

Goiter in a 6-year-old patient with novel thyroglobulin gene variant (Gly145Glu) causing intracellular thyroglobulin transport disorder: Correlation between goiter size and the free T3 to free T4 ratio

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Highlights

- We report a child with a novel Gly145Glu thyroglobulin gene variant.
- Gly145Glu caused an intracellular thyroglobulin transport disorder and enlarged goiter.
- A high freeT3/freeT4 ratio and large goiter compensate for impaired hormone synthesis.

Abstract. Thyroglobulin gene abnormalities cause thyroid dyshormonogenesis. A 6-yr-old boy of consanguineous parents presented with a large goiter and mild hypothyroidism (thyroid-stimulating hormone [TSH] 7.2 μ IU/mL, free T3 [FT3] 3.4 pg/mL, free T4 [FT4] 0.6 ng/dL). Despite levothyroxine (LT4) administration and normal TSH levels, the goiter progressed slowly and increased rapidly in size at the onset of puberty. Thyroid scintigraphy revealed a remarkably high ¹²³I uptake of 75.2%, with a serum thyroglobulin level of 13 ng/ml, which was disproportionately low for the goiter size. DNA sequencing revealed a novel homozygous missense variant, c.434G>A [p.Gly145Glu], in the thyroglobulin gene. Goiter growth was suppressed by increasing the LT4 dose. Thyroidectomy was performed at 17-yr-of-age. Thyroglobulin analysis of the thyroid tissue detected mutant thyroglobulin present in the endoplasmic reticulum, demonstrating that thyroglobulin transport from the endoplasmic reticulum to the Golgi apparatus was impaired by the Gly145Glu variant. During the clinical course, an elevated FT3/FT4 ratio was observed along with thyroid enlargement. A high FT3/FT4 ratio and goiter seemed to be compensatory responses to impaired hormone synthesis. Thyroglobulin defects with goiter should be treated with LT4, even if TSH levels are normal.

Key words: thyroglobulin, goiter, dyshormonogenesis, levothyroxine, FT3/FT4 ratio

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Introduction

Thyroglobulin (TG) is a large secretory glycoprotein synthesized in the thyroid gland. TG functions as a matrix for thyroid hormone synthesis and storage of inactive forms of thyroid hormones and iodine (1). TG gene abnormalities can cause thyroid dyshormonogenesis with an autosomal recessive inheritance (2). The first family with a TG gene variant was reported in 1991 by Ieiri *et al.* (3). Subsequently, 227 human TG gene variants have been identified (4). The prevalence of TG defects is approximately 1 in 100,000 newborns in China (5) and 1 in 67,000 newborns in Japan (6). Most patients with TG gene variants have congenital goiters or goiters appearing in childhood (7). Their biochemical profile is usually characterized by relatively low TG levels for the thyroid size, high serum thyroid-stimulating hormone (TSH) levels, high iodine uptake, normal perchlorate discharge test results, low serum free T4 (FT4) levels, and variable serum free T3 (FT3) levels (7, 8). Some patients reportedly have large goiters without TSH elevation (9–11); treatment for these patients has not been determined.

Here, we report the case of a patient with a large goiter and mild hypothyroidism. The patient harbored a novel homozygous missense variant, c.434G>A [p.Gly145Glu], in the TG gene, which impaired the transport of the mutant TG from the endoplasmic reticulum (ER) to the Golgi apparatus. Despite levothyroxine (LT4) administration and normal TSH levels, the goiter gradually increased in size. During his clinical course, the elevation of the FT3/FT4 ratio was observed along with thyroid enlargement. A high FT3/FT4 ratio and goiter seemed to be compensatory responses to impaired hormone synthesis.

Materials and Methods

TG gene analysis

Informed consent for DNA analysis was obtained from the patient, his parents, and his brother. This study was performed in accordance with the regulations of the Ethical Committee of Dokkyo Medical University. The TG gene was sequenced using genomic DNA extracted from peripheral white blood cells of the patient, his parents, and his brother. The primers and PCR conditions have been previously described (9).

