CASE REPORT

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Thyroid Dyshormonogenesis Due to *Dual Oxidase Maturation Factor* 2 Mutation as Non-Transient Status of Hypothyroidism

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Dual oxidase maturation factor 2 (DUOXA2) is necessary for the enzymatic activity of dual oxidase 2 (DUOX2) to generate hydrogen peroxide production during thyroid hormone synthesis. We describe two Korean children, who were initially suspected to have transient congenital hypothyroidism (CH), but later confirmed to have permanent CH caused by DUOXA2 mutation. Treatment with levothyroxine was discontinued after confirming thyroid-stimulating hormone (TSH) level to be below 10 μU/mL and normal thyroid scan at the first or second trial-off therapy. However, after therapy cessation, TSH elevated to more than 10 μU/mL, and goiter developed in case 2. As a result, levothyroxine was resumed. Next-generation sequencing showed compound heterozygous mutations of DUOXA2 at Y138X and Y246X in case 1 and homozygous mutations of DUOXA2 at Y246X in case 2. In this report, a longer follow-up is recommended even after treatment termination in transient CH, and genetic studies might help assess the permanence of hypothyroidism in cases of mildly elevated TSH after trial-off therapy.

Key Words: Congenital hypothyroidism, Thyroid dyshormonogenesis, DUOXA2

Introduction

Congenital hypothyroidism (CH) is classified into permanent and transient CH.[1)] In the literature, 75-85% of permanent CH cases are caused by thyroid dysgenesis (TD), and the remaining cases are caused by dyshormonogenesis (DH). While most TD cases are sporadic and TD-associated genetic cases are less than 5%, a considerable number of individuals with DH have mutations in genes encoding thyroid hormone biosynthesis.[2,3)] The prevalence of CH has risen from 1 in 6,500 to approximately 1 in 2,000 to 3,000 after newborn screening test (NST).[4)] The increased incidence of CH after NST comes in part from transient hypothyroidism related to maternal and neonatal factors. Transient CH is defined as temporary thyroid hormone deficiency which improves to normal thyroid hormone levels usually in few months.[5)] If the etiologic investigation was not completed in neonatal period, re-evaluation should be planned after 2 or 3 years of age after withdrawal of thyroxine for 4-week period, that is, trial-off therapy.[6)] Transient CH occurs most commonly in premature babies in areas of endemic iodine deficiency.[1,2)]

During thyroid hormone biosynthesis, oxidative iodination of tyrosine residues of thyroglobulin (TG) is essential. This process, called "iodide organification" is catalyzed by thyroid peroxidase (TPO), which requires hydrogen peroxide (H_2O_2) for oxidation. Thyroid oxi-

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dase (THOX) was identified as an H_2O_2-generating enzyme, a member of the family of nicotinamide adenine dinucleotide phosphate oxidase enzymes (NOX-es). However, the name was later changed to dual oxidase (DUOX) because the enzyme was found in other tissues and functioned as oxidases and peroxidases. NOX/DUOX enzymes generate reactive oxygen species in regulated manner. In the thyroid follicle, the isoform DUOX2 is the major source of H_2O_2.[7] Dual oxidase maturation factor 2 (DUOXA2), an endoplasmic reticulum (ER) resident transmembrane protein, allows the process of DUOX2 maturation during co-expression.[8]

Recently, the authors experienced two cases of permanent CH caused by *DUOXA2* mutation. The initial clinical course of the patients mimicked transient CH. However, genetic diagnoses were performed due to persistent and mildly elevated TSH levels after trial-off therapy.

