Natural course of congenital hypothyroidism by dual oxidase 2 mutations from the neonatal period through puberty

Yoshihiro Maruo1, Keisuke Nagasaki, Katsuyuki Matsui, Yu Mimura, Asami Mori, Maki Fukami2 and Yoshihiro Takeuchi

Department of Pediatrics, Shiga University of Medical Science, Tsukinowa, Seta, Otsu, Shiga 520-2192, Japan, 1Department of Pediatrics, Niigata University, Niigata, Japan and 2Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan

Abstract

Aim: We previously reported that biallelic mutations in dual oxidase 2 (DUOX2) cause transient hypothyroidism. Since then, many cases with DUOX2 mutations have been reported. However, the clinical features and prognosis of individuals with DUOX2 defects have not been clarified.

Objective: We investigated the prognosis of patients with congenital hypothyroidism (CH) due to DUOX2 mutations.

Patients: Twenty-five patients were identified by a neonatal screening program and included seven familial cases. Their serum TSH values ranged from 18.9 to 734.6 mU/l. Twenty-two of the patients had low serum free thyroxine (fT4) levels (0.17–1.1 ng/dl). Twenty-four of the patients were treated with L-thyroxine.

Methods: We analyzed the DUOX2, thyroid peroxidase, Na+/I− symporter, and dual oxidase maturation factor 2 genes of these 25 patients by PCR-amplified direct sequencing. An additional 11 genes were analyzed in 11 of the 25 patients using next-generation sequencing.

Results: All patients had biallelic DUOX2 mutations, and seven novel alleles were detected. Fourteen of the patients were able to discontinue replacement therapy, and seven were receiving reduced L-thyroxine doses. Normalization of thyroglobulin lagged several years behind the completion of treatment. Two patients showed permanent hypothyroidism. Except for one case of a learning disability, growth and psychomotor development were normal.

Conclusion: The prognosis of Japanese patients with DUOX2 defects was usually transient CH. Delayed improvement of thyroglobulin indicates that these patients have subclinical hypothyroidism. Hypothyroidism did not recur in patients during the study period (up to 18 years old).

Introduction

Congenital hypothyroidism (CH) is one of the most common endocrine diseases in infants. The incidence of CH reported from worldwide neonatal screening programs is between 1/2000 and 1/4000 (1, 2), and its etiology is heterogeneous. The most common cause of CH is thyroid dysgenesis (ectopy, athyreosis and orthotopic hypoplasia), which accounts for 75–80% of cases (3). Defects in thyroxine synthesis account for 15–20% of cases (3). However, the etiology of many cases is unknown because the underlying cause is not clear.

Recently, the mechanisms of thyroid hormone production and the associated genes have been clarified. The genes associated with thyroid hormone production include thyroid peroxidase (TPO), thyroglobulin (TG),...
sodium iodide symporter (NIS), pendrin (PDS), dual oxidase 2 (DUOX2), dual oxidase maturation factor 2 (DUOX2A), dual oxidase 1 (DUOX1), dual oxidase 1 maturation factor (DUOX1A), and iodotyrosine deiodinase (IYD) (4, 5, 6, 7, 8, 9, 10, 11, 12), and mutations have been detected in numerous undiagnosed cases. Recent reports have shown that a high proportion of CH cases are caused by mutations in hormone-producing enzymes, and DUOX2 mutations are one of the most frequent causes (13, 14, 15, 16).

DUOX2, which is also known as thyroid oxidase 2 (THOX2) (10, 17), spans 6376 nucleotides on chromosome 15 and includes 33 exons. The encoded DUOX2 protein is a 1,548-amino acid polypeptide that includes a 26-amino acid signal peptide. DUOX2 is localized to the apical membrane of thyrocytes, and it is involved in the Ca²⁺/NADPH-dependent generation of H₂O₂. To synthesize thyroid hormone, TPO requires H₂O₂ to catalyze both the iodination of tyrosine residues and the coupling of iodotyrosine residues of TG (18).