Endoglycosidase H (Endo H) treatment

The thyroid gland obtained during surgery was quickly frozen in liquid nitrogen and stored at -80°C . Thyroid tissue was analyzed at Dokkyo Medical University, Japan. Approximately 20 mg of thyroid tissue was homogenized in 100 μL Tris buffer (10 mmol/L, pH 8.0) that contained a cocktail of protease inhibitors (Complete Protease Inhibitor Cocktail Set; Roche, Mannheim, Germany) using 1.5 mL Eppendorf

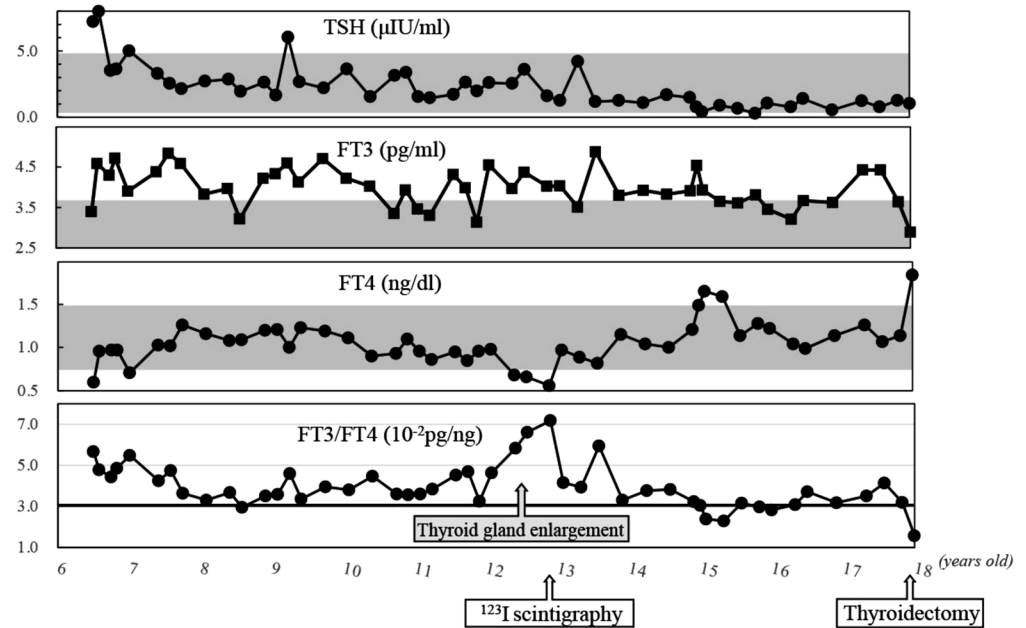
tubes with specialized pestles (Funakoshi Co., Ltd., Tokyo, Japan). The homogenate was centrifuged at $18,000 \times g$ twice for 30 min each time. The supernatant was used as thyroid tissue extract. TG contents in the homogenate were measured using an RIA kit (Eiken Chemical Co., Tokyo, Japan). Aliquots of the thyroid extract containing 2 μg TG were digested with 0.3 mU/L Endo H (Roche, Basel, Switzerland) and then analyzed by 4–15% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (9).

