stopped taking medication by her own accord at 13.5 year of age. Thereafter, the goiter size changed according to the functional status of the gland. At 18 years of age, the nodule size was 1.1×0.9 cm in the same location. At 22 years of age, peripheral calcification appeared around the isoechoic solid nodule previously reported,

without a change in the size (Fig. 3). The radiologist reported category 3 malignancy risk of the nodule according to the Korean Thyroid Imaging Reporting and Data System (K-TIRADS). NGS panel for CH reported two variants of the *TPO* gene and one variant of the dual oxidase maturation factor (*DUOX2*) gene. The first variant of *TPO*, c.[2757del](p.[Met921Trpfs*53]) was a pathologic variant from the asymptomatic father, while the second variant, c.[1580G>T](p.[Trp527Leu]) was likely pathogenic variant from the asymptomatic mother. The heterozygote variant of the *DUOX2* gene with c.[535T>C](p.[Tyr179His]) is value of uncertain significance. The patient is scheduled to undergo regular US to monitor the progression of the thyroid nodule.

Discussion

Thyroid hormone biosynthesis involves the following stages: iodide transport, thyroglobulin synthesis, oxi-

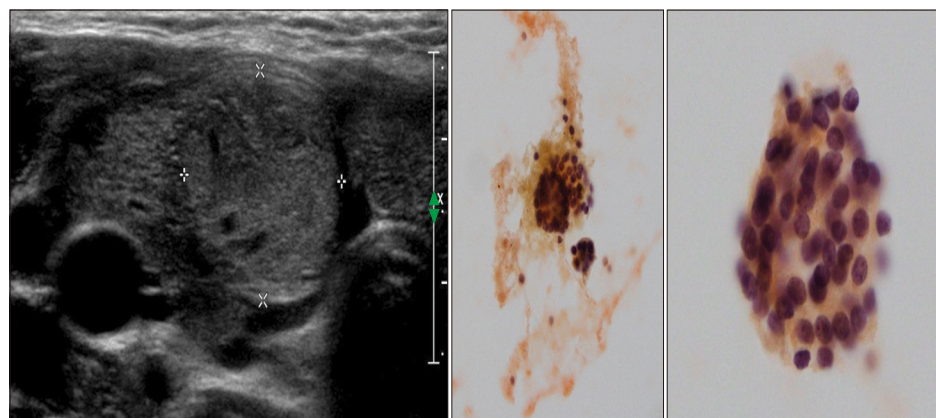


Fig. 2. At 11.6 years of age, an isoechoic solid nodule with a peripheral halo is visible in the right thyroid gland with diffusely enlarged and heterogeneously decreased echogenicity of the entire gland (left). Aspiration biopsy shows a benign follicular lesion favoring nodular hyperplasia (middle and right).

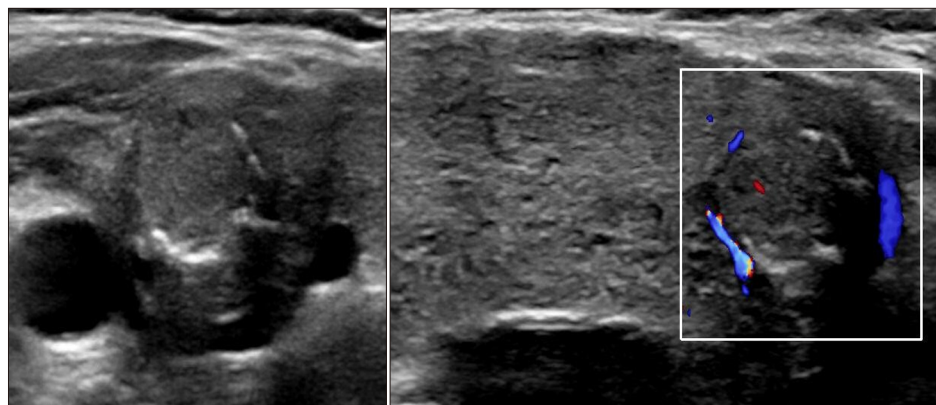


Fig. 3. At 22 years of age, a well-defined isoechoic solid nodule with peripheral calcification in the right inferior thyroid can be seen, along with an enlarged thyroid gland with diffuse heterogenous echogenicity and increased vascularity.