In 2002, Moreno et al. (8) demonstrated that DUOX2 defects cause CH. They found that patients with homozygous DUOX2 mutations showed permanent CH, and patients with heterozygous mutations showed transient CH. Since then, additional CH cases with underlying DUOX2 mutations have been reported (19, 20, 21). In 2008, we reported eight cases of transient CH with biallelic DUOX2 mutations (13). Ohye et al. reported that a homozygous missense mutation (p.R1110Q) causes acquired goiterous hypothyroidism (22). Narumi et al. showed that DUOX2 mutation is the most prevalent cause of CH due to dyshormonogenesis (14). However, many questions remain regarding DUOX2 mutations, the genetic mechanisms, and clinical course of CH. In this study, we examined 25 Japanese patients with CH caused by biallelic DUOX2 mutations including follow-ups until up to 18 years of age.

**Patients and methods**

**Patients**

Twenty-five patients with biallelic DUOX2 mutations were included in the study. Twenty-one patients were female, and four were male. Their present ages range from 3 to 18 years. There were seven familial cases (family 1, cases 1–4; family 2, cases 5 and 6; family 3, cases 8 and 9; family 4, cases 10 and 11; family 5, cases 13 and 14; family 6, cases 16 and 17; and family 7, cases 24 and 25), which included two sets of twins (family 4 (monozygotic twins) and family 6 (dizygotic twins)). All familial cases were siblings. All patients were identified based on elevated thyroid stimulating hormone (TSH) levels in a neonatal screening program, and consequently visited our hospital. All the infants had a gestational age >35 weeks (range, 35 weeks 4 days to 41 weeks 3 days; mean, 38 weeks 4 days ±11.7 days). Their birth weight and length ranged from 2406 to 3460 g (mean, 2870 ±290 g) and 44.0–50.0 cm (mean, 47.4 ±1.4 cm), respectively. The observed birth weights and lengths were appropriate for the gestational ages of the patients (Table 1). The TSH concentration at neonatal screening ranged from 10.0 to 242.8 µU/ml (median, 32.75 µU/ml). Serum concentrations of TSH, free tri-iodothyronine (fT₃) and free T₄ (fT₄) were 25.7–771.0 µU/ml (median, 94.0 µU/ml), 1.89–5.52 pg/ml (median, 3.72 pg/ml) and 0.18–1.50 ng/dl (median, 0.55 ng/dl), respectively (Table 1). TG concentrations were very high, and in most cases, >800 ng/ml, except in two patients (family 7, patients 24 and 25, with 150 and 91.8 ng/ml, respectively). Using a checklist for clinical diagnosis based on the mass-screening guidelines for CH of the Japan Society of Pediatric Endocrinology and Japan Society of Mass Screening, 12 of 23 patients scored above two points for symptoms of CH (e.g., prolonged jaundice, constipation, umbilical herniation, failure to thrive, dry skin, poor activity, macroGLOSSIA, hoarseness, cold extremities, edema, opened posterior fontanellae and goiter; more than two points indicates severe CH). Thyroid ultrasonography showed enlargement of the thyroid gland in 11 of the 21 patients. The other ten patients had an orthotopic thyroid gland without enlargement. Six of the 25 patients showed an absence of distal femoral epiphysis (Table 1).

Except for one patient with transient hyperthyrotropinemia in family 1, all patients were treated with L-thyroxine. The first doses of L-thyroxine were determined by the severity of hypothyroidism (check list, elevation of TSH level and reduction of fT₄ level) at first visit to our outpatient department. The usual dose is 5–10 µg/kg per day. However, case 6 did not show apparent reduction in serum fT₃ and fT₄ levels; a lower dose was used.

The reevaluation and classification of patients as having transient or permanent CH were based on normalization of TSH levels despite withdrawal of L-thyroxine. After withdrawal of therapy, serum TSH, fT₃, fT₄ and TG levels of all patients were followed once or twice a year until now (Table 2).

