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Original Article

Genetic Analysis in Children with Transient Thyroid Dysfunction or Subclinical Hypothyroidism Detected on Neonatal Screening

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Abstract. About 30% of children with elevated TSH levels during neonatal screening have a transient form of disorder. On the other hand, it has been reported that subclinical hypothyroidism persists in late childhood in about 30% of children found to be false-positive during neonatal screening. The aim of this study was to determine whether transient thyroid dysfunction and subclinical hypothyroidism detected during neonatal screening are influenced by genetic background. The TSH receptor (TSHR), thyroid peroxidase (TPO) and dual oxidase 2 (DUOX2) genes, for which it has been reported that heterozygous defects cause neonatal transient thyroid dysfunction, were analyzed. Nine children with transient thyroid dysfunction or subclinical hypothyroidism detected during neonatal screening were studied. One child was heterozygous for a TSHR gene mutation (R450H), and another child was heterozygous for a TPO gene mutation (P883S). No children with mutation of the DUOX2 gene were identified. Genetic background may contribute to development of transient thyroid dysfunction and subclinical hypothyroidism detected during neonatal screening.

Key words: neonatal screening, transient hyperthyrotropinemia, subclinical hypothyroidism, TSH receptor, thyroid peroxidase

Introduction

The prevalence of newborns with elevated TSH levels on neonatal screening has increased. The prevalence of newborns with hyperthyrotropinemia was 1 in 8,000 live births at the beginning of neonatal screening in 1979 (1), and it is now 1 in 2,500 births (2) in Japan. Due to the increase in the prevalence of screening-positive newborns, the number of newborns diagnosed as having transient hypothyroidism or transient hyperthyrotropinemia has increased. It has been reported that about 30% of screening-positive newborns with anatomically normal thyroids have transient forms of the disorder (3). The causes of transient hypothyroidism or transient hyperthyrotropinemia include prematurity, maternal thyroid disease and excess or lack of maternal iodine intake (4). On the other hand, it has been reported that subclinical hypothyroidism persists in late childhood in about 30% of children found to be

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Table 1 Subjects' initial thyroid function results

Subject	Sex	Serum TSH level at screening (μU/ml)	At first visit to the hospital				Initial dose of levothyroxine (l-T4) (μg/kg/d)
			Age (d)	Basal TSH level (μU/ml)	fT4 (ng/ml)	TSH peak after TRH stimulation (μU/ml)	
1	F	42.0	12	17.96	1.71	90.71	3.4
2	F	15.4	17	11.88	1.59	48.76	2.6
3	F	14.5	54	5.5	1.2	NA	no treatment
4	F	26.1	18	32.78	1.75	NA	3.0
5	M	14.5	12	14.32	1.09	52.91	3.8
6	M	43.4	11	19.05	1.85	46.62	3.0
7	M	20.3	38	11.58	1.42	NA	no treatment
8	F	NA	56	4.19	NA	NA	no treatment
9	M	17.9	20	11.8	1.2	NA	no treatment

NA: not available.

false-positive during neonatal screening (5).

It is unclear whether transient thyroid dysfunction and subclinical hypothyroidism represent true clinical conditions. The aim of this study was to determine whether transient thyroid dysfunction and subclinical hypothyroidism detected during neonatal screening are influenced by genetic background. The TSH receptor (TSHR), thyroid peroxidase (TPO) and dual oxidase 2 (DUOX2) genes, for which it has been reported heterozygous defects cause neonatal transient thyroid dysfunction, were analyzed in children with transient thyroid dysfunction or subclinical hypothyroidism detected during neonatal screening.

Subjects and Methods

We recruited nine screening-positive children who had not received levothyroxine (l-T4) replacement or had been able to stop l-T4 replacement after re-evaluation of thyroid function. Subjects with known underlying causes of transient thyroid dysfunction, such as prematurity, Down syndrome, maternal thyroid disease and excessive maternal iodine intake, were excluded. They were continuously observed at Toho University Omori Medical Center or Ibaraki Children's Hospital.

The study was performed with the approval of the institutional review boards of the Toho University School of Medicine and Ibaraki Children's Hospital, and informed consent for participation in this study was obtained from all the subjects' parents. Genomic DNA was isolated from peripheral blood lymphocytes. All exons of the TSHR gene, TPO gene and DUOX2 gene were amplified by polymerase chain reaction (PCR) using primers described in previous reports (6–9). The purified PCR products were sequenced directly by an automated DNA sequence analyzer (ABI 310 autosequencer, Applied Biosystems, Foster City, CA, USA).

Basal TSH levels above 5 μU/ml were considered elevated (5), and peak TSH levels after TRH stimulation above 35 μU/ml in infants and above 25–30 μU/ml in children were considered exaggerated responses (10, 11).

