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 (H₂O₂) 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 ($<35.0 \,\mu\text{U/mL}$).

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.

Jucle	otide	Protein	Exon	PolyPhen-2	SIFT
UOX	X2				
No	onsense mutation				
	c.3540T>A	p.Tyr1180X	26	NA	NA
De	eletions				
	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
M	issense 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
Sp	blice site mutation				
	c.4240-1G>C	IVS30-1G>C		NA	NA
PO					
M	issense 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

^aPreviously described mutation. Abbreviation: NA, not available.

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

no. diaganosis Formula (μU/mL)	Case	Confirmed	Sex	Gene	Genotype	TSH at	At fir.	At first visit		At confirmed diagnosis	sis
DH M DUOX2 Q202RfxX93 / D735N >200 NA NA 36.4 TH M DUOX2 D513N / R1039Q 14.9 37.7 0.82 NA TH M DUOX2 L116Mel / IVS30-IG>C 10.1 17.36 1.56 38.68 DH M DUOX2 L116Mel / IVS30-IG>C 10.1 17.36 1.56 38.68 DH M DUOX2 L116Mel / IVS30-IG>C 10.1 17.36 1.56 38.68 TH F DUOX2 L116Mel / IVS30-IG>C 10.1 17.36 1.56 38.68 TH F DUOX2 L133N / H678R* 46.4 25.4 NA 67.2 DH M DUOX2 R41IK / WT 88.3 68.37 0.63 NA DH F DUOX2 R1110Q / WT 57.9 259 NA 33.6 DH F DUOX2 X1180X / HG78R* 14.4 65.7 0.49 NA	100.	diagnosis				screening	TSH	fT4 (ng/dL)	Iodine-123	Perchlorate	Peak TSH under
DH M DUOX2 Q202RfsXO3 / D735N >200 NA NA 36.4 TH M DUOX2 D513N / R1039Q 14.9 37.7 0.82 NA DH M DUOX2 L112WfsXS / H678R* 18.1 20.8 2.9 30.16 DH F DUOX2 L12WfsXS / H678R* 17.5 9.4 NA 20.4 DH F DUOX2 L133N / H678R* 46.4 254 NA 20.4 TH F DUOX2 R411K / WT 83.3 63.37 0.63 NA DH F DUOX2 R411K / WT 83.3 63.37 0.63 NA DH F DUOX2 R411K / WT 83.3 63.37 0.63 NA DH F DUOX2 R1110Q / WT 57.9 259 NA 33.6 DH F DUOX2 R1110Q / WT 14.1 21.2 1.79.1 NA 31.6 DH						(µU/mL)	(µU/mL)		uptake (%)	discharge rate	TRH provocation
DH M DUOX2 Q2020RfsX93 / D735N >200 NA NA 364 TH M DUOX2 L1160de1/ IVS30-1G~C 10.1 17.36 1.56 38.68 DH M DUOX2 L12WfsX5 / H678R³ 18.1 20.8 2.9 30.16 DH F DUOX2 L12WfsX5 / H678R³ 17.5 9.4 NA 20.4 TH F DUOX2 L133N / H678R³ 17.5 9.4 NA 20.4 TH F DUOX2 R341K / WT 12.6 10.07 2 34.13 DH F DUOX2 R411K / WT 83.3 63.37 0.63 NA DH F DUOX2 R541W / H678R³ 21.2 15.93 1.24 NA DH F DUOX2 R1110Q / WT 57.9 55.9 NA 33.6 DH F DUOX2 Y1180X / H678R³ 14.4 65.7 0.49 NA DH F										(%)	(hU/mL)