dation and organification, H_2O_2 generation, coupling of iodinated tyrosines within thyroglobulin, endocytosis of colloids and hormone release, and recycling of iodide. Defects in biosynthetic pathway cause approximately 10–15% cases of CH. Seven genes are known to cause dyshormonogenesis.⁵⁾

The most common mutated gene in thyroid dyshormonogenesis is *TPO* gene. A study from Slovenia, Bosnia, and Slovakia reported that up to 46% of thyroid dyshormonogenetic participants had a *TPO* gene mutation.¹¹⁾ Since the first report of a biallelic mutation of *TPO* in 1992 (6), 188 (professional ver.) different *TPO* mutations have been identified in HGMD[®] Professional 2021.4 (<http://www.hgmd.cf.ac.uk>). Most patients with *TPO* mutations have congenital goiter. They show profound hypothyroid state, high uptake of radioactive iodine on scintigraphy, elevated thyroglobulin levels, and positive perchlorate discharge test.⁵⁾ However, normal development without goiter was reported in a sibling who received LT4 immediately after birth compared to two other goitrous siblings who received LT4 from 6 months of age.³⁾ Our patient also showed diffuse goiter with increased uptake on ^{99m}Tc scintigraphy and elevated thyroglobulin levels. Unfortunately, the perchlorate discharge test was not carried out because of the risk of radiation exposure.

TPO mutated CH has autosomal recessive pattern of inheritance, although monoallelic *TPO* defect comprises approximately 20%. Maybe unconfirmed cryptic mutations in introns or regulatory regions can explain this phenomenon.¹⁾ The NGS panel for CH in our case revealed three relevant variants, two in *TPO* and one in *DUOX2*. The first *TPO* variant, c.[2757del] (p.[Met921Trpfs*53]), inherited from an asymptomatic father, is a well-known pathogenic frameshift variant that is associated with a stop codon. The incidence of the second *TPO* variant, c.[1580G>T](p.[Trp527Leu]), inherited from an asymptomatic mother, is 0.0058% in the population database (gnomAD); this has been reported as likely pathogenic. The incidence of the variant of the *DUOX2* gene, c.[1580G>T](p.[Trp527Leu]), has been reported to be 0.056% in the population database (gnomAD) or 0.14% (KRGDB). This variant is also classified as value of uncertain significance but is pre-

dicted to be deleterious in silico (SIFT, Polyphen2, MutationTaster), and has been suspected to be related to the disease.

Some investigators have suggested that DNA collection is necessary for future mutational analysis if dyshormonogenetic goiter is suspected in a patient based on the identification of the thyroid gland in US. Genetic counseling, differentiating between transient and permanent forms of CH, and predicting iodine supplementation in iodotyrosine deiodinase (*IYD*) gene defects are possible after molecular diagnosis in patients with dyshormonogenesis.¹⁾ However, the standard treatment remains LT4 replacement.

In a clinicopathological study of 56 cases, 75% of dyshormonogenetic goiters occurred before the age of 24 years. The thyroid glands were enlarged, multinodular, and cellular nodules with solid and/or microfollicular patterns were the most common microscopic alterations. In 18% of cases, the degree of hyperplasia and atypia led to a misdiagnosis of follicular, papillary, or undifferentiated carcinoma.¹⁰⁾ Some investigators have emphasized regular tracing and LT4 medication to prevent thyroid stimulation from elevated TSH.⁴⁾ Our patient exhibited diffuse goiter with normal thyroid function without autoantibodies at the age of 8 years. Unfortunately, normal neonatal screening test results prevented further investigations in this case. Goiter decreased after 3.5 years of follow-up with LT4 medication. However, the goiter aggravated and thyroid function slightly deteriorated after 6 months' off treatment. A diffusely enlarged thyroid gland with solid nodules was observed via US. The pathological diagnosis of the aspirated specimen was a benign follicular lesion favoring nodular hyperplasia rather than papillary carcinoma, although it was non-typical.