Case Reports

Case 1

A 32-month-old boy was referred to our clinic for continued levothyroxine therapy. The patient was born at term without any perinatal problem. There was no family history of thyroid disease. Because of a high TSH level of 36.1 μU/mL at NST, the patient had been prescribed levothyroxine since 2 weeks of age after confirmatory thyroid function test (free T4, 0.5 ng/dL [reference range, 0.9-2.6 ng/dL]; TSH >150 μU/mL [reference range, 0.5-10.0 μU/mL]). Neonatal goiter was not seen in the medical records. The patient's height was 91 cm (10th-25th percentile), body weight was 15.2 kg (75th-90th percentile), and all developmental milestones were normal. Levothyroxine was continued until 3 years of age. After a 30-day trial-off therapy, TFT showed normal free T4 of 0.95 ng/dL [reference range, 0.8-2.2 ng/dL] but elevated TSH of

Table 1. Clinical course and laboratory findings in the two cases

Case no	Age	Free T4 (ng/dL) [Reference]	TSH (μU/mL) [Reference]	Ht/Wt (cm/kg)	Dx	Tx course (Dose μg/kg)
1	NST		36.1 [0.5-10]			
	2 wk	0.5 [0.9-2.6]	>150 [0.5-10]			Start T4 12.5
	2.8 yr	1.51 [0.8-2.2]	0.45 [0.5-4.8]	91/14		On T4 3.57
	3.1 yr	0.95	16.4	ND/15.2	RI	After trial-off
	3.6 yr	1.42	0.84			Restart T4 3.36
	4.1 yr	1.14	1.71	100.1/16.4		On T4 3.05
	4.6 yr	0.99	5.16		US	After trial-off
	5.1 yr	1.05	2.31	ND/18		Off T4
	6.0 yr	0.8	12.28	114.6/19.9		Off T4
	6.1 yr	1.05	2.31			Restart T4 1.39
	7.0 yr	1.29	0.93	120.7/24.9	NGS	On T4 1.00
2	NST	3.3* [>2.3]	76.3 [0.5-10.0]	ND/2.9		
	2 wk	0.29 [0.9-2.6]	>50 [0.5-10.0]	50.6/3.4		Start T4 9.71
	1.5 yr	1.75 [0.8-2.2]	1.62 [0.5-4.8]	85/12		On T4 2.08
	2.1 yr	1.9	9.76	92/13	RI	After trial-off
	2.5 yr	1.0	9.0	ND/14		Off T4
	3.0 yr	1.1	8.6			Off T4
	4.1 yr	0.58	28.8	100.7/16.5	RI	Off T4
	4.3 yr	1.78	0.38	102.7/17		Restart T4 3.03
	14.5 yr	1.22 [0.8-2.3]	1.58	156.8/50.6	US, NGS	On T4 0.98

Dx: diagnostics, Free T4: free thyroxine, Ht/Wt: height/weight, ND: no data, NGS: next-generation sequencing, NST: neonatal screening test, RI: radionuclide scan, T4: levothyroxine, TSH: thyroid-stimulating hormone, Tx: treatment, US: ultrasound scan, wk: weeks, yr: years
*Total T4 (μg/dL).

16.40 μU/mL [reference, 0.5–4.8 μU/mL]. All thyroid auto-antibodies (Abs), including thyroglobulin Ab, microsomal Ab, and thyroid-stimulating Ab, were negative. ^{99m}Tc thyroid scan showed normal uptake. With these results, levothyroxine was administered again with the dosage of 3.36 μg/kg. After confirmation of the normal TFT with negative thyroid auto-Abs after a trial-off therapy attempted at 4.6 years of age, levothyroxine was stopped. However, the patient resumed the medication at 6 years of age due to increased TSH levels up to 12.28 μU/mL (Table 1). On examination, goiter was not seen, and ultrasonography showed normal thyroid volume (1.7 cc each side). The patient was showed normal development during childhood.

We performed next-generation sequencing (NGS) for hypothyroidism at 7 years of age. The hypothyroidism panel covers 23 related genes (i.e., *TSHR*, *PAX8*, *TSHB*, *NKX2-5*, *THRA*, *GNAS*, *FOXE1*, *NKK2-1*, *SLC5A5*, *TPO*, *TG*, *DUOX2*, *DUOXA2*, *SLC26A4*, *IYD*, *SLC16A2*, *THRB*, *POU1F1*, *PROP1*, *LHX3*, *TSHB*, *HESX1*, *TRH*, and *TRHR*) using Miseq (Illumina, San Diego, CA, USA) with 192× depth of coverage and greater than 99% accuracy. Known compound heterozygote mutations of *DUOXA2* with c.413dupA (p.Tyr138*) and c.738C>G (p.Tyr246*) were identified.

