博士論文（要約）

High prevalence of *DUOX2* mutations in Japanese patients with thyroid dyshormonogenesis and transient hypothyroidism

（甲状腺ホルモン合成障害および一過性甲状腺機能低下症日本人患者において *DUOX2* 変異を高頻度に認める）

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Introduction

To date, several genes have been identified as causes of congenital hypothyroidism (CH). Of these, mutation of the *TPO* gene is considered the most common cause of dyshormonogenesis (DH). Recently, mutations in the *DUOX2* gene, encoding dual oxidase 2, a generator of hydrogen peroxide (H_2O_2) required for thyroid hormone synthesis, have been identified as a cause of DH (1). Moreover, mutations in the *DUOXA2* gene, encoding dual oxidase maturation factor 2, have also been identified as a cause of DH.

Moreno et al. (1) speculated that biallelic and monoallelic *DUOX2* mutations would result in permanent CH and transient hypothyroidism (TH), respectively. In contrast, Maruo et al. (2) detected biallelic *DUOX2* mutations in patients with TH. These inconsistent results suggest that the phenotype of *DUOX2* mutations can vary widely, from mild to severe.

In this study, to clarify the prevalence of *DUOX2* mutations in not only patients with DH but also those with TH, and to determine whether there was a relationship between genotypes and phenotypes, we intensively analysed the *DUOX2* gene in a cohort of Japanese patients with DH and TH.

Subjects and methods

Subjects

Forty-eight unrelated Japanese patients who had been given a confirmed diagnosis of DH or TH, were enrolled in our study. After maintenance of normal thyroid function using immediate replacement therapy with levothyroxine in neonatal period, CH pathogenesis was re-evaluated at preschool age on the basis of the TRH provocation test, echography, iodine-123 uptake and sodium perchlorate discharge tests. To determine whether the CH phenotype was permanent (DH) or transient (TH), we used a cut-off value of peak TSH levels under TRH provocation (<35.0 μU/mL).

Our study was approved by the Institutional Review Board of Asahikawa Medical College, and informed consent was obtained from all patients and/or their parents for molecular analysis.

Molecular analysis

Genomic DNA was extracted from peripheral blood leukocytes. All of the coding exons and flanking introns of *DUOX2*, *TPO* and *DUOXA2* were analysed by PCR-direct sequencing.

Results

Molecular analysis

We identified 14 and 3 mutations in *DUOX2* and *TPO*, respectively (Table 1). Of these, 10 *DUOX2* mutations and 3 *TPO* mutations were novel. The *DUOX2* and *TPO* mutations were identified in 11 (22.9%) and 3 (6.3%) patients, respectively. The prevalence of *DUOX2* mutations was higher than that of *TPO* mutations ($p = 0.02$). Of those, 3 patients had biallelic *DUOX2* mutations; in addition, 8 and 3 patients had monoallelic mutations in *DUOX2* and *TPO*, respectively (Table 2). Of 8 patients with monoallelic *DUOX2* mutations, 5 patients also had the H678R variant, which was considered a mild functional single-nucleotide polymorphism (SNP) (3). Furthermore, only 1 patient (case 9) had digenic heterozygous mutations in both *DUOX2* and *TPO*.

Of the 9 and 3 missense mutations in *DUOX2* and *TPO*, respectively, 7 and 2 were predicted to be deleterious by PolyPhen-2 and/or SIFT programs (Table 1). In *DUOXA2*, no mutations were detected.

Clinical evaluation

Of the 48 patients, 30 and 18 were confirmed to have DH and TH, respectively. The laboratory findings at screening, first visit, and admission for confirmed diagnosis are shown in Table 2. The prevalence of *DUOX2* mutations in TH (6/18, 33.3%) was slightly, but not significantly, higher than in DH (5/30, 16.7%) ($p = 0.165$). Of the 3 patients with biallelic *DUOX2* mutations, 1 and 2 patients were diagnosed with DH and TH, respectively. Iodide organification defect was present in only 1 patient with *TPO* mutation but not in any patients with *DUOX2* mutations.

Discussion

Our study demonstrates that *DUOX2* mutations may be the most common cause of DH and TH. The findings in our study are consistent with the previous reports

DUOX2 mutations were identified in not only patients with DH but also those with TH, and they were more common in TH than in DH. These results in our study were comparable to the other Korean cohort in that the prevalence of *DUOX2* variants in TH (50%) was higher than that in DH (29%) (4). Furthermore, no patient with *DUOX2* mutations showed iodide organification defects, suggesting that the phenotype of *DUOX2* mutations may be milder than that of other causes, since TH is a somewhat milder form of hypothyroidism.