Case Presentation

The patient was born as the second child to consanguineous Japanese parents. His parents were cousins with no history of thyroid disease. Neonatal mass screening did not reveal any abnormalities. He visited our hospital at the age of 6 yr because of goiter. He was 107.3 cm tall (-1.88 standard deviation [SD]), weighed 16.2 kg (-1.48 SD), and displayed normal development. He had a large goiter (grade 4 according to the Shichijo classification) that was elastic and soft. There were no suspicious findings of hypothyroidism, such as skin dryness, coldness, or edema. Blood analysis indicated slightly high TSH (7.2 $\mu\text{IU/mL}$, normal range 0.35–4.94 $\mu\text{IU/mL}$), normal FT3 (3.4 pg/mL, normal range 1.71–3.71 pg/mL), low FT4 (0.6 ng/dL, normal range 0.70–1.48 ng/dL), and normal TG (13 ng/mL, normal range < 30 ng/mL). In the thyrotropin-releasing hormone stimulation test, the peak TSH value was 33.5 $\mu\text{IU/mL}$, and total T3 was elevated from 2.03 to 2.67 ng/mL (normal range 0.84–1.52 ng/mL). Tests for thyroid autoantibodies against TG and thyroid peroxidase were negative. Thyroid ultrasonography showed a diffusely enlarged thyroid gland but no nodules.

Based on the above results, we suspected that the patient had mild hypothyroidism, which caused the goiter. LT4 was administered, and his elevated TSH and decreased FT4 levels were within the reference values (Fig. 1). However, ultrasound determined goiter size slowly increased. The LT4 dosage was gradually increased to suppress thyroid growth, although the TSH level remained within the reference values. At the onset of puberty at age 12, the thyroid gland rapidly increased in size (grade 5 Shichijo classification) and partially penetrated the mediastinum (Fig. 2). Serum FT4 levels decreased despite normal TSH and high FT3 levels, and a sharp increase in the FT3/FT4 ratio was observed (Fig. 1).

To determine the cause of the enlarged thyroid gland, LT4 was discontinued for two weeks, and thyroid scintigraphy using radiolabeled iodine (^{123}I) was performed. ^{123}I was diffusely taken up by the entire thyroid gland. The uptake rate at 3 h was markedly high (75.2%). The perchlorate discharge test result was negative. Blood samples taken two weeks after LT4 was discontinued showed a markedly decreased FT4 level of 0.56 ng/mL, despite a normal TSH level of 1.60 μIU



Thyroglobulin (ng/ml)	25	13	13	8		8.8	<5.0	<5.0	<5.0	
Thyroid volume calculated by US (ml)	15.8		20.7	29.8	32	*	*			
Thyroid volume measured by CT (ml)							60.2	61	72.5	72.8
* Total volume cannot be measured due to mediastinal invasion										
LT4 (µg/day)			15	20	25	30	50	75	50	125

Fig. 1. Clinical course and thyroid hormone change. The gray range in the graph indicates the respective adult reference values. FT3, free T3; FT4, free T4; US, ultrasonography; CT, computer tomography; LT4, levothyroxine.

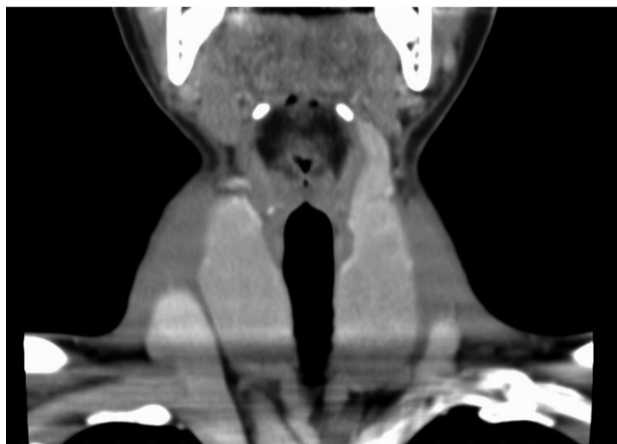


Fig. 2. Contrast-enhanced computer tomography image of a coronary section of the neck of the patient at 17-yr-of-age.

mL and a relatively high FT3 level of 4.02 pg/mL. The FT3/FT4 ratio increased to approximately 7 (10⁻² pg/ng).