TH M DUOX2 DS13N / R1039Q 14.9 37.7 0.82 NA TH F DUOX2 L1150del / IVS30-IG>C 10.1 17.36 1.56 38.68 9.0 DH M DUOX2 L12WisxS / H678R* 18.1 20.8 2.9 30.16 30.16 TH F DUOX2 N43Y / H678R* 46.4 25.4 NA 20.4 80.16 TH F DUOX2 A366G / WT 12.6 10.07 2 34.13 80.2 DH M DUOX2 R41IK / WT 83.3 63.37 0.63 NA 80.2 DH F DUOX2 R54IW / H678R* 21.2 15.93 1.24 NA 33.6 NA DH F DUOX2 R1110Q / WT 57.9 259 NA 33.6 NA DH F DUOX2 Y1180X / H678R* 14.1 21.21 1.0-1.8* NA NA DH F <td>1</td> <td>HQ</td> <td>M</td> <td>DUOX2</td> <td>Q202RfsX93 / D735N</td> <td>>200</td> <td>NA</td> <td>NA</td> <td>36.4</td> <td>NA</td> <td>≥200</td>	1	HQ	M	DUOX2	Q202RfsX93 / D735N	>200	NA	NA	36.4	NA	≥200
TH F DUOX2 L1160del/IVS30-IG>C 10.1 17.36 1.56 38.68 38.68 DH M DUOX2 L12WfsX5/H678R* 18.1 20.8 2.9 30.16 30.16 TH F DUOX2 1133N/H678R* 46.4 25.4 NA 67.2 67.2 TH F DUOX2 A36G/WT 12.6 10.07 2 34.13 87.3 DH M DUOX2 R541W/H678R* 21.2 15.93 1.24 NA 1.24 NA DH F DUOX2 R541W/H678R* 21.2 15.93 1.24 NA 1.24 NA DH F DUOX2 R541W/H678R* 14.4 65.7 0.49 NA 1.24 NA DH F DUOX2 R1110Q/WT 57.9 57.9 66 NA 1.73b 1.73b DH F DUOX2 Y1180X/H678R* 14.1 21.21 1.79·1b NA	2	TH	Z	DUOX2	D513N / R1039Q	14.9	37.7	0.82	NA	<10	11.45
DH M DUOX2 L12Wisx5 / H678R³ 18.1 20.8 2.9 30.16 DH F DUOX2 N43Y / H678R³ 17.5 9.4 NA 20.4 20.4 TH F DUOX2 A366G / WT 12.6 10.07 2 34.13 8.13 67.2 NA 67.2 NA 9.4 NA 67.2 10.4 10.07 2 34.13 10.4 10.07 2 34.13 10.4 10.07 2 34.13 10.4 10.07 2 34.13 10.4 10.07 10.03 10.03 10.03 10.03 10.03 10.03 10.03 10.03 10.03 10.03 10.04 <td>3</td> <td>TH</td> <td>江</td> <td>DUOX2</td> <td>L1160del/IVS30-1G>C</td> <td>10.1</td> <td>17.36</td> <td>1.56</td> <td>38.68</td> <td><10</td> <td>11.32</td>	3	TH	江	DUOX2	L1160del/IVS30-1G>C	10.1	17.36	1.56	38.68	<10	11.32
DH F DUOX2 N43Y / H678R³ 17.5 9.4 NA 20.4 70.4 70.4 70.4 70.4 70.4 70.4 70.4 70.4 70.2 34.13 70.2 71.3 70.0 70.03 NA 71.3 70.03 NA 70.03 <t< td=""><td>4</td><td>DH</td><td>Z</td><td>DUOX2</td><td>L12WfsX5 / H678R^a</td><td>18.1</td><td>20.8</td><td>2.9</td><td>30.16</td><td><10</td><td>75.24</td></t<>	4	DH	Z	DUOX2	L12WfsX5 / H678R ^a	18.1	20.8	2.9	30.16	<10	75.24
TH F DUOX2 1133N / H678R³ 46.4 254 NA 67.2 TH M DUOX2 A36GG / WT 12.6 10.07 2 34.13 53.14 53.14	5	DH	江	DUOX2	N43Y / H678R ^a	17.5	9.4	NA	20.4	NA	64.79
TH M DUOX2 A366G / WT 12.6 10.07 2 34.13 DH M DUOX2 R41IK / WT 83.3 63.37 0.63 NA DH F DUOX2 R541W / H678R³ 21.2 15.93 1.24 NA TH F DUOX2 R1110Q / WT 57.9 259 NA 33.6 TH F DUOX2 Y1180X / H678R³ 14.1 21.21 1.3 NA DH M TPO H407Y / WT 4.1 21.21 1.3 NA DH F TPO G776D / WT 55.9 66 1.0-1.8³ 7-35° Inctional single-mucleotide polymorphism.	9	TH	江	DUOX2	I133N / H678R ^a	46.4	254	NA	67.2	NA	17.53