This study was approved by the ethics committee of Shiga University of Medical Science.
Table 1 Clinical features at neonatal screening and mutations of the dual oxidase 2 gene (DUOX2).

| Case | Family | Sex | Age | Gestational age | Birth weight (g) | Height (cm) | TSH (mU/l) | CL | Epiphysis [mm] | TSH (mU/l) | fT3 (ng/dl) | fT4 (ng/dl) | TG (ng/dl) | L-Thyroxine (µg/kg per day) | Allele 1 | Mutation | Allele 2 |
|------|--------|-----|-----|-----------------|-----------------|-------------|-------------|-----|-------------|-------------|------------|------------|------------|-----------|--------------------------------|---------|----------|---------|
| 1    | 1      | F   | 18y3m | 39w0d          | 3736            | 48          | 36.9        | 16  | 3540        | 1           | 5.6        | 95.4       | 2.59       | 0.43      | ND                  | 7       | c.1435_1440delCTATC-CinsAG | p.L479F | p.L479F
| 2    | 1      | F   | 16y3m | 37w0d          | 3022            | 48.5        | 17.8        | 28  | 4030        | 3           | 5.3        | 233        | 1.89       | 0.19      | >800     | 7.5    | c.1435_1440delCTATC-CinsAG | p.L479F | p.L479F
| 3    | 1      | F   | 14y1m | 37w0d          | 3298            | 49          | 18.5        | 25  | 3298        | 1           | 5.3        | 119        | 3.2        | 0.53      | >800     | 5      | c.1435_1440delCTATC-CinsAG | p.L479F | p.L479F
| 4    | 1      | M   | 9y3m  | 37w0d          | 2854            | 48.5        | 10          | 28  | 3535        | 0           | 4.6        | 25.7       | 2.7        | 1.5       | ND      | ND    | c.1435_1440delCTATC-CinsAG | p.L479F | p.L479F
| 5    | 5      | F   | 11y5m | 38w0d          | 2920            | 48          | 23.7        | 24  | 3980        | 1           | 4.6        | 41.9       | 5.52       | 0.84      | >800     | 5      | c.1588A>T                  | p.K530K | p.[E879K;L1067K] |
| 6    | 2      | M   | 7y8m  | 40w1d          | 3460            | 50          | 18.9        | 15  | 3950        | 0           | 6.3        | 18.9       | 4.3       | 1.33      | ND      | 2.5    | c.1588A>T                  | p.K530K | p.[E879K;L1067K] |

ND, no data; CL, check list; TSH, thyroid stimulating hormone; fT3, free T3; fT4, free T4; TG, thyroglobulin.

1Subjected to mutation screening by the next-generation sequencing.

2Monozygotic twin.

3Dizygotic twin.
Table 2  Prognosis in the patients with biallelic mutations of DUOX2.

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<th>Case</th>
<th>Family</th>
<th>Age</th>
<th>Normalization of thyroid function (months of age)</th>
<th>Withdrawal T4 therapy (age)</th>
<th>Normalization of TG (age)</th>
<th>Recent data</th>
<th>TSH (µU/ml)</th>
<th>FT3 (pg/ml)</th>
<th>FT4 (ng/dl)</th>
<th>TG (ng/dl)</th>
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ND, no data.

\(^a\)LD: mild learning disability.
**Laboratory testing and sequence analysis**

At neonatal screening, TSH was measured by the Cretin TSH ELISA II assay (Eiken Chemical, Tokyo, Japan). During hospital visits, TSH was measured using a fluorescent enzyme immunoassay method (TOSHO, Tokyo, Japan). fT₄ and fT₃ levels were measured with a fluorescent enzyme immunoassay (TOSHO). TG was measured with an immunoradiometric assay (Eiken Chemical).

Genomic DNA was extracted from blood leukocytes using standard techniques after obtaining informed consent from patients and their families to participate in this study. Amplification of exons and exon-intron boundaries by PCR from genomic DNA was performed using the ten pairs of oligonucleotide primers shown in Supplementary Table 1, section on supplementary data given at the end of this article (13). The genomic DNA sequences and cDNA sequences were based on those in the human genome database (NCBI accession numbers CCDS10117.1 and AF267981). The 33 exons of DUOX2 were amplified as ten PCR products. PCR products were purified using the Nucleospin Gel & PCR Clean-up Kit (TaKaRa, Kyoto, Japan), and the sequences of the amplified DNA fragments were determined directly using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems). The sequencing primers are shown in Supplementary Table 1.