We suspected a TG defect based on progressive goiter growth, high iodine uptake by the thyroid gland, low TG levels disproportionate to the thyroid size, and decreased FT4 levels. We analyzed the TG gene at Dokkyo Medical University after obtaining informed consent from the patient, parents, and older brother. DNA sequencing identified a novel homozygous

missense TG gene variant, NM_003235: c.434G>A [p.Gly145Glu] (**Fig. 3**). His parents and older brother were heterozygous for this variant, did not have a goiter, and had normal thyroid function. Since the patient's goiter was considered a compensatory response to impaired hormone synthesis, LT4 administration was increased from 30 to 50 µg and finally to 75 µg per day. The goiter was too large to allow accurate measurement of the thyroid gland using ultrasound. However, after increasing the LT4 dose to 50 µg per day, the thickness of the thyroid isthmus decreased from 7.6 to 4.9 mm, and the thyroid gland visually shrank. Blood tests revealed elevated FT4 levels, whereas the FT3/FT4 ratio was decreased (**Fig. 1**). When the LT4 dose was increased to 75 µg per day to further reduce the size of the thyroid gland, symptoms of hyperthyroidism appeared; these included excessive sweating and irritability. The LT4 dose was returned to 50 µg and maintained at this level.

The patient underwent thyroidectomy at 17-yr-of-age because carcinogenesis rates are high in patients with TG defects, and his goiter was so large that the full extent of the gland could not be ascertained by ultrasound alone. The thyroid gland partially reached the mediastinum, which required an open chest for removal if the goiter grew further. The excised thyroid gland weighed 90 g. Thyroid histopathology showed that the thyroid gland had sparse colloids with some solid parts or papillary growth. No distinct cellular atypia was noted (**Fig. 4**). TG analysis of thyroid tissue was performed

