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Resistance to Thyroid Hormone Caused by a G344R Mutation of Thyroid Hormone Receptor Beta Gene: A Case Report Study

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Abstract

Background: Resistance to thyroid hormone (RTH), is a rare genetic disease. The aim of this research is to study a pediatric case of resistance to thyroid hormone (RTH) and its clinical manifestation.

Methods: An infant demonstrating resistance to thyroid hormone (RTH) and her parents were subjected to THRB gene analysis by PCR amplification of peripheral blood DNA. The sequences were aligned and compared.

Results: A heterozygous mutation, c.1030G>C, was found in exon 10 of THRB gene resulting in an amino acid substitution, G344R, in the encoded protein.

Conclusion: In Chinese children, a heterozygous mutation of c.1030G>C in THRB gene may cause RTHS.

Keywords: Resistance to thyroid hormone; Thyroid hormone receptor beta; Thyroid hormone; Gene mutation

Abbreviations:

RTH: Resistance to Thyroid Hormone; THIS: Thyroid Hormone Insensitivity Syndrome; THRB: Thyroid Hormone Receptor Beta; TSH: Thyroid-stimulating Hormone; PRTH: Pituitary Resistance to Thyroid Hormone; TRIAC: Tri-iodothyronine Acetate

Introduction

Thyroid hormone insensitivity syndrome (THIS), also known as resistance to thyroid hormone (RTH), is a rare genetic disease. The cardinal features of this syndrome of reduced sensitivity to thyroid hormone are elevated serum levels of free thyroid hormone with nonsuppressed TSH, often with goiter and no clear symptoms and signs of thyrotoxicosis [1]. The incidence of RTHS is 1:40,000~1:50,000 in neonates [2], without gender or racial differences.

RTH is an autosomal dominant disease. However, a small proportion of RTHS is sporadic and autosomal recessive [1]. This disease is mainly caused by a genetic mutation in the thyroid hormone receptor beta (THRB) gene, producing a mutant protein with a dominant negative effect on the TH receptor [3]. This receptor is a nuclear protein encoded by the proto-oncogene erbA in normal cells. From the N-terminus to the C-terminus, there are six regions (A-F) that are classified into four functional domains, including a T3 binding region and a DNA binding region.

In humans, THRA and THRB genes are located on chromosomes17q11, 2-21 and 3p22-24, respectively [4]; each encodes two products, known as RTHα1, TRα2, RTHβ1, RTHβ2. RTHα2 cannot bind to TH but competitively binds to the TH response element to inhibit the function of RTHα1 and RTHβ. TRs are widely expressed, but the distribution of different RTH subtypes varies significantly among tissues. RTHα1 and RTHβ1 are distributed in most tissues while RTHβ2 is only expressed in the pituitary gland, hypothalamus, retina and inner ear [5].

Clinically, RTH is characterized by increased serum (TH), while thyroid-stimulating hormone (TSH) is not suppressed. However, the clinical manifestations of RTH vary significantly between families, ages and even between members of the same family. Some patients show manifestations of hyperthyroidism while others exhibit hypothyroidism. Thus, the disease is usually misdiagnosed. In clinical practice, RTH may be diagnosed when one of following conditions is observed [6]:

1. Patients present with thyroid gland enlargement and normal thyroid function, but have increased serum totals of T3, T4, FT3, and FT4 in multiple examinations;
2. Patients present with thyroid gland enlargement accompanied with hypothyroidism and increased serum totals of T3, T4, FT3 and FT4;
3. Patients present with thyroid gland enlargement, clinical manifestations of hyperthyroidism, increased serum levels of T3, T4 and TSH but pituitary tumors are absent;
4. Patients are non-responsive to high-dose TH treatment with hypothyroidism;
5. Patients are susceptible to the recurrence of hyperthyroidism after treatments and the exclusion of pituitary tumors is confirmed; and
6. Patients whose family members have an RTH history.

In addition, mechanistic insights about the contributions of THR beta to various processes, including colour vision, development of the

cochlea and the cerebellum, and normal functioning of the adult liver and heart, have been obtained by either introducing human THRB mutations into mice or by deletion of the mouse THRB gene [7].

The first report of RTH was by Refetoft et al. in 1967 [8], and since has been reported in more than 100 families in China and worldwide. Although initially limited, the knowledge of the molecular pathogenesis, clinical manifestations and therapies for RTH is accumulating. Here, we report a pediatric case of RTH using a family-wide mutation analysis and summarize the clinical characteristics of the patient. Mutation analysis of the THRB gene is not only informative but also helpful for the early diagnosis of RTH, especially for those with mild or no symptoms. These findings present a better understanding of RTH and are also helpful for a preventive treatment approach and familial genetic counseling.