Results

The serum TSH level was 24.3 ± 11.2 μU/ml (n=8) during neonatal screening. The serum TSH and free T4 levels during the first visit to the hospital at 27 ± 16 d of life were 14.3 ± 8.0 μU/ml (n=9) and 1.52 ± 0.25 ng/dl (n=8), respectively (Table 1). Four children had not received l-T4 replacement, but their thyroid functions had been

Table 2 Subjects' follow-up thyroid function results

Subject	Age	Genetic defect	Basal TSH level under no treatment (μU/ml)	At re-evaluation		
				Age	TSH peak after TRH (μU/ml)	dose of l-T4 (μg/kg/d)
1	6yr, 5mo	a single TSHR mutation (R450H)	3.20–6.00	4yr, 0mo	43.7	1.0
2	8yr, 4mo	ND	1.40–2.80	6yr, 6mo	21.7	0.9
3	8yr, 4mo	ND	2.98		NA	no treatment
4	8yr, 6mo	ND	2.50–2.60	7yr, 2mo	18.4	1.3
5	9yr, 3mo	ND	2.10–6.60	5yr, 6mo	29.1	0.8
6	10yr, 4mo	ND	1.40–1.90	9yr, 0mo	20.7	1.0
7	14yr, 1mo	ND	1.20–6.55	0yr, 8mo	26.9	no treatment
8	14yr, 3mo	a single TPO mutation (P883S)	3.01–6.11	10yr, 8mo	30.2	no treatment
9	15yr, 0mo	ND	1.30–5.00	1yr, 5mo	22.5	no treatment

ND: not detected, NA: not available.

evaluated every 6 to 12 mo. Although subject 8 showed normal serum TSH and T4 levels (4.19 μU/ml and 10.9 μg/dl, respectively) during her first visit to the hospital at 56 d of life, her serum thyroglobulin (Tg) level (120 ng/ml) was slightly high. Therefore, we continued long-term follow-up of her. The remaining five children started l-T4 replacement during the neonatal period and stopped it after re-evaluation of thyroid function at the age of 5.5 ± 2.2 yr. After the cessation of l-T4 replacement, their thyroid functions were evaluated every 6 mo. The initial dose of l-T4 replacement was 3.2 ± 0.5 μg/kg/d, and the dose of l-T4 replacement at cessation was 1.0 ± 0.2 μg/kg/d (Table 2). Subject 4 was a younger sister of subject 6. No patients had a family history of thyroid diseases.

Ultimately, subjects 1, 5, 7 and 8 were diagnosed as having subclinical hypothyroidism because of fluctuating basal TSH levels during the no treatment period and/or exaggerated TSH responses to TRH simulation at re-evaluation. All subjects with subclinical hypothyroidism were negative for anti-TPO and anti-Tg antibodies. Subjects 2, 4 and 6 were diagnosed as having neonatal transient hypothyroidism because they showed normal TSH responses to TRH stimulation at re-evaluation and their basal TSH levels had been normal after cessation of l-T4 replacement. Subjects 3 and 9 were diagnosed as having transient hyperthyrotropinemia because they had not received l-T4 replacement and their basal TSH levels had been normal since the early infantile period.

Subject 1 was heterozygous for a TSHR gene mutation (R450H), and subject 8 was heterozygous for a TPO gene mutation (P883S; Fig. 1). No children with mutation of the DUOX2 gene were identified.

Discussion

The candidate genes associated with primary congenital hypothyroidism are divided into two groups. One group consists of genes causing thyroid dysgenesis, and the other group consists of genes causing thyroid dyshormonogenesis (12). Among them, TSHR, sodium iodine symporter (NIS), Tg, TPO and DUOX2 gene defects have been occasionally reported to cause mild hypothyroidism. Subjects who are heterozygous for TSHR gene mutations have been reported to show various phenotypes, from euthyroid to mild hypothyroidism (13–16). Some subjects with