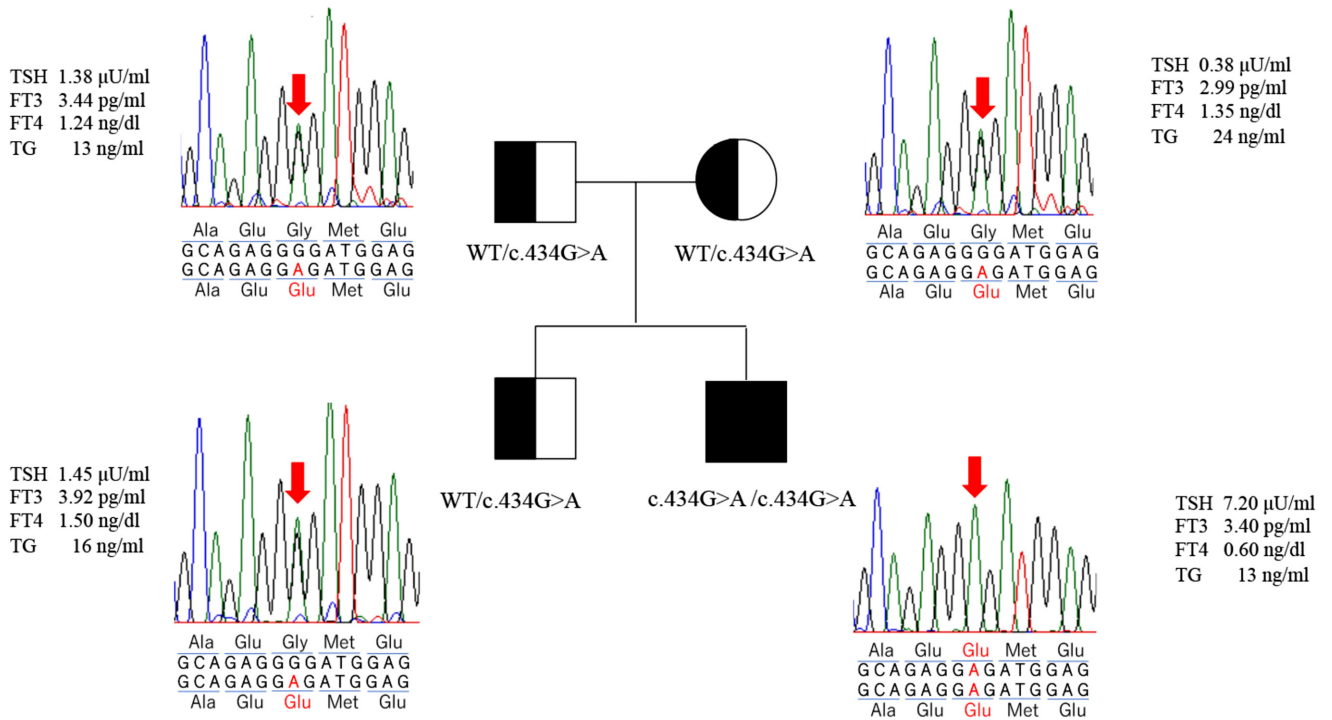


Fig. 3. Variant of the thyroglobulin gene in the patient and his family. Squares and circles represent males and females, respectively. Filled and half-filled symbols denote affected and unaffected heterozygous individuals, respectively. Thyroid hormone levels are shown next to the symbols. FT3, free T3; FT4, free T4; TG, thyroglobulin.

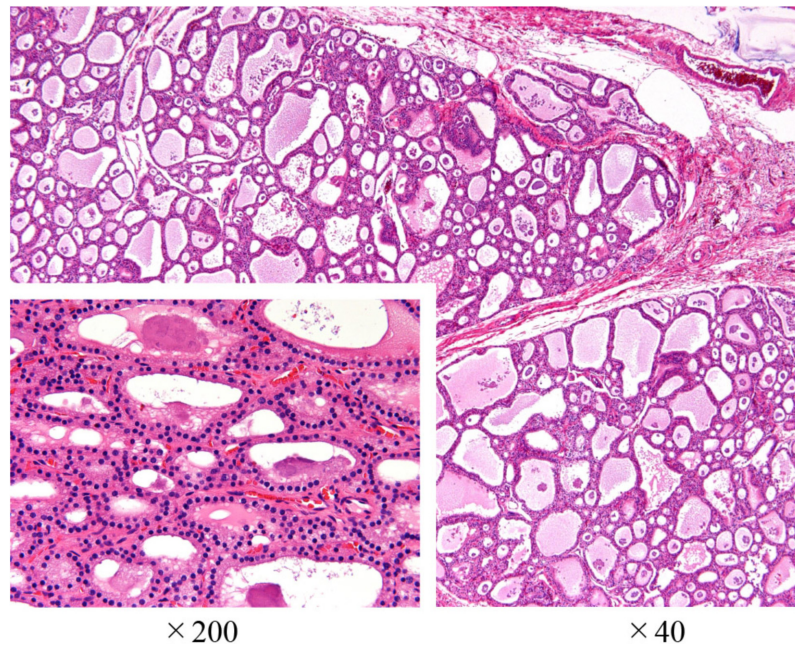


Fig. 4. Hematoxylin and eosin staining of thyroid tissue.

at Dokkyo Medical University. The patient's TG in the thyroid tissue was treated with Endo H and compared with wild-type TG and homozygous Cys1264Arg TG (**Fig. 5**). All TG samples (wild-type, Cys1264Arg, and the patient's) that were not treated with Endo H migrated as a 330 kDa band. After treatment with Endo H, the wild-type TG remained as a 330 kDa band, whereas the Cys1264Arg TG and the patient's TG were digested and

decreased in size (**Fig. 5**). This finding suggests that most of the patients' TG samples were the ER-type. After thyroidectomy, the patient received LT4 at 125 μ g per day, and the FT3/FT4 ratio decreased (**Fig. 1**).