DH M DUOX2 R411K / WT 83.3 63.37 0.63 NA DH F DUOX2 R541W / H678Ra 21.2 15.93 1.24 NA TH F TPO R1110Q / WT 57.9 259 NA 33.6 DH F DUOX2 Y1180X / H678Ra 14.4 65.7 0.49 NA DH F TPO H407Y / WT 35.9 66 31 NA DH F TPO G776D / WT 35.9 66 31 7-35 ^b Inctional single-nucleotide polymorphism. ^b Reference range. Abbreviations: DH, dyshormonogenesis; F, female; M, male; NA, data not available; TH, transient	7	TH	Z	DUOX2	A366G / WT	12.6	10.07	2	34.13	NA	24.9
DH F DUOX2 RS41W / H678R³ 21.2 15.93 1.24 NA TH F TPO R1110Q / WT 57.9 259 NA 33.6 TH F DUOX2 Y1180X / H678R³ 14.4 65.7 0.49 NA DH M TPO H407Y / WT 14.1 21.21 1.3 NA DH F TPO G776D / WT 35.9 66 31 7.35° DH F TPO G776D / WT 210.0° 1.7-9.1° 1.0-1.8° 7.35° mctional single-nucleotide polymorphism.	8	DH	Z	DUOX2	R411K / WT	83.3	63.37	0.63	NA	<10	35.74
TH F DUOX2 R1110Q / WT 57.9 259 NA 33.6 TH F DUOX2 Y1180X / H678R³ 14.4 65.7 0.49 NA DH M TPO H407Y / WT 14.1 21.21 1.3 NA DH F TPO G776D / WT 35.9 66 1.7-9.1³ 7.35° DH F TPO G776D / WT 35.9 66 1.0-1.8³ 7-35° mctional single-nucleotide polymorphism. PReference range. Abbreviations: DH, dyshormonogenesis; F, female; M, male; NA, data not available; TH, transient pothyroidism; WT, wild type. MA 7-35°	6	DH	ഥ	DUOX2	R541W / H678R ^a	21.2	15.93	1.24	NA	<10	60.34
TH F DUOX2 R1110Q / WT 57.9 259 NA 33.6 TH F DUOX2 Y1180X / H678Ra 14.4 65.7 0.49 NA DH M TPO H407Y / WT 14.1 21.21 1.3 NA DH F TPO G776D / WT 35.9 66 31 31 Actional single-nucleotide polymorphism. PReference range. Abbreviations: DH, dyshormonogenesis; F, female; M, male; NA, data not available; TH, transient pothyroidism; WT, wild type. Actional Single-nucleotide polymorphism. Preference range. Abbreviations: DH, dyshormonogenesis; F, female; M, male; NA, data not available; TH, transient pothyroidism; WT, wild type.				TPO	E917K / WT						
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DH M TPO H407Y / WT 14.1 21.21 1.3 NA DH F TPO G776D / WT 35.9 66 31 31 Action of the control	11	TH	江	DUOX2	$ m Y1180X / H678R^a$	14.4	65.7	0.49	NA	<10	13.29
DH F TPO G776D / WT 35.9 66 31 31 31 210.0 lb 1.7-9.1 lb 1.0-1.8 lb 7-35 lb 1.0-1.8 lb 1.0-1.	12	DH	Σ	TPO	H407Y / WT	14.1	21.21	1.3	NA	16.3	63.35
ymorphism. ^b Reference range. Abbreviations: DH, dyshormonogenesis; F, female; M, male; NA, data not available; TH, transient	13	DH	冮	TPO	C276D / WT	35.9	99		31	NA	130
ymorphism. ^b Reference range. Abbreviations:						<10.0 ^b	1.7-9.1 ^b	1.0-1.8 ^b	7-35 ^b	<10 ^b	$10-35^{b}$
ymorphism. ^b Reference range. Abbreviations:											
hypothyroidism; WT, wild type.	^a Funct	tional single-nuc	leotide	polymorphism. 1	^b Reference range. Abbreviations		genesis; F, female; l	M, male; NA, data no	t available; TH, tran	sient	
	hypot	thyroidism; WT.	, wild ty	pe.							