The amplified DUOX2 fragments were subcloned into the pCR 2.1 vector using the TA Cloning Kit (Invitrogen) to verify the mutant sequence in patients with deletion or insertion mutations, and to determine the cis or trans arrangement of the two mutations in one exon in cases 16 and 17. Each PCR product (30 ng), including the regions harboring mutations, was ligated with the vector (50 ng) and transformed into cells using the Competent High JM109 Kit (Toyobo, Osaka, Japan). The DUOX2 sequences of the patients’ relatives were analyzed after obtaining informed consent. *Cis* or *trans* arrangement of the multiple variations was determined by analyzing the DUOX2 genes of the patients’ parents.

To analyze the genes encoding TPO, NIS and DUOX2, all coding exons and surrounding intronic sequences were amplified by PCR and were sequenced directly as described previously (4, 6, 9, 13).

Eleven of the 25 patients (8, 10, 11, 13, 14, 16, 17, 20, 21, 23, and 25), who were enrolled in the study after July 2012, were subjected to mutation screening for DUOX1, DUOX2, DUOX1, DUOX2, GNAS, FOXE1, IYD, NIS, NKX2-1, NKX2-5, PDS, PAX8, TG, TPO and TSHR using the Haloplex Target Enrichment System (Agilent Technologies, Palo Alto, CA, USA). The library was sequenced as 150-bp paired-end reads on a MiSeq next-generation sequencer (Illumina, San Diego, CA, USA). Nucleotide alterations were called by the Surecall system (Agilent Technologies) and SAMtools 0.1.17 software (http://samtools.sourceforge.net) and were confirmed by Sanger direct sequencing.


**Results**

**Identification of variants by DUOX2 sequencing**

All patients had biallelic DUOX2 variants. Twenty-one distinct variants were detected (Table 1). The novel variants are shown in Supplementary Figures 1, 2, 3, 4, 5, 6 and 7, section on supplementary data given at the end of this article. Variants p.V779M (rs145061993) and p.R1492H (rs147945181 and rs144543420) were previously reported in SNP databases. These novel seven variants have not been reported as polymorphisms in the Japanese population. The allelic frequencies of these variants in Japanese individuals are likely to be <0.01 (Human Genetic Variation database, http://www.genome.med.kyoto-u.ac.jp/SnpDB/). The most prevalent allele was c.2033A>G (p.H678R); 10 of the 25 patients had this allele (Fig. 1).

None of the patients had biallelic mutations in any other genes involved in the generation of thyroid hormone (TPO, NIS, and DUOX2). Furthermore, the 11 patients analyzed by next-generation sequencing only had biallelic mutations in DUOX2. Cases 10 and 11 (twins) also had a monoallelic mutation in TG (p.T1498M). Cases 16 and 17 (twins) had a heterozygous mutation in TG (p.R2585W). Both TG variants were reported on the web as rare and probably damaging variants by PolyPhen-2 (http://evidence.pgp-hms.org/TG-T1498M, http://evidence.pgp-hms.org/TG-R2585W). All the parents of the patients
included in this study are carriers of monoallelic DUOX2 mutations. They did not have a history of hypothyroidism.

**Prediction of the functional effects of missense variants in DUOX2 by in silico assays**

Prediction of the functional effects of the seven missense variants by in silico assays showed that p.D115Y, p.A649E, p.E879K, p.R885Q and p.R1492H were probably damaging and that p.V779Q, p.L1080T and p.L1343F are possibly damaging. The results suggested that the seven missense mutations likely have a disease-causing role. In contrast, the three polymorphic variants (p.P138L, p.H678R and p.L1067S) were not predicted to be disease-causing variants in the in silico assays (Table 3).