Discussion

This study presents a case of a novel homozygous

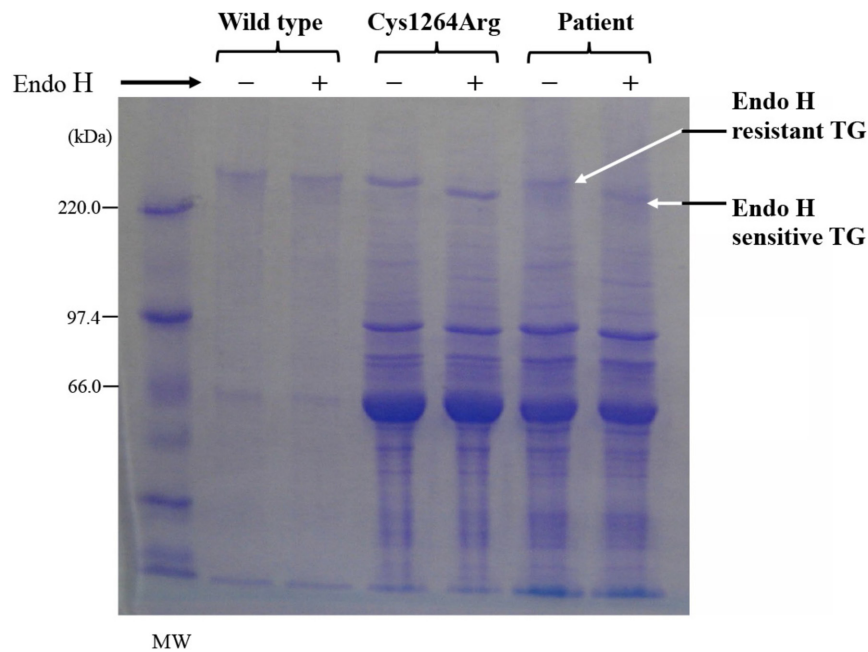


Fig. 5. Digestion of thyroid tissue extracts by Endoglycosidase H (Endo H). Thyroid tissues were obtained from a patient homozygous for Cys1264Arg TG, our patient, and a patient with Graves' disease that did not contain a variant in TG cDNA (wild-type). TG, thyroglobulin; MW, molecular weight marker.

variant (Gly145Glu) in the TG gene that resulted in a large goiter. The heterozygous parents and older brother of the patient did not have goiter. Goiter and a mild hormone synthesis disorder were only present in the homozygous patient. This variant was rare because it was not found in the genome aggregation database (gnomAD) v3.1.2 and v2.1.1 (<https://gnomad.broadinstitute.org>) or the Tohoku Medical Megabank Organization (ToMMo) database 14KJPN (<https://jmorp.megabank.tohoku.ac.jp>). Most TG defect cases are positive for mass screening; however, some are negative. Hishinuma *et al.* reported that 12 of 16 (75%) TG defect cases born after 1979, when newborn mass screening began in Japan, were positive by screening, and 5 of them either did not require LT4 replacement or the replacement was transient (6), indicating that many patients with TG defects in Japan have mild hormone synthesis disorders. In this case, the patient compensated for the hormone synthesis defect by increasing the thyroid volume without TSH elevation, which was probably the reason for the negative mass screening result.

Concerning TG defects, most patients present with ER storage disease, in which the TG three-dimensional structure cannot be constructed due to genetic variants, impairing its transport from the ER to the Golgi (1). The carbohydrate chain of TG is the high-mannose type in the ER, which becomes a complex type while being transported to the Golgi apparatus. Endo H digests high-mannose ER-type oligosaccharides but not complex Golgi-type oligosaccharides (10, 12). The Cys1264Arg variant impairs TG transport from the ER to the Golgi (9, 10), and Cys1264Arg TG can be digested by Endo H.

In this study, Gly145Glu TG was also digested by Endo H, similar to Cys1264Arg TG (Fig. 5), suggesting that Gly145Glu TG is an ER-type, which indicates that the transport of TG from the ER to the Golgi is impaired. Moreover, several protein bands appeared in the middle region of Cys1264Arg and Gly145Glu, unlike those in the wild-type. In Cys1264Arg, these bands have been identified as ER chaperones, including glucose-regulated protein 94 kDa (GRP94) and 58 kDa ER-folding enzyme (protein disulfide isomerase: PDI). These ER chaperones are induced by the unfolded protein response (UPR) (13), suggesting that a similar reaction occurred in Gly145Glu.