Case 2

A 2-week-old female baby was referred for abnormal NST (total T4 3.3 μg/dL [reference range, >2.3 μg/dL]; TSH 76.3 μU/mL [reference range, 0.5–10.0 μU/mL]). The patient was born at term with a birth weight of 2.9 kg. Confirmatory TFT showed hypothyroid (free T4 0.29 μg/dL [reference range, 0.9–2.6 μg/dL]; TSH >50 μU/mL [reference range, 0.5–10.0 μU/mL]). All thyroid auto-Abs were negative. Family history was negative for thyroid disease. Levothyroxine was started and maintained until 2 years of age with the dosage of 2.08 μg/kg. The patient showed normal growth and development. A 30-day trial-off therapy showed normal free T4 and mildly increased TSH (free T4 1.9 μg/dL [reference range, 0.8–2.2 μg/dL]; TSH 9.76 μU/mL [reference range, 0.5–4.8 μU/mL]). ^{99m}Tc thyroid scan showed normal uptake. TFT was followed until 3 years of age with a decreasing TSH value to less than 10 μU/mL, with normal free T4 without medication. However, TFT at 4 years of age showed hypothyroidism with negative thyroid auto-Abs, and mild goiter was visible. Image studies showed diffusely increased uptake on ^{99m}Tc thyroid scan. Levothyroxine was prescribed again (Table 1). Development of the patient was unremarkable during infancy and childhood. At 14 years of age, NGS revealed a known homozygous mutation of *DUOXA2* with c.738C>G (p.Tyr246*). On ultrasonography, thyroid showed slightly increased volume of 10.63 cc with diffuse homogenous echogenicity, that is more than 97th percentile (9.36 cc) of normal at that age.[9]

Discussion

In this study, two cases of permanent CH caused by known *DUOXA2* mutations presented with mildly elevated TSH values of 5–10 μU/mL after trial-off therapy, and consequently, levothyroxine was discontinued. However, after 1.5–2 years' follow-up, TSH increased to more than 10 μU/mL, and goiter developed in case 2. Eventually, they had to resume treatment within a few years after medication cessation, and genetic confirmation was performed.

The widely used classification of CH relies on clinical and biochemical evaluation. However, in cases of incomplete etiologic investigation or doubtful diagnosis during the neonatal period, treatment should be started without confirmation. Therefore, diagnostic re-evaluation can be scheduled after the age of 2 or 3 years with a 30-day trial-off therapy.[5,6] According to recent guidelines from the European Society of Paediatric Endocrinology (ESPE), the thyroid axis, off treatment, should be normally re-evaluated after the age of 3 years because the myelination of the central nervous system is completed by 36–40 months of age, at which point the baby is more likely to be cooperative for thyroid imaging than at the age of 1 or 2 years. However, exceptions favoring earlier re-evaluation than recommended include cases with highly probable transient increase in TSH concentrations from thyroid auto-Abs or eutopic normal-sized thyroid gland in ul-

trasound scans.[10] Most clinicians cannot confirm whether the CH suspected on NST is transient or permanent until later. With the current guidelines, most preterm infants in whom hypothyroidism is most likely transient are treated for a long time.[11] One study has shown that infants with CH requiring lower doses (less than 3.25 μg/kg) seem to have the transient type, and thus, re-evaluation at 12 or 24 months rather than 3 years of age might be possible.[12] Our two cases showed decreased levothyroxine dosage less than 3.25 μg/kg before trial-off therapy.

Approximately 17-40% of children with CH on NST were later determined to have transient hypothyroidism.[11] Many factors, such as maternal antithyroid drugs, maternal thyrotropin receptor-blocking Abs, heterozygous thyroid oxidase 2 deficiency, germline mutations in TSH-R, endemic iodine deficiency, and exposure to excess iodides, may cause transient CH. If the basal TSH values are persistently higher than 10 μU/mL after the first 2 weeks of age, most physicians tend to regard the values as abnormal.[13] However, managing infants with persistently elevated TSH values between 6 and 10 μU/mL after the first month of life is even more controversial.[10] In view of our cases, slightly elevated TSH values less than 10 μU/mL are not always benign and can be a sign of permanent hypothyroidism requiring treatment.