As childhood thyroid nodules are more often malignant (up to 25%), a more aggressive management approach is justified. Cases of thyroid cancer have been reported in CH due to dyshormonogenesis, usually with follicular histology.¹²⁾ Several authors have reported that multinodular goiters associated with dyshormonogenesis can be malignant or proceed to real malignancy. Minimally invasive follicular thyroid carcinoma was reported in *TPO* mutated patient with large

multinodular goiter during a 29-year follow-up. However, genetic or environmental factors may have been implicated in this case because the serum TSH levels were not elevated.¹³⁾ The largest data from 20 *TPO*-deficient patients followed-up for 43 years showed that 61% developed multinodular goiter, thyroidectomy was performed in 24%, and invasive follicular thyroid carcinoma was found in one case. Moreover, elevated lifetime TSH levels was not associated with goiter occurrence.¹⁴⁾ A biallelic thyroglobulin (Tg) mutation was reported in two siblings aged 25 and 31 years. They had similar histories of LT4 medication since childhood, subtotal thyroidectomy for pressure symptoms, and recurrent goitrous hypothyroidism. The older sibling developed a metastasis from microscopic FVPTC after 8 years. In contrast, the younger sibling had a benign dysmorphogenetic goiter on FNAB. The older sibling was frequently noncompliant with LT4. Therefore, malignant transformation of follicular cells by prolonged TSH stimulation was suggested.¹⁵⁾ Another frameshift *TPO* mutated case with complex pathology underwent thyroidectomy.¹⁶⁾ If potential neoplastic changes are truly related to atypical nucleus in dysmorphogenesis, rapid genetic diagnosis would be beneficial for patients with dysmorphogenesis.

TPO employs H_2O_2 as an oxidizing agent for iodine. Timely and spatially regulated activity of NADPH oxidase enzymes (NOX/DUOX) can produce H_2O_2 , a reactive oxygen species (ROS), for various physiologic actions. On the other hand, improper release of ROS from NOX4 is associated with pathologic thyroid tumorigenesis.¹⁷⁾ Therefore, the suppression of TSH may be justified with sufficient LT4 to reduce oxidative stress.

Until now, no clear causal relationship has been identified between CH and thyroid cancer. The most widely known causative genes for thyroid cancer (ex. *RET*, *BRAF*, *RAS*, *TP53* etc.) and the most detected genes for CH (ex. *DUOX2*, *TSHR*, *DUOX2*, *TPO*, *SLC26A4* etc.) are very different, in some reports, *TPO*, *TG*, and *TSHR* mutations that are common in CH have been found in the tissues of thyroid cancer. But, it is difficult to identify whether the cause of malignancy

is directly due to genetic mutation or the chronic increase of TSH and high oxidative stress in CH.¹⁸⁾ Traditional risk factors of thyroid cancer in children are known to be iodine deficiency, autoimmune thyroid disease, and radiation exposure until now. Therefore, somatic mutation and epigenetics contribute much more to the occurrence of malignancy in CH than genetic mutations themselves, because the genes responsible for thyroid cancer are very different from those responsible for CH.

According to the established guidelines for thyroid nodules in pediatric patients, several sonographic features are helpful for selecting patients for FNAB evaluation. Microcalcification, lymph node changes, growing nodule size with LT4 medication, and increased intranodular vascularization on the color Doppler are the sonographic features associated with the increased possibility of malignancy.¹²⁾ Other sonographic findings of higher malignancy risk in pediatric thyroid nodules included taller-than-wide shaped large solid parenchyma with speckled calcification and absence of smooth margin.¹⁹⁾

In our case, a solid nodule was detected at 11.5 years of age, after 3.5 years of LT4 treatment under the impression of dysmorphogenetic CH, and peripheral calcification around the isoechoic nodule appeared another 10 years later without change in the nodule size. FNAB was not performed during the last follow-up US at 22 years of age because the nodule size and calcification pattern had not changed. The indication for FNAB in cases of category 3 is a nodule size of more than 1.5 cm in K-TIRADS.²⁰⁾ However, frequent follow-up monitoring of the nodule over time is necessary.

In conclusion, early molecular diagnosis and sufficient TSH suppression are important management in childhood dysmorphogenesis to prevent thyroid nodule formation. As with the currently recommended guideline, it would be sufficient to perform US every two to three years and FNAB if necessary in patients with CH (especially dysmorphogenetic goiter).

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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