Materials and Methods

Patient description

A 10-month-old female was diagnosed with RTHS in Beijing Children`s Hospital. She was born after full-term gestation. The birth weight and height were 2.4 kg and 45 cm, respectively. There was no history of asphyxia and hypoxia during parturition. She was breastfed normally. On day five after birth, a blood screening showed that the TSH level was 49.7mU/L (0-9mU/L). On day 18, the TSH level was 50.7mU/L. On day 44, venous blood was collected for the detection of thyroid function and the results showed thatFT3 was 12.4pmol/L (3.19-9.15pmol/L), FT4 was 32.2pmol/L (9.1-25.47pmol/L), TSH was 44.1uIU/ml (0.3-5uIU/ml), thyroglobulin antibody (TG-AB) was 6.9% (<30%) and thyroid microsomal antibody (TM-AB) was 4.4% (<20%).

Two magnetic resonance imaging examinations of the pituitarygland were normal. Adrenocorticotropic hormone and prolactin levels were also normal. Her physical appearance, growth and milk intake were normal. Urination and defecation were also normal. Her parents were not close genetic relatives. There was no concomitant increase of serum FT3/FT4 and TSH. Given that the pituitary imaging was normal, RTH was the considered diagnosis for this case.

PCR amplification and sequence analysis

After receiving informed consent from the parents and hospital ethical committee, peripheral blood (2ml) was collected from the patient and both the mother and father. The blood was EDTA-treated and the genomic DNA was extracted using the TIANamp genome extraction kit (Tiangen (Beijing) Biotec China). For PCR amplification, the genomic sequences of THRB gene between exons 4 and 11 were amplified. The PCR primers are shown in Table 1; they were designed based on the RTHβ genome sequence obtained from the GeneBank database using Primer premier 5.0 software (Premier Biosoft International, America). The reaction mixture (50uL total) used for the PCR amplification consisted of 20pmol forward primer, 20pmol reverse primer, DNA template (0.1ug) and Easy Taq Super Mix (25uL).

The conditions for the PCR reaction were as follows: pre-denaturation at 95 for 4 min, followed by 38 cycles of denaturation at 95 for 40s, annealing at 60 for 40s and extension at 72 for 1min. After amplification, PCR products were separated by agarose gel electrophoresis. The bands were observed and retrieved with a gel retrieval kit according to the manufacturer's instructions. DNA sequencing was performed using the ABI 3730XL sequencer. BLAST software was used to compare the target sequence to known sequence obtained from GeneBank to identify potential THRB mutations among the family members.

Exon	Sequence(5` to 3`)	Length (bp)
4	FGCTTCAAGGTAATTAATGCATAT RCAGAAGAAGATGGAAGTACATTT	393
5	FAAGTGGCATGTGAATACTGTC RCAAGTGTCCCAGCACAAC	262
6	FTGGCCTAGCAACCTTAGC RTGAAGAGCACAACCAGACAC	282
7	FTTGAATATGTGGAGCTAGAGG RAGGTGGTATGACAGGGTAGG	391
8	FTGCTGGTGAGGAGAGAAACT RAGTAAAATGAGGCAATAACACC	374
9	FAATGATGTGAAAACTGGAAAC RCGGACAAGCTAATGAATGAT	405
10	FGGTTGTCGAAAGTCTGCAG RGTATCCACCAAAGGCGG	360
11	FTATCTGAACAAAGTACTGCCTC RCGAGAACGAAATGCAATAGT	325

Table 1: Primers used in amplification of the THRB gene. F: Forward primer; R: Reverse primer.

Results

A heterozygous mutation, c.1030G>C, was identified in exon 10 of the THRB gene from the patient (Figure 1A). This missense mutation caused the transformation of Gly to Arg at amino acid 344 and subsequently affected the protein function. No mutation was found in the THRB gene from the parents (Figure 1B and 1C). These results suggest that the c.1030G>C mutation was novel. The child in our study was asymptomatic with normal growth rate and development. Thus, no specific treatment was initiated.

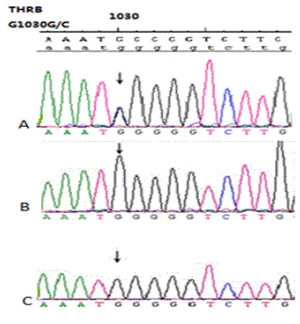

Figure 1: (A) Gene sequencing figure of the patient. The mutational site is indicated by arrow, referenced base sequence is G, and the patient's base sequence is heterozygous G/C. (B) Gene sequencing figure of the patient's father. (C) Gene sequencing figure of the patient's mother.

Discussion

Up to now, there are more than 3000 cases have been identified, 80% of which harbored mutations in the THRB gene [1]. These mutations include missense mutations, deletions and insertions. THRB gene includes 11 exons and encodes 461 amino acids. While exons 1-3 encode the non-translated region, THRB gene mutation hotspots are clustered at the C-terminal lig and binding domainat residues 234-282, 310-353 and 429-461corresponding to exons 7-10 [9]. The genome in this area is CG-rich.