In conclusion, *DUOX2* may be the most common cause of DH and TH. Furthermore, *DUOX2* mutations may result in milder phenotypes than other causes.

References

1. Moreno JC, et al. N Engl J Med 2002;347:95–102.
2. Maruo Y, et al. J Clin Endocrinol Metab 2008;93:4261–7.
3. Narumi S, et al. J Clin Endocrinol Metab 2011;96:E1838–42.
4. Jin HY, et al. Horm Res Paediatr 2014;82:252–60.

Table 1 *DUOX2* and *TPO* mutations detected in this study.

Nucleotide	Protein	Exon	PolyPhen-2	SIFT
DUOX2				
Nonsense mutation				
c.3540T>A	p.Tyr1180X	26	NA	NA
Deletions				
c.34delC	p.Leu12TrpfsX5	1	NA	NA
c.605_621del	p.Gln202ArgfsX93[a]	5	NA	NA
c.3478_3480del	p.Leu1160del[a]	25	NA	NA
Missense mutations				
c.127A>T	p.Asn43Tyr[a]	2	probably damaging	affect protein function
c.398T>A	p.Ile133Asn	4	probably damaging	affect protein function
c.1097C>G	p.Ala366Gly	9	benign	affect protein function
c.1232G>A	p.Arg411Lys	10	benign	affect protein function
c.1537G>A	p.Asp513Asn	12	possibly damaging	tolerated
c.1621C>T	p.Arg541Trp	13	benign	tolerated
c.2203G>A	p.Asp735Asn	17	benign	tolerated
c.3116G>A	p.Arg1039Gln	23	probably damaging	affect protein function
c.3329G>A	p.Arg1110Gln[a]	24	probably damaging	affect protein function
Splice site mutation				
c.4240-1G>C	IVS30-1G>C		NA	NA
TPO				
Missense mutations				
c.1219C>T	p.His407Tyr	8	probably damaging	affect protein function
c.2327G>A	p.Gly776Asp	13	probably damaging	affect protein function
c.2749G>A	p.Glu917Lys	17	benign	tolerated

[a]Previously described mutation. Abbreviation: NA, not available.

Table 2 Clinical and laboratory findings in congenital hypothyroidism patients with *DUOX2* and *TPO* mutations.

Case no.	Confirmed diagnosis	Sex	Gene	Genotype	TSH at screening (μU/mL)	At first visit		At confirmed diagnosis		
						TSH (μU/mL)	fT4 (ng/dL)	Iodine-123 uptake (%)	Perchlorate discharge rate (%)	Peak TSH under TRH provocation (μU/mL)
1	DH	M	*DUOX2*	Q202RfsX93 / D735N	>200	NA	NA	36.4	NA	≧200
2	TH	M	*DUOX2*	D513N / R1039Q	14.9	37.7	0.82	NA	<10	11.45
3	TH	F	*DUOX2*	L1160del / IVS30-1G>C	10.1	17.36	1.56	38.68	<10	11.32
4	DH	M	*DUOX2*	L12WfsX5 / H678R[a]	18.1	20.8	2.9	30.16	<10	75.24
5	DH	F	*DUOX2*	N43Y / H678R[a]	17.5	9.4	NA	20.4	NA	64.79
6	TH	F	*DUOX2*	I133N / H678R[a]	46.4	254	NA	67.2	NA	17.53
7	TH	M	*DUOX2*	A366G / WT	12.6	10.07	2	34.13	NA	24.9
8	DH	M	*DUOX2*	R411K / WT	83.3	63.37	0.63	NA	<10	35.74
9	DH	F	*DUOX2*	R541W / H678R[a]	21.2	15.93	1.24	NA	<10	60.34
			TPO	E917K / WT						
10	TH	F	*DUOX2*	R1110Q / WT	57.9	259	NA	33.6	NA	28.81
11	TH	F	*DUOX2*	Y1180X / H678R[a]	14.4	65.7	0.49	NA	<10	13.29
12	DH	M	*TPO*	H407Y / WT	14.1	21.21	1.3	NA	16.3	63.35
13	DH	F	*TPO*	G776D / WT	35.9	66		31	NA	130
					<10.0[b]	1.7-9.1[b]	1.0-1.8[b]	7-35[b]	<10[b]	10-35[b]

[a]Functional single-nucleotide polymorphism. [b]Reference range. Abbreviations: DH, dyshormonogenesis; F, female; M, male; NA, data not available; TH, transient hypothyroidism; WT, wild type.