NOTE

Two Novel Mutations in the Thyroid Peroxidase Gene with Goitrous Hypothyroidism

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Abstract. We encountered a Japanese patient with goitrous hypothyroidism due to iodide organification defect in the thyroid gland. Sequence analysis identified two novel mutations (E378K in exon 8 and a heterozygous 10 base deletion of the intron 15-exon 16 boundary) in the thyroid peroxidase (TPO) gene. As individuals with goitrous hypothyroidism caused by TPO gene mutation develop thyroid cancer, regular and careful follow-up for such patients must be done.

Key words: Goitrous hypothyroidism, Thyroid peroxidase gene, TPO, iodide organification defect


THYROID peroxidase (TPO) is a key enzyme in the biosynthesis of thyroid hormone. TPO is located on the apical membrane surface of thyroid follicular cells and is responsible for the iodination and coupling of specific tyrosine residues in the thyroglobulin to form thyroxine (T4) and 3,3',5-tri-iodothyronine (T3). The human TPO gene is located on chromosome 2p25 and consists of 17 exons, encoding 933 amino acids. The genetic impairment of the TPO gene is the cause of thyroid dyshormonogenesis among congenital hypothyroidism, characterized by iodide organification defects [1, 2]. So far frameshift mutations, missense mutations, splicing mutations, nonsense mutations and gene deletion of the TPO gene have been reported in congenital hypothyroidism [1–10]. Furthermore, it is of note that mutations of the TPO gene have been identified in follicular thyroid carcinoma and adenoma [4, 11, 12].

In this study, we identified two novel mutations of the TPO gene in a Japanese patient with congenital goitrous hypothyroidism.

Case Report

The patient was born after 40 weeks of gestation by normal vaginal delivery from nonconsanguineous parents. His birth weight was 3750 g and length was 48 cm. There were no abnormal physical findings including goiter. However neonatal mass screening using filter paper for congenital hypothyroidism at 5 days of age demonstrated markedly elevated thyrotropin (TSH) level (120 mU/l, normal range 0.54–10.0 mU/l). For further evaluation, he was referred to our hospital at 10 days of age. At this time, his serum TSH was 256.8 mU/l, T4 4.3 /g109 g/dl (normal range 5.1–11.4 /g109 g/dl), T3 86 ng/dl (normal range 90–170 ng/dl). Under a diagnosis of congenital hypothyroidism, L-thyroxine (L-T4) therapy was started immediately. His developmental milestones were within normal range. During follow up, a small goiter was noticed at 2 years of age. 123I uptake at 3 hours was 48.1% and 75.3% of 123I was discharged at 60 min after the oral administration of KClO4 at a dose of 0.4 g in the perchlorate discharge test, confirming iodide organification defect in the thyroid gland. During thyroid hormone replacement therapy, the goiter remained. At 15 years of age, an ultrasound examination of his thyroid gland showed enlarged goiter (6.5 cm × 6.9 cm) and four nodules in the right lobe and two nodules in...
the left lobe. All these nodules had an inhomogeneous echo structure and showed augmented vascularization in Doppler ultrasonography. At this time, his serum thyroglobulin was high (80.1 ng/ml, normal range <30 ng/ml). Thyroid antibodies were negative. At 18 years of age, the size of his thyroid gland slightly decreased (6 cm × 5 cm) by ultrasound examination. Now he is 20 years old, and the size of goiter is not changed, and several nodules are present.

Informed consent to participate in the study was obtained. Approval of the Hokkaido University School of Medicine ethics committee was also received. Genomic DNA was extracted and each exon of the TPO gene was amplified by polymerase-chain-reaction (PCR) using primers according to previous studies in a Perkin-Elmer Gene Amp PCR System 2400 thermal cycler (PE Applied Biosystems, Foster City, CA) [4, 7]. After amplification, the purified PCR products were sequenced directly with an ABI PRISM Dye Terminator Cycle Sequencing Kit and an ABI 373A automated fluorescent sequencer (PE Applied Biosystems, Foster City, CA) from both strands.

Results

Sequence analysis of the TPO gene demonstrated two novel mutations. One was a heterozygous missense mutation 1222 G→A, that changes a glutamic acid to lysine in exon 8 (E378K) (Fig. 1A). This amino acid substitution was not identified in 50 healthy controls. Two known different heterozygous polymorphisms were also found:1207 G→T and 1283 C→G in exon 8 [7, 8]. The other was a heterozygous 10 base deletion of intron 15-exon 16 boundary (Fig. 1B). His parents were not subjected to DNA analysis.

Discussion

We identified two novel mutations of the TPO gene in one patient with goitrous hypothyroidism. We did
not determine the functional consequences of these two mutations in vitro. However, a deletion mutation at the intron 15-exon 16 boundary might cause abnormal splicing. The sequence of the exon 13–17 is well conserved in TPO, which encodes the membrane spanning part of TPO [1, 2, 7]. Thus, this mutant is not likely to insert the membrane properly. The other missense mutation is E378K. This glutamic acid is highly conserved in the human, pig, mouse and rat TPO. In addition, glutamic acid is acidic, however, lysine is a basic residue. As exon 8 encodes the catalytic center of TPO, this amino acid substitution may affect the enzymatic activity. This aspect must be further investigated.

A number of cases of thyroid cancer developed from dyshormogeneic goiter [11, 12]. Among them, Umeki et al. have described the development of adenomatous goiter during thyroid hormone replacement therapy in a patient with mutations of the TPO gene [13]. Furthermore, thyroid carcinoma arising from congenital goiter due to mutations of the TPO gene was reported [11]. These findings suggest that goiter due to defects of the TPO gene is prone to develop tumor.

In conclusion, we identified two novel mutations of the TPO gene. Regular and careful ultrasound investigation for thyroid cancer in the patients with TPO defects should be performed.

References