In the present study, a heterozygous mutation, c.1030G>C, was found in exon 10 of the THRB gene in the RTH patient. This mutation caused the transformation of Gly to Arg at amino acid 344 (G344R) and was located in one of the mutation hotspot regions. Mutations at amino acid 344, causing the transformation of Gly to Glu or Ala, have been reported previously and both were pathogenic [10]. To our knowledge, the G334R mutation has not been reported previously.

Of note, the mutation was not found in the corresponding loci of the parents. This suggests that the mutation occurred *de novo* in Chinese children. Also, the mutation may have occurred in a parent's gamete and transmitted to the proposita or may have occurred at a very early stage in her embryonic life. Our analysis indicated that the mutation led to an alteration of TR structure. Altered protein structure reduces or even inhibits TR binding to TH. In addition, the mutant receptor may inhibit normal receptor function and interfere with downstream signaling, known as a "dominant negative effect" [3]. Functional studies have shown that the product of the maternal allele was characterized by a dramatic impairment of TRH binding activity and by the loss of inositol phosphate response to TRH stimulation [11].

RTH can be classified into three types according to the degree of tissue resistance to TH [12]:

1. Pituitary resistance to thyroid hormone (PRTH). Patients with PRTH are characterized by hyperthyroidism and above-normal TSH levels without a pituitary tumor;
2. Generalized resistance to thyroid hormone (GRTH) where TH resistance is observed in the pituitary and peripheral tissues;
3. Peripheral thyroid hormone insensitivity syndrome (PerRTH).

This is characterized by non-responsiveness of peripheral tissues to TH while the pituitary is not involved and responding to TH stimuli. Although the TH and TSH levels are normal, patients usually develop manifestations of hypothyroidism. Generally, patients with complete resistance or homozygous gene mutations have more severe clinical symptoms while those with incomplete resistance often have mild symptoms.

The diagnosis of RTH resistance is based on the following findings:

1. High T4 associated with normal TSH levels;
2. No combined pituitary hormone deficiencies;
3. No pituitary lesions with MRI/CT scan;
4. Absent/impaired responses of serum TSH and PRL to TRH stimulation [11].

Thyroid function assays are not specific among the three types of RTHS. Thus, the results could be from elevated TH with elevated or normal TSH. In this case, the patient had no obvious goiter or feeding difficulty and no dermal moisture changes or weight loss. Her growth and development in both height and weight were comparable to her peers. Clinically, she had no typical manifestations of hyperthyroidism and hypothyroidism; the thyroid functional test was also normal with no thyroid gland enlargement. Thus, this child was diagnosed with GRTH.

GRTH is occasionally observed in routine examination at any age. The ratio of female to male incidence is about 1:1. GRTH is characterized by the hyperthyroxinemia without hyperthyroidism and patients usually have normal thyroid function. On occasion, the patient may have even developed hypothyroidism. So far, the transition between RTH subtypes has not been reported.

Currently, there is no effective treatment to cure RTH. For most patients, tissue resistance is compensated by increased biosynthesis of endogenous TH and does not require any therapeutic intervention [13]. The child in our study was asymptomatic with normal growth rate and development. Thus, no specific treatment was initiated. However, for patients with hypothyroidism, especially those developing RTHS during infancy, supplementation with exogenous TH is necessary and the dosage should be customized.

For patients with hyperthyroidism, triiodothyronine acetate (TRIAC) is preferred. TRIAC is ametabolite of TH and can significantly inhibit TSH activity but has no metabolic activity. Thus, it may reduce the serum levels of TSH and TH and alleviate thyroid enlargement and hyperthyroidism. For patients with tachycardia, palpitation and shortness of breath, β blockers are preferred [11-13]. In addition, dopaminergic drugs, such as bromocriptine, and the somatostatin-like drugs can reduce TSH and TH levels, although they may have some long-term side effects. Infantile RTHS should be treated promptly so as to maintain normal growth and development. Of note, normalization of serum TH levels is not a therapeutic indicator; instead, the treatment regimen should be personalized and the clinical presentation should be carefully observed. Follow-up studies including a regular physical examination and detection of TH and TSH should be analyzed to record any early clinical changes.

Conclusion

The clinical manifestations of RTH are diverse. Though it has been extensively studied, it is often misdiagnosed and erroneously treated in China. Early diagnosis can be challenging. THRB gene mutation analysis provides evidence for the molecular diagnosis of RTH for a symptomatic patient. For patients with diverse manifestations of RTH, the therapeutic strategies should be personalized, particularly for infantile RTH. Routine THRB gene mutational analysis is recommended for patients suspected with RTHS. The results of this case study indicate that THRB gene mutations can be detected in asymptomatic patients and supports screening for RTH in newborns to help with early diagnosis and treatment.

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