Thyroid hormonogenesis requires the presence of iodide, TPO, a supply of H_2O_2 (DUOX system), an iodine acceptor of TG, and the rescue and recycling of iodide by iodotyrosine deiodinase (IYD). There are two iodide transporters: sodium iodide symporter (*SLC5A5* encoding NIS) located in the basolateral membranes and Pendrin (*SLC26A4* encoding PDS) located in the apical membrane.[14] TPO and DUOX2 enzymes are involved in the redox steps of thyroid hormone synthesis. Catalyzing iodination of tyrosine residues and subsequent coupling of iodotyrosine chain within TG by TPO require H_2O_2 supplies from DUOX2.[8] After ER-to-Golgi transition and N-glycosylation in Golgi, DUOX1/2 enzymes are translocated to the plasma membrane. During the process of maturation, transport, and translocation, DUOX1/2 requires the co-expression of DUOXA1/2, respectively. The formations of heterodimers between DUOX and DUOXA proteins are indispensable to produce H_2O_2.[8,15]

The incidences of DH-associated mutations are strongly influenced by ethnicity. TPO defects are frequent in European and Pakistani individuals, but less frequent in East Asian individuals.[2] DUOX2 mutations are frequently reported in East Asians. Moreover, DUOXA2 mutations are not uncommon in East Asian individuals.[16]

We have found two known pathological mutations in *DUOXA2* gene, one compound heterozygote mutation and one homozygous mutation. *DUOXA2*:c.738 C>G (p.Tyr246*) is a pathogenic mutation reported in a Chinese patient with a partial iodine organification defect, who was homozygous for a C-to-G transversion at nucleotide 738 of *DUOXA2* gene that resulted in a tyr-to-ter substitution at codon 246 of the protein (Y246X). The truncated DUOXA2 protein, which lacked transmembrane helix 5 and the C-terminal cytoplasmic domain, was inactive in reconstituting DUOX2 *in vitro*.[17] *DUOXA2*:c.413dupA (p.Tyr138*) is also a known pathogenic mutation of protein (Y138X) reported in a Chinese patient with mild symptoms of CH.[18] This Chinese patient had the same compound heterozygous *DUOXA2* gene mutations as case 1 in this case report.

Clinical and functional data for cases with *DUOXA2* mutations are rare, but most cases appear to have mild or transient CH, and loss of function may be associated with either normal protein expression or decreased expression levels of unstable DUOXA2.[19] However, at least two Korean cases of *DUOXA2* gene mutation showed persistent goiter and hypothyroidism, requiring permanent treatment similar to our cases.[20] One had the same compound heterozygous mutation as our case 1, and the other had homozygous mutation of p.Tyr138*. Both cases showed TSH values of 5.26 and 6.02 μU/mL, respectively, after trial-off therapy, but goiter appeared and TSH values increased to more than 10 μU/mL after 1-2 years' off therapy. This is similar situation to our cases. Therefore, CH caused by *DUOXA2* gene mutation could be misled to the transient type at trial-off therapy.

This study has some limitations. The first limitation

is the duration and age of trial-off therapy. We used "30 days" during the trial-off therapy, according to the guidelines. If we had used 6 weeks' off-medication protocol instead of 30 days, TSH values might have increased much earlier and higher. The recent ESPE guidelines recommended "4-6 weeks" for the phase-out period. In case 2, we performed the trial-off therapy at age 2 because the dosage could be reduced up to 2.08 μg/kg and the thyroid gland was eutopic and normal in size. However, the ESPE guidelines changed from 2-3 years to 3 years. Therefore, we suggest that re-evaluation of the thyroid axis would be better performed after 3 years of age and a 6-week trial-off therapy.

In conclusion, considering our two cases mimicking transient CH at the trial-off therapy, transient CH should be carefully confirmed if TSH values are in between 5 and 10 μU/mL. Therefore, a regular TFT follow-up is recommended after termination of treatment in transient CH, and genetic studies might help assess the permanence of hypothyroidism in cases of mildly elevated TSH after trial-off therapy.

Conflicts of Interest

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

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