CLINICAL REPORT

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Delayed Onset Congenital Hypothyroidism in a Patient With *DUOX2* Mutations and Maternal Iodine Excess

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Congenital hypothyroidism (CH), one of the most common congenital endocrine disorders, causes irreversible intellectual disability in untreated patients. Today, the vast majority of patients receive early diagnosis and treatment in the context of newborn screening for CH, and achieve satisfactory cognitive development. However, a subset of patients with delayed onset are undetectable by newborn screening, and miss benefit from early intervention. Here, we report on a delayed-onset CH patient that had two contributing factors in the pathogenesis of CH simultaneously, i.e., a genetic defect and iodine excess. The patient was exposed to excessive iodine in utero because her mother consumed massive amounts of seaweed during pregnancy. Surprisingly, the patient had a negative result in newborn screening, but developed overt CH at age 3 months. She received thyroxine supplementation until when normalization of the thyroid function was confirmed at age 3 years (i.e., transient CH). Mutation screening for *DUOX2*, a causative gene for transient CH, showed biallelic mutations (p.[E327X] + [H678R]). This report provides a new example of environmental modification of phenotypes of CH due to a genetic defect, which can potentially distort screening results.

Key words: congenital hypothyroidism; neonatal screening; false negative reactions; iodine; DUOX2

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INTRODUCTION

Congenital hypothyroidism (CH) is the most common congenital endocrine disorder, affecting about 1 in 3,000 newborns worldwide. In developed countries, majority of patients are detected through newborn screening, in which blood samples on filter paper obtained at age 2–5 days are analyzed. Owing to early diagnosis and treatment, most patients can achieve normal or near normal intellectual outcome today. However, a subset of patients with delayed onset are undetectable by screening, and miss benefit from early intervention.

Inborn errors of thyroid hormone production are collectively referred to as thyroid dyshormonogenesis. Thyroid dyshormonogenesis is a relatively infrequent form of CH, accounting for about 15–20% of cases. Patients with thyroid dyshormonogenesis have a goiter, which results from hyperstimulation of the thyroid by thyroid stimulating hormone (TSH). Genetic defects of a molecule within the thyroid hormone synthesis pathway, such as sodium-iodine symporter (NIS) (*SLC5A5*, OMIM*601843), pendrin (*SLC26A4*, OMIM*605646), dual oxidase 2 (*DUOX2*, OMIM-*606759), thyroid peroxidase (*TPO*, OMIM*606765), and thyroglobulin (*TG*, OMIM*188450), cause thyroid dyshormonogenesis that is inherited as an autosomal recessive trait [Park and Chatterjee, 2005]. These genetic defects account for at least 70% of

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thyroid dyshormonogenesis [Narumi et al., 2011], while inappropriate iodine status (i.e., deficiency and excess) can cause transient CH resembling thyroid dyshormonogenesis. In this article, we report a patient with CH due to *DUOX2* mutations, the most common form of thyroid dyshormonogenesis [Narumi et al., 2011], with a delayed onset. Of interest, the patient was compromised by extreme iodine excess during pregnancy, and had a negative result in newborn screening for CH.

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The patient (a 6-year-old girl) was the first child of healthy nonconsanguineous Japanese parents. Reportedly, the mother of the patient consumed as much as 50–100 g of several kinds of seaweed everyday from the third trimester of pregnancy to 3 months after delivery, in order to "maintain good health". The estimated iodine intake level was 20–40 mg/day, which greatly exceeds the recommended dietary allowance (0.24 mg/day) and tolerable upper intake level (2.2 mg/day) for pregnant women in Japan according to the Dietary Reference Intakes 2010 by Ministry of Health, Labour and Welfare. During the period of seaweed overconsumption, she had neither goiter nor symptoms suggesting hypothyroidism, although we did not test her thyroid function. She did not overconsume seaweeds during the other pregnancy.

The patient was born at 38 weeks with a weight of 2,580 g, and was breastfed. Dried blood samples obtained at age 5 days were subject to newborn screening, in which blood-spot TSH level was measured. The result was negative (screening cutoff TSH level, 10 mU/L). At age 3 months, she had persistent jaundice and poor weight gain (weight, 5,210 g; −2.0 SD). Routine blood tests revealed hyperbilirubinemia (total bilirubin, 6.7 mg/dl) accompanied by a slightly elevated serum aspartate aminotransferase level (105 U/L; ref, 5–45). The thyroid function test showed that she had overt hypothyroidism: TSH, 492 mU/L (ref, 0.3–4.2) and free thyroxine, 0.2 ng/dl (ref, 1.0–1.8). No thyroid autoantibodies were detected. Urinary iodine level was not measured. Thyroid ultrasonography showed a slightly enlarged gland (+1.8 SD [Yasumoto et al., 2004]). Replacement therapy with levothyroxine was initiated, and hyperbilirubinemia and hypertransaminasemia improved subsequently. We reevaluated her thyroid status at age 3 years with discontinuation of therapy. She had normal thyroid function (TSH, 2.3 mU/L; free thyroxine, 1.7 ng/dl), with a normal-sized gland (0.0 SD) on ultrasonography. Thyroidal ^{123}I uptake was normal (24.9% at 24 hr; ref 8–40), with normal perchlorate discharge rate (8.3%; ref <10). The Wechsler Preschool and Primary Scale of Intelligence score evaluated at age 4 years was 93. At last clinical visit, she maintained a normal TSH level without therapy, was growing normally and was developing satisfactory.