**Clinical course of patients with biallelic mutations in DUOX2**

After beginning L-thyroxine treatment, serum TSH, fT3 and fT4 levels in most patients improved within 2 months.
For most patients, the L-thyroxine dose could be reduced by about 2–4 years of age, except for the twins in cases 10, 11 and 21. Twenty-one of the 24 patients were able to receive reduced doses of L-thyroxine. Fourteen patients ranging in age from 2 years 1 month to 9 years 4 months were able to stop L-thyroxine treatment (median, 8 years 2 months). For the remaining seven patients, L-thyroxine was withdrawn by the conclusion of the study (ages 3–8 years). For the twins (cases 10 and 11) with compound heterozygous mutations p.R885Q and p.L1343F, it was necessary to increase the L-thyroxine dose to control the elevation of TSH and permanent CH. For patient 21, with p.H678R and p.V779M, L-thyroxine also could not yet begin to be withdrawn. Elevated serum TG levels persisted after terminating L-thyroxine treatment. However, TG levels usually normalized after the patients reached 10 years of age (cases 1, 2, 3, 5, 12, 13, 15 and 20; Table 2). This indicates that the patients with DUOX2 mutations have subclinical hypothyroidism until puberty. After withdrawal of therapy, all patients were followed until recently (for 9 months–13 years) (Table 2). During puberty, none of the transient cases showed sign of hypothyroidism (elevation of TSH or reduction of fT4 level).

The oldest patient, who was compound heterozygous for p.L479SfsX2 and p.K628RfsX10, showed no signs of hypothyroidism after finishing treatment for the remainder of the study period (i.e., until she reached 18 years of age), and her TG level remained in the normal range.

The growth of the patients was within the normal range, and psychomotor development was also normal except for patient 5, who exhibited a mild learning disability (Table 2).

### Discussion

After the discovery of the connection between DUOX2 deficiency and CH, many patients with DUOX2 mutations have been reported (Fig. 1) (8, 13, 14, 15, 16, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28). However, some questions still remain. For example, which DUOX2 mutations result in permanent CH and which result in transient hypothyroidism? What is the natural course of DUOX2 deficiencies?

Moreno et al. (12) reported that biallelic DUOX2 mutations cause permanent CH and monoallelic mutations cause transient CH. However, there is a manuscript supporting the hypothesis that carriers of monoallelic mutations only suffer from transient CH (29). This recent study also suggested the involvement of a monoallelic variant of DUOX2 in the development of transient CH. Other reports have shown that biallelic DUOX2 mutations cause mild to moderate CH (13, 14, 15, 16, 19, 22, 30). Recently, some studies have shown that DUOX2 defects are the predominant cause of CH associated with dyshormonogenesis (13, 14, 15, 16, 25, 26).

In our analysis of 25 Japanese patients with CH carrying biallelic DUOX2 mutations, 13 patients finished L-thyroxine replacement therapy prior to reaching 10 years of age, and eight patients, ranging from 3 to 8 years old at the end of the study period, were receiving reduced L-thyroxine doses. This result indicates that in the Japanese population, DUOX2 mutations can cause transient CH, even combinations of inactivation mutations (i.e., nonsense and frameshift mutations) as observed in family 1 (cases 1, 2, 3, and 4) and case 19. Moreover, case 4 in family 1, with compound heterozygous p.L479SfsX2 and p.K628RfsX10 mutations, showed only transient hyperthyrotropinemia, whereas the patient’s three elder