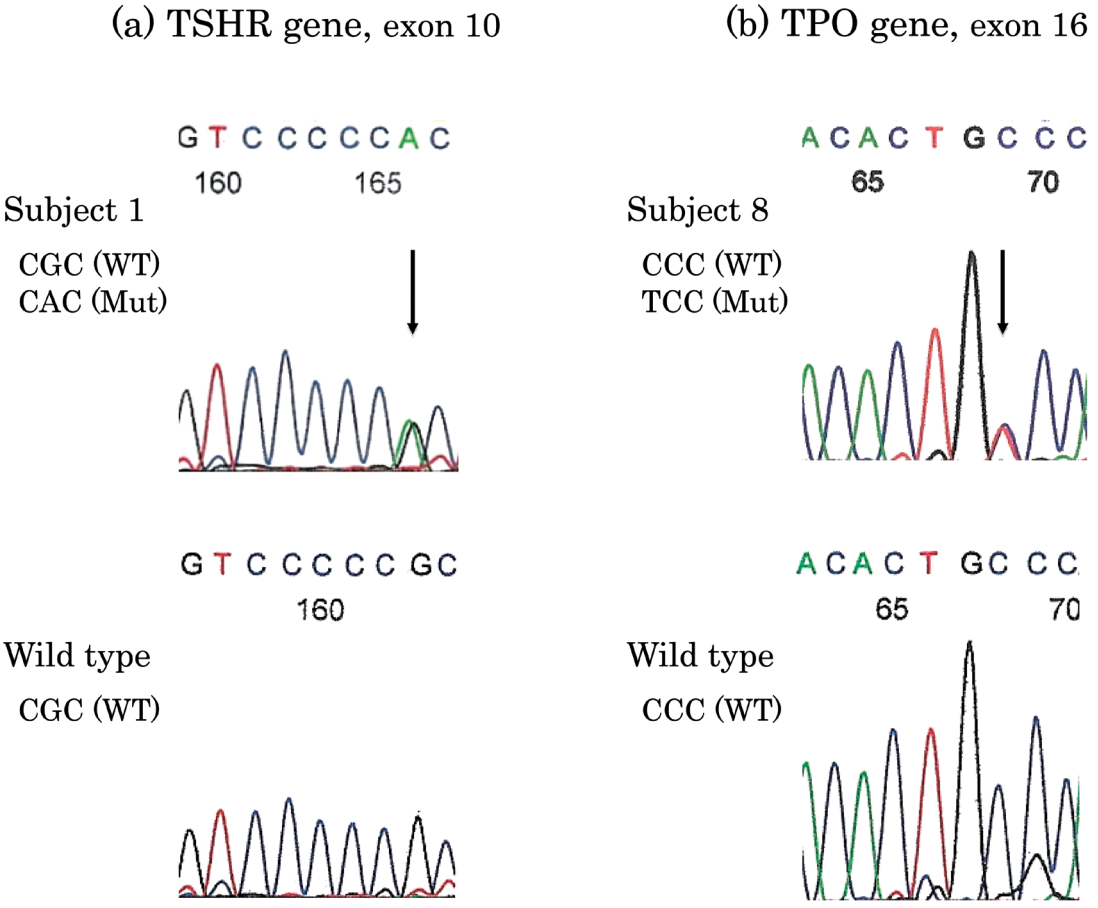

Fig. 1 Sequence analysis of a TSHR gene in subject 1 (a) and a TPO gene in subject 8 (b). (a) The mutant site (G to A) is indicated by the arrow. This mutation is accompanied by amino acid substitution (R450H). (b) The mutant site (C to T) is indicated by the arrow. This mutation is accompanied by amino acid substitution (P883S). Mut, mutation; WT, wild type.

heterozygous TSHR gene mutations were identified during neonatal screening, and the others were identified in the course of evaluation for common nonspecific symptoms. Subjects with biallelic inactivating mutations of the NIS gene and Tg gene have also been reported to show various phenotypes, from euthyroid to severe hypothyroidism (17–20). Niu *et al.* reported that the heterozygous carrier rate for TPO gene mutations is significantly higher in babies with neonatal transient hypothyroidism than in normal babies and suggested that the presence of heterozygous TPO gene mutations contributes to development of neonatal transient hypothyroidism (21). Several reports suggest that biallelic inactivating mutations in the DUOX2 gene are associated with severe and permanent congenital hypothyroidism and that heterozygous mutations of DUOX2 gene are associated with neonatal transient hypothyroidism (9, 22). However, Maruo *et al.* reported that subjects with compound heterozygous DUOX2 mutations detected during neonatal screening also showed transient hypothyroidism (23).

In the present study, the TSHR, TPO and

DUOX2 genes were analyzed because monoallelic mutations of these genes might cause transient neonatal thyroid dysfunction. Two of nine children with transient thyroid dysfunction or subclinical hypothyroidism detected during neonatal screening had heterozygous gene mutations, one for a TSHR gene (R450H) and one for a TPO gene (P883S). The R450H mutation of the TSHR gene is common in Japan (15), and the P883S mutation of the TPO gene has been reported in Japanese patients (24). Since NIS and Tg genes were not analyzed in the present study, some of the remaining seven children may have had mutations of these two genes. Although further examinations are necessary, genetic background may contribute to development of the transient thyroid dysfunction and subclinical hypothyroidism detected during neonatal screening.

Thyroid function is influenced by several factors, such as general condition, age and amount of iodine intake. It is well known that patients with subclinical hypothyroidism can develop overt hypothyroidism during puberty and pregnancy due to the increase in thyroid hormone requirement. Excessive iodine intake usually suppresses thyroid function. However, large amounts of iodine intake have been reported to cover the development of hypothyroidism in patients with biallelic inactivating mutations of the NIS and DUOX2 genes (17, 25). Moreover, it has been reported that, if left untreated, goiters caused by elevated TSH stimulation in patients with defects of the TPO gene and Tg gene are prone to development into thyroid cancer (26, 27). Therefore, it is important to continue long-term follow-up of children with transient thyroid dysfunction and subclinical hypothyroidism detected during neonatal screening to avoid missing the development of overt hypothyroidism and goiters, given that they may have background gene defects.

In conclusion, two of the nine screening-positive children who had not received l-T4 replacement or had been able to stop the treatment had genetic mutations. It is possible that genetic background contributes to the development of transient thyroid dysfunction and subclinical hypothyroidism detected during neonatal screening. The affected children may develop overt hypothyroidism when their thyroid hormone requirements increase. Therefore, long-term follow-up of screening-positive children is required.

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