Mutation Analysis

DNA samples were collected with written informed consent from the proband, her parents, and her sister. Coding exons and flanking introns of *DUOX2* were analyzed by standard PCR-based sequencing as previously described [Narumi et al., 2011]. The patient was compound heterozygous for a novel nonsense mutation (c.978G>T; c.979G>T, p.E327X) and a known functional single nucleotide polymorphism (c.2033A>G, p.H678R) [Narumi et al., 2011] (Fig. 1A). E327X is a null mutation lacking all functional domains of the DUOX2 protein (Fig. 1A). The DUOX2 allele harboring H678R was shown to have about 60% of residual function in our previous study [Narumi et al., 2011]. The mother and younger sister of the patient were heterozygous for E327X, while the father was heterozygous for H678R (Fig. 1B). These three individuals had normal thyroid function.

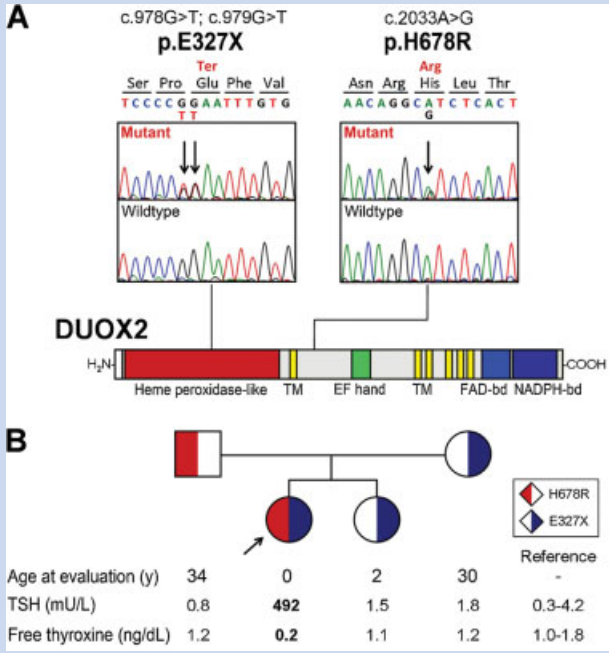

FIG. 1. A: Partial sequences of polymerase chain reaction products of patients are shown along with a schematic diagram of the dual oxidase 2 (DUOX2) protein. Arrows indicate mutated nucleotides. Note that all functional domains of DUOX2 are deleted by the E327X mutation. FAD-bd, flavin adenine dinucleotide binding domain; NADPH-bd, nicotinamide adenine dinucleotide phosphate binding domain; TM, transmembrane segment. B: A pedigree of the patient is shown with clinical parameters, which are aligned with each symbol. Values outside the normal range are typed in boldface.

DISCUSSION

The Japanese consume 1–3 mg of iodine per day [Zava and Zava, 2011], which is one of the highest iodine intake in the world. Perinatal exposure to large amounts of iodine, e.g., maternal ingestion of an iodine-rich drug, is an established cause of transient CH [Theodoropoulos et al., 1979]. The role of milder iodine excess in the pathogenesis of CH remains to be clarified. Nishiyama et al. [2004] studied urine iodine (UI) levels of 34 newborns that were positive at screening for CH in Kumamoto prefecture, Japan, and found that 15 babies had UI levels of greater than 20 μg/dl (a value corresponding to +2 SD of control newborns). Those 15 patients had only slightly high UI level (i.e., mild iodine excess), indicating