### Table 3: Prediction of functional effects of missense variants of DUOX2 by in silico assays.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Polyphen2*</th>
<th>Mutation tester</th>
<th>SIFT Human Protein</th>
<th>SIFT Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.D115Y</td>
<td>Probably damaging</td>
<td>Disease causing</td>
<td>Damaging</td>
<td>0</td>
</tr>
<tr>
<td>p.A649E</td>
<td>Probably damaging</td>
<td>Disease causing</td>
<td>Tolerated</td>
<td>0.07</td>
</tr>
<tr>
<td>p.V779M</td>
<td>Possibly damaging</td>
<td>Disease causing</td>
<td>Damaging</td>
<td>0.01</td>
</tr>
<tr>
<td>p.E879K</td>
<td>Probably damaging</td>
<td>Disease causing</td>
<td>Damaging</td>
<td>0.04</td>
</tr>
<tr>
<td>p.R885Q</td>
<td>Probably damaging</td>
<td>Disease causing</td>
<td>Tolerated</td>
<td>0.06</td>
</tr>
<tr>
<td>p.I1080T</td>
<td>Possibly damaging</td>
<td>Disease causing</td>
<td>Damaging</td>
<td>0</td>
</tr>
<tr>
<td>p.L1343F</td>
<td>Possibly damaging</td>
<td>Disease causing</td>
<td>Damaging</td>
<td>0</td>
</tr>
<tr>
<td>p.R1492H</td>
<td>Probably damaging</td>
<td>Disease causing</td>
<td>Polymorphism</td>
<td>0.06</td>
</tr>
<tr>
<td>p.P138L</td>
<td>Benign</td>
<td>Benign</td>
<td>Polymorphism</td>
<td>1</td>
</tr>
<tr>
<td>p.H678R</td>
<td>Benign</td>
<td>Benign</td>
<td>Tolerated</td>
<td>0.32</td>
</tr>
<tr>
<td>p.L1067S</td>
<td>Benign</td>
<td>No data</td>
<td>Tolerated</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*The PolyPhen-2 algorithm calculates the naive Bayes posterior probability that a given mutation will be damaging and qualitatively predicts that it will be benign, possibly damaging or probably damaging, corresponding to posterior probability intervals (0, 0.2), (0.2, 0.85), and (0.85, 1), respectively.
sisters showed apparent transient CH. These results indicate that DUOX2 mutations cause mild to moderate forms of hypothyroidism, including transient CH and transient hyperthyrotropinemia. A possible mechanism by which DUOX2 mutations cause transient CH is as follows. Two dual oxidases in thyrocytes, DUOX1 and DUOX2, generate H2O2 for organification and coupling of tyrosine (10, 17). However, the expression levels of these two DUOXs differ; the DUOX1 expression level is one-fifth that of DUOX2 (31). Even if DUOX2 enzyme activity is lost, a low level of H2O2 may be maintained by DUOX1 throughout life. The requirement for thyroid hormone during the neonatal period (10–15 μg/kg) is 5–7 times higher than that of adults (2 μg/kg) (32, 33); then, the thyroid hormone requirement gradually decreases after the infantile period. The H2O2 supply from DUOX1 alone may be inadequate during the neonatal and infantile periods, so individuals with DUOX2 deficiencies are liable to show signs of CH. As the thyroid hormone requirement decreases over the course of development, DUOX1 production of H2O2 is sufficient to maintain thyroid hormone synthesis, irrespective of DUOX2 mutations (Fig. 2). However, the members of Family 1 were not analyzed by next-generation sequencing. The presence of other unidentified defects may be involved in the difference in the severity.

In addition to the frameshift mutations (p.S199WfsX121, p.R268SfsX51, p.L479SfsX2, p.K628RfsX10, and p.G948VfsX47) and the one amino acid deletion variant (p.L1160del), prediction of the functional effect by in silico assays suggested that seven of the missense variants – p.D115Y, p.A649E, p.V779M, p.E879K, p.R885Q, p.L1343F, and p.R1492H – were disease-causing variants. In contrast, the in silico assays suggested that the three polymorphic variants – p.P138L, p.H678R, and p.L1067S – were silent polymorphisms. This was consistent with a previous expression study (25). Previous studies showed that p.H678R did not reduce H2O2 production (14, 34). The most prevalent variant detected in this study was p.H678R. Previously, Narumi et al. (14) reported that this variant is a polymorphism in the Japanese population and that it has an interactive effect with other mutations leading to a mild phenotype. Therefore, patients who are compound heterozygous for p.H678R and other pathological variants might exhibit mild clinical manifestations due to DUOX2 defects. However, even patients with a combination of nonsense and frameshift mutations (i.e., cases 1, 2, 3, 4 and 19) showed transient CH. The location of the mutations within the DUOX2 protein varied (Fig. 1). Moreover, some patients (cases 10 and 11; compound heterozygotes p.R885Q and p.L1343F alleles) also exhibited permanent CH. Therefore, it is difficult to detect associations between DUOX2 mutations and prognosis.