We found that the clinical feature of the Gly145Glu variant was a large progressive goiter with normal FT3 and low FT4 levels without TSH elevation. Some studies have demonstrated that TG gene variants impair TG transport from the ER to the Golgi apparatus in the patient's thyroid tissue, including Cys1264Arg, Cys1996Ser, and Gly2375Arg (9, 10, 14). In these prior studies, the patients had a large goiter and underwent thyroidectomy, as in our case. Their blood tests revealed normal TSH, normal FT3, and low FT4 levels.

The most interesting findings in our case were changes in the movement of thyroid hormones and goiter size. At the initial examination in our case, the FT3 was normal despite low FT4, and the FT3/FT4 ratio was 5.6 (10^{-2} pg/ng) (Fig. 1). This ratio was evidently higher than the reference value of 3.03 ± 0.38 (10^{-2} pg/ng) reported in normal children (15). Thereafter, the FT3/FT4 ratio decreased slowly with LT4 administration. However, when the thyroid gland rapidly enlarged during puberty, the FT4 decreased to below reference values despite high

FT3, and the FT3/FT4 ratio increased rapidly (Fig. 1). Increased type 2 iodothyronine deiodinase (D2) activity has been demonstrated in thyroid tissue with Cys1264Arg and Cys1996Ser variants. It has also been reported that thyroidal D2 activity is responsible for the higher FT3/FT4 ratio in patients with defective intracellular TG transport (14). In our case, an elevated FT3/FT4 ratio was observed with thyroid enlargement, whereas an increase in LT4 dosage stopped thyroid enlargement and decreased the FT3/FT4 ratio. Furthermore, the increase in LT4 dosage did not increase FT3 but instead decreased it, suggesting that T3 synthesis in the thyroid gland was decreased. These findings suggest that increased thyroidal D2 activity and thyroid volume are compensatory responses to abnormal hormone synthesis.

Interestingly, this compensatory response occurred in the absence of elevated TSH levels. A large goiter with normal TSH, FT3, and low FT4 levels is recognized as an iodine deficiency goiter (16, 17). In iodine-deficient rats, thyroid volumes are increased, and T3 is preferentially produced over T4 in the thyroid gland to avoid T3 deficiency in systemic tissues (18). This response occurs regardless of TSH level, suggesting that the thyroid gland has a TSH-independent self-regulating function (19). As with iodine deficiency, there may be thyroid autoregulation in TG deficiency to compensate for impaired hormone synthesis. Conversely, strong suppression of TSH by LT4 administration reduces

the goiter size in TG defects, suggesting that even mild stimulation of TSH within the normal range may have some effect on goiter.

A high prevalence of thyroid cancer has been reported in patients with giant goiters carrying TG gene variants (20). The cancer is thought to be caused by repeated thyroid proliferation (12). To prevent thyroid cancer development, it is necessary to stop the growth of goiter, which requires administration of LT4, even when TSH levels are normal. However, it is unclear how much LT4 should be administered. It may be advisable to administer a dose that reduces the proliferation of the thyroid gland and corrects the imbalance between FT3 and FT4.

Conclusion

We report a case of a novel homozygous missense variant (p.Gly145Glu) in the TG gene that impaired the transport of TG from the ER to the Golgi apparatus. TG defect with Gly145Glu was characterized by an elevated FT3/FT4 ratio and a large goiter without elevated TSH levels. To treat goiter with a TG defect, it is necessary to administer a dose of LT4 that can suppress goiter growth and correct the imbalance between FT3 and FT4.

Conflict of interests: The authors have no conflicts of interest to declare.

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