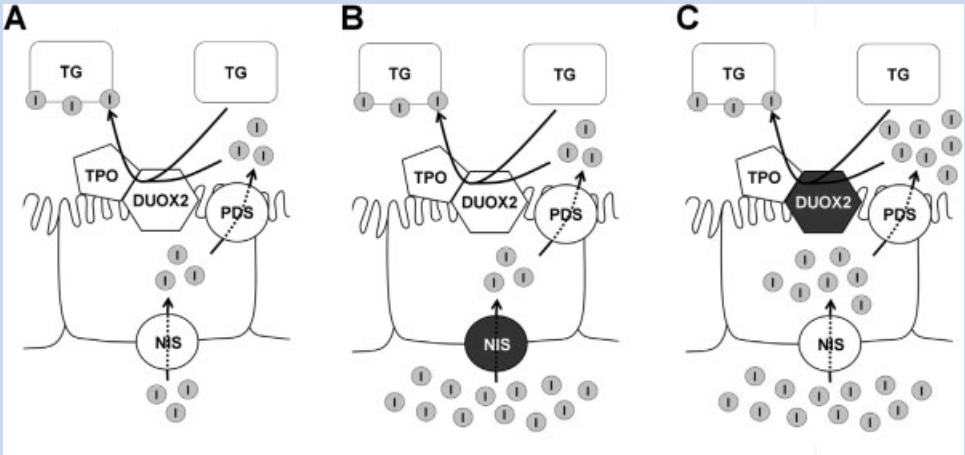

FIG. 2. Schematic diagrams showing a part of the thyroid hormone synthesis pathway. A: Iodine in blood (shown in lower part) is transported into thyroid cells through sodium-iodine symporter (NIS), and then transported into follicular lumen through another transporter named pendrin (PDS). At the apical surface of thyroid cells, iodide is incorporated to thyroglobulin (TG) by thyroid peroxidase (TPO). This reaction requires hydrogen peroxide, which is produced by dual oxidases 2 (DUOX2). B: In a thyroid with the NIS defect, excessive amount of iodine normalizes the defective iodine transportation. C: In a thyroid with the DUOX2 defect, iodine excess presumably compensates the defective iodination of TG.

that mild iodine excess can increase risk of CH. Considering that most newborns with a UI level above +2 SD (constituting 2.3% of population by definition) were assumed to have normal thyroid function, those 15 patients would be affected by other genetic and/or environmental factors making them susceptible to mild iodine excess. In any case, it has been widely accepted that iodine overload in the fetus and newborn negatively affects thyroid function. Therefore, it is notable that thyroid function was not impaired but rather restored by iodine excess in the report of patient with a *DUOX2* defect.

The thyroid hormone-producing capacity varies dramatically in the neonatal period. Several factors influence neonatal TSH and thyroid hormone levels, e.g., TSH surge at birth (followed by transient elevation of thyroid hormone), maturity of hypothalamus-pituitary-thyroid axis, iodine status (deficiency/excess), etc. Timing of onset of TSH rise can be affected by these factors, resulting in negative newborn screen in a subset of patients. Importantly, such false negatives are not rare: A study by Northwest Regional Newborn Screening Program, where second screening at age 2–6 weeks is routinely implemented, showed that the false negative rate of first screening was 7.6% [Hunter et al., 1998]. The public health impact of these false negatives remains to be clarified.

Mechanisms underlying delayed TSH rise are largely unknown except for premature birth. One important model is the genetic defect of NIS, which transports iodine from blood to thyroid cells. The NIS defect, a form of thyroid dyshormonogenesis, has high variability in age at disease onset, ranging from neonatal to adult age [Spitzweg and Morris, 2010]. Of interest, phenotypic expression of the defect is influenced by iodine status: Iodine excess alleviates defective hormone production (Fig. 2) [Matsuda and Kosugi, 1997]. Correspondingly, in our patient with the *DUOX2* mutations, large amount of transplacentally transferred iodine likely prevented her from developing hypothyroidism immediately after birth. A similar mutation-carrying case with a negative screening result has been reported by Vigone et al. [2005], although the level of iodine excess and severity of hypothyroidism differs considerably. The mechanism of alleviation of the *DUOX2* defect by iodine excess is unclear. Considering that thyroid peroxidase and *DUOX2* coordinately incorporate iodine into thyroglobulin at the apical membrane of the thyroid cells, excessive amount of iodine might compensate the defective iodination process (Fig. 2). Alleviation of hypothyroidism by iodine excess has also been reported in a patient carrying mutated dehalogenase 1 [Moreno et al., 2008], the molecule involving in intrathyroidal recycling of iodine. We speculate that similar phenotypic modification could be observed in other forms of thyroid dyshormonogenesis, such as the pendrin defect and the DUOX maturation factor 2 defect.

The main limitation of the present study is the lack of data about urine iodine determination of the proband. In this case, magnitude of iodine excess cannot be discussed in a quantitative manner.

We suspect that not only the NIS defect but also the *DUOX2* defect can have a delayed onset, probably associated with individual iodine status. Because *DUOX2* mutations are the most frequent genetic cause of CH, the defect could be an important source of false negative screen, especially in areas where baseline iodine intake level is high. Future studies targeting screening-negative CH cases will be required to develop more effective screening programs.

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