The results of the next generation sequencing of 11 patients, showing no other biallelic mutations in thyroid hormonogenesis genes, indicated that a DUOX2 defect is an important cause of hypothyroidism. However, modulators other than the genetic polymorphism are thought to exist that lead to the variety observed in the patients’ clinical manifestations. Permanent CH cases (cases 10 and 11) had biallelic mutations in DUOX2 and a monoallelic mutation in TG. However, cases 16 and 17 with transient CH had a combination of the biallelic mutations in DUOX2 and the monoallelic mutation in TG. A recent report revealed that the combination of biallelic mutation in DUOX2 with the monoallelic mutation in a different thyroid hormone-generating enzyme might cause CH. (35). However in some cases, the combination of biallelic mutation in DUOX2 with the monoallelic mutation in a
different thyroid hormone-generating enzyme might cause permanent CH.

Another factor in patients with DUOX2 deficiencies that might affect the differential prognosis between transient CH in Japanese and permanent CH in Europeans is the iodine content in soil. In Europe, the iodine content in the soil is low, whereas in Japan, it is plentiful (36). This difference might explain the different phenotypes observed in Europe and Japan, as a higher iodine supply might increase thyroid hormone production.

In our study, there was an unbalanced sex ratio, with 21 females and four males. It was not clear why such sex-based differences occurred. However, in family 1 (compound heterozygous for p.L479SfsX2 and p.K628RfsX10), the three elder sisters showed apparent transient hypothyroidism, whereas the younger brother showed only transient hyperthyrotropinemia. These cases suggested that the symptoms of the DUOX2 defect might be different in each individual, even in those with same mutations.

TG levels were elevated for 3–10 years after the L-thyroxine treatments ended (Table 2). After the start of puberty, when the L-thyroxine requirement is reduced to that of adults, the TG level improved to within the normal range. The delayed improvement of the TG level suggests that patients with DUOX2 defects might exhibit insufficient thyroid hormone production. After stopping the L-thyroxine treatment, TG levels improved, and hypothyroidism did not recur in the patients from puberty to 18 years of age (cases 1, 2, 3, 5, and 20). However, Ohye et al. showed that a patient who was homozygous for p.R1110Q developed acquired goiterous hypothyroidism (23). The goiter appeared when the patient was in her 20 s, and hypothyroidism developed when the patient was in her 40 s. Abe et al. (22) reported a 39-year-old mother with a homozygous mutation (p.H678R) and a mild phenotype that also showed elevated TSH (15.6 U/ml). Accordingly, our patients with transient CH might develop hypothyroidism later in life. Given the increased need for thyroid hormone during pregnancy, pregnant women with a history of transient CH due to biallelic mutations in DUOX2 should be observed carefully, especially after withdrawal of L-thyroxine. Further observations are necessary to clarify the clinical course of DUOX2 defects.

In this study, the growth and development of patients identified by neonatal screening were largely normal. In contrast, a previous report of Turkish patients with homozygous p.R434X showed mental retardation due to delayed diagnosis at 6 months of age (26). Therefore, early diagnosis by neonatal screening programs and early treatment are important for patients with DUOX2 defects, even for transient CH.

Conclusion

Japanese patients with DUOX2 defects primarily showed transient CH. Many patients completed L-thyroxine replacement therapy before reaching puberty. As some patients showed permanent CH and transient hyperthyrotropinemia, DUOX2 mutations might be associated with various phenotypes. To clarify the phenotypes and clinical course of DUOX2 mutations, additional data and patient observations are necessary.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-15-0959.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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